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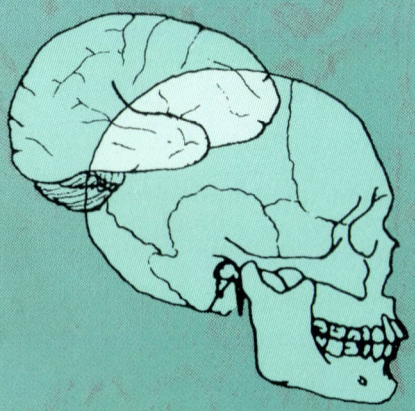

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RESEARCH REPORT

A COLUMNAR-SUPPORTING MODE OF ASTROGLIAL ARCHITECTURE IN THE CEREBRAL CORTEX OF ADULT PRIMATES?

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Neuronal modular (columnar) organisation of the cerebral cortex may represent an evolutionary acquisition that could optimize communication and information processing with the least volume compromise in terms of wiring. Yet, for such columns to be functionally operative adequate isolation from neighbouring units would be required, otherwise their spatial definition could be compromised. Evidence for "cable-like" processes stemming from astroglial cells has been recently presented although their existence has been forgotten for more than a century. These processes tend to form a sort of "palisade" or "brush" whose spatial distribution appears to correlate with the distribution of apical dendrites within the supragranular cortical layers. Patterned neuronal organization in the striate cortex is associated with a patterned distribution of GFAP-IR processes, both at the cellular and cell-aggregate levels. It can be tentatively proposed that evolutionary pressures resulted, in primates, in the progressive appearance of an increased length of astroglial processes in the supragranular region which may be associated with optimization of cerebral cortex modular (columnar) organization. It is interesting that this cortical region has undergone the larger growth among mammalian species during evolution, and would bear a crucial role in corticocortical interactions.

Key words: astroglia, cerebral cortex organization, columnar organization, primate evolution, cell processes

INTRODUCTION

Morphological observations of the elements of the nervous system often provide significant hints on its functional organization. Most notable, the anticipatory views on several fundamental issues of central nervous system organization by Cajal and several other celebrated members of the early days of Neuroscience were based on morphological analysis. Even in the early days of the "nervenkitt", daring (for the time) opinions on the possible fundamental role of glia have been recorded. Several names could join this group, such as Lugaro (1907), Virchow (1842) and Dutrochet (1842) (cf. Varon and Somjen 1976), Nageotte (1910), Andriezen (1893), Retzius (1894), Schlacher (1894) (cf. Dierig 1994), Achucarro

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(1913) and del Río Hortega (1942) among others. Yet, it was not until recent times that speculations on possible role(s) of astroglia was supported by adequate, powerful procedures that allowed for both an aggregated and single cell functional analysis. But stilt the emerging predominant view has been one of "mass operation", of an "expanded syncitium" able to transfer local perturbations of the extracellular environment or of local neuro-glial interactions into relatively distant loci within such "syncitium". Important as it may be this concept, coupled to the notion of "conditional" gap junctions that may modify the actual functional geometry of such "syncitium" (Salm and McCarthy 1990; Giaume and McCarthy 1996) it seems that to some extent this almost excluding concept is also the product of our classical views on the morphology of the mature astrocyte. According to doctrine, from a strictly morphological point of view a mature astrocyte of the gray substance is essentially characterized by a stellate configuration of its processes, which may be interactive with several structures of the brain parenchyma and adapted to them (see e.g. Reichenbach 1989). This basic view is supported by *in vivo* and *ex vivo* research performed almost systematically in our largely preferred laboratory species of the mammalian order *rodentia.* This astroglial prototype and its aggregates may recognize functional regional variations. Yet, two basic properties seem to withhold: its stellate individual morphology and its syncitial aggregation. Of course, some variations could take place in terms of its ultimate branching pattern, or preferred direction of its processes (as the subpial ones), or the expression of membrane receptors, or of its cytoskeletal proteins. These variations imply a certain degree of non-homogeneity within the "panglial syncitium". The possible impact of the former on its actual, probably dynamic, spatial configuration is still under study.

If we agree to make two apparently reasonable assumptions, i.e., that glia is intimately interactive with neurons in terms of their growth, fate and function, and that in case of the cerebral cortex its neuronal architecture is based on a modular (columnar) architecture, an apparent inconvenient combination of patterns would seem to emerge. On the astroglial side a syncitial configuration, in which because of its very nature a principle of relatively equipotent tissue volume, coupled to the ability of transfering ions/molecules through relatively long distances within the neuropil, would rule from an operative stand point. Local concentration of extracellular ions/molecules and uneven distribution of membrane receptors in glial cells could somewhat modulate the geometrical expression of this functional syncitium. On the neuronal side, an organization that in the cerebral cortex seems to operate based on a columnar distribution of its elements has been proposed both on ontogenetic and evolutionary grounds (Rakic 1995; Bugbee and Goldman-Rakic 1983). Such modular architecture would result, in large brained species, in an economy of wiring spenditure for communication purposes (Ringo 1991). Viewed in such terms, cortical columns (whether micro- or minicolumns) (Powell and Mountcastle 1959, 1997; Szentágothai and Arbib 1974; Szentágothai 1978) may represent an evolutionary acquisition that could optimize communication and information processing with the least volume compromise in terms of wiring. Yet, for such columns to be functionally operative adequate isolation from neighbouring units would be required, otherwise their spatial definition could be compromised. Consequently, ideally, neuronal columns should be mirrored by similarly organized glial compartments. It remains to be proven how this neuron-glial interaction takes place at the cell-aggregate (modular) level. Yet the proposed "panglial syncitium", appears to insert within the overall cortical organization a factor that could promote "fading" of columnar spatial definition. This, in turn, would tend to increase "noise" in the system, rather than "enhanced identity" of such cortical columns. Presumably, as a consequence of such disparate arrangement, variations in the dynamic state of local elements

within any conical neural circuit would have a distinct probability of affecting the function of somewhat distant neuronal columns, an effect that may represent an unwanted factor if the processing power of the cortical architecture relies on associations of "independent" columnar operations. Based on this rationale it could be proposed that among other, as yet unknown, possible resources for evolutionary maximization of cortical function, one possibility would rest on the development of cellular mechanisms that may enhance individual columnar operation and, consequently, information processing capabilities. One such possibility would be to incorporate means for "columnar-based" neuron-glial interaction. This could be provided by apposing to the vertical neuronal organization cable-like threads of astroglial processes that could follow the fundamental receptor segment of neuronal processes (apical dendrites of pyramidal neurons). This would provide a spatially oriented (columnar-like) resolution of neuronal ionic/molecular requirements (K^+, glutamate, Na^+) and hence reduce "volume" operations in favour of more discrete columnar operations. Another interesting possibility is that astroglial long processes could preferentially route intracellular Ca^{2+} waves along them, thus hypothetically providing means for directed signalling pathways across gap junctions.

HISTORICAL BACKGROUND

Evidence for such kind of "cable-like" processes stemming from astroglial cells has been recently presented (see below), although their existence has been forgotten for more than a century. According to available records, Martinotti (1889) (see also Cajal 1913) may have been the first who described in the human brain what Andriezen (1893) called the "caudate glial cells", a kind of glial cell whose soma was located in the subpial region (or, for the most part in lamina I), and its long processes reaching the third cortical layer. One year later this observation was also illustrated by Retzius (1894). It is not completely clear to the author whether Martinotti was actually referring to this type of glial cells, too, in what Cajal (1913) mentions as "glial cells with long radiations". It is intriguing why the spanish school of morphology did not make special note on these processes. To claim solely technical reasons would seem ludicrous based on their high inventive and exploratory activity in neuromorphology, and the early descriptions by Andriezen. Perhaps certain bias of the time, such as the intriguing and imposing presence of astrocytes with perivascular endfeet or the controversy with the German school regarding the nature of the astroglial syncitium, and the absence of a conceptual frame on the modular organization of the cerebral cortex, acted as disclaimers. Figure 1 are photomicrographs taken from histological preparations of Pio del Rio Hortega (preparations kindly provided by Drs. F. Cuevas, Argentina and A. Vianni 'Pio del Rio Hortega Museum', Valladolid, Spain) processed with triple impregnation, silver carbonate stain. It can be observed in such Figure numerous thin, very delicate processes that seem to form the background and from which it may prove difficult to ascertain their length, main direction and singularity. These processes are in fact candidates of being 'interlaminar' in nature. In spite of my effort I could not succeed in tracing original Andriezen's slides, so one has to rely on the author's statement and drawings (no photomicrographs being available) that his technique (a modified Golgi's stain) did allow for a better visualization of such glial processes. Since the relative success of that kind of techniques often depended on "hands on tricks" or details, the final quality of tissue stain was often variable and heavily dependent upon the operators expertise. At any rate, Andriezen's and Retzius' drawings are quite demonstrative

of the type of astroglia with "caudate" (interlaminar?) processes. Most notably (see below), all previous observations on this type of glia were made on primate material (human).

OUTLINE OF RESULTS AND DISCUSSION

My first encounter with these processes was rather serendipitous, on occasion of looking at possible astroglial compromise in MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) - parkinsonian monkeys. Their appearance after GFAP-IR labelling just did not fit with "what the books use to tell". From there on, the search was performed in various mammalian orders and species, and at various ages (Colombo and Puissant 1994 a, b; Colombo et al. 1995; Colombo 1996; Colombo et al. 1997a, b, c, 1998, 1999a, 2000). Although mostly of descriptive nature, the observations made so far do seem to fit within a novel and potentially interesting conceptual framework regarding participation of the astroglial architecture in cerebral cortex organization and its evolution within the primate Order. The following account attempts to briefly describe such observations and suggest a theoretical framework where to tentatively place them.

1. In addition to what could be called the "general mammalian astroglial architecture" (the so-called "panglial syncitium") of the cerebral cortex, composed of typical stellate astrocytes (intralaminar astrocytes) (Fig. 2A), the anthropoid species show a manifest vertical, radial distribution of long astroglial processes (Fig. 2 B), most consistently among *catarrhines*. These processes tend to form a sort of "palisade" or "brush" whose spatial distribution seems to correlate with apical dendrites within the supragranular cortical layers, and penetrate at least two cortical lamina (hence called astrocytes with interlaminar processes) (Colombo 1995, 1996; Colombo et al. 1995, 1998, 1999, 2000).

2. Interlaminar processes so far could only be systematically labelled using antibodies against non-phosphorylated GFAP (Biogenex, Sternberger Monoclonal Inc.). Usually they could not be labelled with a-Vimentin (Dako), a-Glutamine Synthetase (Chemicon) (also kindly supplied by H. Tumani, Germany, and I. Boksha, Russia) nor a-S100 (Sigma). Antibodies directed to phosphorylated GFAP residues (kindly supplied by Dr. M. Inagaki, Japan) appear to label only occassionally the full extent of these long glial processes, although a faint labelling of terminal segments (with appearence of puncta) could be observed using a cocktail of antibodies to several GFAP residues (unpublished observations).

3. GFAP-immunoreactive interlaminar processes, at least as they could be studied in *ceboidea* monkeys, are postnatal events of the cerebral cortex (Colombo et al. 1997b). Their long processes do not originate from modified radial glial cell processes. Also, at least in *ceboidea* their emergence takes place in the form of patches along the cortical perimeter, suggesting that full maturity of the cerebral cortex may be attained in a spatially discontinuos fashion.

4. Truly GFAP-IR interlaminar processes first appear in some prosimian species, but not all (they are present in *Microcebus murinus* but not in *Eulemur fulvus)* (Colombo et al. 1998; 2000). Long astroglial processes are present in the periamygdaloid temporal cortex in species of *chyroptera* and *insectivora,* but only occasionally go clearly beyond the thick lamina I in this region. Isolated long processes traversing lamina I and barely entering lamina II could be, occassionally, seen in the dog irrespective of cerebral cortex region. See Fig. 3 for a general view of the species distribution of these processes in our screening.

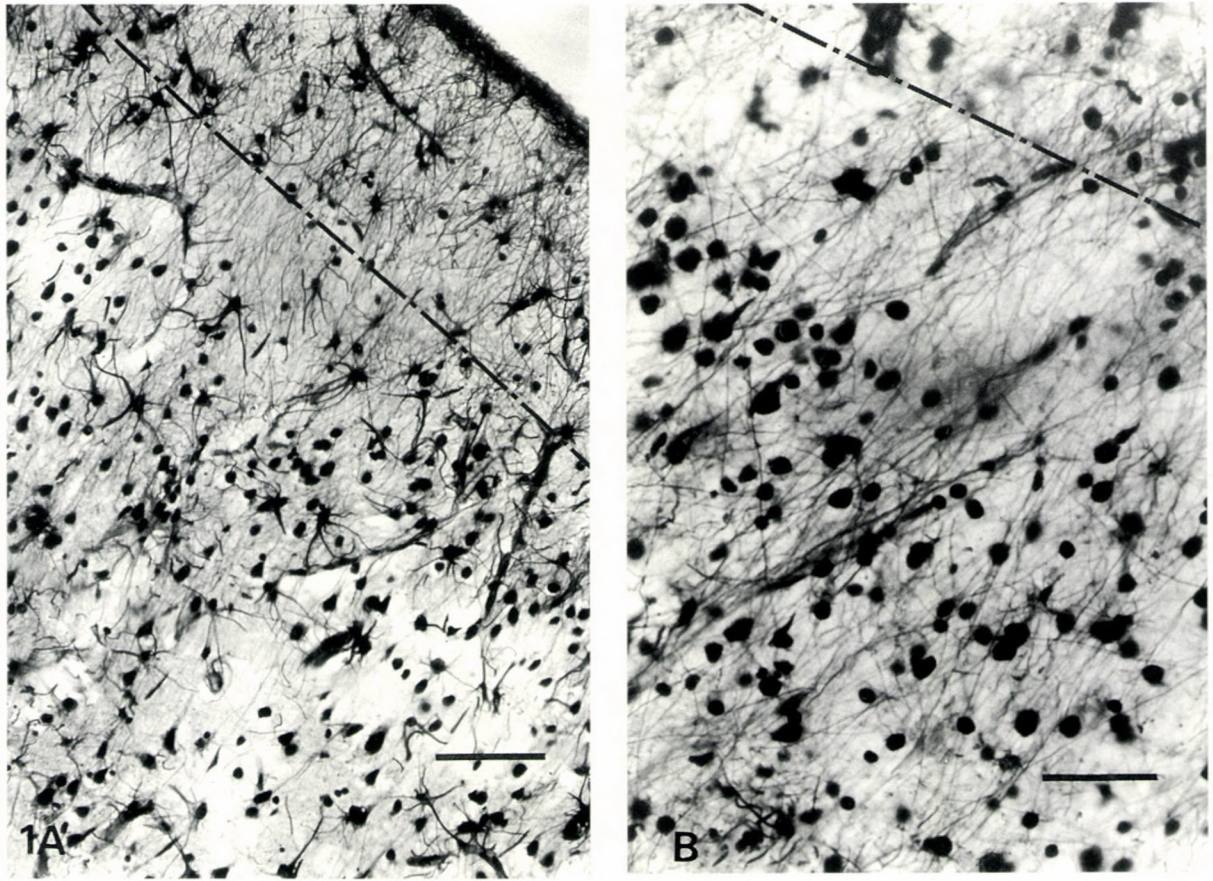
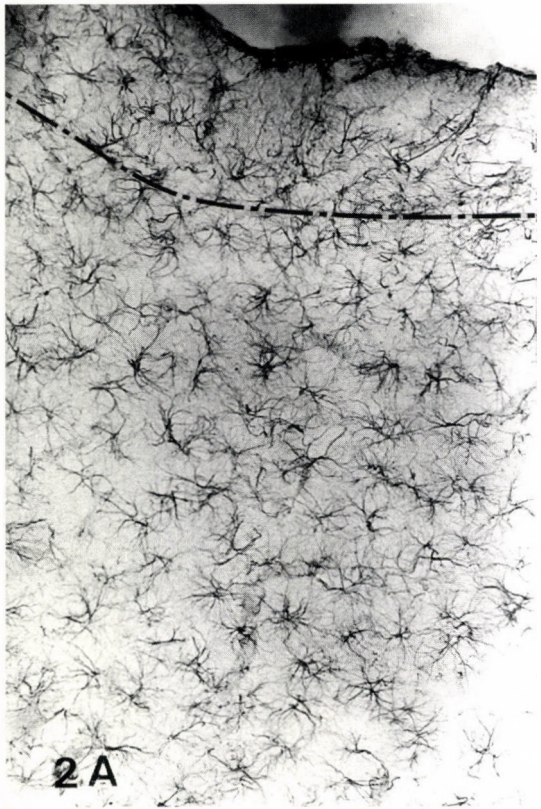


Fig. 1. Photomicrographs obtained from a histological preparation (unknown date) by Pio del Río Hortega. Adult human cortex, triple impregnation (silver carbonate). Note delicate processes on the background of what appears as a strong staining of glia. Broken line indicates limit of lamina I. Bar = (A) 100 μm, (B) 50 μm



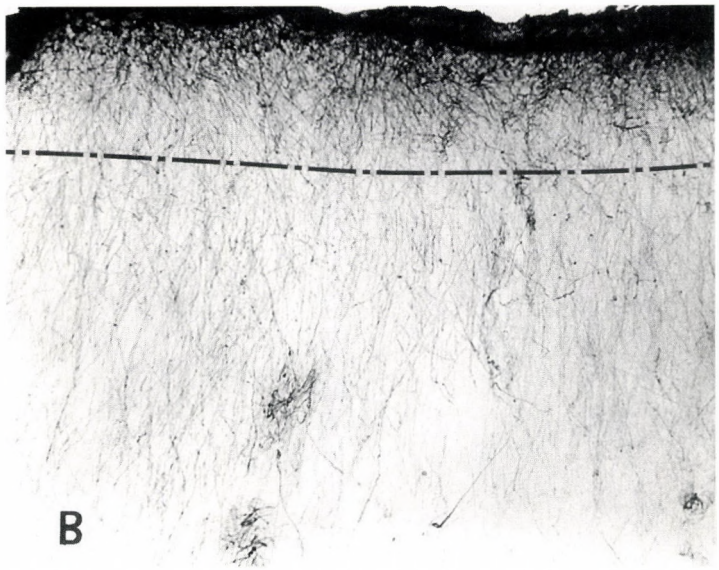


Fig. 2. Photomicrographs illustrating typical differences between the "rodent-like" (or, rather, "general mammalian-like") type of GFAP-IR astroglial architecture in (A), and "primate-like" in (B). (A) Occipital cortex from adult rat; (B) Occipital cortex from adult *Saimiri boliviensis*. Broken line indicates limit of lamina I. Bar = 100 μm

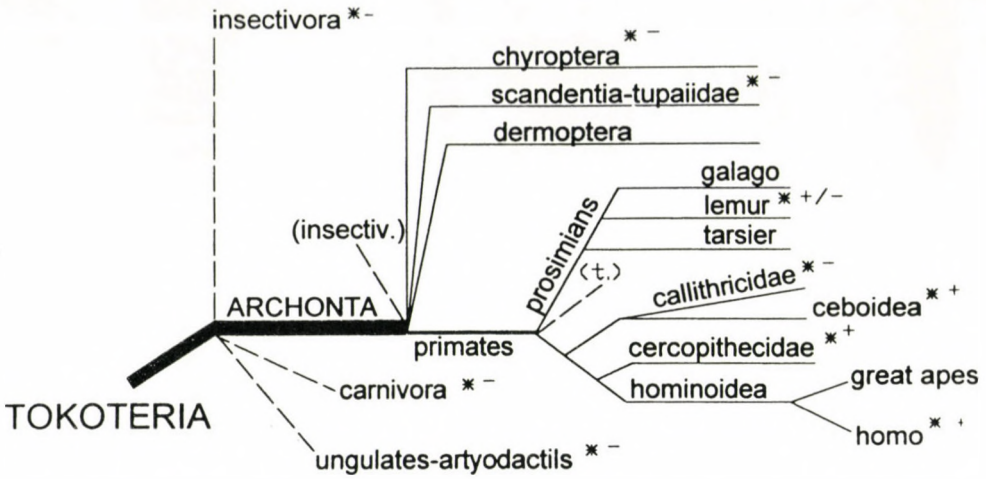


Fig. 3. Scheme of phylogenetic tree intended to illustrate the species studied (*) and whether they presented interlaminar processes (+) or not (-). Scheme after Hershkovitz 1977, Horovitz and Meyer 1997, Napier and Napier 1985, Kaas 1996. Note that tarsiers (t.) and *insectivora* (insectiv.) have been placed in two different loci of the phylogenetic tree, due to lack of agreement among authors

5. Presence and density of the "GFAP-IR interlaminar palisade" appears to increase, while interindividual and interspecies variations appear to diminish, as one proceeds from prosimians, to New World monkeys (marmosets do not seem to display this type of processes), to Old World monkeys, to human. In general *catarrhine* species show abundant and consistent presence of these interlaminar processes among species and among individuals (Colombo et al. 2000). Hence, it appears as if they would have first appeared late in evolution, perhaps some 55 million years ago, and consolidated with the emergence of *catarrhines* approximately 30 million years ago.

6. GFAP-IR interlaminar processes appear to accompany apical dendrites in supragranular layers (see scheme in Fig. 4). The interlaminar "palisade" generally stops abruptly at or close to lamina IV. Lamina I is covered by a dense syncitial arrangement superimposed to the fine terminal arborizations of the apical dendrites with numerous short processes abutting on the *glia limitans.*

7. In spite of abundant research and accumulated data on patterns of neuronal organization of the brain across mammalian species, practically no information is available regarding a possible astroglial counterpart of such repetitive entities. In architectonically highly differentiated cortical regions, as in the striate cortex (Hubel and Wiesel 1968; Horton and Hubel 1981; Wong-Riley and Carroll 1984), GFAP-IR processes also show a patterned distribution both at the cellular and cell-aggregate levels (Colombo et al. 1999a). In this study it was documented that the striate (V1) cortex of adult *ceboidea* monkeys shows clear GFAP-IR astroglial patterns, which were disclosed in coronal and tangential sections. This took place in the form of honeycomb-like cells whose size frequency distribution was similar to the

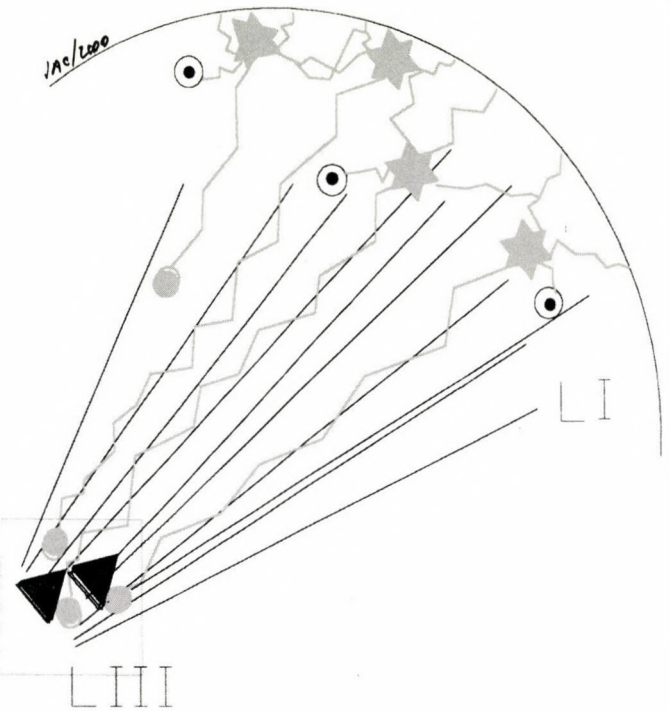


Fig. 4. Highly schematic diagram illustrating the spatial overlap of apical dendrites (in black) and interlaminar processes (in red). Blood vessels are depicted as black doughnuts. Large (red) dots = terminal masses. L I and L III = cortical Lamina I and III. Black triangles = lamina III pyramidal cells

distribution of distances in-between fascicles of apical dendrites (with its peak ranging between 27-32 μm; see also Peters (1994) and Peters and Sethares (1996). Also, at the cellular level glial processes appear to follow the presence of fascicles of apical pyramidal dendrites (Fleischhauer et al. 1972) or "pyramidal columns" (Peters 1994). At the cell-aggregate level, dense patches of GFAP-IR material (process endings) forming aggregates 200-500 μm in diameter were observed in tangential sections of lamina I-III (Fig. 5A), with processes frequently terminated in a twisted segment (Fig. 5B), often decorated at its end with a spheroid mass (Fig. 5C).

The mentioned GFAP patterns appear to be affected following suppression of visual input. The dependency of some of these events on neuronal input in the visual system was explored following surgical visual deprivation in an adult *Cebus apella* monkey (Colombo et al. 1999b). This intervention resulted in evidence of bilateral degeneration of the optic nerve three months later and changes in the GFAP-IR interlaminar processes in the striate cortex. These changes consisted in reduction of the GFAP-IR interlaminar processes so that the dense patches were no longer present in horizontal sections, nor the characteristic honeycomb-like cells (Fig. 6). In neighbouring sections MAP-2b did not show appreciable changes. These results suggest that

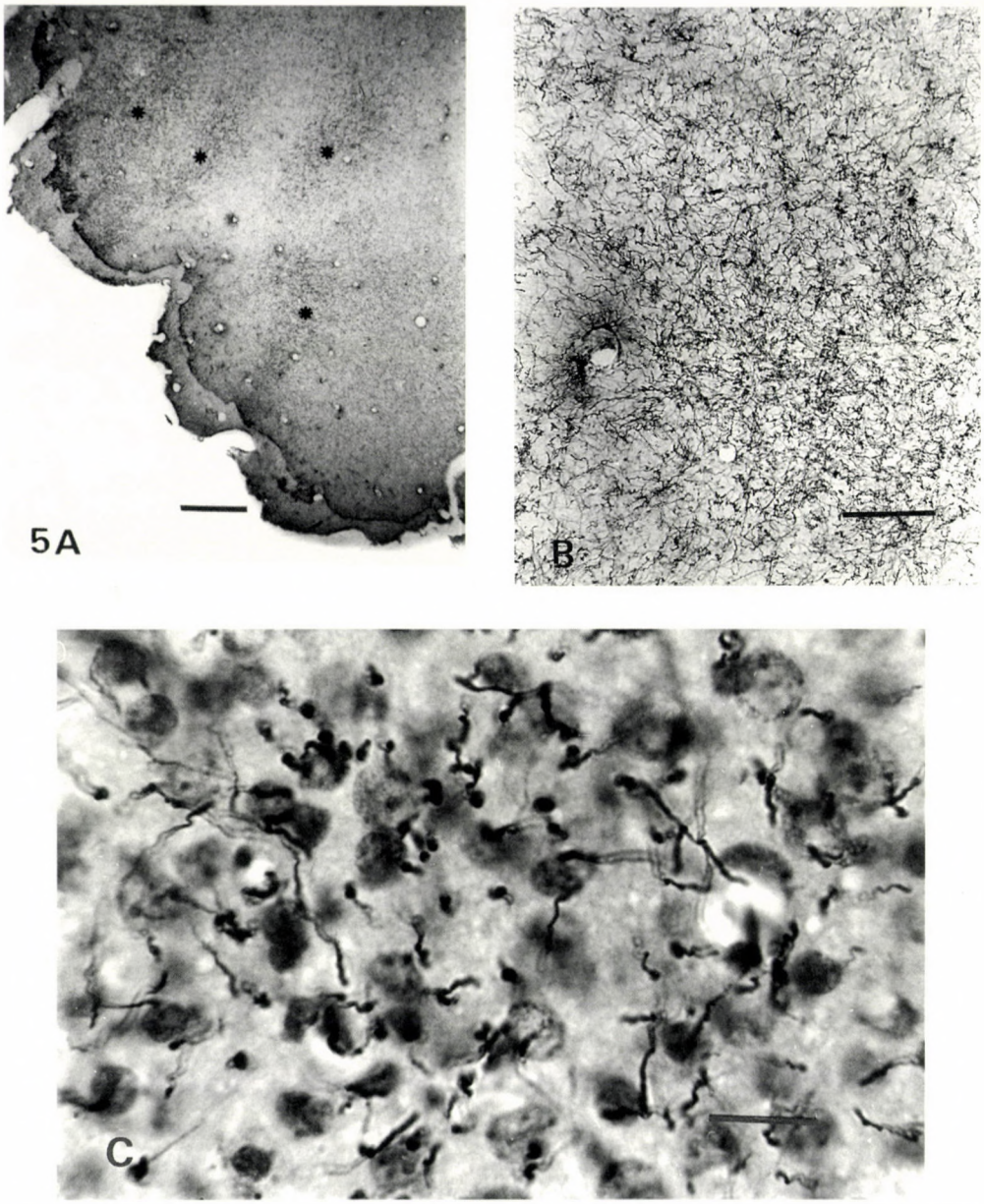


Fig. 5. Tangential sections of striate cortex from an adult *Saimiri boliviensis* monkey, reacted for GFAP. In (A), note patches (asterisks) of high density packing of GFAF-IR elements. In (B), at higher magnification, a patch can be seen composed of terminal segments of highly curly appearance, which may be better seen at the periphery of the patch. In (C), with Nissl counterstain, note curly terminal segments and the numerous "masses" decorating them. Bars = (A) 200 μm, (B) 100 μm, (C) 20 μm

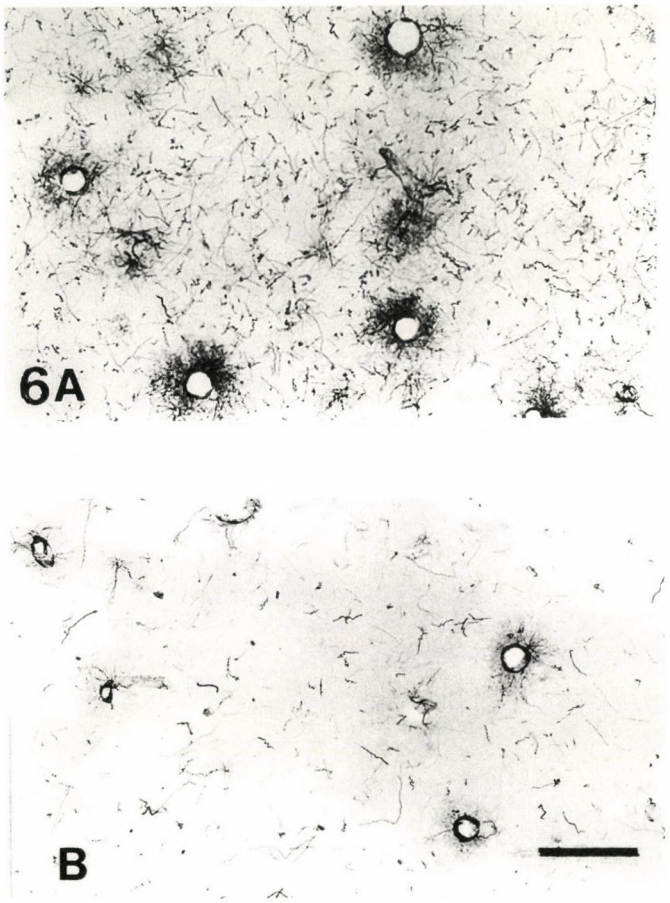


Fig. 6. Alteration of the honeycomb lattice of GFAP-immunoreactive elements, observable in tangential sections of striate (V1) cortex in a *Cebus apella* monkey, following surgical visual deprivation (reproduced with permission of the J. Brain Res.) (Colombo et al. 1999b). (A) Intact. (B) Following three months of surgical visual deprivation. Bar = 100 μm

GFAP-IR astroglial processes are an integral part of the modular organization of the cerebral cortex, and that they are susceptible to changes in neuronal activity.

8. Non-primate mammalian cortices with similar overall or laminar thickness do not express interlaminar astroglial processes. Consequently, the "astroglial interlaminar palisade does not seem to be generally associated with increased thickness of the cerebral cortex or its constituent lamina, nor to the lissencephalic or gyrencephalic condition of the cortical surface. Hence, interlaminar processes are not a universal cellular adaptation to increased length of apical dendrites within the mammalian class or, in general, with increase in cortical thickness.

9. It can be proposed that evolutionary pressures on primates resulted in the progressive appearance of an increased length of astroglial processes in the superficial part of the cortex,

the supragranular region. It is interesting that this region has undergone the larger growth among mammalian species during evolution (Zilles et al. 1982; Marín-Padilla 1998), and that would bear a crucial role in cortico-cortical interactions (Jones et al. 1975; Rockland and Pandya 1979; Tigges et al. 1981; Levitt et al. 1993; Kritzer and Goldman-Rakic 1995) (see scheme in Fig. 7). It is precisely this region of cortical cytoarchitecture that has shown a consistent development of astroglia with interlaminar processes.

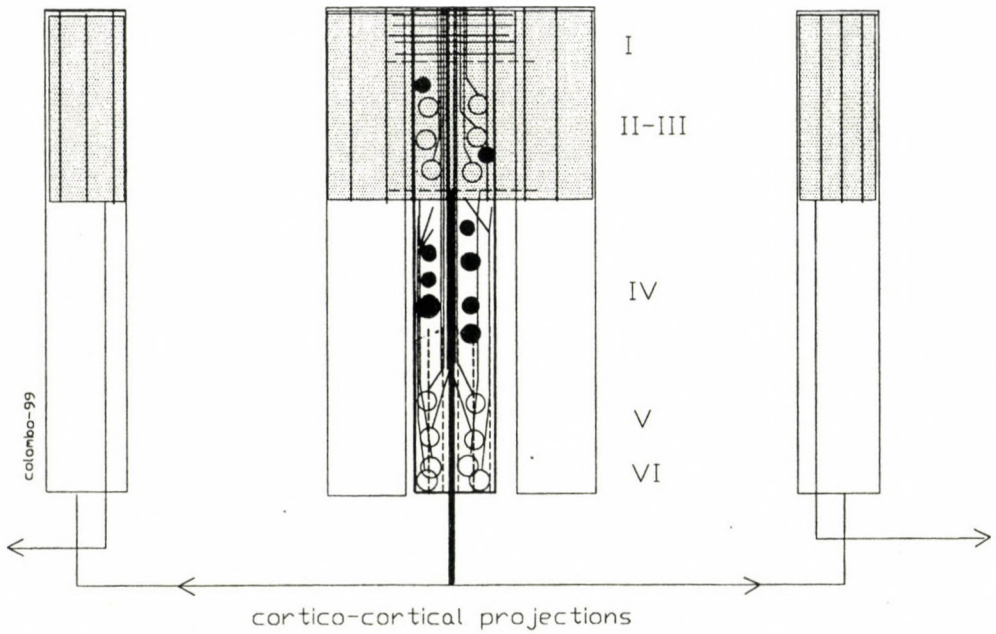


Fig. 7. Scheme illustrating possible arrangement of cortico-cortical neuronal processes stemming from the supragranular region (gray area) and associating distant cortical columns, in order to stress the hypothetical relationship of glial interlaminar processes with the function of such cortical communication processing set up. Vertical (red) lines= interlaminar processes. Inside central column= empty circles, pyramidal cells; solid circles, granule cells; black lines, apical dendrites from pyramidal cells; (red) dotted lines in the inferior portion, glial processes occasionally observed in deeper lamina. I - VI = cortical lamina. Although the schematic distribution of cellular elements inside the central column partially includes suggestions made by Peters and Sethares (1996) for the visual cortex, for the purpose of this illustration it is only anecdotic, and no cortical regional specialization of columnar set up of cellular events is necessarily implied

10. Certain neurodegenerative processes associated with severe cognitive alterations (e.g., Alzheimer's disease) show disorganisation and eventually disappearance of the "immunoreactive interlaminar astroglial palisade" which is usually replaced by a reactive astroglial syncitium (Colombo, Quinn and Puissant, submitted). Mechanical lesioning also induces a (perhaps transitory) loss of the interlaminar palisade (Colombo et al. 1997c). Hence, these glial cell processes do not appear to survive mechanical lesioning or degenerative

conditions and, at best, in the former case may reappear once post injury reactive processes have receded.

CONCLUSIONS

In conclusion, it seems as if the growth of interlaminar processes in primate species would be locked-in to changes operating mostly in lamina I-III. The fact that growth of these supragranular laminae have been linked with cortical development in primate species may not be coincidental, and is probably signalling a conceded evolutive neuron-glial event. In fact, what appears to be a close association between astroglial interlaminar processes and apical dendrites suggests that elongation of the latter during primate brain evolution would have been met using a complementary strategy in addition to the astrocyte net. That is, by associating long, mostly radially oriented astroglial processes capable perhaps of acting as sink elements for extracellular ions along the apical dendritic path. This would avoid or reduce excessive amount of overlap or sharing of the astroglial individual buffer capacity amongst local and distant (net mediated) neuronal elements, and perhaps also imposing a preferred directionality to intercellular communication based on Ca^{2+} waves (see scheme in Fig. 8). These dynamic possibilities of interlaminar processes will have to be experimentally tested before their full significance could be determined. Yet, although speculative at present time, such physical relationship and possible functional advantages of the "primate-like" astroglial model offer new conceptual possibilities which ought to be further explored. In order to analyze dynamic characteristics of these long processes. Due to their exlusively primate origin, adequate experimental procedures ought to be developed. One such possibility could be "tissue printing" procedures aimed at acutely sampling the cerebral cortex and also allowing for short-term storage under cell culture conditions (Colombo, Napp, Yáñez and Reisin, submitted).

Finally, within an evolutionary context, these observations point to the fact that astroglial architecture of the cerebral cortex would have undergone significant changes in certain "niches" of the mammalian radiation, departing from the exclusively "general mammalian" astroglial layout. Although still on speculative grounds, those changes would have aided in optimizing the performance efficiency of "largely neocortical" brains such as those of primates.

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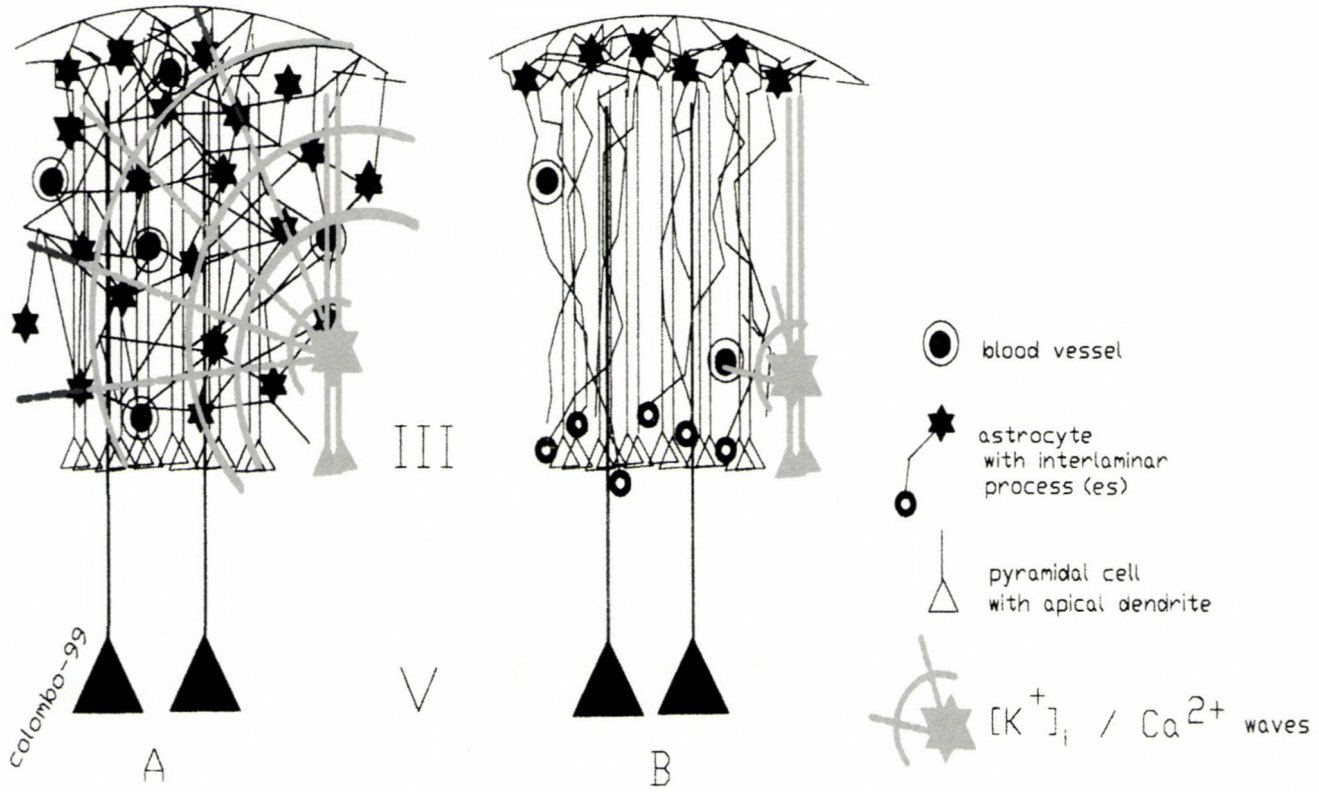

Fig. 8. Highly digrammatic illustration of one hypothetical functional consequence of the presence of interlaminar glial processes in the cerebral cortex of adult primates: spread of ionic/molecular perturbations (K^+/Ca^{2+} waves) subsequent to the local activation of cellular elements. (A) Purely syncitial glial arrangement. (B) Purely interlaminar glial arrangement. In grey, activated elements and hypothetical spread. II, V = cortical lamina

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RESEARCH REPORT

REVERSAL OF HYPERALGESIA BY TRANSPLANTATION IN LATERAL HYPOTHALAMIC LESIONED RATS

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Lateral hypothalamus (LHA) plays a very important role in the modulation of nociceptive behaviour. The stimulation of LHA is known to produce analgesia of both tonic and phasic pain. The present study reports hyperalgesia induced by lateral hypothalamic lesions and the effect of fetal (gestation day 16) hypothalamic transplant on the nociceptive response to phasic thermal noxious stimulation {tail flick latency (TFL)} in LHA lesioned rats. The TFL decreased significantly (12.91±3.91 sec to 10.51±1.23 sec) following LHA lesion. However, after transplantation, the TFL did not change. This is the first report of a hypothalamic transplant inducing recovery of a nociceptive response.
Key words: lateral hypothalamus, phasic pain, hypothalamus transplant, hyperalgesia.

INTRODUCTION

The role of lateral hypothalamus (LHA) in pain modulation has recently been established on the basis of five physiological criteria (Dafny et al., 1996). The unpublished data from our laboratory and published data from other laboratories have shown analgesia following LHA stimulation in conscious and behaving or anaesthetised models. Sinha et al. (1999) observed analgesia in the tooth pulp jaw-opening reflex following LHA stimulation in rats. Carr and Uysal (1985) also reported an increase in the threshold for eliciting poststimulus vocalization following LHA stimulation. Behbehani et al. (1988) reported an increase in the TFL following LHA stimulation. The involvement of LHA in the modulation of pain response is also suggested by histological demonstration of neural connections between LHA, PAG and dorsal horn of spinal cord (Bester et al., 1997).

A large number of studies have reported recovery of the lost functions by transplanting diencephalic tissue in the ventricles or at other hypothalamic sites, namely, ventromedial

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nucleus of hypothalamus (VMN), preoptic area and suprachiasmatic nucleus (SCN) of the rats (Aguilar-Roblero et al., 1994; Bartholomew and Floody, 1997; Sollars and Pickard, 1998). Jain et al. (1999) have reported recovery of feeding behaviour in lateral hypothalamic lesioned rats by fetal hypothalamic transplants.

However, it is not known if LHA lesioned rats have an alteration in the nociceptive behaviour. The present study was designed to establish 1) the effect of LHA lesion on nociceptive behaviour and 2) the probability of recovery in response to noxious stimulation with neural tissue transplant at the LHA lesioned site.

MATERIALS AND METHODS

Animals: Adult male wistar rats, 200-300 gm body weight, were housed in separate cages in an animal room having controlled temperature (26±2°C) and lights on from 05:00 to 019:00 h. Food and water were available to them *ad libitum*.

Surgery: Bilateral LHA lesions were produced in ten rats by anaesthetizing them with ketamine (75 mg/kg, i.p.). The LHA (AP 2.3 mm, ML 2 mm, and DV 8.5 mm) (Paxinos and Watson, 1982) was lesioned bilaterally by passing direct current (2 mA) for 10 sec, using bipolar concentric electrodes (tip diameter 100 µm). In five sham operated rats, the electrode was lowered into the LHA and removed without passing current. Following surgery all the rats were injected with gentamycin (8 mg/kg i.p. for five days) for prophylaxis. The rectal temperature and general condition were monitored continuously.

In a separate group of ten rats LHA was lesioned and immediately fetal (gestation day 16) hypothalamus was transplanted at the lesion site, as described by Das et al. (1974). The fetal tissue was procured from the ketamine anaesthetised (75 mg/kg b.wt.) pregnant rat. The uterus was incised, one viable fetus was separated at a time. The brain of the fetus was removed rapidly and placed in chilled sterile lactated Ringer's solution. The hypothalamus was separated from the cerebral hemispheres and the hindbrain. The tissue was made free of any meningeal membranes or choroid plexus attached to it. Approximately 2 µl of fetal tissue suspension was withdrawn in a glass capillary (inner diameter 0.5 mm) fixed to a tuberculin syringe and stereotaxically injected into the previously lesioned LHA site. The glass capillary was left in the same position for 5 minutes after injecting the donor tissue to prevent backflow of injected tissue. It was then slowly and gradually removed. The skull was cleaned and skin sutured.

During recovery, all the operated rats were tube fed 2 ml of Cerelac (a baby food, Nestle, India) solution, thrice a day, to maintain their body weight. After 4-5 days of recovery from surgery tail flick latency was recorded. Serial tests were not performed in view of the difficulty in maintaining the LHA operated rats. It was for the same reason that fetal transplant was grafted immediately into the lesion site in the lesion-transplant group without performing the TFL test.

Behavioural test (Tail flick latency): Each rat was conditioned in the restrainer for 30 min. The tail was cleaned with spirit. Radiant heat (45±2 °C) was focused onto the ventral surface of the tail (approx. 5 cm from the caudal end of the tail) and the TFL was noted using the Tail Flick Analgesia Monitor. The procedure was repeated thrice at an interval of 5min. The cut off time was set at 30 sec to avoid tissue damage. The mean of three observations was taken as the basal

TFL (Prado and Roberts, 1985). It was recorded before and after lesion-transplantation in the same rat.

Sacrifice and Histology: At the end of the study the rats were sacrificed by anaesthetising them with ether and perfusing the aorta with 10% buffered formalin. The brain was removed and coronal sections of 10 μm thickness were cut. The brain sections were either stained with cresyl violet (for verification of the transplant) or haematoxylin-eosin (for verification of the lesion).

Statistical Analysis: "Student's t-test" was used to find out the significant variation, if any, between the basal data of the two groups. Then "paired t-test" was employed to compare the basal data with experimental.

RESULTS

On histological examination LHA lesion as well as the transplant tissue (viable neurons, ghost cells and glial cells) were observed bilaterally in all the rats (Fig. 1). However, because of the difficulty in maintaining the LHA operated rats, the tail flick latency could be recorded in 8 LHA lesioned and 4 LHA lesion-transplanted rats. The results of these rats are presented below.

The tail flick latencies obtained on presentation of thermal noxious stimulation are presented in Table 1. Sham operation did not affect the tail flick latency. The basal values in the sham operated group ranged from 10.83 sec to 13.19 sec with a mean of 12.59±2.3 sec. Following sham operation the TFL changed to 13.0 ±1.55 sec, the values ranging from 11.3 sec to 13.4 sec. In the LHA lesioned group, the basal values ranged from 8.72 sec to 15.80 sec with a mean of 12.91±3.9 sec. Following LHA lesion a significant decrease ($t=3.9$, $p=0.003$) in the TFL (10.51±1.2 sec) was observed, indicating hyperalgesia. In a separate group of 4 rats in which fetal hypothalamus was transplanted at the LHA lesioned site, the basal values ranged from 12.35 sec to 14.87 sec. No statistically significant difference in the tail flick latency was observed amongst the basal values of sham operation, lesion and lesion-transplant group ($t=0.56$, $p=0.59$). No change in the tail flick latency was observed following fetal hypothalamic transplant at the LHA lesioned site, when it was compared with pre-lesion-transplant data (14.06±1.17 sec and 14.71±2.55 sec, respectively). This indicates absence of hyperalgesia when fetal hypothalamus transplantation was done at the lesioned site.

Table 1 Effect of bilateral sham operation, LHA lesion and lesion-transplant on the tail flick latency (TFL) in sec. (Mean±S.D.)

Groups	Basal TFL	Experimental TFL
Sham op	12.59±2.3	13.0±1.55
LHA Lesion	12.91±3.9	10.51±1.2**
LHA Lesion-Transplant	14.06±1.17	14.71±2.55

* indicates the comparison of experimental TFL with the basal. ** $P<0.01$

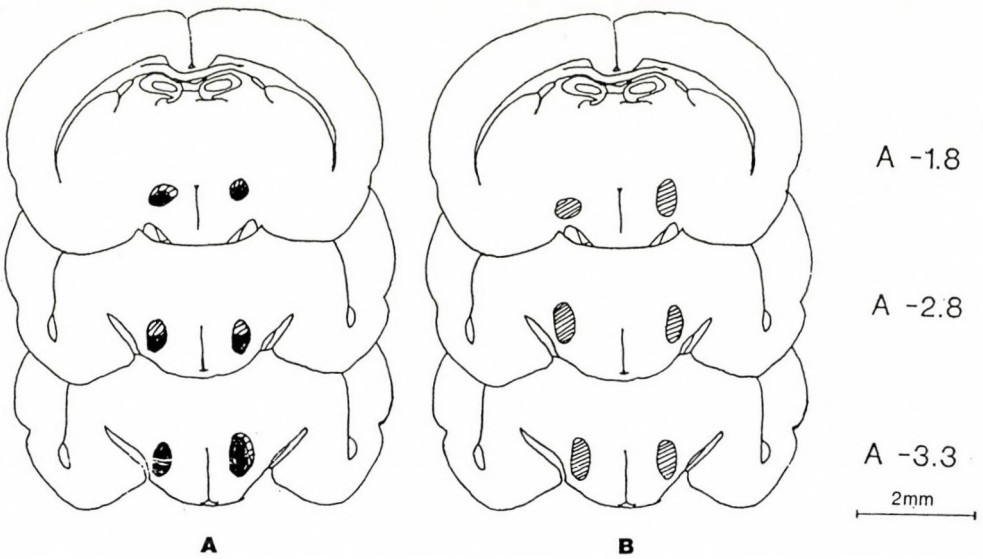

Fig. 1. Camera Lucida drawings showing the site and extent of transplant tissue (A) and LHA lesion (B) at three levels i.e. A -1.8, A -2.8, A -3.3

However these rats showed behaviour which is characteristic of LHA lesioned rats. They were generally inactive and kept sitting in one corner of the cage most of the times. When they walked, if at all, they walked with a hunched posture, carrying their head low to the ground. Some of the rats cleaned their sitting place. The grooming behaviour in these rats was markedly reduced. To maintain these rats they were tube fed with "cerelac" solution and water. The stools of these rats were loose and tarry.

The results indicate recovery of the response to thermal noxious stimulus following fetal hypothalamus transplant at the lesioned site.

DISCUSSION

Bilateral LHA lesion significantly reduced the TFL, which completely recovered with hypothalamic transplantation. Researchers have generally used LHA stimulation model for studying its role in nociception. It is suggested in the literature that LHA modulate both phasic and tonic pain leading to antinociception. Aimone et al. (1988), Behbehani et al. (1988) and Dafny et al. (1996) reported an increase in the TFL following LHA stimulation, indicating analgesia. However, Dafny et al. (1996) stimulated at a higher temperature of 50-52 °C and Aimone et al. (1988) stimulated tail at 6-7 points, each at a distance of 2.5 cm. We confined ourselves to stimulation at only one site with a lower temperature of 45 °C. Carr and Uysal (1985) also reported an increase in the threshold for eliciting poststimulus vocalization following LHA stimulation. These studies suggest analgesia following LHA stimulation. Our lesion model confirms hyperalgesia following bilateral LHA lesion.

Fetal hypothalamic transplantation in our rats produced a complete recovery of the phasic thermal pain when tested on the fourth day. This recovery may be due to release of neurotrophic factors from the site of injury of the host or from the grafted tissue (Alvarado-Mallart and Sotelo, 1993). Degeneration of the host tissue stimulates the production of neurotrophic, activating factors and the expression of neuromodulators (Tomey and Heckroth, 1993; Bulloch et al., 1998; Heckroth et al., 1998). The expression of CGRP (Borlongan et al., 1998), a neuromodulator, has been shown to minimise the neuronal loss by inducing the damaged cells to undergo apoptosis. Thus preventing an acute phase response and attenuating the noxious signal. The host microenvironment has also been shown to have the potential to induce grafted neurons to either secrete neurotransmitters (Miyazono et al., 1995), which helps in the amelioration of behavioural deficits, or to differentiate progressively into fully mature adult CNS neurons. The neurotrophic factors released by the transplanted tissue attenuates the neuronal loss by arresting degeneration of the adult host tissue and by encouraging sprouting of the intact axon (Trok et al., 1996). We have observed viable cells in the histological sections, which may have contributed in the formation of synaptic connections. Sollars and Pickard (1993) demonstrated that anterior hypothalamic grafts of mouse and rat origin send axons into the surrounding hamster neuropil within two days of implantation into the host third ventricle and becomes extensive within two weeks. However, detailed histological analysis, to observe the degree of integration, remain to be done.

Although Aguilar-Roblero (1994) concluded from studies on suprachiasmatic nucleus (SCN), that the graft location as well as the graft connections with the host brain are not crucial for the restoration of circadian function but it is essential that the graft has SCN like tissue. In the present study we transplanted fetal hypothalamus from which LHA differentiates. So it is quite possible that the transplanted tissue had released growth-promoting factors which initiated this recovery process.

CONCLUSION

Thus, it is concluded from the present study that fetal hypothalamic transplants can induce recovery of the phasic nociceptive response to thermal noxious stimulation.

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RESEARCH REPORT

MENINGEAL ALLOGRAFTS OF THE 6-DAY-OLD RAT PINEAL: A MODEL FOR A PINEAL DEPRIVED OF INTRACEREBRAL INNERVATION

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Pineals from 6-day-old rats were transplanted into an incised bed of the parietal cortex of adult rats, of which 29 survived 4-5 weeks after transplantation. The pinealocytes and capillaries in the grafts were comparable in structure to those in the control. Grafts were demarcated from the host cortical tissue by a double, meninx *plus* gland-capsule sheath through which no nerve ingrowth was seen into the graft from the host brain. On the other hand, sympathetic nerves originating from the cervical ganglia reached the grafted pineal along the perivascular spaces of blood vessels, as is the case *in situ*. On this basis, the present meningeal graft is thought to be a model of the pineal gland surviving without its intracerebral neural control.

Key words: pineal, transplantation, innervation, rat

INTRODUCTION

The mammalian pineal gland is innervated from two major sources. Through the habenular stalk, bundles of intracerebral adrenergic and serotoninergic afferents reach the gland, while sympathetic adrenergic and peptidergic fibres derived from the cervical ganglia follow the distribution of arteries and arrive to the pineal via perivascular spaces (see for references Oksche and Prévet, 1981). This dual innervation represents the direct neural control of the organ (Nonaka et al., 1990), which is phylogenetically of photo-endocrine nature as indicated also by its development from the diencephalon, similarly to the retina. Accordingly, in lower vertebrates a pronounced photosensitivity of pineal function has been observed which in higher vertebrates is replaced by neural control (see for references Vígh and Teichmann, 1988). Since innervation is heterogeneous as far as its sources and transmitters are concerned, a selective analysis would be required to elucidate neural mechanisms underlying the control of the pineal

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gland. Among methods available, *in vitro* studies either of cultured pinealocytes (Araki et al., 1992, Kus et al., 1994, Olcese and Munker, 1994) or that of the superfused pineal tissue (Reuss et al., 1993, Santana et al., 1994) are relevant to the totally denervated situation. On the other hand, the extirpation of the superior cervical ganglion (Maurel et al., 1992) deprives the gland from its extracerebral input. Another promising approach is the intracerebral grafting of the immature pineal (Nonaka et al, 1990. Wu et al., 1993) by which it may be revealed how reinnervation affects function. Nevertheless, it may also be of interest to see the contribution of different types of afferents to pineal function. To this end, we attempted to develop an experimental model of the pineal which lacks innervation from intracerebral sources, while possessing an extracerebral innervation.

MATERIALS AND METHODS

Forty intact (non-pinealectomized), adult Wistar rats (200 g body weight) of either sex were used as recipients. Craniotomy was performed under deep ketamine-xylazin anaesthesia on the left parietal bone exposing a 2×2 mm area. An incision was made through the meninges in the cortex to form a tissue bed. In pilot experiments whole encapsulated pineals were transplanted from 1- to 10-day-old rats. Based on survival rates obtained in the different age groups, for further transplantations the 6-day-old pineal was used as a routine. After placing the graft into the cortex, the opening of the skull was covered with the bone plate removed, and the skin sutured. Wound healing was secured with local and intramuscular antibiotics treatment. No immunosuppression was employed. Operated animals were kept under 12 h light-dark cycles. Four to eight weeks after transplantation 29 grafts survived. Grafts were fixed by the trans-aortic perfusion of the recipient animal with Zamboni's fixative. Two recipients were perfused at dawn, the rest at late afternoon. The grafts were removed with a small amount of surrounding cortical tissue and processed for electron microscopy. From resin-embedded blocks semi- and ultrathin sections were cut. Semithin sections were stained with 1% toluidine-blue. Pineal glands of intact 6-day-old, 5-week-old and adult rats, and the own pineals of recipients were used as controls, processed as above. Semi- and ultrathin sections were studied with light- and electron microscopy, respectively.

RESULTS

Of 40 transplants 29 survived as judged by graft appearance and microscopic structure. No particular change in behaviour of the recipients was observed.

3.1. Graft appearance

Surviving pineal grafts were clearly visible in the cortical bed (Fig. 1). They were usually covered by a meningeal scar and were found slightly smaller as compared to their size at the time of implantation. Some grafts filled out the entire cortical bed, while in others a partial adherence was observed with a loose vascular space under the graft.

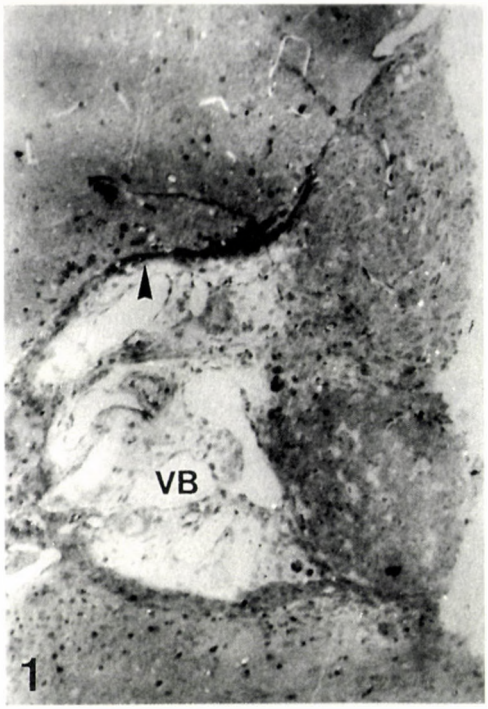

Fig. 1. Survey micrograph of a 6-day-old pineal 5 weeks after its *in toto* transplantation into the adult cerebral cortex. At its circumference the graft adheres tightly to the host tissue, while at its base a vascular bed (VB) is visible. Note the pial lining (arrows) of the vascular bed and graft-host border. Semithin section stained with 1% toluidine-blue. Mag: × 40

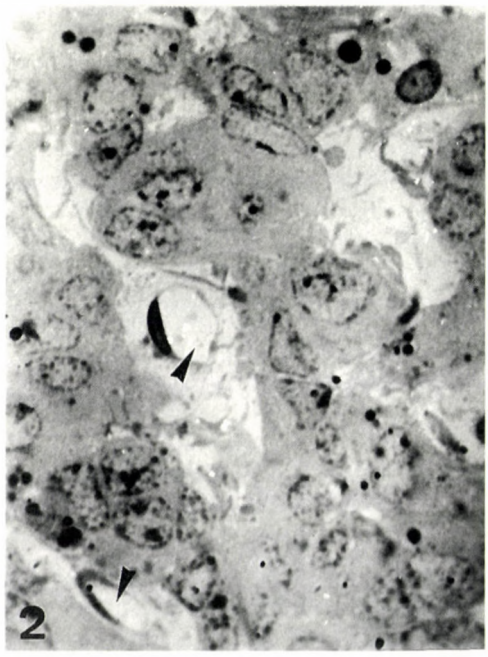

Fig. 2. The graft tissue is permeated by capillaries (arrows) situated in a wide pericapillary space around which pinealocytes can be seen with heterochromatic nuclei and pale cytoplasm. Semithin section stained with 1% toluidine-blue. Mag: × 450

3.2. Light microscopy

Light microscopy showed that transplanted pineals retained their characteristic cellular structure. The epitheloid type of cell organization was evident, the tissue was densely vascularized. Mitotic endothelial cells were also observed within the graft. Similarly to the cell types of the intact gland, large and small pinealocytes were distinguished also in the grafts. Most frequently the large type was encountered. This comprised cells oval or slightly angular in shape having heterochromatic nuclei (Fig. 2). The cytoplasmic staining of pinealocytes in the grafts was comparable to that in the controls. The occurrence of cytoplasmic lipid droplets in graft pinealocytes also corresponded to that in the adult controls, whereas in the grafts a negligible amount of acervulus was seen. Although we implanted the grafts directly into a cortical incision, the pia grew in all specimens onto the cut surface to form a continuous lining of the incision bed. Moreover, the grafted pineals retained their own capsule, thus becoming hermetically demarcated from the cortical tissue by a double connective tissue sheath (Fig. 3). In the loosely adhering grafts the underlying cleft between the pial and capsular layers contained blood vessels and reactive macrophages. Within this cleft, blood vessels were the only connections between host and graft tissues. The pineal tissue showed no significant difference in tightly (Fig. 4) and loosely adhering grafts.

3.3. Electron microscopy

As revealed by electron microscopy, the cytoplasm of graft pinealocytes was found to be rich in organelles. Particularly, an abundance of mitochondria was encountered indicative of an intense metabolic activity in these cells. Synaptic ribbons as specific features of photoreceptor-related pinealocytes, were looked at with special attention. While in the control, synaptic ribbons occurred either alone or in pairs, in the graft cells they appeared in small groups or clusters and were frequently seen to be associated with the cell membrane (Fig. 5). In the graft pinealocytes, ribbons were usually longer, and synaptic vesicles attached to the synaptic rods were more numerous than in the control. Preliminary observations suggested that grafts fixed at early dawn contained more synaptic ribbons associated with the plasma membrane then those fixed at late afternoon. This finding, however, requires further statistical support.

In the perivascular spaces of the graft, small bundles of unmyelinated nerve fibres were observed ensheathed by Schwann-cell cytoplasm (Fig. 6). Varicosities filled with clear, small synaptic vesicles occurred adjacent to major blood vessels but some were seen close to the pinealocytes. Varicosities containing dense-core vesicles were not encountered.

A similarity between grafts and controls was observed in the endothelium of the capillary sinusoids, which was fenestrated also in the grafts (Fig. 6), corresponding to the classical fenestrated type of capillary of endocrine organs.

DISCUSSION

The successful grafting of embryonic and one-day-old pineals has recently been reported on (Nonaka et al., 1990, Wu et al., 1993). Nonaka et al. (1990) described the histology of newborn pineals transplanted into the parietal cortex and interhemispheric fissure. Studying the reinnervation of their grafts, these authors arrived at the conclusion that ingrowing nerve fibres were of similar nature to those innervating the *in situ* pineal gland including the recently

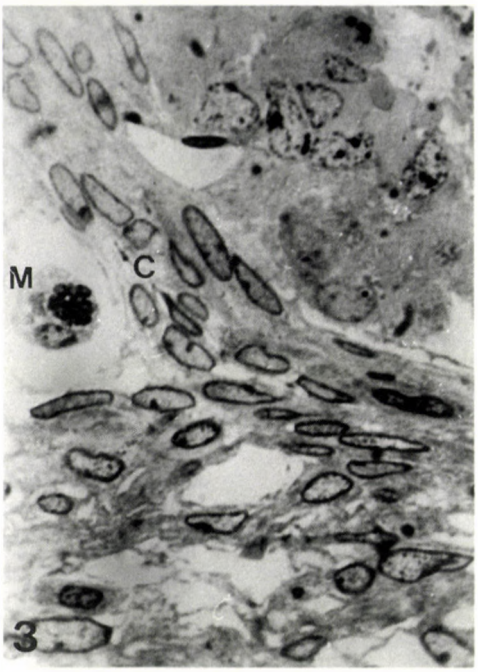

Fig. 3. The capsule of the gland (C) fuses with the ingrown pial lining (P) of the incision bed. M: activated macrophage. Semithin section stained with 1% toluidine-blue. Mag: × 450

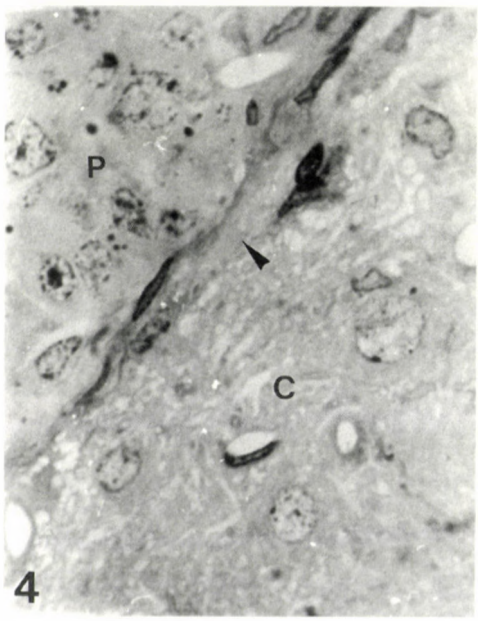

Fig. 4. The border between the grafted pineal (P) and the host cortex (C). The separation of the two tissues by a meningeal barrier (arrow) is evident. The cortical tissue is reaction-free. Semithin section stained with 1% toluidine-blue. Mag: × 450

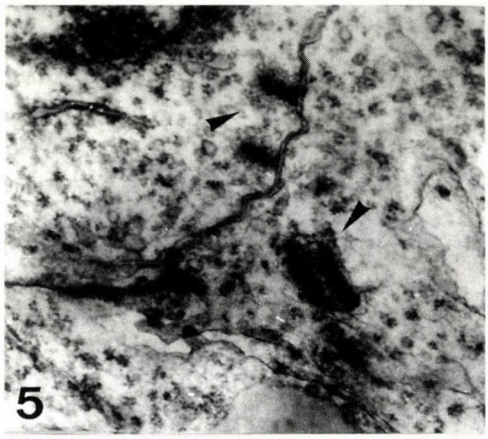

Fig. 5. Electronmicrograph of pinealocyte portions from the graft displaying aggregated (arrow) and membrane-associated (arrowheads) 'synaptic ribbons'.
Mag: × 26,000

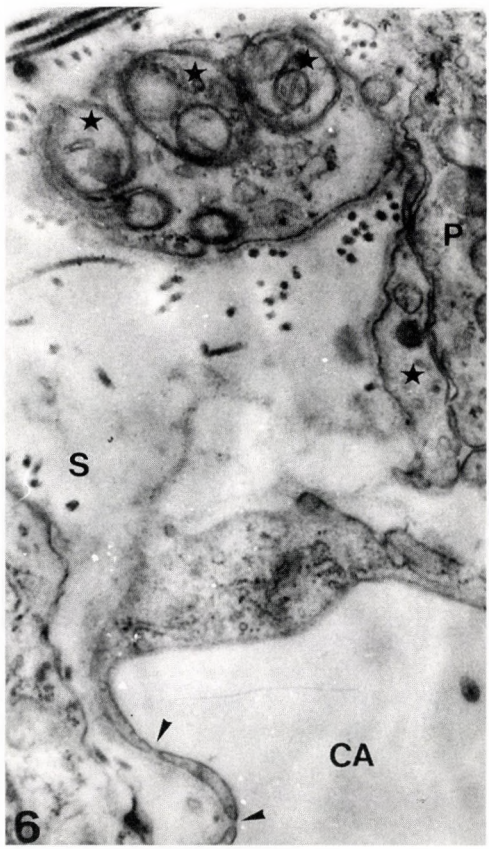

Fig. 6. Electronmicrograph from a pericapillary space of a graft capillary (CA) having a fenestrated (arrows) endothelium. Nerves (arrowheads) can be seen either attached to pinealocytes (P) or ensheathed by a Schwann-cell process.
Mag: × 18,000

described brain-derived adrenergic and extrinsic peptidergic innervations (Reuss and Moore, 1989, Lin et. al., 1994, Moller et al., 1994). In our experiments first we attempted to find the latest age at which the pineal gland still survived transplantation, following the reasoning that since cyclic melatonin synthesis underlying rhythmic release (Pickard and Tang, 1994) develops with postnatal age (Vígh and Teichmann, 1993), in the early postnatal period pinealocytes should be still immature enough to possess some degree of plasticity. Surveying the period up to postnatal day 10, the 6-day-old pineal showed the best survival rate. Accordingly, our experiments extended the age of transplantation well into the postnatal period of the donor and have demonstrated that when grown on the pia and soaked in the cerebrospinal fluid of the subarachnoid space, an environment favourable for differentiation could be ensured. It has to be noted that the viability of transplanted pineals older than 6 days declined rapidly with age. The thorough histological and electron microscopic investigation of 6-day-old pineals 4-6 weeks after neocortical transplantation suggested that they not only survived, but also preserved all structural features characteristic for the control including the revascularization by fenestrated capillaries. The presence of fenestrated capillaries in pineals grafted into various intra- and extracerebral sites has been pointed out by Wu et al. (1993). Our observations suggest that revascularization of the graft takes place from the pia growing in to cover the surface of the incision bed. The presence of mitotic endothelial cells and the fenestrated nature of capillaries in the graft indicate that a proliferation of the graft capillaries may also be instrumental in establishing a vascular link with the host tissue.

A remarkable feature of our grafts was their demarcation by meningeal and capsular cell layers from the cortical tissue. The thick double connective tissue barrier between host and graft tissues excluded neural connections with the host cortex. Indeed, even the most careful electron microscopic examination could not demonstrate nerve fibres entering the graft from the host cortex. On the other hand, a clear-cut vascular connection of the graft was observed with both the meninges and the host tissue established by blood vessels carrying nerves in their adventitia and then, after having divided into smaller twigs, in their perivascular space. These nerves around the blood vessels originate, like all perivascular plexuses within the skull, from the cervical sympathetic ganglia. Electron microscopic observations of Schwann-cell ensheathed nerve bundles and small, clear vesicular terminals in the perivascular spaces of the graft lend further support to our claim that this type of graft is a pineal gland possessing an exclusive cervical autonomous (sympathetic) innervation by contrast to the dual (autonomous and intracerebral) innervation of the *in situ* organ.

The experimental potentials of this model await exploitation. So far the preliminary observation was made that synaptic ribbons in the graft pinealocytes react to dark-light cycles, a phenomenon described for the *in_situ* pineal and also for *in vitro* models (Araki et al., 1992, Reuss et al., 1993). This is promising as to future functional studies with this type of semi-innervated pineal graft.

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PRELIMINARY NOTE

THE EFFECTS OF CENTRALLY ADMINSTERED NEUROPEPTIDE Y ON THERMOREGULATION

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Neuropeptide Y (NPY) is a likely common mediator for different target points of energy metabolism, such as food-intake and thermoregulation. This peptide has been shown to increase food-intake and energy conservation when injected into different sites of the hypothalamus (1, 2). Fasting levels of NPY proved to be higher than normal, and evidence of increased action of NPY in starvation has also been found. Cold adaptation appears to influence NPY-induced food-intake *(3),* although opinions vary as to NPY participates in the development of cold induced hyperphagia or not, since central NPY levels failed to increase in hyperphagic, cold-acclimated rats (3, 4). The reported effects of NPY on thermoregulation vary according to the site of the central nervous system where the neuropeptide was injected (1, 5).

In the present study the central thermoregulatory effects of NPY have been investigated. Adult female Wistar rats (n=20) were used in our experiments. One group of the animals was kept at an ambient temperature of 22-26 C^0, another group was maintained in a cold chamber of 3-5 C^0 for at least three weeks before the tests, forming a non-adapted (NA) and a cold-adapted (CA) group, respectively. The rats were kept in individual cages where laboratory chow and water were available *ad libitum*. The lights were on between 6 am and 6 pm. Each animal had a stainless steel guide cannula implanted into the right lateral cerebral ventricle under ketamine-xylazine anesthesia at least one week before the tests started. Before the tests an injection cannula attached to a long pp 10 polythene tube containing NPY was introduced into the guide cannula under ether anesthesia. During the test sessions the animals were placed into a semi-restraining wire cage and, together with it, into an open circuit metabolic chamber, where colon temperature (indicating core temperature), tail-skin temperature (indicating peripheral heat-loss mechanisms) and CO_2 production (indicating metabolic rate) were continuously measured by using copper-constantan thermocouples and a Kipp-Noyons diaferometer and recorded by a dynograph. Neuropeptide Y (Bachem) was injected intracerebroventricularly (ICV) in doses of 500 ng, I ag, 2 jag or 10 pig in a volume of 5 µl The tests were carded out at mild cold and at thermoneutral ambient temperatures, i.e. at 15 ^{0}C and 25 ^{0}C for CA rats, and at 20 ^{0}C and 30 ^{0}C for NA rats, respectively. Student's t-test was used for the statistical analysis of the data.

The presented experiments showed that ICV administration of NPY was followed in both groups by a dose-dependent decrease in metabolic rate and consequently a significant dose-dependent decrease in core temperature in cold environment (see Fig. 1). This acute effect was often followed by a late hyperthermic period. The decrease in body temperature in the cold was more enhanced in the CA than in the NA group. At thermoneutral temperatures, although the

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metabolic rate decreased, NPY failed to depress Tc either in the NA or in the CA group. No consistent changes of Ts were observed in either group.

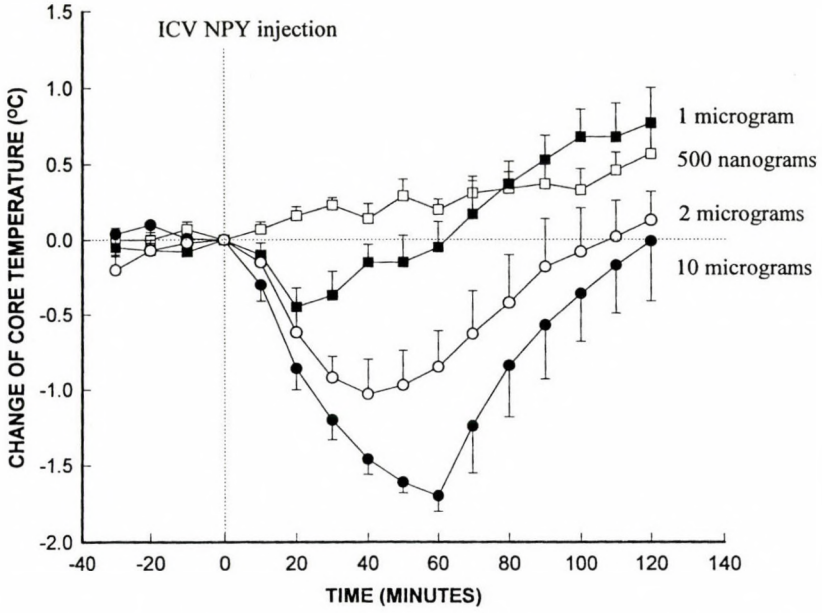

Fig. 1. In a cold environment (ambient temperaturel 5 °C) an intracerebroventricular (ICV) injection of neuropeptide *Y* (NPY) at doses of 1, 2 or 10 μg (n = 6, 6, 7, respectively) was followed by an acute dose-dependent decrease in core temperature of cold-adapted rats, as shown by the change in their colon temperature. ICV NPY at a dose of 500 ng (n=7) failed to induce any change in core temperature. There was no significant difference among the initial core temperatures of the different groups (mean value: 37.67±0.06 S.E. °C)

The results suggest that, although NPY may participate in the regulation of body temperature through decreasing metabolic rate, it does not induce a regulated decrease in Tc, since peripheral heat loss mechanisms are hardly affected. NPY therefore does not appear to be a principal regulator of body temperature but rather a link between the regulatory systems of food-intake and body temperature. (Supported by OTKA T020277, T02651 1.)

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PRELIMINARY NOTE

EFFECT OF LONGEVITY TREATMENT WITH (-)DEPRENYL ON LIFESPAN AND SEXUAL BEHAVIOR IN FEMALE RATS

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Previously it was found that longevity (-)deprenyl treatment has increased the sexual activity and lifespan in male rats (Knoll et al. 1989), moreover there is a close correlation between the sexual activity and lifespan in male rats (Knoll 1997).

A series of experiments with female rats has been carried out by us in order to find out whether there is a similar connection between the sexual activity and lifespan. Preliminary data were published (Dalló and Köles 1996). The copulatory activity of female rats consists of lordotic posture to the male's mount. Intact females which failed to show lordotic behavior in ten consecutive cycles are considered as sexually inactive (44 females were inactive out of 131). The females which displayed lordosis six or more times out of twenty consecutive cycles, were considered as sexually active (25 out of 162).

We also carried out experiments in castrated female rats with and without hormone (estradiol and norgestrel) replacement. Wistar females with 250-300 g body weight were used to our experiments.

The females in all the three groups were treated with (-)deprenyl in a dose of 0.25 mg/kg s.c. three times a week till decay, and in the control groups with saline. Sexual activity was tested weekly with cage adapted males. We found that in sexually inactive females there is no difference between the lifespan of the saline and that of the (-)deprenyl treated group. Similar result was obtained in sexually active females (Fig. 1) though sexually active females lived for shorter time than inactive ones.

In castrated females without hormone substitution, however, a significant difference exists in lifespan: saline treated females lived shorter than the (-)deprenyl treated ones. As to the total number of sexual activity during lifespan see Table 1.

Table 1. Total number of sexual activity during lifespan. (-)Deprenyl treatment 0.25 mg/kg s.c. three times a week till decay

	Saline	(-)Deprenyl
Intact, sexually inactive females	38.05 +/- 4.21 (N = 22)	39.95 +/- 4.21 (N = 22)
Intact, sexually active females	40.33 +/- 4.99 (N = 12)	30.54 +/- 3.81 (N = 13)
Castrated females without hormone substitution	0 (N = 9)	0 (N = 9)

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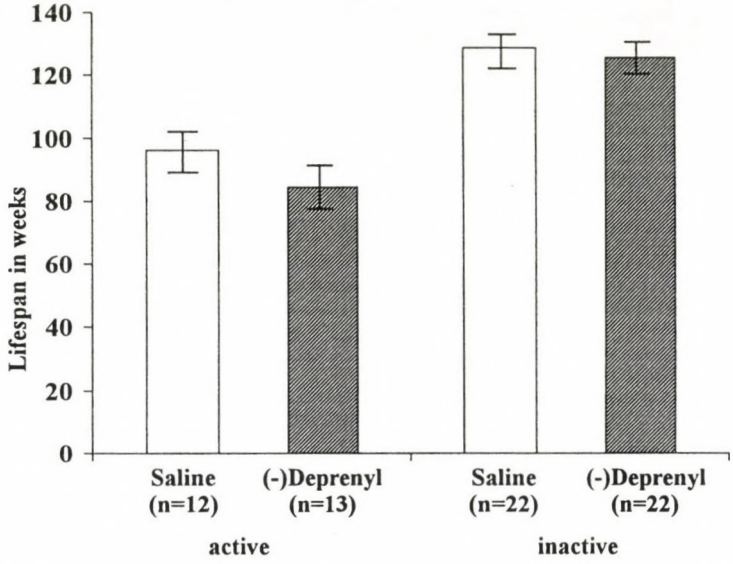

Fig. 1. Effect of (-)deprenyl treatment on lifespan in intact sexually inactive and sexually active female rats

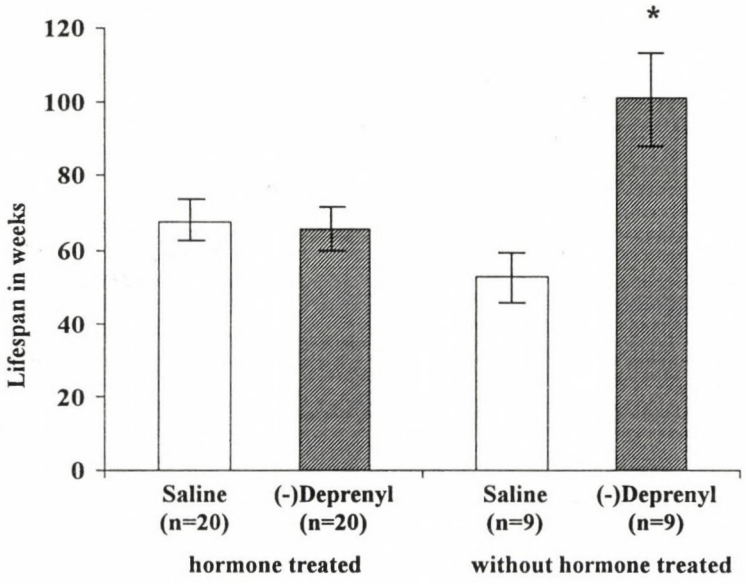

Fig. 2. Effect of (-)deprenyl treatment on lifespan in castrated hormone treated (estradiol and norgestrel) and in castrated without hormone treated females. Student two-way of significance *$p<0.05$

To summarize our data: (-)deprenyl is effective in the extension of lifespan in female rats only when sexual behavior and gonadal hormones are quite absent. Contrary to the males, in (-)deprenyl treated female rats there is no correlation between sexual activity and lifespan. Thus these data support the view that there is a sex difference in the aging process: in males the sexual activity is important for survival while in females it is not. Which other pharmacologic effect of (-)deprenyl is responsible for the observed data, it is the topic of another intensive research (Kitani et al. 1998).

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PRELIMINARY NOTE

BEHAVIOURAL CONSEQUENCES OF METHAMPHETAMINE-INDUCED NEUROTOXICITY IN RATS

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Increasing popularity of illicit use of amphetamine derivatives and increasing number of reports about their toxic reactions in humans inspired a great deal of research on the mechanisms by which these compounds might affect the central nervous system. High doses of methamphetamine (MA) are neurotoxic both to dopaminergic and serotonergic systems in rat brain, resulting in a decrease in tissue levels, reuptake mechanism and activity of synthetic enzymes as well (Wagner G.C. et al. 1980, Ricaurte G.A. et al. 1982). In comparison to the neurochemical deficits, relatively less reports are published regarding the behavioural consequences of neurotoxic MA treatment in rats.

Aim of the present study was to investigate short-term and long-term behavioural consequences of MA-induced neurotoxicity in rats.

Male Wistar rats (Charles Rivers, Budapest) received four injections of (+)MA (10 mg/kg every 2 h) or saline. Body weight and body temperature were measured immediatelly before the first, and 30 min after the last injection. In agreement with the well-known acute effect of amphetamine and amphetamine-like compounds, a decrease of the body weight (22.9 ± 2.7) and a high increase of the body temperature (2.23 ± 0.29) were observed. These differences were significant ($t=4.52$ and 4.58, respectively, $p<0.001$, two tailed Student's *t*-test) compared to the differences measured in saline treated animals.

Spontaneous motility of the animals was checked on the 3rd and 13th day MA postinjection in an "Animal Activity Measurement System" (Timár J. et al. 1993). The time spent on locomotor activity, and the time spent without any activity (resting) was recorded during a 5 min observation period. Results are summarized in Fig. 1. Locomotor activity of MA-treated animals was significantly lower on the 3rd postinjection day ($t=2.62$, $p<0.05$, two tailed Student's *t*-test), but not on 13th day. Parallelly with this, of course, a significant increase was observed in the time animals spent in "resting" on the 3rd day. There was no significant difference in the rearing behaviour.

Performance of the animals in a step-through passive avoidance task was measured in an apparatus consisting of two boxes (one of them illuminated), separated by a guillotine door. On the 3rd postinjection day (training) the animals were placed into the light compartment, and the latency time to the step into the dark compartment with all four feet was measured. If the rat stepped through, the door was closed, and received a foot-shock (1.2 mA) for 10 sec and than was removed from the apparatus. Forty-eight hours later (Day 5) the retention was checked, by measuring the latency time again, the maximum of which was 180 sec. As Fig. 2. demonstrates, there was no difference in the latency time on Day 5, indicating the lack of

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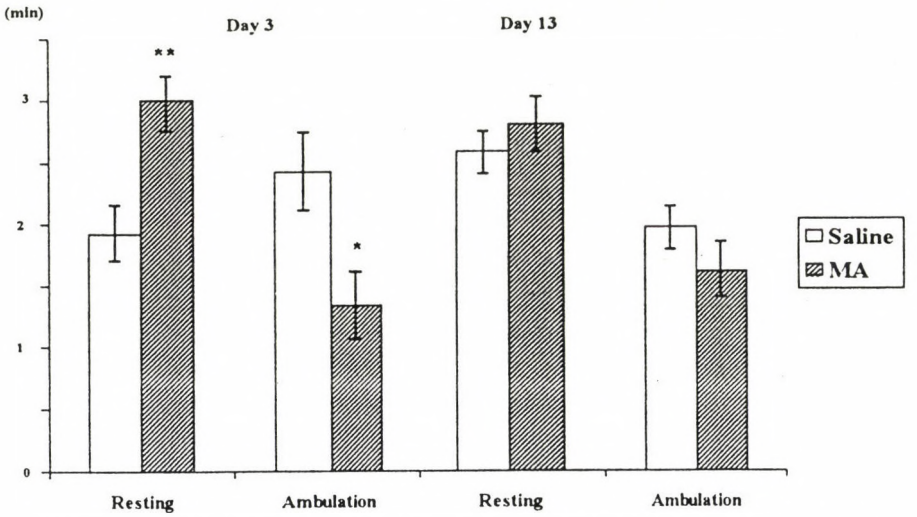

Fig. 1. Effect of (+)-methamphetamine in neurotoxic dose (4 × 10 mg/kg s.c. in every 2 h) on spontaneous motility of rats on the 3rd and 13th days postinjection. Values are expressed as mean ± S.E.M. of time spent with (locomotion) or without activity (resting). *p<0.05, **p<0.01 compared to saline treated animals (two tailed Student's *t*-test). n=10/group

impairment of memory retention in MA-treated animals. However, the latency time was significantly longer during the training, in agreement with the decreased spontaneous locomotion we observed (Fig. 1).

The effect of directly acting DA and 5-HT agonist was checked 1 week and 4-5 weeks after MA treatment. The apomorphine (APO)-induced stereotyped behaviour and the 5-methoxydesmethyltryptamine (5-MeODMT)-induced 5-HT-syndrome were rated by scores based on the intensity of the typical stereotyped movements. The results, summarised in Table 1 indicate that the sensitivity of dopaminerg receptors to APO and that of the serotonergic receptors to 5-MeODMT failed to change.

Table 1. Effect of (+)methamphetamine in neurotoxic dose (4 x 10 mg/kg s.c. in every 2 h) 1 week and 4-5 weeks after treatment on apomorphine- and 5-MeODMT-induced stereotyped behaviour.

		APO	5-MeODMT
		(0.3 mg/kg s.c.)	(3 mg/kg s.c.)
1 week after	MA	14.4 ± 1.9	11.7 ± 3.0
MA treatment	S	11.5 ± 2.5	10.2 ± 1.7
			(0.5 mg/ kg s.c.)
4-5 weeks after	MA	17.8 ± 2.9	5.5 ± 0.9
MA treatment	S	16.2 ± 3.1	4.2 ± 0.6

Values are expressed as mean ± S.E.M. of scores of intensity of behavioural patterns. n=10/group

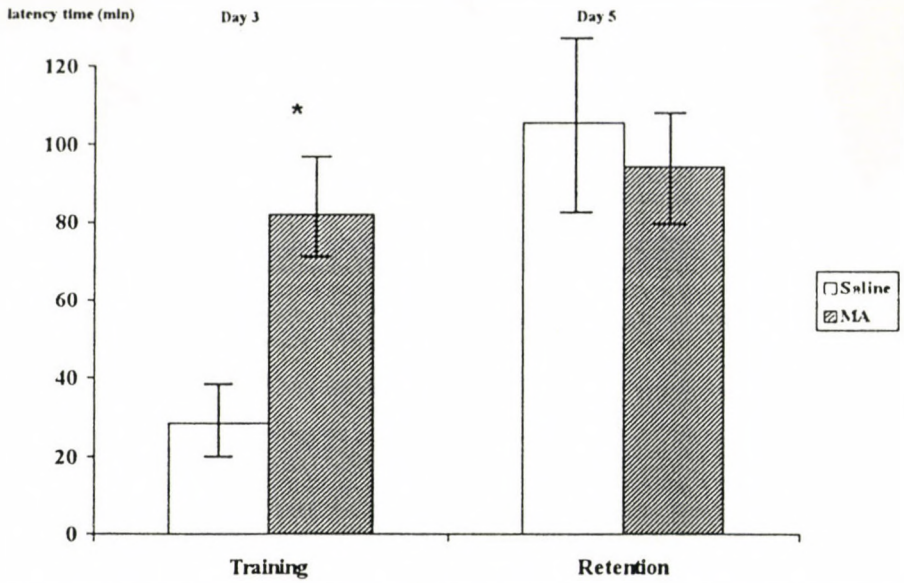

Fig. 2. Effect of (+)-methamphetamine in neurotoxic dose (4 × 10 mg/kg s.c. in every 2 h) on passive avoidance behaviour in rats. The training was performed on the 3rd and the retention was measured on the 5th postinjection day. Values are expressed as mean ± S.E.M. of latency time.*$p<0.05$, n=10/group compared to saline treated animals (Mann Whitney U-test). In brackets number of animals

Summarising our preliminary data, we may conclude that high dose MA treatment produces a transient reduction of the spontaneous motility, but fails to impair the cognitive behaviour measured in a passive avoidance task. By measuring the effect of APO and 5-MeODMT, we tried to follow the changes in the sensitivity of DA and 5-HT receptors, however, we could not detect any difference. The lack of demonstrable changes in the receptor-sensitivity may be due to the adaptive responses reported to normalize synaptic levels of DA after high dose MA treatment (Robinson T.E. et al. 1990), however, it must be kept in mind that the dose of MA administered in these experiments (40 mg/kg as total dose) was relatively low.

Acknowledgement. This work was supported by OTKA T-025-424, Grant in Hungary

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PRELIMINARY NOTE

PROCTOLIN-IMMUNOREACTIVE NEURONS IN THE CENTRAL NERVOUS SYSTEM OF *PORCELLIO SCABER*

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Proctolin is a pentapeptid identified initially as a stimulatory agent of the proctodeal nerve in cockroach[3]. Its occurrence in the central nervous system (CNS) of other invertebrates e.g. oligochaetes, decapod crustaceans is also documented[2]. This compound acts on both visceral and sceletal muscle enhancing both duration and magnitude of muscle tension[1].

Proctolinergic system of isopod crustaceans has not been described yet. To reveal the cellular distribution of proctolin in the CNS of *P. scaber* (Crustacea, Isopoda) we employed diaminobenzidine-visualized immunocytochemistry applying highly specific antisera. Proctolin-like immunoreactivity, both in perikarya and in nerve fibres, was seen in all ganglia, showing unequal distribution pattern. One half of the immunoreactive cells located in the brain, forming seven neuron groups: two in the optic lobes, two in the anteromedial region of protocerebrum, one in the deuterocerebrum and two in the tritocerebrum (Fig. 1).

In the ventral nerve cord the highest number of cells occurred in the 1st and 7th thoracic ganglia and the terminal ganglion (Fig. 2). Darkly stained varicose fibres, seen in every connectives, could be followed passing through the whole length of the CNS. Side branches and many bead-like processes were seen in the central neuropile of each ganglia of the ventral nerve cord and also of the brain. Positive fibres were also detected in the segmental nerves, for example in the thick nerve roots of the terminal ganglion.

The distribution of the proctolin-like immunoreactive structures in the CNS of *P. scaber* is similar to those which were found in decapods. This fact suggests that the organisation of proctolinergic system remained the same in crustaceans further proctolin may have the same functions in their CNS.

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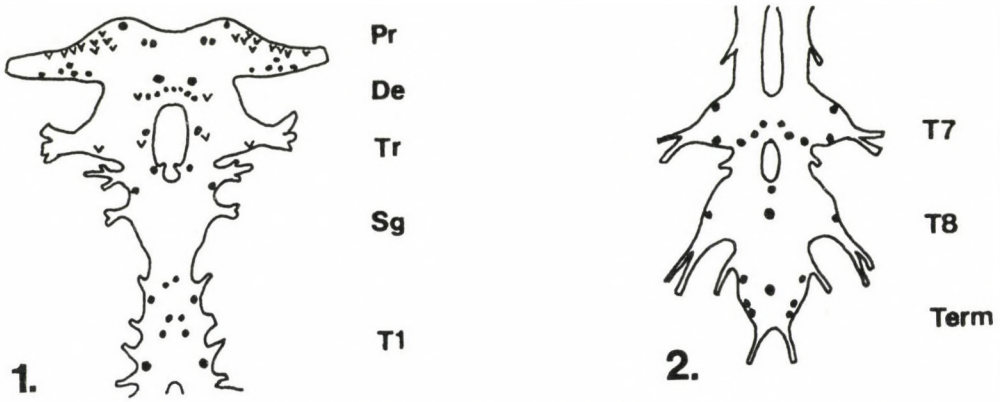

Figs 1-2. Approximate position of strongly (filled) and moderately (empty) stained perikarya in the anterior (Fig. 1) and posterior part of isopod CNS. Pr: protocerebrum, De: deuterocerebrum, Tr: tritocerebrum, Sg: subesophageal ganglion, T1: 1st thoracic ganglion, T7-T8: 7th and 8th thoracic ganglia, Term: terminal ganglion

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PRELIMINARY NOTE

NEURON CLUSTERS OF SEGMENTAL NERVES OF THORACIC GANGLIA IN *PORCELLIO SCABER*: RETROGRADE LABELLING WITH LUCIFER YELLOW

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The central nervous system (CNS) of isopod crustaceans is organised from a cerebral and subesophageal ganglion, eight thoracic ganglia and a compressed mass of six abdominal neuromeres. While detailed anatomy and histology of their CNS is described (Schmitz, 1989), our knowledge about position of neurons, which send their axons via segmental nerves to the peripheral organs, is sparse. Hitherto neuron clusters of segmental nerves of the fourth (Molnár and Pollák, 1998) and eighth thoracic ganglion (Hunyadi et al., 1998) have only been investigated. In this study neuron clusters of segmental nerves of thoracic ganglia have been traced by retrograde filling in *Porcellio scaber* (Crustacea, Isopoda).

Lucifer yellow CH (LY; Sigma Co., St. Louis, USA) was applied to the cut end of segmental nerves of anaesthetised animals (n: 5). Backfilling was carried out by iontophoresis for 60 minutes at 4-6 °C in the dark. After this, each filled sample was kept in isopod-ringer for 10-12 hours then the whole CNS was dissected and was fixed in 4% paraformaldehyde dissolved in phosphate buffer (PB). Each CNS was washed with several changes of PB, mounted in glycerol and coverslipped for immediate photography using LY filter set of a Nikon epifluorescence microscope.

LY was quite suitable for backfilling segmental nerves of *P. scaber*, labelling brightly distinct neuron processes and somata. In the best samples the fluorescent dye could be followed throughout several ganglia of the CNS suggesting that both projection neurons and their central synaptic connections could be clarifyed with this method. The backfills were about 90% reproducible and some variation in staining pattern of neuron set was occurred thus each map presents composite results from five experiments.

Distribution pattern of neurons projecting via segmental nerves of the 8th thoracic ganglion differs from those sets of neurons found in other thoracic ganglia. Positions of labelled cells in the 8th thoracic ganglion were the same as found in our earlier investigations (Hunyadi et al., 1998).

The same neuron subset belongs to each segmental nerve of thoracic ganglia from 1st to 7th, respectively. Via the 1st segmental nerve several perikarya, situated not only in the traced but also in its closest neighbouring ones, were labelled. These cells form four neuron groups in the traced ganglion: an anterior and a posterior set in the ipsilateral, further an anterior subset in the contralateral hemiganglion as well as a small neuron cluster situated in its central part. Well-labelled nerve fiber branches forming tree-like structures in both anterior and posterior

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neighbouring ganglia could be filled via the 1st segmental nerve (Fig. 1). Neuron clusters of the 2nd segmental nerve situated in the anterior and posterior part of the ipsilateral hemiganglion. No neurons situated in the neighbouring ganglia were filled throughout this nerve. The tree-like arborisation of nerve fibres was only seen in the closest anterior ganglion (Fig. 1). Via the 3rd segmental nerve 3 neurons in the posterior neighbouring ganglion and several cell somata in the traced one could be filled (Fig. 2).

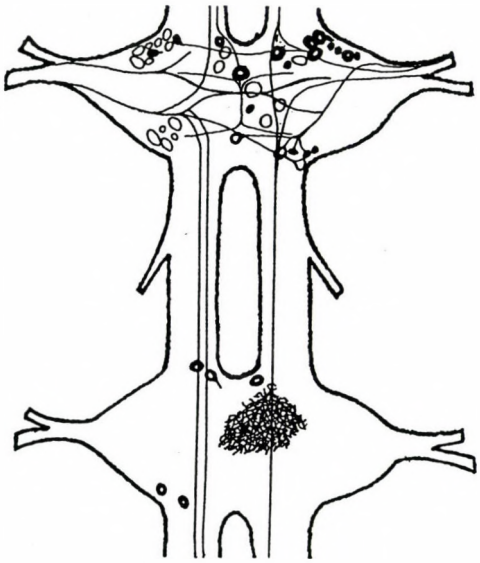

Fig. 1. A representative camera lucida drawing showing the neuron clusters of the 1st (black) and 2nd (empty) segmental nerves of a thoracic ganglion. Note the occurrence of well-labelled tree-like structures in neighbouring ganglia

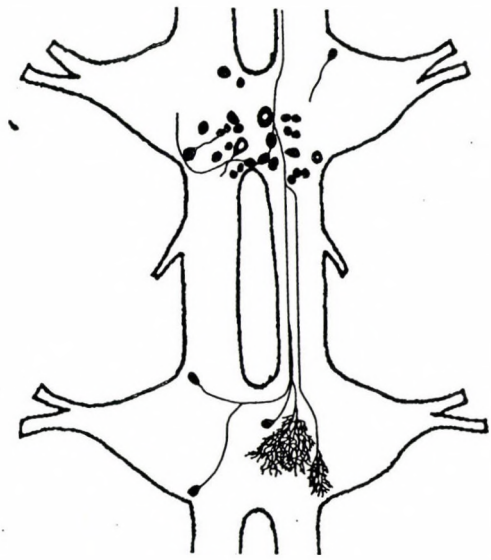

Fig. 2. Neuron clusters of 3rd segmental nerves of a thoracic ganglion

Our results show that the organisation of neuron clusters of segmental nerves of thoracic ganglia in *P. scaber* is relatively simple since the most complex cluster consists of a few dozen central neurons. To reveal the exact targets of these neurons intracellular staining is needed. The superficial positon and large soma size of several perikarya suggest that there is a good possibility to describe the neural circuit design of these ganglia.

Acknowledgement. This study was supported by the Hungarian National Research Fund (OTKA No. T 026652).

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PRELIMINARY NOTE

INCREASED EXTRACELLULAR CONCENTRATION OF TRANSMITTER AMINO ACIDS FOLLOWING 4-AMINOPYRIDINE TREATMENT IN RAT STRIATUM

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Excitatory amino acids (EAA) such as glutamate (GLU) and aspartate (ASP) act as a main mediators of excitatory signals in the mammalian central nervous system. Amino acids may take part in seizure induction, propagation and termination. GLU and ASP exert an excitatory function and are epileptogenic, when administered to the brain [Choi, 1988]. The release of these EAAs from the nerve terminals may be Ca^{++}-dependent and Ca^{++}-independent, although the mechanisms are not fully verified. K^+ itself stimulates the EAA efflux from the brain slices [Patterson et al., 1995] and 4-aminopyridine (4-AP) as a potassium channel blocker also stimulates neurotransmitter release through plasma membrane depolarisation, and activation of the voltage-gated Ca^{++} channel [Basavappa et al., 1994]. 4-AP is a convulsive substance which blocks voltage dependent K^+ channels, facilitates inward Ca^{++} movement [Rogawski and Baker, 1983]. The neurochemical events in 4-AP seizure have not been monitored *in vivo* previously, therefore we used intracerebral microdialysis sampling method to reveal, how the EAA levels in rat striatum change after treatment with 4-AP. Parallel with detection of EAA the catecholamine content of same samples obtained by *in vivo* microdialysis were determined, and at the end of experiments, rats were perfused transcardially with 4% formaline in 0.1 M sodium phosphate buffer, pH 7.2. Coronal sections of removed brains were cut and c-fos immunohistochemistry have been performed. In this study we present the results of changes in amino acid release induced by 4-AP in the rat striatum.

Adult male Wistar rats (LATI, Gödöllő, Hungary) weighing 200-250 g were housed in a light- and temperature-controlled room (lights on between 6:00 a.m. and 6:00 p.m.; 23 °C) and had free access to food and water. Injection with 4-AP in the awake animals produced intense motor alteration (tremor, shivering, generalised tonic-clonic seizure) [Mihály et al., 1990], therefore the microdialysis experiments were carried out in anaesthetised rats. The animals were kept and handled during the experiments in accordance with the instructions of the Albert Szent-Györgyi Medical University Ethical Committee for the Protection of Animals in Research.

Microdialysis probe was implanted into the right striatum (AP: + 0.7 mm, ML: +2.77 mm, DV: -6 mm to bregma, according to the atlas of Paxinos and Watson) under Nembutal (35 mg/kg) anaesthesia and continuously perfused with modified Ringer solution (composition: 140 mM NaCl, 3.0 mM KCl, 1.2 mM Na_2HPO_4, 0.27 mM NaH_2PO_4, 1.0 mM $MgCl_2$, 7.2 mM glucose, 1.2 mM $CaCl_2$, pH=6.8-7.2) at flow rate of 2 µl/min delivered by a CMA 100 microinjection pump. After the 2 h equilibrium period perfusates were collected into polyethylene vials every

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30 min throughout the experiments. Two baseline samples were obtained before applying of 4-AP (5 mg/kg, i.p.).

Immediately following collection, dialysates were assayed for levels of glutamate (GLU), aspartate (ASP), arginine (ARG), citrulline (CIT) and γ-aminobutyric acid (GABA) via high-performance liquid chromatography with electrochemical detection (HPLC-EC) following pre-column o-phtalaldehyde-sulfite (OPA) derivatization at room temperature for 15 minutes. Separation of amino acids was achieved using a reverse-phase column (250×4 mm, Nucleosil 5C18) and a mobil phase consisted of 100 mM monosodium phosphate and 0.5 mM EDTA with 25% methanol (v/v) water adjusted to pH 4.95 with 85% phosphoric acid. Total elution time was 20 min. The column temperature was kept at 25°C.

Statistical analysis was performed by the GLM procedure of the SAS system. ANOVA was used for comparisons an experimental groups with the control group at each test (30 min, 60 min, 90 min, 120 min, 150 min) using Tukey's Studentized Range (HDS) Test.

4-AP significantly increased the extracellular level of ARG at 60 min. The dialysate GLU concentration was elevated at every test time. There was no statistical difference in ASP concentration between control and 4-AP treated groups. GABA reached a maximum at 60 min, while CIT was elevated at 90 min following 4-AP administration.

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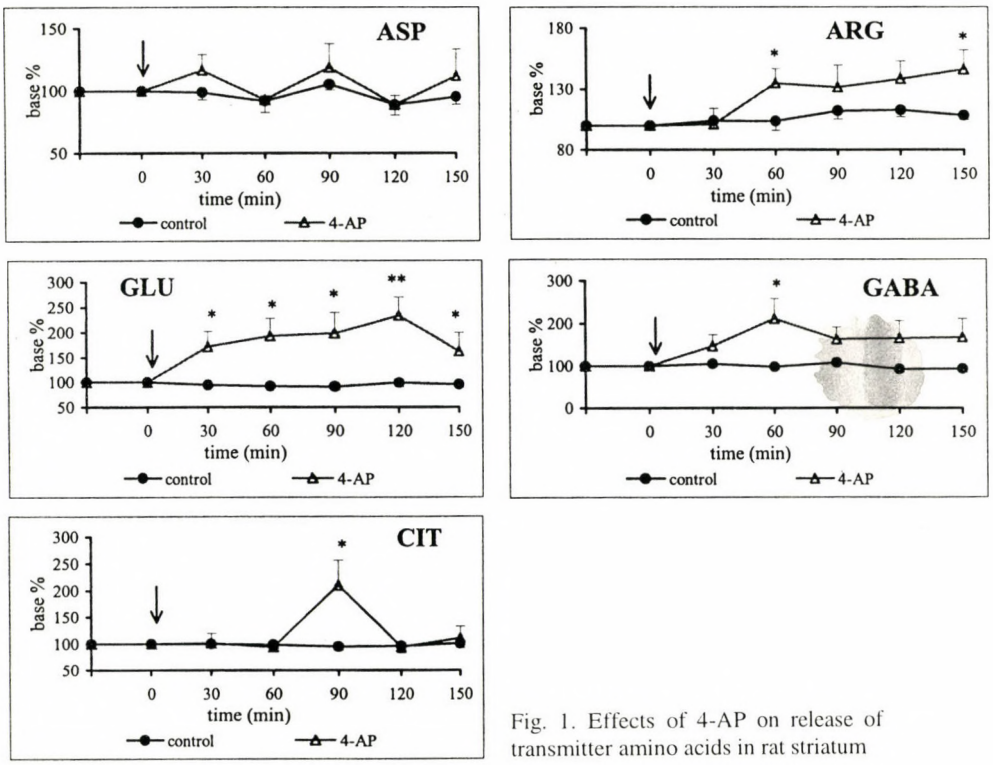

Fig. 1. Effects of 4-AP on release of transmitter amino acids in rat striatum

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PRELIMINARY NOTE

STRIATAL CATECHOLAMINE RELEASE INDUCED BY 4-AMINOPYRIDINE

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The voltage-sensitive K^+-channel blocker 4-aminopyridine (4-AP) is commonly used depolarising agent in the neurobiological experiments. It facilitates inward Ca^{++} movement [Rogawski and Baker, 1983; Thesleff, 1980] and causes neuronal hyperactivity [Mihály et al., 1983; Mihály et al., 1985]. 4-AP is a potent convulsant, induces characteristic electrophysiological changes, which are comparable to clinical convulsions [Szente and Boda, 1994]. We have shown that 4-AP seizures induce vasogenic brain edema with protein extravasation. This extravasation was completely prevented by diphenylhydantoin (DPH). The beneficial effect of DPH can be explained by the membrane-stabilising action of the drug [Mihály et al., 1990]. We also demonstrated that in 4-AP induced focal seizures c-fos protein was detectable in the neocortex and in some allocortical areas of the treated hemisphere [Mihály et al., 1997]. 4-AP stimulates neurotransmitter release via plasma membrane depolarisation and subsequent activation of voltage-gated calcium channels [Basavappa et al., 1994]. 4-AP can increase the spontaneous basal release of noradrenaline (NA) [Huang and Dai, 1991] and 5-hydroxytryptamin (5-HT) [Schechter, 1997] in rat hippocampal slices.

There is no direct *in vivo* evidence on neurotransmitter release and c-fos activity induced by 4-AP. Therefore we were studied the changes of striatal catecholamine levels following 4-AP treatment and compared these data to c-fos immunoactivity induced by 4-AP. In this paper we present the results of our *in vivo* microdialysis studies, concerning the striatal catecholamine release caused by 4-AP.

Adult male Wistar rats were used in all experiments. Rats were housed in temperature-controlled room (23 °C) under 12 h light/12 h dark cycle. Food and water were freely available. The animals were kept and handled during the experiments in accordance with the instructions of the Albert Szent-Györgyi Medical University Ethical Committee for the Protection of Animals in Research.

The rats were anaesthetised with Nembutal (35 mg/kg), placed in a stereotaxic frame. The body temperature was maintained at 37 °C using a heating pad. The microdialysis probe was implanted into a right striatum (AP: +0.7 mm, ML: + 2.77, DV:-6 mm to bregma according to the atlas of Paxinos). The dialysis probes were perfused with physiological saline (pH=6.8-7.2, composition:140 mM NaCl, 0.27 mM NaH_2PO_4, 1.2 mM Na_2HPO_4, 3.0 mM KCl, 1.0 mM $MgCl_2$, 7.2 mM glucose, 1.2 mM $CaCl_2$) at a rate 2 µl/min. After 2 h equilibration period two baseline samples were collected. After that 4-AP (5 mg/kg, i.p.) was administered, and 5 samples collected to monitor the effects of the 4-AP on catecholamine release.

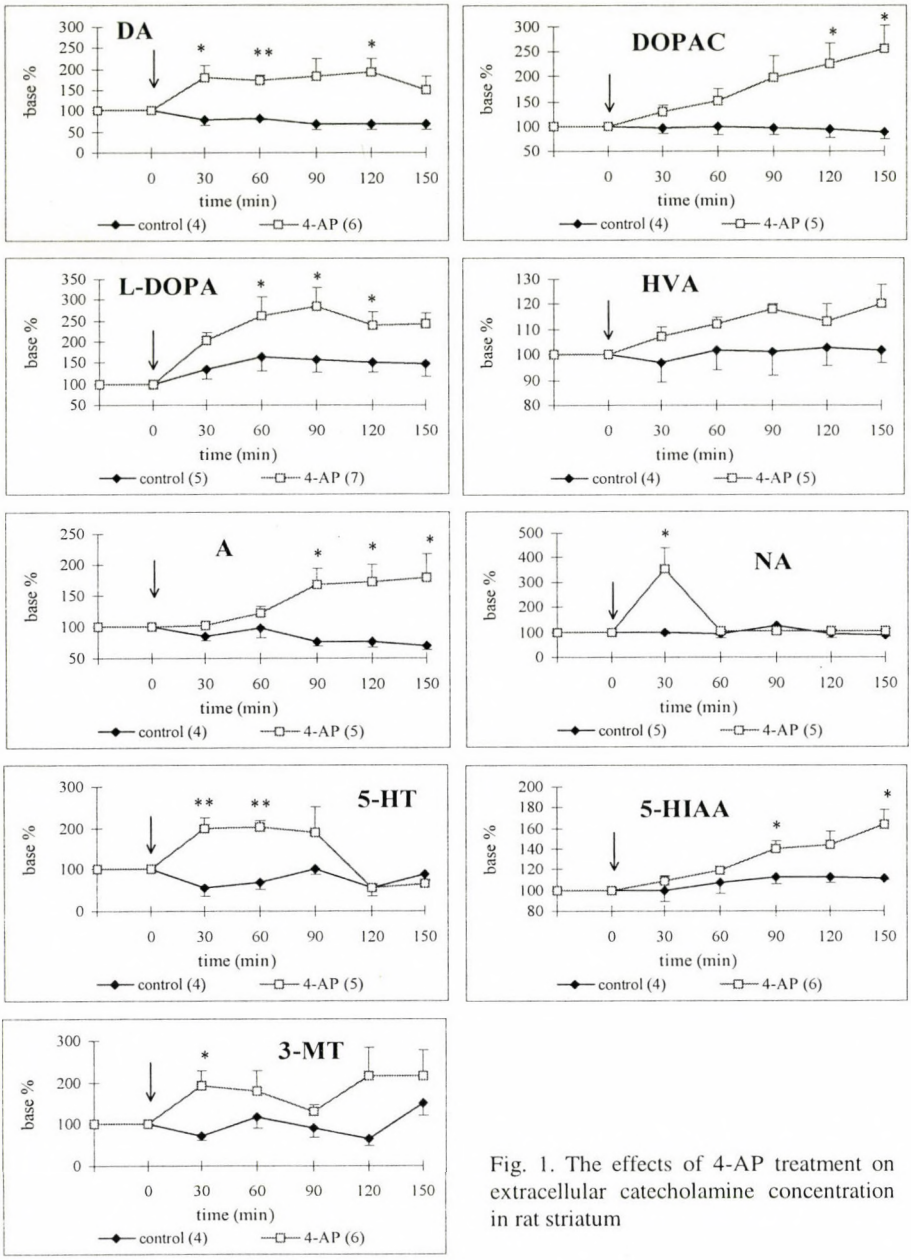

Fig. 1. The effects of 4-AP treatment on extracellular catecholamine concentration in rat striatum

Brain dialysate samples were assayed for levels of noradrenaline (NA) adrenaline (A), l-dihydroxyphenylalanine (L-DOPA), dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), 5-hydroxyindolacetic acid (5-HIAA), 3-methoxytryptamine (3-MT), 5-hydroxytryptamin

(5-HT) and homovanillic acid (HVA) by high-performance liquid chromatography with electrochemical detection (HPLC-EC). Separation of samples was achieved using a BST (60×4 mm) Hypersil 3ODS reverse-phase analytical column and BST (20×4 mm) Hypersil 3ODS guard column and a mobile phase consisted of 100 mM Na_2HPO_4, 0.7 mM EDTA, 0.7 mM OSA with 12% methanol (v/v) water adjusted to 2.90±0.05 pH with 85% phosphoric acid. The column temperature was 25 °C. Total elution time was 15 min.

At the end of experiments the microdialysis probe was taken off, and the animals was transcardially perfused with cold 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Brains were removed, coronal sections were cut for c-fos immunohistochemistry.

The results are expressed as a means ± S.E.M. of percentage of basal levels (n=4-6). Statistical significance was determined by one-way ANOVA, followed by the Tukey test. A p value less than 0.05 was regarded as significant.

The 4-AP treatment caused the increased release of every catecholamine, except HVA in rat striatum. The concentration of DA, L-DOPA and 3-MT remained increased during the whole test period. The amount of striatal DOPAC, 5-HIAA and A showed a steady increase. The extracellular level of 5-HT and NA was raised significantly immediately after 4-AP administration.

The present data show the changes of *in vivo* catecholamine release during seizures induced by 4-AP. The relation between 4-AP-induced neurotransmitter release, seizure spread and c-fos immunohistochemistry will be analysed in further experiments.

Ackowledgements. The work was supported by grants from the Hungarian Ministry of Social Affairs and Health (ETT T-02-670/96), OTKA (T-022230, T 26584, T 6084), FKFP (0091/1997) and MTA-AKP (96-330 3,2).

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PRELIMINARY NOTE

EFFECTS OF ACETYLCHOLINESTERASE INHIBITORS ON THE METABOLISM OF AMYLOID PRECURSOR PROTEIN *IN VITRO*

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On the basis of the cholinergic theory of Alzheimer's disease (AD), the inhibition of acetylcholinesterase (AChE) is the one of the most beneficial tools to improve the memory and cognitive functions in patients with AD [5]. The principal mechanism of AChE inhibition involves an increase in the availability of acetylcholine at cholinoceptive synapses. There are controversial data concerning the relationship of the cholinergic hypofunction, AChE inhibition and the metabolism of amyloid precursor protein (APP) [3, 4]. Therefore, the first goal of this study was to determine whether the processing of APP can be regulated indirectly by different AChE inhibitors *in vitro* on an embryonic septal culture.

There are two alternative pathways in the metabolism of APP: non-amyloidogenic cleavage, resulting in soluble APP extracellularly, and amyloidogenic proteolysis, where degradation may lead to formation of the toxic amyloid fragment [2]. There is a suggestion that protein kinase C (PKC) activation plays a potential role in routing APP into the two alternative pathways [1]. Within this context, we wished to establish whether the alterations in APP level induced by AChE inhibitors are mediated by the activation of PKC.

Septal neurons from 18-day-old embryonic rats were cultured for 7 days *in vitro* under serum-free conditions. Cells were incubated for 2 h at 37 °C with different concentrations of reversible (ambenonium) and irreversible (metrifonate) AChE inhibitors. The enzyme inhibitory effects of ambenonium and metrifonate were controlled by the measurement of AChE activities spectrophotometrically and by visualization morphologically. After treatments, the changes in the APP levels were investigated by means of APP immunoblotting. The stimulation of PKC was also tested by PKC immunoblotting.

Both biochemical measurements and enzyme histochemistry proved the inhibitory effects of different concentrations of ambenonium and metrifonate. 10^{-7} M ambenonium inhibited the enzyme activity by 95%, while 10^{-6} M metrifonate did so by 76%. All four concentrations (10^{-3}, 10^{-5}, 10^{-7} and 10^{-9} M) of ambenonium resulted in an elevation of the APP level. The same effect was observed when cells were treated with different concentrations (10^{-4}, 10^{-5} or 10^{-6} M) of metrifonate (Fig. 1). The PKC level of neuronal cultures also increased after the treatment with reversible and irreversible (Fig. 2) AChE inhibitors. The maximal effect was observed for 10^{-3} M ambenonium or 10^{-5} M metrifonate, as concerns effects on both APP and PKC levels.

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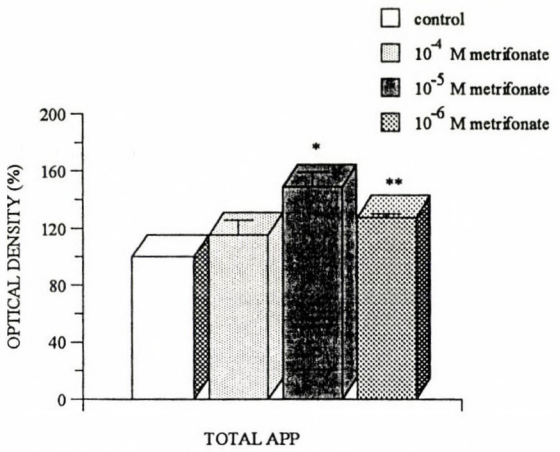

Fig. 1. Effect of metrifonate on levels of APP in septal neuronal cultures 2 h after treatment. Data are expressed as percentages over the level of control cultures. Each value is the mean ± S.D. of data obtained from three different experiments

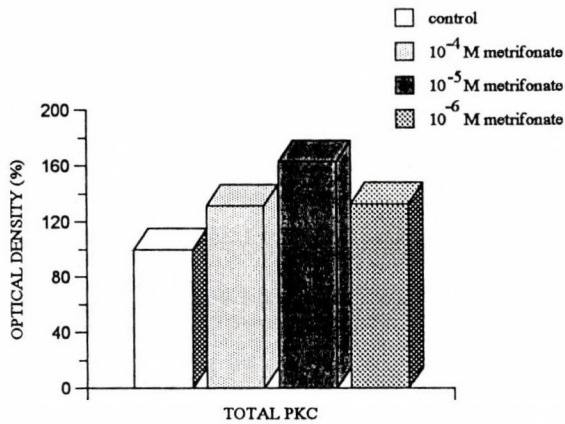

Fig. 2. Effect of metrifonate on levels of PKC in septal neuronal cultures 2 h after treatment. Data are expressed as percentages over the level of control cultures. Each value is the mean of data obtained from two different experiments

In summary, our results confirmed the hypothesis that the inhibition of AChE can readily stimulate the processing of APP in cultured septal neurons. Our data also demonstrate that AChE inhibitors can modulate the level of PKC. We suggest that regulation of the APP metabolism may occur indirectly, via the activation of PKC. Since PKC activation stimulates the non-amyloidogenic secretory pathway of APP processing, we conclude that the beneficial

effects of AChE inhibitors in AD therapy may be due to the stimulating cholinergic function and the increase of non-amyloidogenic APP processing.

Acknowledgement: This work was supported by OTKA (Grant. No. T022683, T26470), ETT (Grant No. 584/1996, T-04 652/96, T-04 117/97) and the Bolyai fellowship.

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RESEARCH REPORT

INVESTIGATION OF THE DEVELOPMENT OF A VENTRAL NERVE CORD GANGLION IN *EISENIA FETIDA*: A HISTOLOGICAL AND HISTOCHEMICAL STUDY

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A considerable amount of information is available on the anatomical and neurochemical organisation of the central nervous system (CNS) of adult oligochaete worms as *Lumbricus terrestris* and *Eisenia fetida* (Günther, 1971a, b; Lyckman et al., 1992). The expression of fixation resistant NADPH-diaphorase (NADPHd), which is known to be identical with neural nitric oxide synthase (NOS) and catalyses nitric oxide (NO) production (Dawson et al., 1991), has been shown recently in annelids (Elofsson et al, 1993). The role of NO in synaptic plasticity, axon outgrowth and differentation of neurons in the CNS of both vertebrates and invertebrates has been suggested by several studies (for review see Bicker, 1998). Though oligochaete CNS is relatively simple in organisation and offers some possibilities to observe the developmental changes of any ganglia, little is known about gangliogenesis in any earthworm species.

To study developmental changes in the subintestinal ganglia, 1- and 3-month-old specimens of *Eisenia fetida* Sav. (Annelida, Oligochaeta) kept at standard laboratory conditions were used in our experiments. After anaesthesia with chilling and carbon dioxide their ventral nerve cord (VNC) ganglia, situated in the 19-25th segments, were dissected and used for histological and histochemical procedures. Both qualitative and quantitative histology further NADPHd histochemistry were focused on the 19th VNC ganglion. Student's t-test was applied for statistical analysis.

Microscopic structure of the 19th VNC ganglion in both age groups was similar to the ganglion structure compiled from anatomical investigations of adult specimens (Günther, 1971a, b). However, smaller cell number and the less complex organisation of neuropile and dorsal giant axons in juvenile worms was apparent with qualitative light microscopy (not shown). Quantitative morphology has proved that the total cell number of the 19th VNC ganglion was significantly lower in 1-month-old worms than in 3-month-old ones (Fig. 1). This finding suggests that the cell number of this, and probably every, VNC ganglion increases gradually during development of the earthworms.

NADPHd activity has been screened throughout the 19th VNC ganglion of both age groups. Moderate or weak NADPHd-staining was characteristic on 1-month-old worms, while strong labelling was the typical in 3-month-old animals, further the number of their stained somata was lower in younger animals than in older ones.

Two pairs of fibre-branches were strongly stained in all ganglia derived from either young or mature worms. Based on their anatomical positions we identified these structures as the

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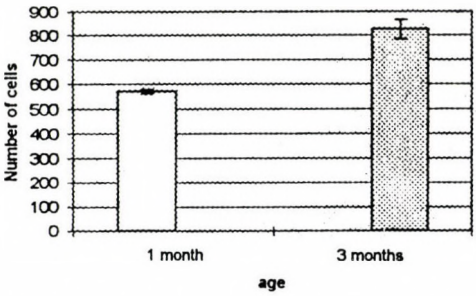
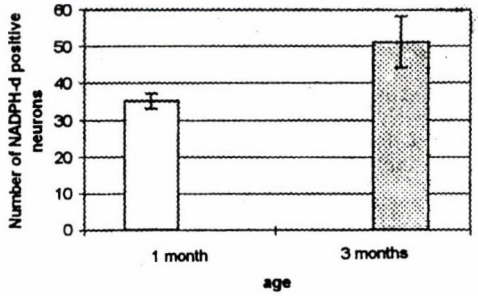
ventrolateral and ventromedial sensory longitudinal axon bundles described by Günther (1971a, b) earlier.

Several perikarya had specific NADPHd-staining in the 1st subintestinal ganglion of 1-month-old worms, however, except of three pairs of strongly labelled somata situated near the fibre-like structures, most of them were moderately or weakly stained. In 3-month-old specimen not only the number of NADPHd-positive cell somata (Fig. 2), but also their staining intensity increased significantly.





Figs 1–2. Diagrams showing total (Fig. 1) and NADPHd-positive (Fig. 2) cell number of the 1st subintestinal ganglion in *E. fetida*

Present results show that no changes could be observed in some NADPHd-positive structures, namely sensory longitudinal axon bundles, in the VNC ganglia, while the number of NADPHd-positive cells increased significantly during the development of *E. fetida*. Since the total cell number of the ganglion also increased we could propose that NO mediates the axon outgrowth and development of new synaptic connections between neural cells in earthworms as was found in other species (Bicker, 1998).

Acknowledgement. This study was supported by the Hungarian National Research Fund (OTKA No. T 026652).

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PRELIMINARY NOTE

THERMAL ADAPTATION STATE INFLUENCES NPY-INDUCED FOOD INTAKE

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Neuropeptide Y (NPY) is synthetized in the arcuate nucleus, the projections of which reach the paraventricular nucleus (PVN), dorsomedial nucleus (DMN), medial preoptic area, that are all important regions involved in the regulation of food intake and energy balance, at least partly through NPY release from these nuclei. During starvation NPY production and action are increased and, besides hunger (what means an increased food intake when food becomes available), a characteristic energy conserving behavior can also be clearly demonstrated: metabolic rate and sympathetic activity decrease and energy storage is activated. Exogenous NPY applied centrally is known to elicit food intake, and it is being reported (1) that upon the immediate action of centrally injected NPY heat production is also depressed, what is occasionally coupled with slightly increased heat loss.

Some data (2, 3) suggested a decreased release (and local accumulation) of NPY in the PVIN and DMN during cold exposure, supposedly to allow or promote an enhanced thermogenesis in the brown adipose tissue or other tissues, what is necessary for the maintenance of homeothermy. Other data (4) demonstrated that during chronic cold exposure no NPY changes were detected in any hypothalamic regions, although plasma leptin fell. It was concluded that, in contrast to starvation-induced hyperphagia, in cold-induced hyperphagia NPY-containing neurons are probably not activated.

In the present studies female Wistar rats were maintained, for at least a month, at room temperature of 22-25^{0}C (non-adapted, NA group) or in a cold room of 3-5^{0}C (cold-adapted, CA group), with lights on between 6 a.m. and 6 p.m. They had laboratory chow and water *ad libitum*. The animals were habituated to handling and daily measurements of body weight. Each animal had a stainless steel guide cannula implanted intracerebroventricularly (ICV) under ketamine-xylazine anesthesia, at least one week before the feeding tests. Testing consisted of an ICV injection of 0, 2, or 10 pig NPY in 5 k^1 0.9% NaCl (using slight immobilization), that was given to normally satiated rats at 9 a.m. Immediately after the injection pellet-food was offered, feeding started and, in the course of this, both fractional and cumulative body weight changes were measured every 30 mm for a total of 3 hours.

The ICV injections of NPY were followed by a dose-dependent increase of food intake and body weight both in NA and in CA rats. The effect of an ICV injection of 2 pig NPY on food intake of CA and NA rats is shown in Fig. 1. In CA animals the feeding responses always significantly exceeded those seen in NA animals, irrespective whether the food was offered at the usual cold room or in a warm environment. This suggests that the greater responsiveness to NPY in CA rats was not due to the actual thermal environment, rather to the adaptation state.

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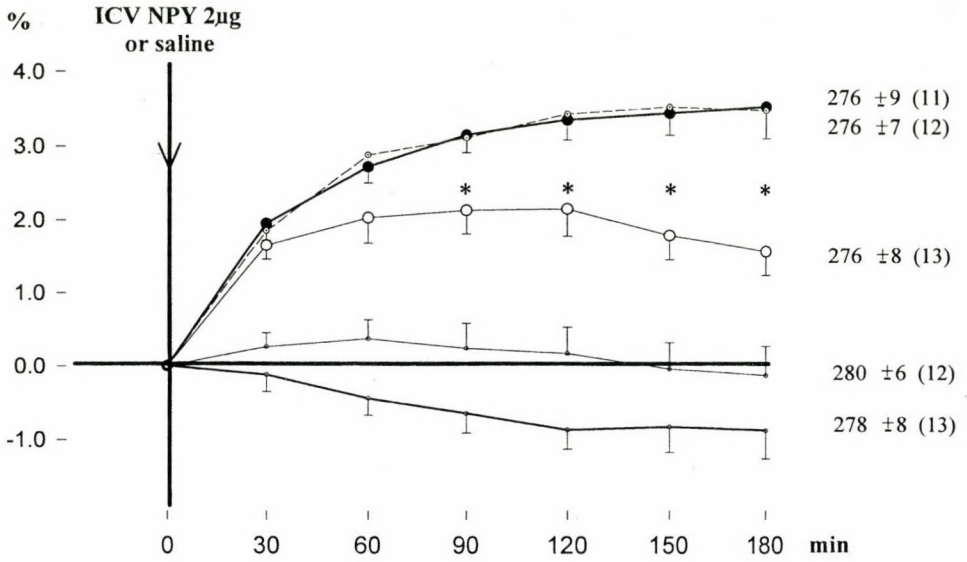

Fig. 1. Cumulative body weight changes in NA (o) and CA (•) rats (expressed as % of the initial body weight), following an ICV injection of 2 pig NPY. The interrupted line refers to the NPY-response of CA rats offered food at room temperature. The thin and thick continuous lines represent similar body weight changes in NA and CA rats, respectively, following ICV injection of µl physiological saline. The average starting body weights and the number of rats are indicated for each group

In conclusion, the adaptation state seems to have a strong influence on the sensitivity of the central regulatory processes of food intake: cold-adaptation increases the sensitivity to NPY. This coincides with our finding that the thermoregulatory sensitivity is also increased in cold-adaptation, but is at variance with the opinion that NPY is not involved in cold-induced hyperphagia. Since, in the cold, NPY induces hypometabolism and hypothermia (1), the apparent hypermetabolism and lack of hypothermia in CA animals needs further investigation.

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PRELIMINARY NOTE

COMPARISON OF PINEAL AND RETINAL PHOTORECEPTORS BY CALCIUM HISTOCHEMISTRY

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The mammalian and human pineal organ develops from the photoreceptor pineal complex of lower vertebrates during evolution. The role of the pineal is to adapt the rhythmicity of the organism to the environmental circadian and circannual light rhythms by means of hormonal and neural efferentation (Vollrath, 1981; Vígh and Vígh-Teichmann, 1988).

In submammalian vertebrates, the pineal organs have a structure similar to that of the retina. They contain cone-like and rod-like photoreceptors. In these cells, in our earlier works, we have demonstrated immunoreactive opsins and other substances known to have a role in the photochemical transduction. We also studied the content of free calcium ions of pineal structures around pineal acervuli. We assumed that the pineal calcification present in some birds, mammals and the human pineal is due to the accumulation of high number of calcium excreting cells in (Vígh and Vígh-Teichmann, 1989, 1992; Vígh et al., 1998).

The comparison of mammalian pinealocytes and the photoreceptor pineal cells of submammalians suggested that the calcium excretion may be connected to the photoreceptor origin and function of pineal cells. Therefore, in the present work, we compared the fine structural localization of calcium ions in the pineal and retinal photoreceptors.

Pyroantimonate cytochemistry was used for electron microscopical demonstration of free calcium ions. The pineal organ and retina of various vertebrates were fixed in a mixture of 1% pyroantimonate and 2% glutaraldehyde solved in buffer, and embedded in araldite as described earlier (Vígh et al., 1998). The following animals were investigated: 5 carps (*Ciprinus carpio*), 8 frogs (*Rana esculenta*), 6 lizards (*Lacerta viridis*), 4 ducks (*Anas platiryncha*) and 10 laboratory rats. The materials were cut on a Reichert Ultracut S ultramicrotome, the ultrathin sections were examined and photographed in an OPTON electron microscope.

Indicating the presence of free calcium ions, Ca-pyroantimonate deposits were found to be localized alongside the cell membrane of pinealocytes and retinal photoreceptors. The deposits accumulated intercellularly in the mammalian pineals (Fig. 1). Also microacervuli showing intense pyroantimonate reaction were found intercellularly. Larger acervuli exhibited concentric layers with different calcium content. In submanimalian vertebrates, outer segments of pineal rods reacted stronger with pyroantimonate than cones. A similar difference was detected in the calcium content of retinal cones and rods. In dark adapted pineal and retinal photoreceptors. Ca-pyroantimonate deposits were localized intracellularly, while in light adapted ones extracellularly. In cross-sections of retinal photoreceptor disks, the pyroantimonate reaction also appeared alongside the membrane invaginations called "incisurae" (Fig. 2). Granular areas near incisures also contained calcium.

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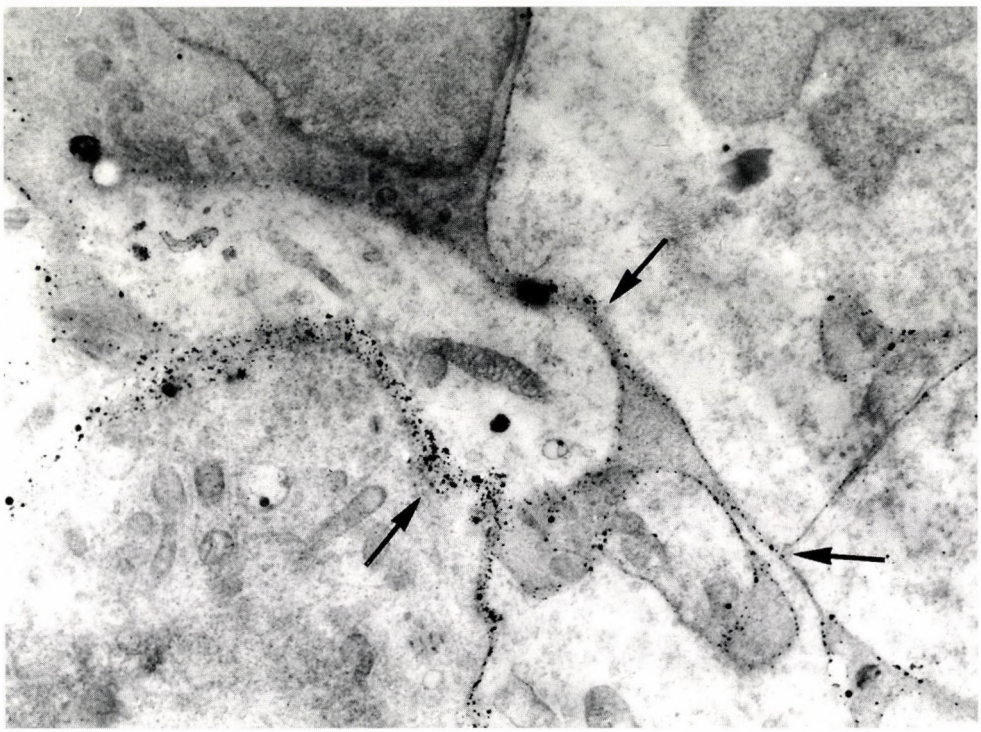

Fig. 1. Free calcium ions (black dots of Ca-pyroantimonate deposits) intracellularly alongside the cell membrane (at arrows) in the pineal organ of the rat. Mag: × 18,000

Our results show that comparing the localization of free Ca ions, there is a multiple similarity between retinal and pineal photoreceptors: a/ The calcium is localized to the cell membrane of retinal and pineal photoreceptors. b/ The rod outer segments contain more calcium ions than in cones. The later result may be explained by the higher activity of rods or by the higher number of photoreceptor membranes in rods than cones. c/ The excretion of calcium ions is connected to photoreceptor activity. Concerning the retina, a similar result was found by Atanassious and coworkers (1984).

The localization of free calcium alongside the incisurae of retinal rod discs indicates the importance of these invaginations in the ion exchange of the outer segments during photoreception. The significance of free calcium content of granular areas near incisurae is not yet known.

In the mammalian pineal organ as well, calcium ions are associated to the cell membrane. An accumulation of calcium ions was detected in the intercellular space, showing the role of intercellular spaces in the pineal calcification. Microacervuli appear in enlarged intercellular spaces filled by free calcium ions. The concentric lamellation of pineal concrements and the different calcium content of the lamellae may be the result of changing calcium concentration of intercellular space due to different cellular activity (seasonal photoperiodicity?). The results

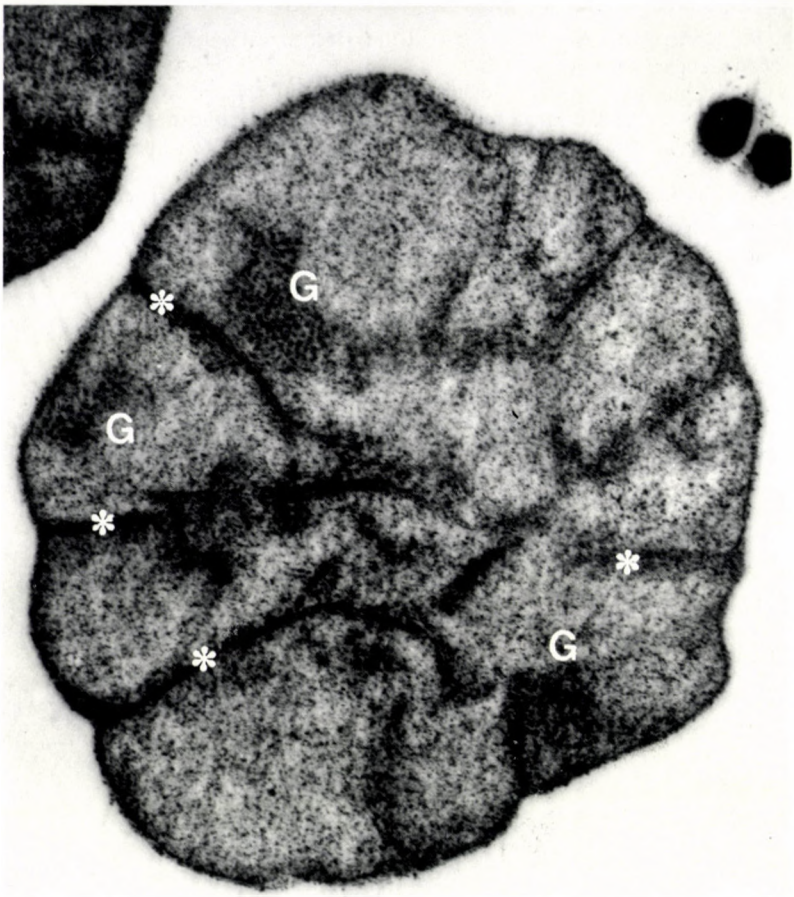

Fig. 2. Ca-pyroantimonate deposits in the outer segment and concerning the incisurae (asterisks) of the retina of the frog (*Rana esculenta*), G: granular areas containing calcium near incisurae. Mag: × 11,300

of the comparison of pineal organ and retina by calcium histochemistry confirm our view about the connection of pineal calcification and the photoreceptor origin of mammalian and human pinealocytes.

Acknowledgements. This work was supported by grants (No. T-20364 and T-1 7703) of the Hungarian Scientific Research Fund (OTKA) and by the Hungarian (OMFB) - Portuguese (ICCTI) scientific, technological interstate cooperation.

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PRELIMINARY NOTE

PATTERN OF NADPH-DIAPHORASE CONTAINING STRUCTURES IN THE SUBINTESTINAL GANGLIA OF *LUMBRICUS TERRESTRIS*

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NADPH-diaphorase (NADPHd) is the marker enzyme of the nitric oxide synthase (NOS) which synthesises the gaseous neurotransmitter, nitric oxide (NO) (Dawson et al., 1991). In the absence of specific antibodies against invertebrate NOS NADPHd histochemistry is widely used for identification of the putative NO-producing structures of invertebrates (for review see Bicker, 1998).

Putative NOS expressing neurons of annelids were also in the focus of some investigations (Elofsson et al., 1993; Leake and Moroz, 1996). While NADPHd-positive structures of the central nervous system (CNS) in leeches have been identified and functionally characterised (Leake and Moroz, 1996), in earthworms only the presence of NADPHd activity was shown without any detail (Elofsson et al., 1993). The aim of this study was to show the presence and topographical distribution of NADPHd activity in the ventral nerve cord (VNC) ganglia of an earthworm species.

Adult specimens (n: 5) of *Lumbricus terrestris* (Annelida, Oligochaeta) collected from their natural habitat were used in all experiments. After anaesthesia with carbon dioxide and chilling the VNC ganglia situated in the 50-60th segments were cut off and used for observation applying a standard protocol of NADPHd histochemistry (Vincent, 1992). Preparing controls NADPH was omitted from the incubating solution and no staining was observed in the samples.

The NADPHd stained structures were easily identified in both whole mount preparations and their serial sections. Strong, moderate and week staining of somata were also occurred in each ganglion. While the number and positions of neurons that belong to the former groups were the same in each ganglion weakly stained perikarya were randomly occurred in the ganglia even if the samples derived from the same incubation. However, the presence of the consistently stained somata in each ganglion proves that distinct sets of neurons could be labelled with this method.

The most intense staining was detected in three pairs of perikarya situated on the ventrolateral surface of VNC ganglia. Less intense and weak labelling was found in several other neurons located on the ventrolateral, lateral and especially on the dorsolateral part of the ganglion (Fig. 1).

The most conspicuous NADPH-d labelled structures of VNC ganglia are the longitudinally directed fibre branches that are characterised by a constant diameter and run without interruption from ganglion to ganglion. In whole mount preparations, because of their overlapping, the exact number of fibre bundles is hard to defined, however, in the best whole

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mounts two pairs of them were clearly visible (Fig. 2). Based on their anatomical position they were identified as ventrolateral and ventromedial sensory longitudinal axon bundles (SLBs) described by Günther (1971a, b) earlier. A direct connection of the SLBs to the labelled fibres of the segmental nerves could be observed. The NADPH-d positive fibres of the 1st and 3rd segmental nerves connect to the thick fibre bundles with a T- or Y-shape ramification while the fibres of the 2nd segmental nerves connect to the more medial thinner structures (Fig. 2).

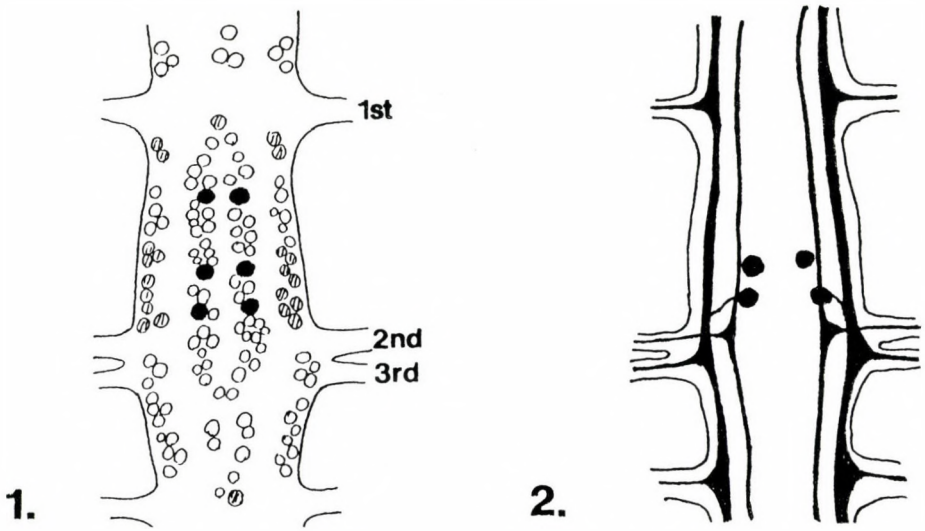

Fig. 1-2. Camera lucida drawings of NADPHd-positive perikarya (1) and fibre-like structures (2) in a ventral nerve cord ganglion. Anterior is the top. 1st, 2nd, and 3rd: segmental nerves; black: strong, hatched: moderate, empty: weak staining

Our results prove the presence and high activity of NADPHd in a distinct neuron set and ventrolateral and ventromedial SLBs of the VNC ganglia in *L. terrestris*. This finding shows that the putative NO-producing (NADPH-diaphorase positive) neural structures mediate sensory information to the earthworm CNS, however, it is not clear yet which type of sensory information is processed by these structures. Though the anatomical positions of two pairs of heavily labeled neurons strongly resemble to the location of the well-known central tactile and pressure sensitive receptor cells in *L. terrestris* (Günther, 1971a, b), at this stage of our experiments we could only present the distribution pattern of NADPH-d positive neurons without their functional characterisation. To verify the exact physiological significance of NADPH-d expressing structures in the earthworm CNS further investigations are in progress.

Acknowledgement. This study was supported by the Hungarian National Research Fund (OTKA No. T 026652)

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OBITUARY

ATTILA BARANYI (1951–2000)

The news was shocking: our recent colleague Attila Baranyi, one of the best-known neurobiologists in Hungary, died in a fatal traffic accident in Budapest on 8 March 2000.

Attila Baranyi was born son of a miner in Egerbocs on 4 August 1951. While at grammar school in Eger, he won first place in the national student competition in biology, and was therefore entitled to enter any Medical School or Faculty of Biology without entrance examination. He decided to study biology at the Faculty of Natural Sciences of the József Attila University in Szeged.

His scientific career started at the Department of Comparative Physiology at József Attila University. While still a student, Attila investigated the inhibitory interactions among the different cortical areas of the cerebral cortex in the cat. He received his M.Sc. in 1975, and joined the group investigating the cellular basis of Pavlovian conditioning. After introducing the method of intracellular recording from the neurones of the cerebral cortex, he performed numerous successful experiments. These led to the finding that a special kind of neural processes, *heterosynaptic facilitation,* underlies the formation of new synaptic connections among nerve cells during the conditioning procedure. He was awarded his degree of C.Sc. on the basis of these experiments in 1985.

As a young and enthusiastic researcher, he decided at the very outset of his scientific career that he would be as good as possible. Despite the fact that his research circumstances were much more modest than those in Western Europe or in the United States, he very quickly made his way to rank among the scientists who were widely acknowledged in his field. The late

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1970s and early 1980s was the most fruitful period in his scientific career: He published his results in the best journals, e.g. Nature, Brain Research, etc.

At the invitation of Professor Woody, he spent two years (1981-83) in the Neuropsychological Institute at the UCLA, investigating the cellular mechanisms of learning. After these successful years, which provided him with a firm background for his further development, he returned to Szeged. Later, he again visited and worked at the UCLA (1986-89), when he collaborated in the characterization of the different functional groups of cortical neurones. These results were published in the J. of Neurophysiology and served as the basis of his D.Sc. thesis, which he defended in 1992. He subsequently visited Yves Fregnac in Paris and participated in experiments aimed at ontogenetical aspects of the organization of the visual cortex and the origin of certain cortical rhythms.

In collaboration with Hungarians and researchers from abroad, Attila Baranyi published numerous papers on a great variety of themes: synaptic plasticity, the effects of ethanol and β-amyloids on neuronal activity, etc. He was a member of various Hungarian and international neurobiological societies and president of the MITT Congress held in 1995 in Szeged.

In the 1990s, he shared his time and energy between research and teaching. In 1994, he became Head of the Department of Comparative Physiology at József Attila University, and in the same year took over the leadership of the Faculty of Biology, too.

In 1996, he suddenly decided to leave his *alma mater,* which had been the site of so many of his successes, and took up a position in the pharmaceutical industry in Budapest. He was making plans to renew his research activity and to restart his scientific career in the United States when this fatal accident took him from us.

All who had the pleasure of knowing him and his exceptional experimental skills and scientific intelligence will deeply regret that he has left so early.

József Toldi

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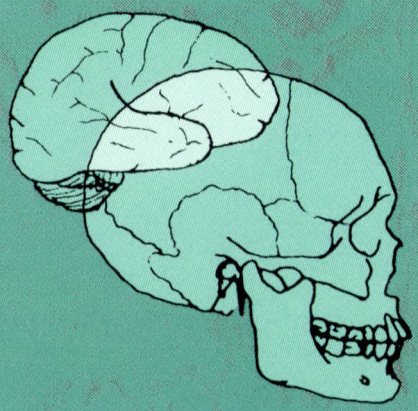

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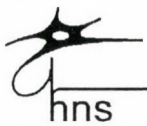

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RESEARCH REPORT

RESPIRATORY RESPONSES TO ELECTRICAL STIMULATION OF THE BASAL GANGLIA IN CATS

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The aim of the present study was to investigate the involvement of basal ganglia in altering respiration. The experiments were carried out on cats using the electrical stimulation techniques. The electrical stimulation of the basal ganglia caused locus-dependent changes both in the tidal volume and the rate of respiration. The most frequent effect of stimulation was hyperpnea and decrease in the tidal volume in awake, freely moving cats. An increase in the tidal volume appeared only as rebound-like aftereffect. The stimulation that caused hyperpnea in awake cats elicited smaller changes under chloralose or ketamine-induced anaesthesia, and, in certain cases, the stimulation produced apnea. After locally applied neurotoxic damage, the electrical stimulation failed to induce significant respiratory changes. It is suggested that the fast, small amplitude ventilation is, probably, produced by high excitation that shifts the respiratory rhythm generator towards the upper limits of its regulatory range. The slowing of ventilation might be produced by an inhibitory mechanism, which is able to cause even apnea under anaesthesia.
Key words: basal ganglia, electrical stimulation, hyperpnea, respiratory rate

INTRODUCTION

It is widely accepted that a complex medullary rhythm generator regulates the ventilation [Cohen, 1974, Gordievskaya and Kireeva, 1999, Hukuhara, 1974, 1988, Koshiya and Smith, 1999, McCrimmon et al. 2000, Rybak et al. 1997]. Also a vast amount of data demonstrates that suprapontine brain mechanisms are able to modulate the activity of the medullopontine respiratory centres [Cohen and Hugelin, 1965, Gallego and Gaultier, 2000, Horn and Waldrop, 1998, Kojima et al. 1965, Millhorn et al. 1987, Orem and Netick, 1986, Xu et al. 1993]. It is assumed that the suprapontine mechanisms are important to initiate the adaptation of the respiration to the metabolic demands of the actual behaviour. However, our knowledge is not complete to answer the question that exactly which suprapontine brain structures and how modulate the respiration. In a series of investigation we have studied the electrically elicited

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cardiorespiratory changes from the nuclei regarded as input (caudate nucleus, putamen) and output (globus pallidus, subthalamic nucleus, substantia nigra) of the basal ganglia circuitry [Ángyán, 1994, Ángyán and Ángyán 1999a, b]. The basal ganglia circuitry plays an important role in motor control, primarily in initiating and programming the somatomotor movements. Our previous investigation revealed that basal ganglia are able to modulate also the cardiorespiratory functions. However, in those studies the emphasis was put on the cardiovascular effects. Therefore, this paper summarises the characteristics of the electrically elicited alterations in ventilation from the basal ganglia in freely moving and narcotised cats.

MATERIALS AND METHODS

Animals: Ten adult cats of either sex, weighing 2-4 kg, were used. All experiments were performed following the animal ethics rules according to the European Communities Council Directive (86/609/EEC).

Surgery: Under pentobarbital sodium (iv. 40 mg/kg) anaesthesia bipolar electrodes were implanted chronically in the caudate nucleus (CD), putamen (Put), substantia nigra (SN), globus pallidus (GP), and subthalamic nucleus (Sub). The conventional stereotaxic techniques were applied. The stereotaxic coordinates of Snider and Niemer's brain atlas were used [Snider and Niemer, 1961]. During the same operation a thermocouple was placed into the nasal orifice through the frontal sinus [Ángyán and Szirmai, 1967]. The electrodes were made of insulated nikrothal wires of 0.3-mm diameter, and were uninsulated 0.5 mm long at the tips. The tip separation was 1 mm along the axis. The free ends of the electrodes and of the thermocouple were soldered to a miniature socket of 12 links. The socket was mounted on the skull with stainless steel screws and dental acrylic cement. The socket could be connected with the stimulator and the input of amplifiers by a light, flexible cable allowing free locomotion of the cat also in the experimental box.

In five cats also a guide cannula for injecting kainic acid was implanted. This cannula was made from injection needle of 0.5 mm diameter. The tip of the guide cannula was placed 0.5 mm above the target locus. Before and after the microinjection of kainic acid a stainless steel wire was placed into the cannula.

Stimulations: The freely moving animal was stimulated with 10 sec long train of rectangular pulses at 100 Hz and 0.3 ms pulse width. The stimulus intensities were determined by means of the elicited behavioural effects. The intensity that caused the first observable effect (orientation, alertness) was regarded as threshold. The intensity producing some postural changes (head turning, tilting of the body, etc.) was used as medium intensity, while the intensity that elicited locomotion (mostly circling) served as high intensity. These intensities varied between 0.1 and 1.0 mA, and remained constant throughout the experiments. The minimum interval between two stimulations was 60 sec. To verify the stability of the responses, stimulations were repeated three to five times, with each stimulus intensity at each electrode site. The experiments were carried out on the cats placed into an experimental box of 70×60×70 cm height, supplied with a Plexiglas door.

Recordings: Polygraph recordings were done. The respiration was recorded by the thermocouple as a change in the temperature of tidal air. The properly placed thermocouple detects the frequency of respiration reliably, only the mouth-licking produces disturbing artefacts. The polygraph was connected to a computer through an interface for automatic evaluation of the signals. The arterial blood pressure was recorded by a cannula inserted into

one of the common carotid arteries. The details of this method was described previously [Ángyán, 1994].

Neurotoxic damage: For microinjection of kainic acid the stainless steel wire was replaced by the injection needle which was moved 0.5 mm downward from the end of the guide cannula. 0.2 μg kainic acid in 1 μL isotonic saline was infused unilaterally into the globus pallidus by means of a Hamilton syringe over a 2 min period in awake, freely moving animal. The needle was carefully removed from the guide cannula 5 min later, and the stainless wire was placed back in the guide cannula. The first symptoms of the neurotoxic damage appeared 30–60 min after the injection.

Anaesthesia for electrical stimulations: α-Chloralose (0.08 g/kg iv.) or ketamine (4 mg/kg i.m.) was injected.

Statistical analysis: Respiratory rate (RR) values were calculated as an average value measured over 10-sec epochs derived from the respiratory recordings during a 10-sec period immediately before stimulation, during a 10-sec stimulation period, and again 10 sec immediately after stimulation. All statistical comparisons were made using a standard Student's t-test. The RR obtained from the pre-stimulation period served as control and was expressed as 100%.

Histology: After finishing the experiments the position of the electrode tips was examined on serial transverse sections of the brain stained with cresyl violet and Luxol fast blue.

RESULTS

Locus-dependent respiratory responses to electrical stimulation were obtained from the different nuclei of the basal ganglia. Both the rate and the amplitude of ventilation altered significantly at all loci. However, the thermocouple, used for recording the change in the temperature of tidal air is inappropriate for precise measurement of the tidal volume. Therefore, only the approximate volume changes could be established. The tidal volume decreased during stimulation at all loci. An increase in the tidal volume occurred only as a rebound-like aftereffect of the stimulation with medium or high intensity, most frequently at the globus pallidus. The respiratory changes were associated with pressor responses that are described elsewhere [Ángyán, 1994, Ángyán and Ángyán 1999a, b]. The electrically elicited effects from the same point in the same animal remained constant throughout the experiments.

The respiratory rate increased parallel with the stimulus intensity; the higher stimulus intensity the higher respiratory rate was obtained (Fig. 1). The increase in the respiratory rate was significant during stimulation with medium and high intensities at all loci, except the caudate nucleus (Fig. 2). The SN stimulation elicited the highest increase in RR, while the lowest changes were obtained from the caudate nucleus. During ketamine-induced anaesthesia the CD and Put stimulation often failed to alter the respiration. At the other loci the electrical stimulation elicited an increase in the respiratory rate also under anaesthesia. However, it is interesting, that in some cases the stimulation caused apnea both under chloralose and ketamine-induced anaesthesia. The apnea persisted till the end of the stimulation. Apnea was elicited frequently from GP, but it might be obtained also from Sub and SN. The apnea occurred in 58% of medium and high intensity stimulations of the GP, while in 26% and 19% of Sub and SN stimulation, respectively.

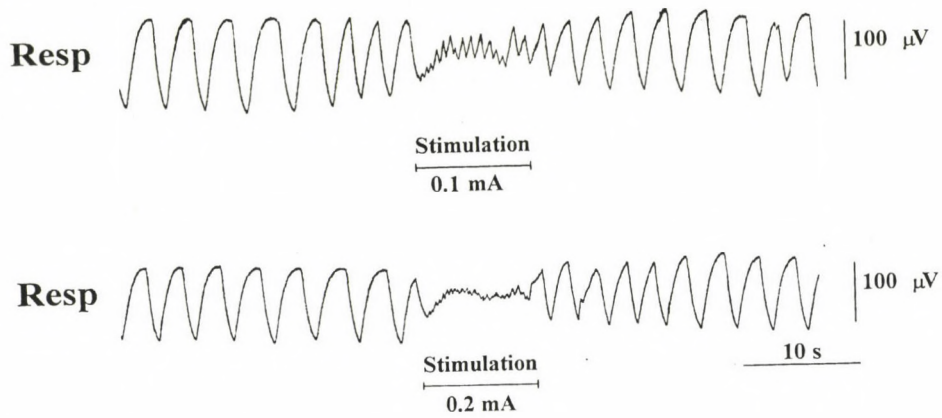

Fig. 1: Stimulation of the globus pallidus in freely moving cat with higher stimulus intensity elicited higher increase in the respiratory rate

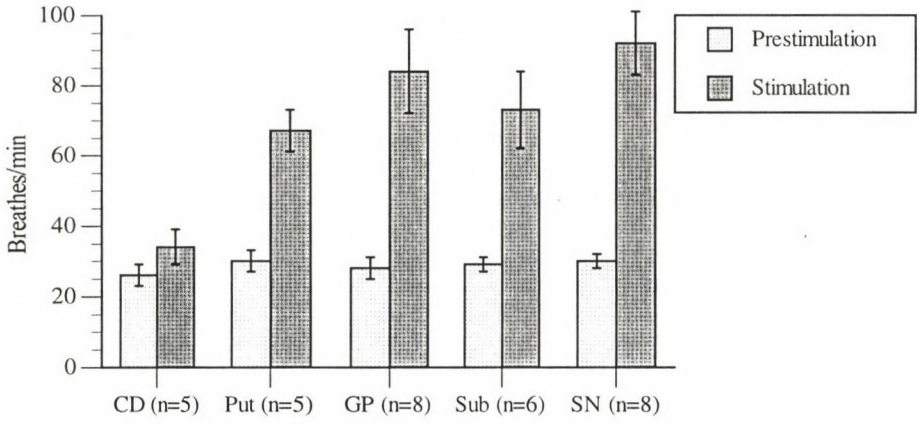

Fig. 2: Respiratory rate responses to electrical stimulation with medium stimulus intensity of the basal ganglia in freely moving cats. The increase in respiratory rate was significant at $p<0.001$ level, except the effects of CD stimulation that was not significant statistically. Abbreviations: CD: caudate nucleus, Put: putamen, GP: globus pallidus, Sub: subthalamic nucleus, SN: substantia nigra

The analysis of the respiratory curves on the polygraph recordings gives some insight into the dynamic changes of respiration caused by the stimulation. At the onset of stimulation hyperpnea appeared with a latency depending on the stimulus intensity (Fig. 3). The latency was measured by the computer as 10% shortening of the interpeak interval between two subsequent waves on the respiratory recording. Two kinds of responses could be observed

during stimulations of SN, GP and Sub. First, the hyperpnea lasted to the end of the stimulation. In some cases the high frequency, low amplitude ventilation produced a respiratory curve similar to the apnea (Fig. 1). Second, a reduction of respiratory rate might be observed during pallidal and subthalamic stimulations. It means that the frequency of ventilation reduced as compared to the value at the beginning of stimulation. The latency of the decrease in respiratory rate varied with the stimulus intensity (Fig. 3). However, the respiratory rate never decreased to the prestimulation level during the stimulation. Therefore, the means for stimulation effects were significantly higher than the prestimulation values (Fig. 2). This type of response was obtained from four GP and three Sub loci.

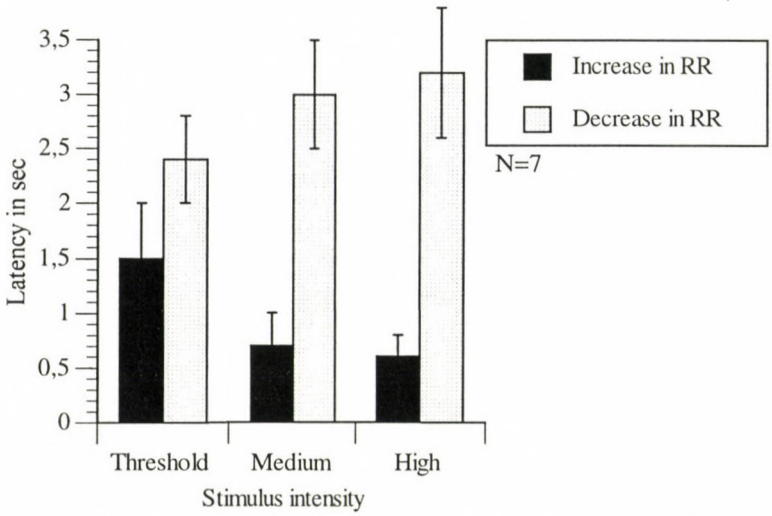

Fig. 3: Latencies of the changes in respiratory rate during stimulations with different intensities

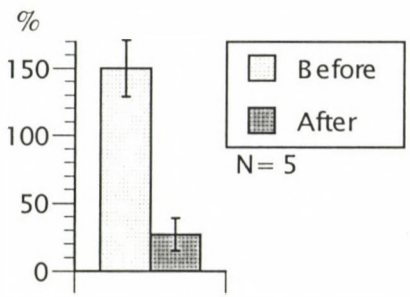

Fig. 4: Respiratory responses, in % of the prestimulation value, to the electrical stimulation of the globus pallidus before and 24 hours after local microinjection of kainic acid

The effects of the neurotoxic damage were tested at the GP. The electrical stimulation elicited smaller respiratory changes 24 hours after microinjection of kainic acid into the locus of stimulation (Fig. 4). The difference between the respiratory changes obtained before and after the microinjection of kainic acid was highly significant statistically ($p<0.001$).

The histological verification of the electrode tips localised 5 electrodes in CD and Put, 8 in GP, 6 in Sub and 8 in SN. The electrodes found outside of the target nucleus were omitted from this study. The neurotoxic lesions in the GP caused a significant loss (>80%) of cells in about 2 mm^2 area around the tip of the cannula.

DISCUSSION

The above results show clearly that the basal ganglia are able to alter the ventilation. However, it is possible to argue that the basal ganglia effects on the respiration are not mediated neuronally, rather current spread from the locus of stimulation to the respiratory centres in the brainstem causes the respiratory changes. The evidences against this possibility are the different effects obtained from the adjacent loci, furthermore, the fact that electrical stimulation failed to produce respiratory changes after neurotoxic damage of the stimulated loci.

It is well established that the respiratory centres in the brainstem consist of several functional subsystems executing different functions [Gordievskaya and Kireeva, 1999, Hukuhara, 1988, McCrimmon et al. 2000]. Therefore the question arises what functions of the respiratory centres are modulated by the basal ganglia stimulation. The fact that the frequency of ventilation was altered by the electrical stimulation indicates that the generation of respiratory rhythm might be modulated by the basal ganglia. This effect is predominantly facilitatory producing hyperpnea. The increase in the respiratory rate and decrease in the tidal volume may cause a respiratory disturbance similar to the apnea. This level of activation indicates that the respiratory rhythm generator is shifted to the upper limit of its regulatory range. The relative slowing of ventilation during stimulation points to the activation of the bradypnoe area. Rostral and caudal bradypnoea areas were localised by McCrimmon et al. [2000], but on the basis of the present results it is not possible to determine which one was activated. The apnea that occurred in anaesthetised animals was probably caused by direct activation of the respiratory motor neurones. Another explanation is offered by the observation that respiratory instability is a common feature of sleep [Neubauer, 1995]. Notwithstanding that sleep and narcosis are different phenomena, it is possible to speculate that the narcotic promotes respiratory instability, and it is increased by the basal ganglia stimulation up to complete arrest of ventilation. However, if it would be the case then stimulation at all basal ganglia have to cause apnea.

In conclusion, the basal ganglia circuitry is able to alter both the respiratory rate and the tidal volume. The alterations in respiration depend on the locus and the intensity of stimulation. The effects that basal ganglia exert on respiration suggest that the basal ganglia play a role in initiating the respiratory adaptation to the somatomotor movements.

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RESEARCH REPORT

THE ROLE OF THE MAMILLARY BODY IN THE PROPAGATION OF THE ICTAL ACTIVITY

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To determine the importance of fornix and mamillary body atrophy in the secondary generalization of the complex partial seizures fornix and mamillary bodies of 11 hippocampal sclerosis patients with secondary generalization (SG) and 3 without secondary generalization (WSG) were retrospectively evaluated using MRI images. Small fornix and/or mamillary body was not found in WSG group. In SG group the frequencies of small fornix and mamillary body were 64% and 45%, respectively, all being ipsilateral to sclerotic side. The frequency of small fornix in the SG group was statistically higher than WSG group ($p<0.01$). All small mamillary bodies were accompanied by ipsilateral small fornices. Considering the spatially decreasing frequency and the ipsilaterality of the hippocampal sclerosis, forniceal atrophy and mamillary body atrophy, a temporo-spatial relationship of the above-stated structures that may link focal seizures to secondary generalization was postulated.

Key words: epilepsy, temporal lobe, hippocampal sclerosis, mamillary body, seizure propagation, MRI

INTRODUCTION

The term epilepsy implies episodic seizure disorders with diverse pathologies that have been classified in accordance to the location and extent of the seizure process within the brain. Fundamentally, seizures are of two types: partial or generalized. This classification is based on the fact that the extent of cortical involvement and the neuroanatomic mechanism of expression differs between two groups.

Hippocampal sclerosis, also known as mesial temporal sclerosis or Ammon's horn sclerosis is characterized by neuronal loss and gliosis and is the most common pathology (50-70%) found in refractory epilepsies (Bronen et al., 1991; Bronen, 1992). This pathology causes complex partial seizures (CPS) also known as psychomotor epilepsy. Above-mentioned sclerotic hippocampal formation is identifiable in 60-80% of CPS patients and according to the

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epileptogenic focus concept, it results the ictal activity (Babb et al., 1984; Babb and Brown, 1987; Gloor, 1987). Because of the recruitment of deeper brain elements that affect the concious behavior the anatomy is more involved in the expression of partial complex seizures.

Hippocampus, fornix and the mamillary body are the components of a limbic circuit. In this circuit hippocampal fibrils extend to the mamillary body via fornix. In the recent past, mamillary body atrophy was encountered at autopsy in patients with epilepsy and it was suggested that the neuronal injuries of the hippocampus caused by mesial temporal sclerosis might result in ipsilateral fornix and mamillary body atrophy by deafferentiation of them (Lindboe at al., 1989; Zola-Morgan et al., 1989; Squire et al., 1990; Meldrum and Bruton, 1992). Different research groups addressed to the frequency of the fornix and the mamillary body atrophy in patients with temporal lobe epilepsy in order to establish their value in preoperative lateralization of the mesial sclerosis (Baldwin et al., 1994; Kim et al., 1995; Mammorian et al., 1995; Altintas et al., 1996). Their results, although vary in frequency, show a significant relation between the hippocampal sclerosis and atrophy of the connected structures. The temporal and spatial significance of these findings merits further research and consideration (Mirski, 1993).

Clinical and experimental studies on the mamillary system support the hypothesis that this system, mediates, in part, propagation and expression of some type of seizures (Green and Morin, 1953; Jinnai et al., 1969; Mirski and Ferrendelli, 1986; Miller et al., 1987; Mirski et al., 1984; Mirski et al., 1984, 1987; Mullen et al., 1967; Sussman et al., 1988). The mamillary system may possibly serve as a link mediating paroxysmal activity between the hippocampus and large cortical and subcortical areas and may influence the clinical expression of the temporal seizures by causing a secondary generalization: an event that is frequently encountered in the anamneses and examinations of CPS patients.

The purpose of the present study is to determine retrospectively the frequency of the asymmetrically small fornix and mamillary body in mesial sclerosis patients with and without secondary generalization by using magnetic resonance (MR) imaging in order to determine whether there is a relation between forniceal and mamillary body atrophy and the secondary generalization of the complex partial seizures.

MATERIALS AND METHODS

Materials

Two patient groups were used to determine the relationship between the frequency of the fornix and the mamillary body asymmetry and the secondary generalization of the temporal seizure.

One patient group (hippocampal sclerosis patients without secondary generalization, WSG) consisted of 3 unilateral hippocampal sclerosis patients with well-lateralized intractable temporal lobe epilepsy and without generalization of their ictal activity. In this group there were 2 men and 1 woman in the age range of 35–40, mean age being 38. The other patient group (hippocampal sclerosis patients with secondary generalization, SG) included 11 unilateral hippocampal sclerosis patients with well-lateralized intractable temporal lobe epilepsy and with the secondary generalization of their ictal activity. In this group there were 5 men and 6 women in the age range of 27–40, mean age being 32.2.

All patients had preoperative computed tomography, MR, posirton emission tomography and/or single photon emission computed tomography studies. They were also assessed with electrophysiological (long-term electroencephalographic EEG monitoring and/or electro-corticographic and deep electroencephalographic recordings with event related potential studies) and neuropsychological studies. Patients with evidence of bilateral mesial sclerosis were excluded from the study. All patients were operated and had pathological results consistent solely with hippocampal sclerosis.

MR Imaging Technique

All patients were examined in 1.5 Tesla superconductive imager (Gyroscan II, Philips, Netherlands) with fast-spin-echo (FSE) T2-weighted, spin-echo (SE) T1-weighted and inversion recovery (IR) T1-weighted images in the axial planes and spin-echo T1-weighted, fast field echo (FFE) T1-weighted and/or FSE T2-weigted images in the oblique coronal planes.

Measurement technique

With the exclusion of the hippocampal regions on the MR images, fornices and mamillary bodies were visually evaluated. When evaluating the fornix, the size was determined on coronal FSE images using large window level and by evaluating the transverse diameter of both fornices at the level of the rostral crus just posterior to the body of the fornix. In this point, both cruses approach the midline to make the forniceal bodies and run perpendicular to the scan plane (Mark et al., 1993). Therefore, both fornices can be well-outlined separately and can be accurately compared. The interslice gap that was used eliminates the cross-talk effect which would cause partial voluming. In order to compensate the large variation of the superior and inferior borders of the cruses, forniceal evaluations were performed in the horizontal axis. Coronal GRE MR images were also used when necessary. Fornices were classified as asymmetrically small or equal in size.

In order to evaluate the mamillary bodies which are 4-5 mm in diameter, both the size and the localization of the mamillary bodies were compared on axial and coronal images under appropriate windowing. Mamillary bodies were classified as asymmetrically small or equal size types. When evaluating the mamillary bodies, artificial asymmetry caused by asymmetrical positioning of the head or the dolichoectasia of the basillary and posterior cerebral arteries are potential pitfalls (Mammorian et al., 1995). Such cases were evaluated in different planes or excluded from the study. As reactive astrogliosos of the mamillary body is not noted in patients with hippocampal sclerosis, a change in the mamillary body signal was not expected and not taken into the consideration (Lindboe et al., 1989).

Mamillohalamic tracs were also visualized in the IR and T2-weighted axial images as they were reported in the studies (Shen et al., 1991). They are bilaterally compared where available but not statistically evaluated.

Statistical analysis

The frequencies of the asymmetrically small fornices and mamillary bodies were compared statistically in complex partial epilepsy patients and in secondarily generalized epilepsy patients using Fisher's Exact test.

RESULTS

Asymmetrically small fornix was not found in WSG group, whereas it was found in 64% (7 of 11) of the SG group, all ipsilateral (Table 1). The frequency of an asymmetrically small fornix in the SG group was statistically higher than that in the WSG group (Fisher's Exact test, $p< 0.01$).

Table 1. The frequency of asymmetrically small fornix in complex partial epilepsy patients and in secondarily generalized epilepsy patients

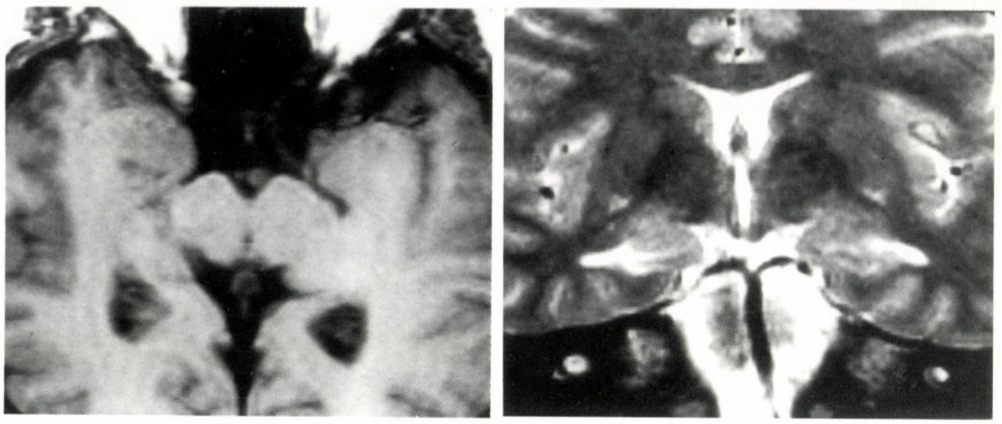
	COMPLEX PARTIAL EPILEPSY PATIENTS	SECONDARILY GENERALIZED EPILEPSY PATIENTS	TOTAL
SMALL	0	7	7
EQUAL	3	4	7
TOTAL	3	11	14

Asymmetrically small mamillary body was not found in the WSG group, whereas it was found in 45% (5 of 11) of the SG group, all ipsilateral (Table 2). The frequency of an asymmetrically small mamillary body in the SG group was obviously higher than that in the WSG group although a statistical study was not carried to avoid a type 2 error which would be caused by the small data set for WSG group.

Table 2. The frequency of asymmetrically small mamillary body in complex partial epilepsy patients and in secondarily generalized epilepsy patients

	COMPLEX PARTIAL EPILEPSY PATIENTS	SECONDARILY GENERALIZED EPILEPSY PATIENTS	TOTAL
SMALL	0	5	5
EQUAL	3	6	9
TOTAL	3	11	14

All patients with asymmetrically small mamillary body also had asymmetrically small fornix on the same side, whereas none had asymmetrically small fornix in the other side (Fig. 1).



A B

Fig. 1. (A) Axial T1-weighted (A) and coronal T2-weighted (B) magnetic resonance images of two different patients, both demonstrating asymmetrically small mamillary bodies. Smaller mamillary bodies were ipsilateral to the sclerotic hippocampi

DISCUSSION

Focal epilepsies, in which a structural disease is frequently present, display manifestations consistent with involvement of only a portion of the cortex and its corresponding functional systems. The spread of such seizure to adjacent cortical regions is presumed to be via local synaptic connections. The seizure may spread along the motor strip in case of Jacksonian epilepsy (simple partial) or may additionally spread along the deeper brain elements as in case of temporal epilepsy (complex partial). Because of the recruitment of deeper brain elements the concious behavior is also get affected and the anatomy is more involved in the latter. Usually hippocampus and amygdala and their connections play a major role in the expression of partial complex seizures. MR have been useful in some cases of partial seizures in locating brain disease, especially hippocampal sclerosis (Bronen et al., 1991; Bronen, 1992; Jackson et al., 1993; Kuzniecky et al., 1987; Jack et al., 1990; Tien et al., 1993).

Generalized seizures characterized with the involvement of the entire cerebral cortex. In such epilepsies there is loss of consciousness and generalized convulsive or paralytic motor phenomena. Their stereotypical electrical and behavioral expressions makes us think that they are well-orchestrated propagation of neuronal synaptic activity. Although the functional anatomy involved in initiating and mediating the convalsive process has not been well-defined, specific subcortical synaptic paths such as ventral anterior, centromedian and lateral thalamic nuclei, fields of Forel, interpedincular complex, and substantia nigra appear to conduct this propagation (Mirski, 1993). Because of the rapid and uniform brain excitation that occurs during a generalized seizure there must be some major neuroanatomic structures and pathways connecting cortical and subcortical brain into the seizure process.

Because of its location within the posterior hypothalamus, the mamillary system is placed in a unique position to act as a synaptic link between brain stem and forebrain in the propagation of paroxysmal electrical activity. Primary afferents to the mamillary body are postcomissural fornice from subiculum and pathways from brain stem ventral and dorsal tegmental nuclei.

Primary efferents from the mamillary body are mamillothalamic tract to anterior thalamic nucleus and pathways to ventral and dorsal tegmental nuclei. From the anterior thalamus the mamillary system projects rostrally to the cingulate gyrus, which has diffuse projections to most of the cerebral cortex and to many subcortical regions. Adjacent to mamillary bodies are median forebrain bundle projecting from rostral midbrain to septal nuclei, and the periventricular nuclei with the afferents from central gray of the midbrain (dorsal longitudinal fasciculus). The described paths form a circuit first decribed by Papez consisting hippocampus-mamillary body-anterior thalamus-cingulate gyrus-hippocampus circle (Papez, 1937). But as a direct communication between the cingulate cortex and hippocampus is not very clear and as anterior thalamus has direct reciprocal contact with the hippocampus the above-mentioned circuit has been revised (Dagi et al., 1983). As evident from the above description, the mamillary body fullfils the need for synaptic pathways by having well-defined connections with thalamus, hippocampus and brain stem, and with possible interactions with more diffuse ascending and descending projection systems.

The electrical stimulation of the fornix, mamillary body or anterior thalamus causes cortical electroencephalographic synchrony and seizure-like discharges in the cats (Murphy et al., 1945; Green et al., 1953). Focal destruction or chemical inhibition of the mamillary bodies raised the seizure threshold to the chemical stimulation (Jinnai et al., 1969; Mirski, in press or 1993). In induced seizures very selective metabolic enhancement in dorsal and ventral tegmental nuclei-tegmentomamillary tracts-mamillary body-mamillothalamic tracts-anterior thalamic nuclei was showed using ^{14}C-deoxyglucose radiography (Mirski and Ferrendelli, 1986). Interruption of the tegmentomamillary or mamillothalamic tracts resulted in anticonvulsant effect against phantylentetrazol (Mirski and Ferrendelli, 1984; Mirski and Ferrendelli, 1987). Muscimil injection into the anterior thalamic nuclei raised the seizure threshold, whereas kainic acid lowered (Mirski and Ferrendelli, 1986; Miller, 1987). Posterior hypothalamotomy or the lesioning or the stimulation of the anterior thalamic complex causes substantial diminution in seizure frequency (Sano et al., 1972; Mullen et al., 1967; Sussman et al., 1988). These clinical and experimental studies on the mamillary system support the hypothesis that the mamillary system, mediates, in part, propagation and expression of some type of seizures.

It was suggested that the neuronal injuries of the hippocampus that are caused by mesial temporal sclerosis or ischemic and toxic damage of the Ammon's horn might result in ipsilateral fornix and mamillary body atrophy by deafferentiation of them (Meldrum et al., 1992; Zola-Morgan et al., 1989; Squire et al., 1990; Lindboe et al., 1989; Charness et al., 1987). The infrequent association of the mamillary body atrophy was explained by the autoradiographic studies on animals where the efferent connections of the mamillary body was shown mainly to originate from the subiculum and by some evidence that shows the subiculum is not involved in mesial sclerosis (Meibach et al., 1975; Swanson et al., 1975; Babb et al., 1984). Still when considering the frequency of the mamillary asymmetry it should be kept in mind that in contrast to Meibach's and Swanson's findings earlier studies had revealed that the fornix originated largely in Ammon's horn (Raisman et al., 1966), and that mesial temporal sclerosis could be much widespread in some epileptic cases.

As evident from the above findings, the forniceal and the mamillary body atrophy may be seen in patients with temporal lobe epilepsy. In some cases, mamillary body atrophy ipsilateral to the seizure side, unaccompanied by hippocampal sclerosis was observed, although not frequently (Mammorian and Brown, 1993). Its fusions and hypoplasias can be rarely seen in the autopsies accompanying the gross brain abnormalities, whereas its unilateral absence was

not demonstrated (Pitella and Marciel, 1985). In some series, mamillary body asymmetries were observed ipsilateral to the pathologies of the medial temporal lobe or middle cranial fossa (Hyoung Kim et al., 1995; Mammorian et al., 1995). These findings implicate that the mamillary body asymmetry is neither a normal variant nor a unique to patients with mesial temporal sclerosis. There are findings which show that the mamillary body asymmetry is not the result of the recurrent seizures (Mammorian et al., 1995). The relationship between the grade of the sclerosis and the accentuation of the mamillary asymmetry is questionable and merits further consideration. Still this finding does not avoid the mamillary body playing a secondary role temporal lobe epilepsy.

Although there seems to be a substantial relation between the hippocampal damage and mamillary body atrophy, in histologic studies of the mamillary bodies in hippocampal sclerosis patients, atrophy and neuropil loss are found, but neuronal perikarya were spared (Lindboe et al., 1989). It is not clearly seen, whether hippocampal injury may directly cause dysfunction of mamillary bodies or not. Changes that are observable in both structure may also depend on same initiator in a complex matter. When considering the spatially decreasing frequency and the definite ipsilaterality of the hippocampal sclerosis, forniceal atrophy and the mamillary body atrophy a temporo-spatial relationship must exist between hippocampus-fornix and the mamillary body. Noted solely in secondarily generalized epilepsy patients that path should link the focal seizure to secondary generalization. It was also noted that all of these patients had secondary generalization only in the earlier period of their medical history. Considering that finding, one might think that such link functions early in the epileptic life, than become nonfunctional as the mamillary body gradually atrophies. But considering that we do not have earlier MRI scans of these patients showing the status of the mamillary bodies, such hyphothesis must be tested in the context of the prospective studies with MRI scans and the pathological samples of the mamillary bodies.

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RESEARCH REPORT

POST-EMBEDDING DOUBLE-GOLD LABELING IMMUNOELECTRON MICROSCOPIC CO-LOCALIZATION OF NEUROTRANSMITTERS IN THE RAT BRAIN

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In this report, we describe a new quantitative electron microscopic protocol based on the use of double colloidal-gold post-embedding immunostaining procedure as markers to analyze the subcellular distribution of enkephalin (ENK) and GABA neurotransmitters simultaneously in the same ultrathin tissue sections in the periaqueductal gray area (PAG) of the rat brain. Double-gold particle signals were significantly improved using a number of technical adjustments for the immunogold electron microscopic co-localization technique of ENK and GABA. The GABA-like neuronal elements were immuno-reacted with 20 nm gold particles and the enkephalin-like immunoreactive neurons were labeled with 10 nm gold particles. This double labeling was more apparent in tissue sections that were deactivated for the gold staining of the first antibody. Excellent double labeling was obtained when we blocked antigenicity of the first antiserum with hot (80^0C) paraformaldehyde fumes. To minimize the clumping of the second gold particles around the first gold particles used against the first antibody, we tried different staining order for the neurotransmitters tested in this study. It was necessary to use a detergent (Triton X-100) at very low concentration (0.1%) instead of etching to expose the antigenic determinants of the neurotransmitters and at the same time to reduce the deleterious effects on the morphology of the tissue sections. Furthermore, the high-glutaraldehyde fixation and the decrease in the interval between cutting and labeling of the ultrathin sections significantly improved the results obtained in this study. Double labeling of sections with ENK and GABA produced co-localization in 23.1% and 1.2% of the immunoreactive axonal terminals and dendrites, respectively. Most of the double-labeled terminals contained more GABA-like than ENK-like immunolabeling. Half of the axon terminals [51%] and dendrites [56%] in the ventrolateral PAG were not labeled with either of GABA or ENK immunoreactivity. This procedure was found to be completely compatible with good double immunolabeling and ultrastructural preservation.
Key words: central gray, pain, GABA, enkephalin, electron microscopy, gold particles

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INTRODUCTION

Many studies designed to investigate neurotransmitter distribution in certain brain regions have been published within the past few years. In these studies a variety of biochemical, physiological and morphological methods were used to localize more than one neurotransmitter in the same tissue sections in different brain regions but each of them has its specific set of potentials and limitations (Ottersen, 1991). Double-labeling techniques using pre-embedding staining for a specific antigen followed by post-embedding immunogold labeling for a second neurotransmitter or using a combination of anterograde or retrograde labeling followed by gold immunolabeling of a neurotransmitter have been used widely in the past decade (Smith and Bolam, 1991; Wang et al., 1994). Gold immunolabeling methods have during recent years been of enormous value for detailed studies of the distribution of neuroactive substances like amino acids, peptides and GABA in the central nervous system (CNS).

The demonstration of neurotransmitters in axon terminals is the most convincing immunocytochemical evidence for identification of their presence in a particular pathway. This can be only shown with electron microscopy. However, pre-embedding immunocytochemistry is highly fixation sensitive and suffers from problems of interpretation related to limited and unpredictable antibody penetration (Priestly and Cuello, 1983). The need for low-glutaraldehyde fixation and the lengthy processing prior to osmication step using this method produce low ultrastructural morphology; moreover, it is not suitable for quantitative study of neurotransmitter. In contrast, post-embedding immunocytochemistry minimizes these difficulties permitting quantitative study of relatively well-preserved tissue at the electron microscopy level (Ottersen, 1989; Tracey et al., 1991). More importantly, electron microscopy post embedding immunocytochemistry allows the simultaneous localization of two or more antigens.

In order to visualize and quantitate two different neurotransmitters at the electron microscopic level with good ultrastructural preservation, the use of double immunolabeling was introduced. This procedure provides good ultrastructural features of the neurotransmitter distribution and allows the determination of the role of neurotransmitter immunoreactive neuronal elements in the synaptic circuitry of the brain region under investigation. The aim of this present study, therefore, is to improve the double post-embedding immunogold labeling procedure in order to achieve a better resolution and a more representative image of the distribution coexistence of ENK and GABA. In this paper we discuss the steps that we believe are required for successful and consistent double post-embedding immunogold staining for GABA and enkephalin.

MATERIALS AND METHODS

Fixation and preparation of the tissue

Male Sprague-Dawley rats (n = 5) weighing 250-275 g were deeply anesthetized with 35% chlorohydrate. The animals were perfused transcardially as previously described (Lee et al., 1992; Renno et al., 1997). Briefly, the chest was opened and a cannula was rapidly inserted through the heart and wedged in the ascending aorta. Within 10-15 sec after thoractomy, rats were perfused with 50 ml calcium-free Tyrode's solution followed by 2.5% glutaraldehyde, 0.2% paraformaldehyde and 0.2% picric acid in 0.1 M Sorensen's buffer. Perfused brains were

removed and further postfixed overnight at 4^0C by immersion in the same fixative used for perfusion. The tissue was osmicated, en bloc stained with uranyl acetate in NaOH maleate buffer (pH 6.0), dehydrated and then embedded in Spur's/Epon (6:4) resin between diethyldichlorosilane-treated glass microscope slides. The slides were separated and the tissues were examined by light microscopy. Areas representative of ventrolateral PAG were excised. These portions of the PAG were glued to Epon blocks, trimmed and sectioned (silver-gold interference color). The ultrathin sections were mounted on formvar coated nickel grids for immunogold staining.

Sera and Specificity Controls

Dr. B. Maley (University of Kentucky, Lexington, KY) kindly provided antibody to GABA that has been previously characterized (Maley and Newton, 1985; Newton and Maley, 1987). Staining by this antiserum was not diminished after pre-absorption with glutamate, aspartate, taurine, glycine or alanine. Pre-absorption of the primary antibody with synthetic GABA or pre-absorption of the secondary antibody with normal rabbit serum abolished immunostaining (Williams and Beitz, 1990b). The ENK antibody (No 153) was kindly provided by Dr. R. Elde (University of Minnesota, MN). This antiserum cross-reacted less than 1% with [Leu]enkephalin and less than 0.01% with ß-endorphin when tested by radio-immunoassay. However, the antiserum was unable to distinguish between [Met]enkephalin and [Leu]enkephalin when employed in immunohistochemical procedures (Williams and Beitz, 1990b).

Omission of the second primary antiserum abolished labeling with the respective gold particles size, indicating a complete saturation of antigenic epitopes on the first layer primary antibody. The immunolabeling pattern for the individual neurotransmitter mimicked that observed in parallel experiments with single labeling of adjacent sections (not shown). There was no overlap between the two gold particle sizes that were used. According to quality control certificate from the manufacturer (E&Y laboratories, INC., San Mateo, CA) the mean size (nm ± S.D.) of the "10-nm" and "20-nm" particles were > 95% 10 nm ± 3 and > 95% 20 nm ± 5, respectively.

Post-embedding double-immunogold labelling of ultrathin sections

After testing all possible sequences of GABA and ENK antisera and gold sizes to ensure that the immunolabeling patterns observed in the ultrathin sections were representative of the antigens, we adopted the following protocol. Briefly, the sections were washed in TBST pH 7.6 buffer (0.05 M Tris pH 7.6 with 0.9% NaCl and 0.1% Triton X-100) and then drained by touching the edge of grids to kimwipe, followed by incubation in anti ENK primary antibody (diluted 1:2000 in TBST pH 7.6) overnight in a moist chamber at room temperature. The next day, the sections were washed twice in TBST pH 7.6 buffer for 5 min each followed by a third wash for 30 min. Next the sections were conditioned in TBST pH 8.2 buffer (0.05 M Tris pH 8.2 with 0.9% NaCl and 0.1% Triton X-100) for 5 min. Grids were then incubated with goat anti-rabbit IgG conjugated to 10 nm gold particles (diluted 1:20 in TBST pH 8.2 buffer) for 1 h, washed twice in TBST pH 7.6 for 5 min each and rinsed in ddH_2O. After completion of ENK labeling protocol, the dried sections were incubated in paraformaldehyde vapors in 80^0C oven for 1 h to deactivate binding sites. Next, the sections were washed in the TBST pH 7.6 buffer for 5 min and immunostained with GABA antiserum (diluted 1:500 in TBST pH 7.6 buffer) overnight in a moist chamber. The following day, the grids were washed as described above after applying the ENK antibody. After washing the sections, they were incubated with

goat anti-rabbit IgG conjugated to 20 nm gold particles (diluted 1:20 in TBST pH 8.2 buffer) for 1 h. Finally, the sections were washed in TBST pH 7.6 buffer for 5 min, thoroughly rinsed in ddH_2O and counter-stained with uranyl acetate and lead citrate.

Sampling of profiles and quantitation of post-embedding immunolabeling

After adapting the above-described staining protocol, two sections each from immuno-labeled portions of ten tissue blocks, two from each of five rats, were subjected to quantitative morphometric analysis. Random electron micrographs were taken from the ventrolateral area of the caudal third of the PAG.

Ultrathin sections were examined under a JEOL 1200EX II transmission microscope at 60 kV. Sections were photographed randomly at a plate magnification of ×25,000, and printed at a final magnification of ×35,000. All gold particles, including those over mitochondria, were counted over each profile (neuron somata, dendrites, axon terminals, or glial cell bodies). Aggregates of two or more gold particles were counted as one. Aggregates could be recognized from the uniform small distance between the particles. Occasionally, closely but independently attached particles may have been counted as one. This would occur mainly in densely labeled structures resulting in the under-estimation of particle density. Since, at the most, only a few percent of the particles were aggregates, this under-estimation would not affect the differences between cell profiles in the present study. For quantitative analysis, the number of gold particles per square micrometer for each structure was calculated according to the morphometric technique of Bendayan et al. (1980) as previously described (Lee et al., 1992; Renno et al., 1997; Renno et al., 1999). Briefly, the surface area (Sa) of individual histological structures was first measured using a digitizing tablet (Jandel Scientific. Sigma Scan V3.90) and associated microcomputer (WSYE pc+). An electron micrograph was positioned on the tablet and the perimeter of the profile to be measured was traced. The number of gold particles (Ni) present over individual neuronal profile was then counted manually, and the density of labeling (Ns) calculated as Ns = Ni/Sa for each gold particle size. A background gold particle density per μm^2 was calculated for each animal from immunohistochemically processed sections in which the ENK and GABA primary antibodies were replaced with normal rabbit serum. This background density of gold particles was then subtracted from the calculated ENK or GABA gold particle densities to obtain a final density per μm^2. Neuronal structures were identified according to the criteria set forth by Peters et al., 1991. A one way analysis of variance (ANOVA) was applied to assess significant differences between the mean values of gold particels among the different neuronal structures quantified. The Scheffe F-test was used to deterimine individual probability values for multiple comparisons. In addition, the surface density of gold was quantitated over individual neuronal and non-neuronal (glial) processes identified on the basis of their fine structural features and their locations in the PAG in order to determine whether the observed differences in ENK or GABA immunolabeling were statistically significant. All gold particles were counted over each profile, and the results were published earlier (Renno et al., 1999).

RESULTS

I) Etching

Most of the post-embedding immunolabeling protocols (Varndell and Polak, 1984; Bendayan and Zollinger, 1983) use oxidizing agents on tissue section to remove osmium and plastic. This etching procedure improves antigenic immunoreactivity by exposing antigenic sites and restoring their affinity for antibody. In this study several etching protocols (Ottersen et al., 1988) were tested. Treating the sections with 10% hydrogen peroxide for 15 and 30 min produced low tissue contrast and numerous holes (Fig. 1A and B). When grids were etched with saturated sodium metaperiodate or with 1% periodic acid followed by saturated sodium metaperiodate, severe tissue destruction and many holes with non-specific gold labeling for both ENK and GABA were apparent (Fig. 1C and D). Incorporating Triton X-100 in our buffers (DeZeeuw et al., 1988) produced excellent tissue preservation of the different subcellular organelles and sharp cellular membranes with great improvement in double immunolabeling of ENK and GABA (Fig. 1E).

II) Order of first and second gold particle sizes

Small (10 nm) gold particles tended to cluster around the large 20 nm gold particles when the sections were incubated first with GABA antiserum followed by 20 nm gold conjugated antibody and then stained with second ENK antiserum followed by 10 nm gold conjugated IgG (Fig. 2A). Similar clustering were noticed when ENK antiserum was used first followed by secondary large gold conjugated IgG then stained with GABA antiserum followed by 10 nm gold conjugated IgG (Fig. 2B). Regardless of the gold particle size used, it seems that using GABA antiserum first causes non-specific clustering and clumping of ENK antibody (Fig. 2C and D). Blocking the sections with 2% normal goat serum generally decreased the non-specific gold labeling of ENK (Fig. 2E and F) but the GABA staining was not intense enough and remained clustered around the small gold particles.

III) Deactivation for double immunolabeling

Because of the fact that all antisera used in this study were from rabbits, deactivation of any rabbit IgG molecules remaining from the first primary antibody treatment was required for the successful labeling with the second primary antibody. Several experimental techniques of deactivation were tested. The degree of deactivation was measured by counting small (10 nm; from the first incubation in secondary antiserum) and large (20 nm; from the second incubation in secondary antiserum) gold particles and calculating the percentage of gold particles that were large (Table I). Thirty-five percent of the gold particles were large after drying the sections at room temperature (20^0C) (Fig. 3A). Immersing the grids in 4% paraformaldehyde solution at room temperature decreased gold particles to 21% (Fig. 3C); while heating the tissue sections at 80^0C reduced this count to 10% (Fig. 3B). However, drying grids at 80^0C over paraformaldehyde crystals significantly reduced the large gold particles to 1% that is comparable to background levels (Fig. 3D). Therefore, this last step was routinely used in our double-immunolabeling protocol. Heating over paraformaldehyde crystals appears to slightly enhance immunoreactivity for the second antibody and significantly reduce the non-specific staining of the second antibody. In addition, for double labeling with GABA and ENK, we routinely used ENK as the first primary, and the anti GABA as a second primary antibody (Renno et al., 1999).

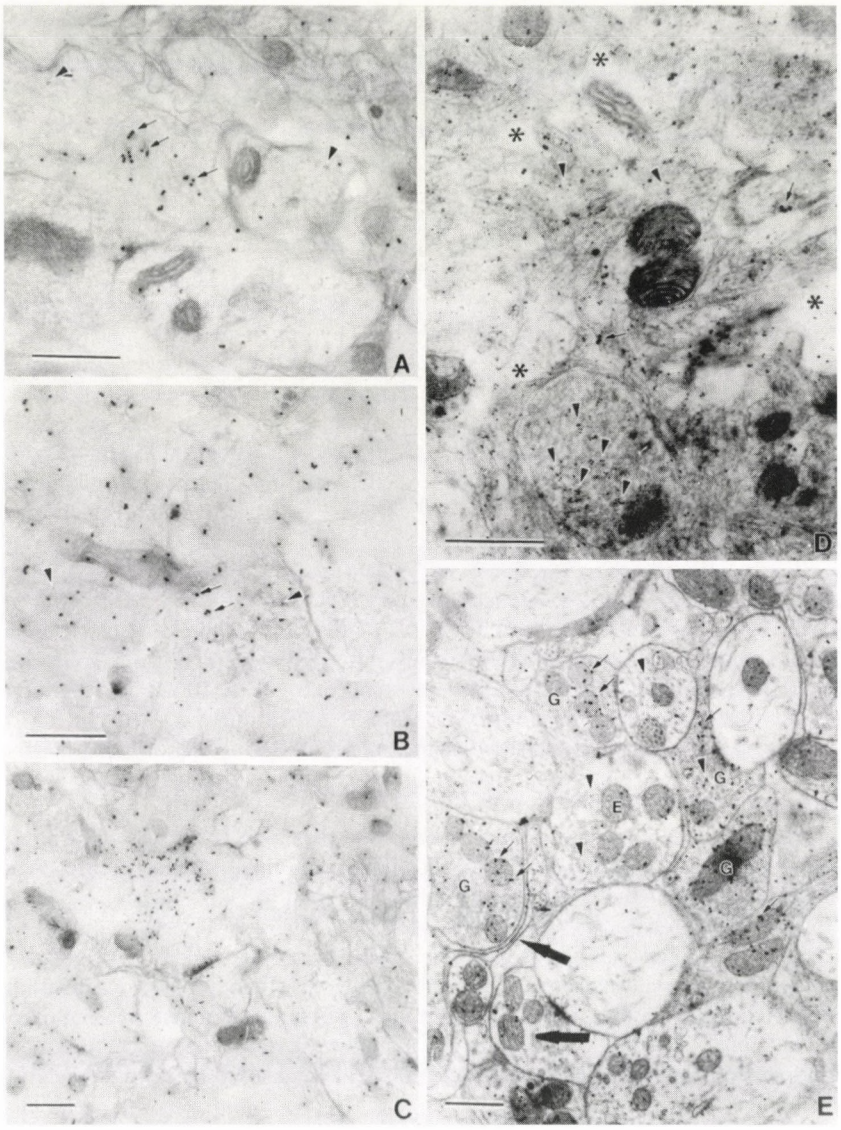

Fig. 1. Effect of etching on tissue from the caudal ventrolateral PAG area of a rat. Immunolabeling runs are for enkephalin (10 nm gold particles) (arrowheads) followed by GABA (20 nm gold particles) (arrows) in all sections. **A and B:** sections were treated with 10% hydrogen peroxide for 15 and 30 min, respectively. Note the low tissue contrast and numerous holes produced as a result of the etching technique. A ×43,000: B ×40,000. Scale bar = .2 μm. **C:** Tissue sections were etched with saturated sodium metaperiodate for 15 min. Severe tissue destruction and holes with non-specific labeling of both enkephalin and GABA were apparent. ×23,000. Scale bar = .5 μm. **D:** Sections were etched with 1% periodic acid for 8 min, rinsed in double distilled water and etched again in saturated sodium metaperiodate for 15 min. Note that the tissue contrast got better, however, holes (asterisks) due to etching procedure still exist. Immunolabeling improved significantly specially for enkephalin (arrowheads). GABA (20 nm) (arrows) was completely abolished. ×49,000. Scale bar = .2 μm. **E:** sections processed without etching. Tissue sections were immersed for 5 min in TBST pH 7.6 buffer containing Triton X-100 before double labeling with GABA and enkephalin. Note that this procedure provided excellent tissue preservation of different subcellular organelles and sharp cell membranes (large arrows) in addition to good GABA (G) and enkephalin (E) immunolabeling of some axonal terminals. ×29,000. Scale bar = .5 μm

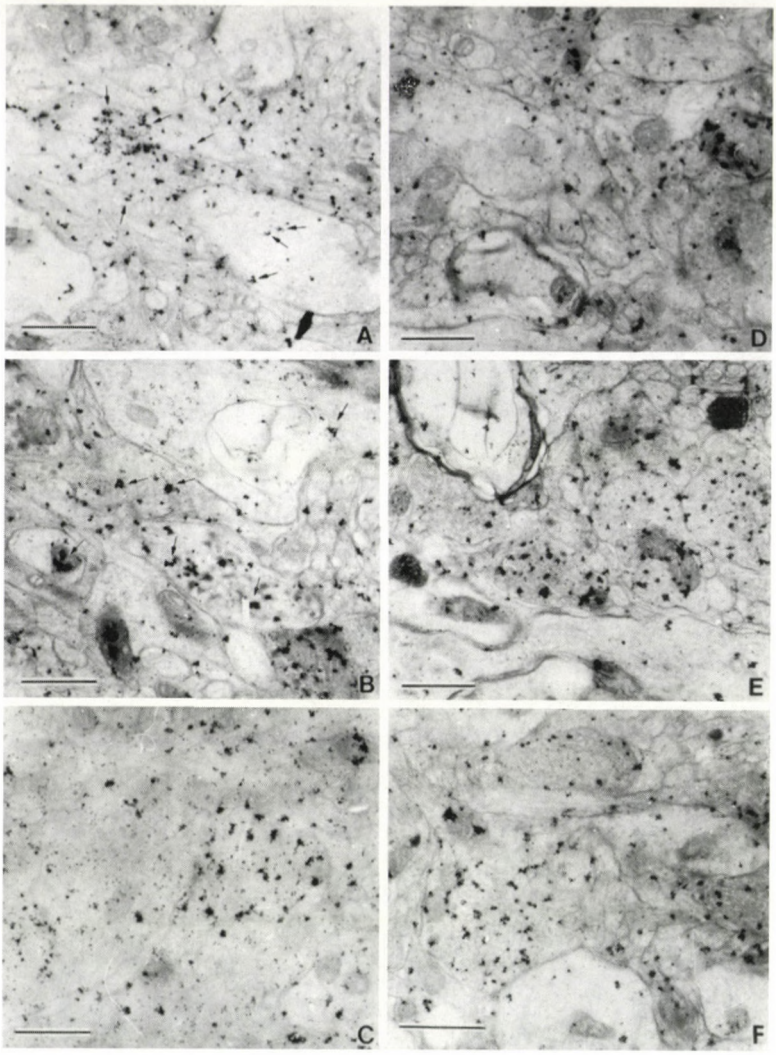

Fig. 2. Effect of the order of using first and second gold particle sizes. **A:** An example of sections that were incubated first with GABA antibody followed by 20 nm gold conjugated antibody and then stained with the second enkephalin antiserum followed by 10 nm gold conjugated IgG. Note that the small 10 nm gold particles (arrows) tend to cluster around the large 20 nm gold particles. ×40,000. In **B:** enkephalin antiserum was used first followed by 20 nm gold conjugated IgG. Tissue was next stained with GABA antibody followed by 10 nm gold conjugated anti-IgG. Note that the 10 nm gold particles (arrows) tend to cluster around the large 20 nm gold particles whenever 10 nm gold conjugated IgG was used after the large 20 nm gold conjugated IgG. ×40,000. **C and D:** Two examples of electron micrographs of sections double immunolabeled with GABA antibody followed by secondary IgG conjugated to 10 nm gold particles then stained with enkephalin antibody followed by anti IgG conjugated to 20 nm gold particles. It seems that using GABA antiserum first tend to non-specifically attract enkephalin antibody. In **C**, the tissue sections were not deactivated or blocked with normal goat serum before staining with the second enkephalin antibody while in **D**, the sections were blocked with 2% normal goat serum for 20 min. ×40,000. **E and F**: Sections were stained first with ENK antibody followed by 10 nm gold particles then stained with GABA followed by anti IgG conjugated to 20 nm gold particles. In **E**, sections were blocked with 2% normal goat serum for 20 min after staining with ENK and its secondary conjugated antibody (10 nm gold particles). ×40,000. In **F**, tissue was blocked with 2% normal goat serum before and after staining with the first antibody (ENK, 10 nm) followed by GABA antibody (20 nm). Note the general decrease in non-specific labeling of enkephalin in both cases. However, GABA gold labeling was still not intense enough and clumped around enkephalin (10 nm gold particles). ×45,000. Scale bars = .2 μm

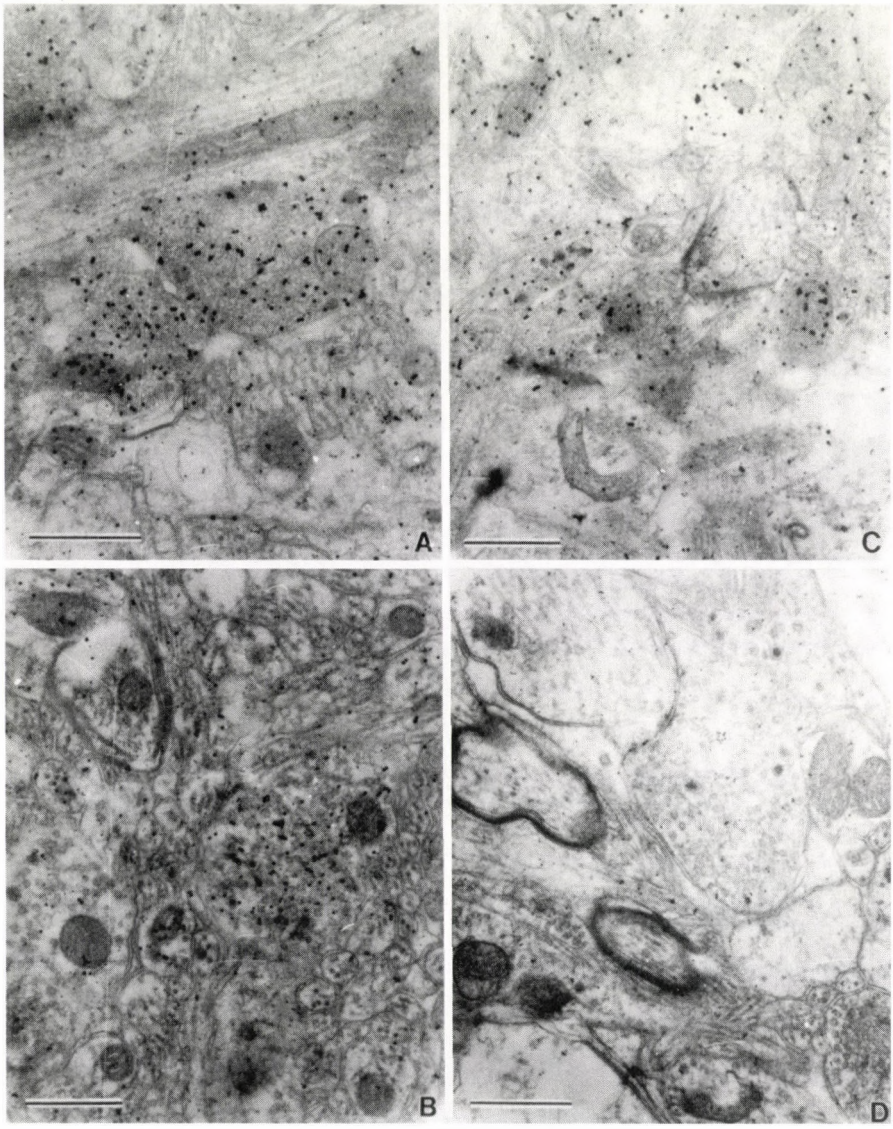

Fig. 3. These electron micrographs illustrate the effect of different procedures to deactivate gold staining of primary antibody (anti-enkephalin binding sites remaining after incubation in goat anti-rabbit IgG coupled to 10 nm gold particles). Deactivation of these sites was evaluated by counting larger particles after incubation in goat anti-rabbit IgG conjugated to 20 nm gold particles. **A:** Deactivation by drying the sections in air at 20^0C for 1 h after applying the anti-enkephalin antibody followed by goat anti-rabbit IgG conjugated to 10 nm gold particles. ×54,000. **B:** drying in air at 80^0C for 1 h. 45,000X. **C:** deactivation by incubating the sections in 4% paraformaldehyde solution for 1 h. ×44,000. **D:** drying in air at 80^0C for 1 h over paraformaldehyde crystals. Counts representing these different deactivation treatments are given in Table I. ×46,000. Scale bars = .2 μm

IV) Primary antibody concentration

After working out the double-immunolabeling procedure as described in the method section above, we then tested the GABA and ENK antisera in dilutions ranging from 1:100 to 1:10,000 (Fig. 4). Enkephalin immunolabeling had high background when the primary antibody was more concentrated than 1:1000 (Fig. 4A and B); while GABAstaining had high background when the primary antibody was more concentrated than 1:100 (Fig. 4D). Good signal-to-noise ration was obtained with dilutions between 1:2000 and 1:500. However, best results with the ENK and GABA antibodies were obtained with dilutions of 1:2000 and 1:500, respectively (Fig. 4C).

V) Specific Controls

Routine control experiments (blocking with the appropriate antigen, omission of primary antisera or substitution of primary antisera with non-immune sera) confirmed the specificity of the immunolabeling. The ENK and GABA antisera used in this study have been shown to possess considerable specificity for ENK and GABA, respectively (Maley and Newton, 1985; Newton and Maley, 1987; Williams and Beitz, 1990a and b). The specificity of the staining, which was observed at the electron microscopic level, is further demonstrated by the following. First, there was no staining when the ENK and GABA antisera was absorbed by pre-incubation with 100 μM GABA (Fig. 5A) and 20 μM ENK (Fig. 5B), respectively. Second, tissue sections were treated with pre-absorbed ENK antiserum (pre-absorbed with 20 μM of ENK; 1:2000) followed by secondary anti IgG conjugated to 10 nm gold particles. These sections were then stained with GABA antibody preabsorbed with 100 μM GABA followed by a secondary anti IgG conjugated to 20 nm gold particles (Fig. 5C). These pre-absorption tests of GABA and/or ENK antisera significantly reduced the gold labeling density of GABA or ENK to background levels. Third, the labeling was not observed when normal rabbit serum was substituted for ENK and GABA antisera during double-staining procedure (Fig. 5D). All these control experiments support the conclusion that the anti-ENK and anti-GABA sera used in this study recognize ENK and GABA, respectively, and corroborate previous results (Maley and Newton, 1985; Newton and Maley, 1987; Williams and Beitz, 1990a and b). At the ultrastructural level only background labeling (6.7 ± 3.3 particles/μm^2; both 10 nm and 20 nm gold particles were counted) was observed in sections treated with normal rabbit serum. This immunogold background labeling was subtracted from the counted values to provide an index of specific labeling in the ventrolateral PAG area.

VI) Quantification of Enkephalin and GABA-like immunolabeling

A complete and detailed description of the ENK and GABA immunolabeling analysis was recently published (Renno et al., 1999). In short, electron microscopic examination of sections through the caudal third of the ventrolateral PAG revealed a low background density of 10 nm and 20 nm gold particles distributed over the entire tissue section. Within labeled profiles (perikarya, dendrites, and axon terminals), however, the gold particles are found at much higher density; thus, immunolabeled profiles are readily distinguished from background. Morphological analysis of the data shows two distinct populations "labeled," or "immunoreactive," profiles contain more than 30 particles/μm^2 and "unlabeled," profiles contain less than 10 particles/μm^2. These unlabeled profiles are not statistically significant from gold density labeling of glial cells (ENK = 7.3 ± 3.9 particles/μm^2; GABA = 10.3 ± 4.1 particles/μm^2). Using these criteria, 247 vesicle-containing axon terminals and 165 dendrites were classified as GABA, GABA and ENK, or ENK-immunolabeled in the ventrolateral region of the PAG (Renno et al., 1999).

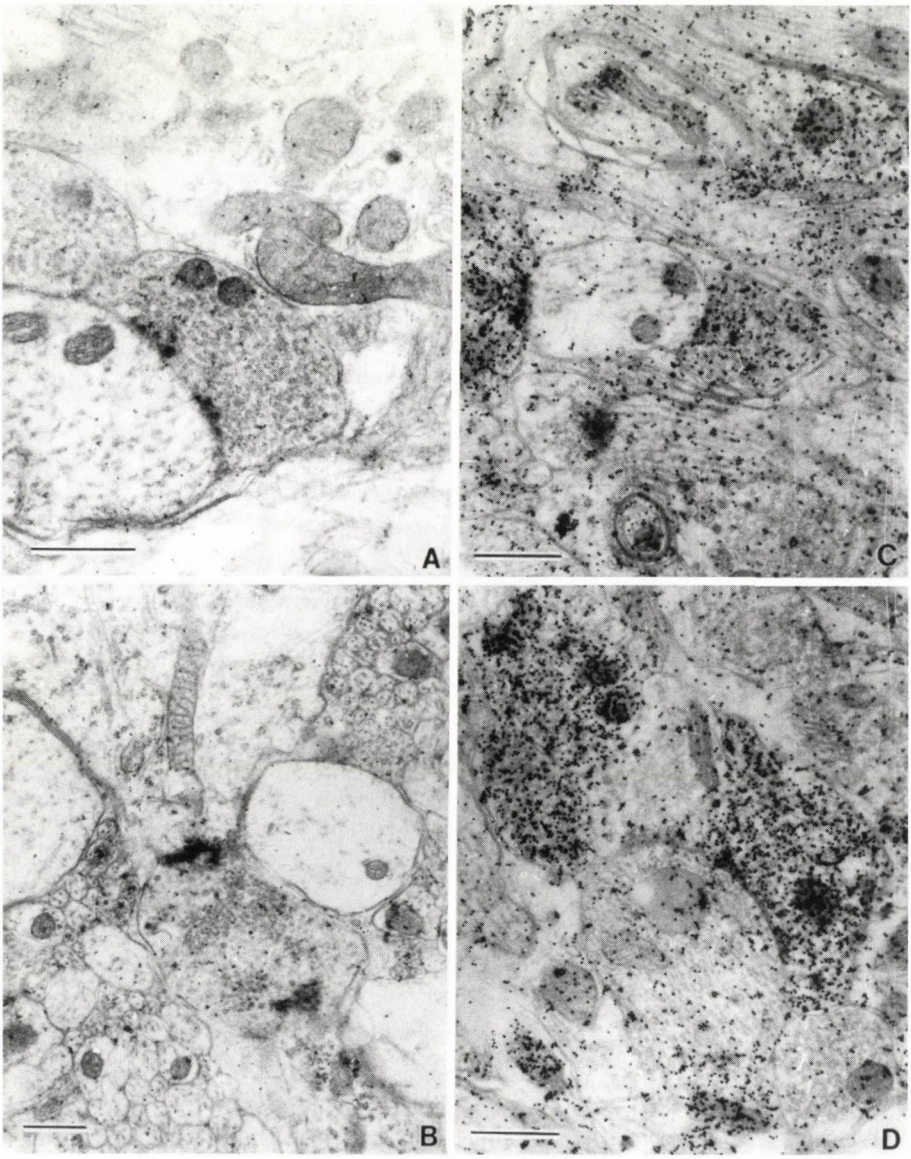

Fig. 4. Electron micrographs showing the effect of different dilutions of GABA and enkephalin antibodies on different grids from the same tissue block and run simultaneously. **A:** enkephalin 1:10,000 and GABA 1:5,000. ×48,000. Scale bar = .2 µm. **B:** enkephalin 1:5,000 and GABA 1:1,000. ×28,000. Scale bars = .5 µm. **C:** enkephalin 1:2,000 and GABA 1:500. **D:** enkephalin 1:1,000 and GABA 1:100. Note that the number of gold particles (20 and 10 nm) diminishes with increasing dilutions. C and D: ×37,000; Scale bars = .2 µm

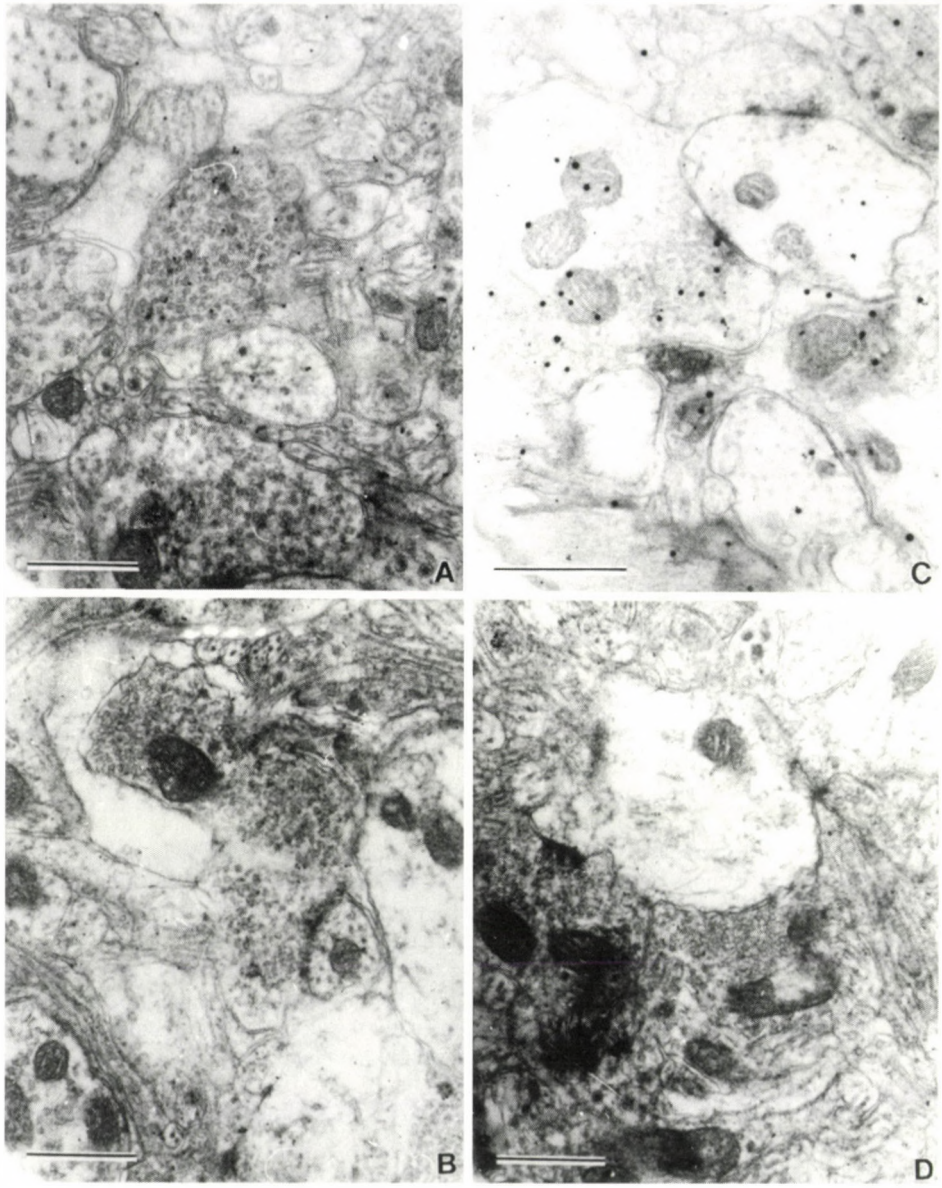

Fig. 5. Effects of pre-absorption. Electron micrographs of some control sections incubated in one of the present experiments from the ventrocaudal PAG of the rat brainstem. Pre-absorption of either GABA, ENK or both antibodies with their respective antigen decreased immunolabeling significantly to background levels. **A:** GABA antiserum (1:500) was incubated with 100 µM of GABA prior to immunogold staining. ×48,000. **B:** Anti-ENK antibody (1:2000) was incubated with 20 µM prior to immunocytochemical staining. ×47,000. **C:** Sections treated with ENK antibody (1:2000) preabsorbed with 20 µM of ENK followed by secondary anti-IgG conjugated to 10 nm gold particles (arrowheads) then sections were stained with GABA antibody (1:500) preabsorbed with 100 µM GABA followed by a secondary anti-IgG conjugated to 20 nm gold particles (arrows). ×60,000. **D:** Control section in which the antiserum was replaced with normal rabbit serum. Note that only a small number of backgrounds gold particles (arrowheads) are evident in this micrograph. ×46,000. Scale bars = .2 µm

Table 1. Effect of deactivation procedures on the percentage of gold particles remained after staining with the secondary antibody conjugated to 20 nm gold particles

Treatment	Percent of 20 nm gold particles
20° C (RT™)	35%
4% PF© at RT	21%
80° C	10%
PF crystals at 80° C	1%

The degree of deactivation was measured by counting small (10 nm; from the first incubation in secondary antibody) and large (20 nm; from the second incubation in secondary antibody) particles and determining the percentage of large 20 nm gold particles. Data were obtained from ultrathin sections of five rats.

™ RT: Room Temperature

© PF: Paraformaldehyde

DISCUSSION

Double immunostaining is an important technique studying interactions between various neurotransmitters in the central nervous system, especially at synapses. Until now, several methods have been used to deal with such studies at the ultrastructural level. These include mainly pre-embedding method by the PAP-silver gold intensification plus PAP technique (Wang and Nakai, 1993) and a combination of the pre-embedding PAP complex and post-embedding immunogold labeling procedures (Wang et al., 1994). However, these methods have their own shortcomings. For instance, quantification of two neurotransmitters at the same time in the same tissue section is not feasible with such procedures. Non-specific staining due to strong DAB reaction used in the above methods is more common in addition to the fact that the PAP-DAB reaction often causes much strong adhesion to the postsynaptic membrane that makes it difficult to distinguish if the synapse is symmetrical or not. Therefore, because of these technical limitations, one cannot verify the co-existence of two neurotransmitters or quantify them simultaneously on the same tissue section with great confidence.

In the course of recent studies it has become clear that using an electron microscopic post-embedding double immunogold technique can minimize many difficulties of the combination of pre- and post-embedding staining method. This procedure offers a very high resolution and allows the antigens distributions to be studied within individual cell compartment, and penetration problems are eliminated since the antibody-antigen reaction is restricted (in both neurotransmitters) to the section surface (Bendayan et al., 1989). Moreover, the double-immunogold procedure permits the immunolabeling of two antigens to be quantified in a relative as well as in absolute manner (Ottersen, 1989).

One of the important application of post-embedding immunogold method is the possibility to distinguish between different "pools" of neurotransmitter, because neurotransmitter immunoreactivity in different neuronal elements can be quantified and examined at high anatomical resolution. For example, most studies employing this method conclude that putative excitatory nerve terminals contain about two to five times higher levels of glutamate than other neuronal elements (Somogyi et al., 1986; Ji et al., 1991). Double-labeling immunoelectron microscopy demonstrates the functional relationship between two transmitters quantitatively and qualitatively.

Technical consideration

Many previous studies have shown that the density of immunogold labeling can be used for rough quantitative determination of the concentration of fixed neurotransmitters in ultrathin section, with a lateral resolution of about 25 nm (Ottersen, 1989; Ottersen et al., 1992). It should be stressed, however, that quantitative results obtained with the immunogold method should always be analyzed with caution, as the labeling intensity can be influenced by several factors. These include effects of osmium treatment, steric hindrance, and the availability of proteins with lysine residues in different tissue compartments (Ottersen, 1989). Variability in the labeling efficiency caused by structural and biochemical differences between different neuronal elements is difficult to determine. It is therefore possible that the protein rich mitochondrial membranes would favor the retention of GABA and/or ENK antigens during fixation, which would have produced an artifactual labeling. The possibility of the presence of GABA and ENK in the mitochondria is highly unlikely because they are not metabolic substances like other single amino acid neurotransmitters (glutamate, aspartate, glycine and taurine). Therefore, it is believed that this mitochondrial labeling may be due to the possibility that the mitochondria offer particularly favorable fixation conditions for GABA and ENK, and that this may have contributed to the labeling in this compartment. Many investigators believe that mitochondrial labeling of single amino acid neurotransmitter may be due to the presence of metabolic pool of these amino acids such as glutamate, aspartate, glycine and taurine, while the GABA immunogold labeling in mitochondria is mainly due to leakage of GABA into the mitochondria during fixation (Broder and Mihailoff, 1990; Ji et al., 1991; Ottersen et al., 1992). Mitochondrial proteins probably act as a substrate for the chemical coupling of GABA by the fixative. However, this does not change the selectivity of the method. Similarly, the mitochondria may bind high numbers of gold particles due to GABA and/or ENK molecules becoming cross-linked to mitochondria proteins during fixation (Smith and Bolam, 1990). Another factor to consider is a possible redistribution of antigens between compartments during fixation process. It is, however, unlikely that a fixation artifact would underlie the different levels of GABA and/or ENK labeling in different profiles. All profiles would be expected to be similarly affected by the fixation.

The application of different controls for specificity is critical in GABA and ENK double post-embedding gold immunolabeling electron microscopy. At sites with low gold particle densities, in particular, it needs to be resolved whether the particles represent nonspecific binding to resin or brain tissue, or whether they indicate the occurrence of low concentrations of authentic GABA and/or ENK. In this regard GABA immunocytochemistry has been shown to give differentiated pattern of immunolabeling that is consistent with the near selective localization of GABA to GABA containing cells (Maley and Newton, 1985; Newton and Maley, 1987; Williams and Beitz, 1990a). Similarly, ENK immunocytochemistry has also been shown to give a differentiated pattern of immunolabeling (Williams and Beitz, 1990b). There is ample reason to conclude that the immunolabeling observed in this study quantitatively and qualitatively signals the presence of fixed GABA and ENK. First, GABA antiserum has been tested against several small molecules endogenous to the brain (see Results) and did not produce significant cross-immunoreactivity with any of them (Maley and Newton, 1985; Newton and Maley, 1987; Williams and Beitz, 1990a). Second, pre-absorption experiments in which the GABA and ENK primary antisera were exposed to an excess of the appropriate antigen abolished immunostaining. Third, method specificity was controlled by the application of rabbit non-immune serum, as well as by processing of a series of immunoreactions in which

various stages were omitted from the regular staining sequence. Fourth, the fact that a single ultrathin section contained heavily gold labeled terminals adjacent to unlabeled terminals or dendrites provided some control assurance that unlabeled profiles were not false negatives. Fifth, the different sequence order of ENK and GABA primary antibodies and secondary colloidal gold (10 nm and 20 nm) conjugated anti-rabbit serum were also tested. All above control experiments confirmed the specificity of our post-embedding double-staining procedure. In summary, it appears very likely that the immunogold labeling of the profiles in this study reflects the presence of authentic GABA and ENK, rather than cross-reactivity or nonspecific binding of antibody molecules.

The background density of the gold particles was calculated for each animal from immunohistochemically processed sections in which the GABA and ENK primary antibodies were replaced with normal rabbit serum. This background density (5.8 ± 2.9 particles/μm^2) was then subtracted from the calculated GABA and ENK gold particle densities to obtain final density per μm^2. In addition, the gold particle density was assessed over clear plastic areas (0.2-1.2 gold particles/μm^2) and the higher value was used to account for the possibility that GABA and ENK antibodies might show some affinity for the tissue and the plastic (embedding media) areas. Some authors working with amino acid neurotransmitters where they have to differentiate between the metabolic Vs neurotransmitter pools estimate the background labeling by measuring areas occupied by the lumen of blood vessels and counting the number of gold particles contained within these profiles or directly from tissue free resin. The immunolabeling of glial cells then can be considered positive staining for the presence of metabolic pool of these amino acids. Any thing 5 to 10 times above the background labeling in the synaptic terminals (in this case 5-10 times more gold particles than those found over glial cells) is considered positive staining for the amino acid neurotransmitter pool.

GABA and ENK immunolabeling

Although both neurotransmitters have been shown to be localized in the PAG of the rat midbrain (Reichling and Basbaum, 1990a, b; Williams and Beitz, 1990a and b; Wang et al., 1994), the morphological and quantitative relations between GABAergic and ENKergic neuronal elements in the caudal ventrolateral PAG are not well described. Therefore, in order to understand the normal PAG circuitry involving GABA and ENK, we have utilized double-immunogold post-embedding technique at the electron microscopic level to examine the relationships between these two neurotransmitters in the PAG neuronal elements (Renno et al., 1999). In the present investigation, it is possible that the frequency of synaptic contacts between GABA and/or ENK immunoreactive profiles within the ventrolateral PAG area was underestimated due to our random sectioning. It is very likely that many planes of sectioning could have failed to include regions of membrane containing specific synaptic specializations formed by the terminals. Even in sections that include synaptic specializations, some synapses could have been overlooked if the plane of section was oblique to the plane of the synaptic cleft (Williams and Beitz, 1990a and b).

Our data support previous observations reported by Williams and Beitz (1990a and b) and Wang et al., (1994) that the ENKergic terminals synapse on GABA containing dendrites. Enkephalinergic axon terminals contained small, clear, round vesicles in addition to the dense-cored vesicles. However, these studies could not show the labeled dense-cored vesicles due to heavily loaded silver-gold particles (Wang et al., 1994) and tetramethylbenzidine crystals (Williams and Beitz, 1990a and b) which made it difficult to see the dense-cored vesicles.

We found 43.3% of all terminals in the caudal ventrolateral PAG are GABA immunoreactive; this figure is close to previous estimates in this region. Reichling and Basbaum (1990a) reported that 39% of PAG terminals are GABA immunoreactive. This is also consistent with other brain regions. In agreement with our previous study (Williams and Beitz, 1990a), it seems that the GABAergic terminals in the caudal ventrolateral PAG out number the ENKergic terminals in this midbrain region by approximately four to one, excluding those terminals which contain both neurotransmitters. This ratio may reflect the presence of different PAG connections controlled by GABA or other types of antinociceptive circuits may exist.

In conclusion, the post-embedding double-immunogold method eliminates many of the limitations and shortcomings of pre-embedding immunostaining procedure, permitting quantitative study of well-preserved tissue at the electron microscopic level. Electron microscopy post-embedding double immunocytochemistry allows simultaneous localization of two neurotransmitters (or antigens), in addition, the immunolabeling can be performed on material labeled with neuroanatomical tracers. Furthermore, this experimental procedure has demonstrated that a large number of axon terminals in the caudal ventrolateral PAG are GABA immunolabeled (Renno et al., 1999). However, ENK immunoreactive axon terminals are almost half of that of GABA. In short, the introduction of post-embedding double immunolabeling provides a powerful new tool for studying the CNS pathways. This procedure should contribute greatly to the analysis of the cytochemistry and connectivity in the CNS and other systems.

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RESEARCH REPORT

DEVELOPMENT OF THE EQUINE BRAIN MOTOR SYSTEM

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The purpose of the present study was to demonstrate the structural maturation of the horse brain in the critical period of development emergence of coordinated locomotion. Equine brains from 14 days before expected birth to adulthood were fixed in formaldehyde and embedded in paraffin. After taking the outer parameters of the brains, full series of large-area coronal sections were prepared on a special microtome and stained with Nissl's cresyl violet and Haidenhain's iron-haematoxylin. Microscopic images of sections were digitized and were subjected to computer-aided image analysis.

The gross morphology of the brains and the image analysis of histological preparations suggest that in the perinatal period studied there is no substantial increase in brain size and mass, while the amount of Nissl substance and myelin grows rapidly till postnatal day 45. Then a relative decrease of both is observed till adulthood accompanied by a doubling of brain size and mass.

It is concluded that during the maturation of the equine brain, decisive changes of the motor system such as up-regulation of protein sysnthesis and full myelination of motor tracts takes place during the critical period of onset of coordinated locomotion.
Key words: brain, horse, development, motor system

INTRODUCTION

The body of knowledge collected on the horse gait during several centuries developed into a separate field of animal sciences called hippology. Hippology had its climax in the 18-19th centuries particularly when horse racing was institutionally introduced in a number of countries as a means to further improve the efficiency of horse breeding and to produce horses bred with the purpose to satisfy best the most special needs of economy as well as of imperial armies. The ultimate goal of hippology was to analyze the function of equine locomotor apparatus to an extent which would allow to select the best horses for a given purpose on one hand, and to obtain a full and perfect control over the animal's behaviour on the other. The unconditional and immediate execution of instructions to make quick movements, turns, jumps or even some movements unusual for the untrained animal such as reversing, turning around lying down, etc. was a general requirement of the military horse. These abilities were life-saving under circumstances of a fight with hand-weapons and could increase enormously the value of a horse. The military training was a crucial moment of preparation for wartime. Beside warfare,

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the best trained horses were also used in equestrian competitions in peacetime. These competitions require extraordinary movements, postural positions and subtle moments of the locomotor apparatus.

Hippology, however, could never develop beyond the confines of mere observation. Around the middle of the 19th century pioneer efforts were taken in cinematography which had, among other things, a dynamical impact on the development of motion analysis. Several attempts have started in different technical directions. The first scientific descriptions and notations of the equine gait were published from the 1870s. The very first correct step succession at walk have been illustrated in hoof diagrams by Wilhelm Baumeister in 1870 (Baumeister, 1870).

As to predictions, gait analysis could not live up to the expectations so far. The reasons may lie in the differences in the understanding of structural basis of locomotion control by the central nervous system in different species. While pathways of skeletal muscle innervation both in the central and peripheral nervous systems have for long been clarified in the human and some easily investigable species such as the cat, dog and laboratory rodents, the equine central motor system is practically unexplored. Lacking detailed information on the topography and development of the motor pathways of the horse there is no biological basis of functional assessment, consequently no objective methods of improvement or selection of foals can be introduced. Veterinary textbooks describe the gross surface anatomy of the horse brain and spinal cord but when considering the internal structure of the brain of domestic mammals they rely on descriptions derived from other species, usually small domestic mammals. This has practical reasons. The brains of the cat, dog, sheep, etc. are small enough to be fixed and embedded as a whole and cut with a conventional microtome. The method of tract-tracing is based on series of sections through the entire brain from where the course of specially stained fibres can be reconstructed. In addition to inconveniences caused by the huge body mass, the high costs of keeping and the fact that the horse is much too valuable to be sacrificed for experimental purposes, all experience suggests that the sampling of horse brains of given ages is a long and cumbersome procedure. Moreover, the size of the equine brain matches with that of the young human brain giving no chance for serial sectioning either in a conventional microtome or a cryostat.

Surveying the theoretical gaps in knowledge the question boils down to the poor understanding of the topography and development of the major motor pathways in the horse. Tract-tracing, in general, is based on the demonstration of bundles of myelinated fibres. A number of staining technologies have been developed to visualize the myelin sheath (see Pannese, 1994). On the other hand, formation of the myelin sheath around an axon is a process taking place in the late embryonic and early postnatal period (Río Hortega, 1930). There are, however, wide interspecies variations in the timing of myelination. Some animals are born with an almost unmyelinated central nervous system, while others in differently advanced stages of myelinization. Some pathways are notorious by their late myelination being protracted into early adulthood. This applies particularly to the motor system. It is also important to note that the acquisition of the myelin sheath also means the functional maturation of an axon since it becomes only then capable of a full velocity (saltatory) impulse conduction (Lillie, 1925; Huxley and Stämpfli, 1949). This is clearly reflected by the pattern of movements of the newborn. Species with more advanced myelination of their motor pathways at birth develop earlier their independent locomotion than the ones with neonatally poorly myelinated motor tracts.

This parallelism between the degree of neonatal locomotion and the advance of myelination of the motor pathways encouraged us to take myelination as a major morphological reference

to compare motion patterns with. Our present objective was therefore to describe the motor system of the adult horse brain and to correlate it with brains from various developmental periods of horse embryos and foals.

MATERIALS AND METHODS

Histology

Equine brains were removed from the skull in the late prenatal and the postnatal period up to 2 years of age. The following brains from half/quarter-bloods were used in this study:

- Brain 1. Fourteen days before calculated birth
- Brain 2. Seven days before calculated birth

Fetal brains were obtained from newborns lost in premature births

- Brain 3. Newborn,

Newborn brain was obtained from an animal born in due time but lost.

- Brain 4. Four-day-old
- Brain 5. Six-week-old
- Brain 6. One and a half-year-old
- Brain 7. One and a half-year-old

Postnatal brains were obtained from animals in which infectious and/or neurological diseases were excluded. Lost animals were kept at 4 C° temperature. Sampling was carried out 2-4 hours after death. After sawing-off the calvaria, the brains were carefully lifted, cranial nerves and hypophyseal stalk were transected, and the medulla was truncated at the level of C2. Then the brain could be removed from the skull without major surface damages. Brains were cleaned from the meninges, weighed (14 days before birth: 305 g, 7 days before birth: 303 g, newborn: 312 g, 4-day-old: 311 g, 6-week-old: 326 g, 545-day-old: 608 g, 560-day-old: 615 g) and their rostrocaudal extent was measured.

Whole brains were fixed in 4-10% formaldehyde buffered at pH 7.4 with phosphate buffer. The duration of fixation was 3-8 months depending on the size of the brain. Following fixation, the rostrocaudal extent of brains was measured again, photographed (Fig. 1) and immersed for a week into 1% eosin solution. Then they were dehydrated in graded ethanol, each phase lasting 2-5 days, and embedded through chloroform in paraffin. Twenty-micron thick serial sections (full series) were cut in the frontal plane from the entire specimen with a TETRANDER Large-Section Microtome. In 500 μm distances, three subsequent sections were mounted on large-area slide glasses. Every first section was processed for Nissl's cresyl violet staining to reveal cytoachtecture, every second for Haidenhain's iron-haematoxylin for the demonstration of myelin sheath, and every third was left blank. The rest of the sections were stored on special plates covered with foils. They were kindly archived by the specially air-conditioned section-store of the O. and C. Vogt. Institute for Brain Research at the Heinrich Heine University of Düsseldorf (Germany).

The blanks, due to the pre-embedding eosin treatment showed in light pink the section contours helping to remove excess paraffin from the slides which greatly facilitated of further processing.

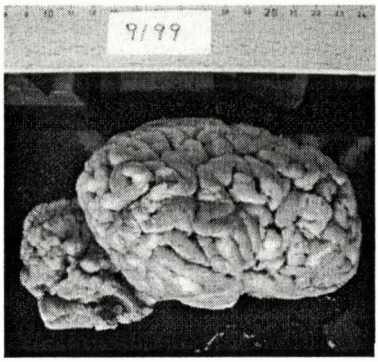

Fig. 1. Lateral view of an adult horse brain followed by fixation

Image analysis

The comparison of results in biological and medical research requires a strong emphasis on objectivity. Visualization of results is biased by a number of factors but detailed description of the applied equipment, technique and protocols makes production of results easier and improves objectivity. Nowadays, when several parameters are provided by international industrial standards, scientific data are more compatible. Notwithstanding, a high degree of subjectivity burdens evaluation. Standardization of evaluation of biological features and phenomena is now a routine in applied mathematics.

In length measurement also the number of pixels are calculated along the selected line. When measuring the perimeter of a shape the problem of pixel relationship might occur. The major question of neighborhood analysis is how the contour pixel should touch the perimeter of the shape: with one or two sides or with the corner. Especially in case of small shapes the particles touching only the edge with their corner can multiply the number of pixels calculated when perimeter is measured. In most of the image analysis softwares the parameter "particles touching the edge" can be switched on or off. Both settings can be applied but it is not recommended to switch within one series.

Histological slices were digitized with a flatbed scanner at 150 dpi resolution as 8 bit gray depth. In the NIH Image 1.62 image analysis software (Wayne Rasband, National Institutes of Health, USA) the size of digitized images was readjusted to the same proportion to meet the requirements of comparison of dimensions.

Contour and area measurements and related calculations

The sharpest contours were seen in the Nissl stained material. A rough contour of each section defined at segmentation of low magnification gave the outer perimeter (C_{out}). At higher magnification all the sulci became clearly identifiable and their total perimeter (C_{tot}) could be drawn. The relationship of the outer perimeter to the total perimeter is similar to the arachnoid and pial surfaces. The depth of the sulci defines the amount of gray substance or gyrification in other terms. The quotient of C_{out} and C_{tot} is the gyrification index (GI; Wosinski et al., 1996).

$$GI=C_{out}/C_{tot} \qquad (1)$$

Beside the perimeter, the area of each section has been determined. Principal elements of the brain such as the hemispheres, cerebellum and the brainstem were measured discretely. Where principal elements join each other (cerebellum and brainstem, hemispheres and mesencephalon), the line connecting the dorsal and ventral end-points of the full hemispheric surfaces showed the demarcation line between brainstem and hemispheres. The quotient of area and perimeter of demarcated parts was calculated in each section. The value of this quotient varies depending on the degree of convolution of the surface: it is smooth and low in the brainstem and high at the hemispheres.

Measurement of territorial amount of myelin

The total area of each section was measured on the Nissl stained sections, while myelin stained sections were segmented for defining the total territory of myelin by sections. This way the sum of the myelin amount could be calculated and related to the entire brain size by adding the values obtained in the individual section.

Since areas of hemispheres, cerebellum, brainstem and mesencephalon have been quantified discretely, the same myelin determination was performed on each demarcated unit. The highest grey-value territory within the section was taken as 100 (the most advanced region of myelination), and the rest was expressed as percent of the total.

Within the sections the tracts which may play important role in the execution and coordination of functions of the locomotor system were segmented manually. We concentrated our attention on the following areas:

- internal capsule
- the brainstem portion of the pyramidal (corticospinal) tract
- the extrapyramidal nuclei and tracts
- the corpus callosum

The territory of the measured regions and the amount of myelin related to the reference areas were calculated. The two major reference areas were the cerebral cortex as a minimum and the optic nerve as a maximum density of myelination. In equines the optic pathway appears to be fully myelinated by birth (Froriep, 1891; De LaHunta, 1997) therefore it seems to be an appropriate reference area in sections where it occurs. In specimens where no optic fibres were available amount of myelin was related to the portions of the best myelinated regions.

Cytoarchitectonics of motor centres

The cytoarchitectonics of the primary motor cortex (Brodman 4 - precruciate gyrus) and the secondary motor cortex (Brodman 6 - postcruciate gyrus) was studied. Density of pyramidal cells of layers 3 and 5 was measured by segmentation of their cell bodies and expressed as percentage of total cortical area.

In the cerebellum, the perinatal migration of granular cells was investigated from the external granular cell layer through the ganglionic layer to the final (internal) granular layer. The amount of cells was determined with densitometric segmentation in the external granular and molecular layers.

The density of Purkinje cells was estimated by calculating the number of cells intersecting a line drawn along the layer of Purkinje cells.

Observation of brain slices on MRI sequences

Series of MRI images were taken from both of the two adult brains. After the removal from the skull a 6 months long period of fixation followed. MRI sequences consisted of 256 coronal

sections. A three-dimensional reconstruction of sequences was made in NIH Object Image 1.62 software (Wayne Rasband, National Institutes of Health, USA) in order to assist identification of anatomical features. Unlike in histological sequences, positioning of slices following each other was correctly adjusted in the MRI series, therefore their three-dimensional reconstruction could be easily performed. The benefit of the MRI reconstruction - as compared to surface pictures of the brains (Fig. 1) - was the possibility to define the relationship of features to the inner structures which are not visible in the surface pictures (such as the primary motor cortex to the corpus callosum).

Statistical analysis

For statistical analyses the Student's T-test and the χ^2-test were applied. For correlations between gait analysis data sets regression tests were used.

RESULTS

The development of cytoarchitecture and myelination

Plots of Nissl preparations

In computer plots of Nissl stained sections the most important motor areas were compared in rostrocaudal series of prenatal, early postnatal and young adult brains. As early postnatal samples, brains of fetuses 14 days before the expected birth were taken, the early postnatals were 45-day-old and the young adults were 545 and 560 days old. The motor areas studied were the primary motor area of the cerebral cortex, the corpus striatum, the motor nuclei of cranial nerves in the upper and lower brainstem, and the cerebellum.

The primary motor cortex (Fig. 2) showed in the prenatal brains (Fig. 2A) an advanced gyrification. The superficial, cell-poor layer (stratum zonale, lamina I) was well visible, while the cell-rich layers (external granular, pyramidal, internal granular, and ganglionic layers, laminae II-V) appeared on the plot as a dark strip. The dimensional changes between prenatal and early postnatal cortices (Fig 2A and B, respectively) were negligible. Growth in size could be seen to occur after the early postnatal period (Fig. 2C) This growth was substantial and as indicated by the measurements of diameters (Fig. 16), was fairly proportionate. In addition, the intensity of Nissl staining increased till day 45, and then it decreased to the adult level.

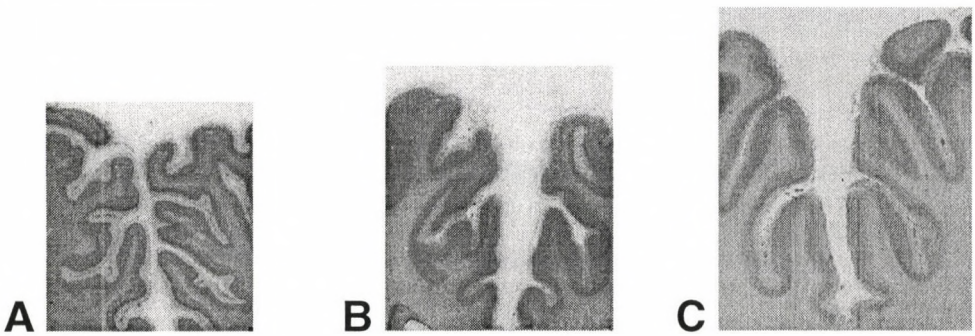

Fig. 2. Computer plots of Nissl stained sections of the primary motor cortex.
A: prenatal, B: early postnatal, C: adult. Mag: ×2

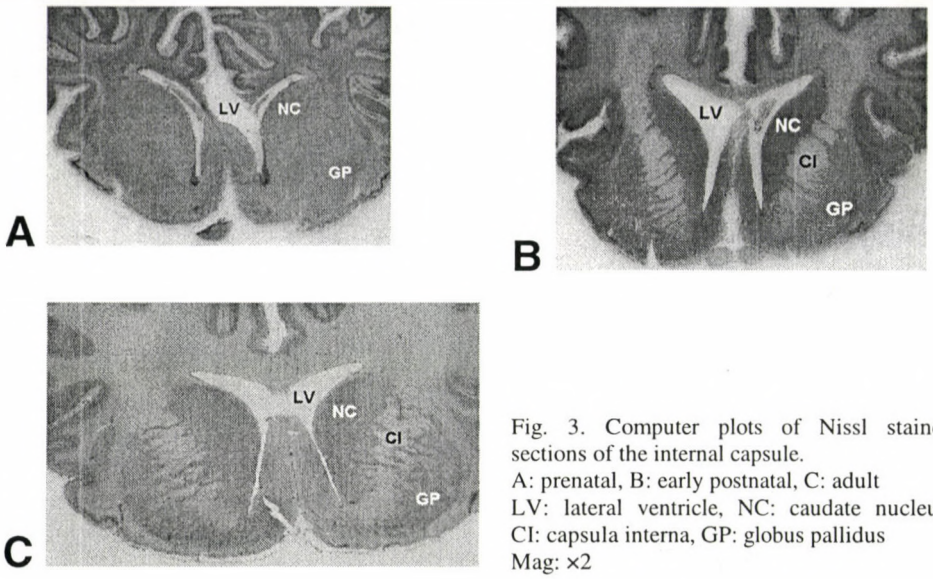

Fig. 3. Computer plots of Nissl stained sections of the internal capsule.
A: prenatal, B: early postnatal, C: adult
LV: lateral ventricle, NC: caudate nucleus, CI: capsula interna, GP: globus pallidus
Mag: ×2

When comparing the region of the striatum (Fig 3A, B, C), in the prenatal brain there was no internal cellular structure visible at all. By contrast, in the early postnatal (Fig. 3b) and adult (Fig. 3c) brains the cytoarchitectonic details of the striatum could clearly be distinguished. The caudate nucleus was distinct, the internal capsule was seen permeated by the typical striae, and the lentiform nucleus was well circumscribed. Also in the striatum, the strongest Nissl staining occurred in the 45-day old brain. The intensity of Nissl staining declined thereafter. The midbrain (Fig. 4) displayed no internal structure in the Nissl stained prenatal brains (Fig. 4A), whereas in the early postnatal midbrain the cytoarchitectonic parcellation was quite remarkable (Fig. 4B). The periaqueductal central grey matter was clearly delineated, the colliculi were darkly stained. The tegmental nuclear groups and the substantia nigra could also be recognized. An even more differentiated structure appeared in the adult (Fig. 4C) where the cellular layers of the rostral colliculus could be distinguished, the lateral geniculate body also appeared, moreover the substantia nigra and the tegmental nuclear groups were also evident. The periaqueductal grey was seen to continue ventrally into the raphe nuclei. It is noteworthy that also at this sectioning-level, the Nissl staining of the adult was less intense than in the perinatal midbrains.

In the cerebellum (Fig. 5) it was interesting to observe that the external granular layer of the cerebellar cortex was almost absent already in the prenatal brain (Fig. 5A). Sporadic clusters of small cells were found under the pial surface (Fig. 5B) but these did not show the typical features of an external granular layer, i.e. mitoses were not encountered and the cells seemed to be aligned in a premigratory position. This was in good accordance with the fact that in the adult (Fig. 5C), apart from a substantial growth in the number of cerebellar folia which resulted in a massive volume increase of the entire cerebellum, there was no appreciable thickening of the internal granular layer, or any other layer of the cerebellar cortex or white matter. This suggested a prenatal cytoarchtectural maturation of the cerebellum supported by the fact that in

this region there was no intensification of the Nissl staining during the developmental period covered by our studies.

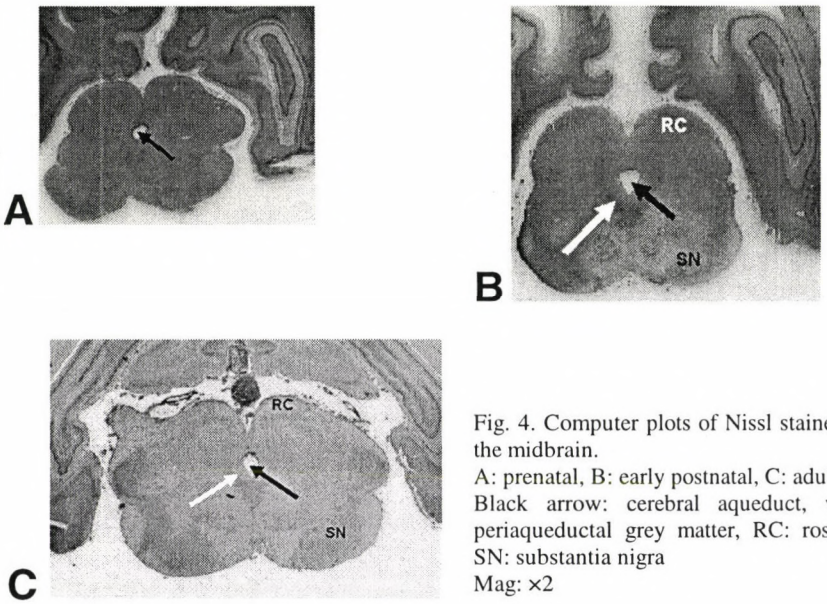

Fig. 4. Computer plots of Nissl stained sections of the midbrain.
A: prenatal, B: early postnatal, C: adult
Black arrow: cerebral aqueduct, white arrow: periaqueductal grey matter, RC: rostral colliculi, SN: substantia nigra
Mag: ×2

The medulla (Fig. 6) again showed the typical temporal sequence of Nissl staining intensification as observed for most motor areas having a peak intensity in the early postnatal brain. Whereas cytoarchitectural detals could only be guessed in the prenatal medulla (Fig. 6A), they were quite marked in the early postnatal preparation (Fig. 6B). This applies particularly to the spinal trigeminal nucleus, the vagal and hypoglossal nuclei and the caudal olivary nucleus. The layered structure of the caudal olivary nucleus was conspicuous in the early postnatal (Fig. 6B), and to a lesser extent in the adult oliva (Fig. 6C).

Taking into consideration that the Nissl staining showed in all regions a similar tendency of intensification till postnatal day 45 from where it decreased till adulthood, we thought it necessary to examine under a higher microscopic magnification the cytoplasm of motor neurons. In agreement with the overall appearance of staining reflected by the computer plots. individual cells showed parallel differences in their content of Nissl material. In the prenatal brain (Fig. 7A) motor neurons contained loosely packed, distinct Nissl bodies in their cytoplasm. At postnatal day 45 (Fig. 7B) the cytoplasm of motor neurons was so densely packed with cresyl violet-stained material that at lower magnifications it gave the impression of a solid cytolpasmic staining and it was only under higher power that the rough granular nature of the cresyl-violet staining could be recognized. In the adult motor neurons (Fig. 7C) Nissl bodies were again discernible as distinct cresyl violet stained blocks.

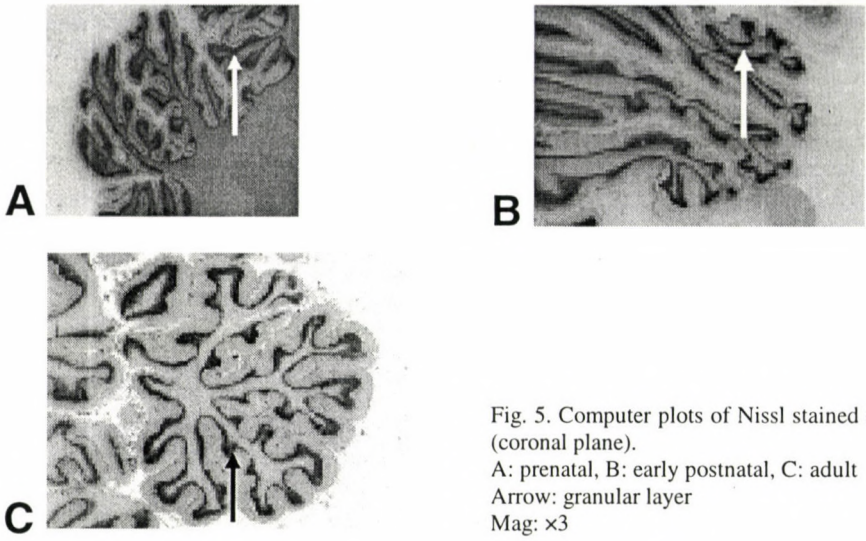

Fig. 5. Computer plots of Nissl stained cerebellum (coronal plane).
A: prenatal, B: early postnatal, C: adult
Arrow: granular layer
Mag: ×3

It is also worth of notion, that in the preantal brain the cells of brainstem motor nuclei are smaller and more densely packed than in the early postnatal brain. From this time, packing density of cells decreased till adulthood, while cell-size remained unaltered.

In this series of observations the major motor pathways were looked at concentrating on the pyramidal tract, the extrapyramidal system, cerebellum and brainstem. The main commissural bundle, the corpus callosum was also investigated.

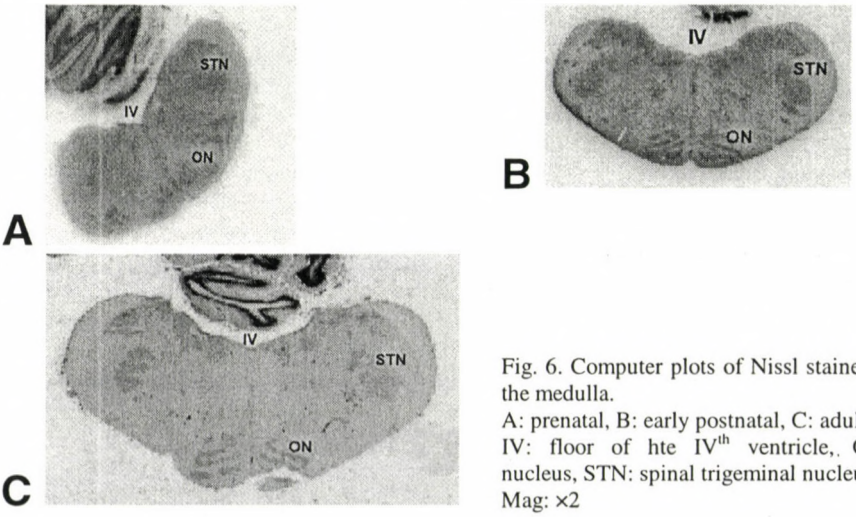

Fig. 6. Computer plots of Nissl stained sections of the medulla.
A: prenatal, B: early postnatal, C: adult
IV: floor of hte IVth ventricle,. ON: olovary nucleus, STN: spinal trigeminal nucleus
Mag: ×2

Between 14 days before and 45 days after birth, there was a significant increase in the amount of myelinated fibres in the white matter of motor cortex and particularly in the internal capsule (Figs 8A, B). Advance in myelination could be seen best in the ansa lenticularis which is still poorly myelinated on prenatal day 14 (Fig. 8A), whereas its myelination is substantial by postnatal day 45 (Fig. 8B). In addition, the grey matter strips which combine the caudate nucleus and the putamen to form the corpus striatum, are thinner at postnatal day 45 than prenatally. In the adult (Fig. 8C), due to a general growth of the brain the myelinated pathways are less compact and the putamen and globus pallidus can be distinguished on the basis of their myelin-content.

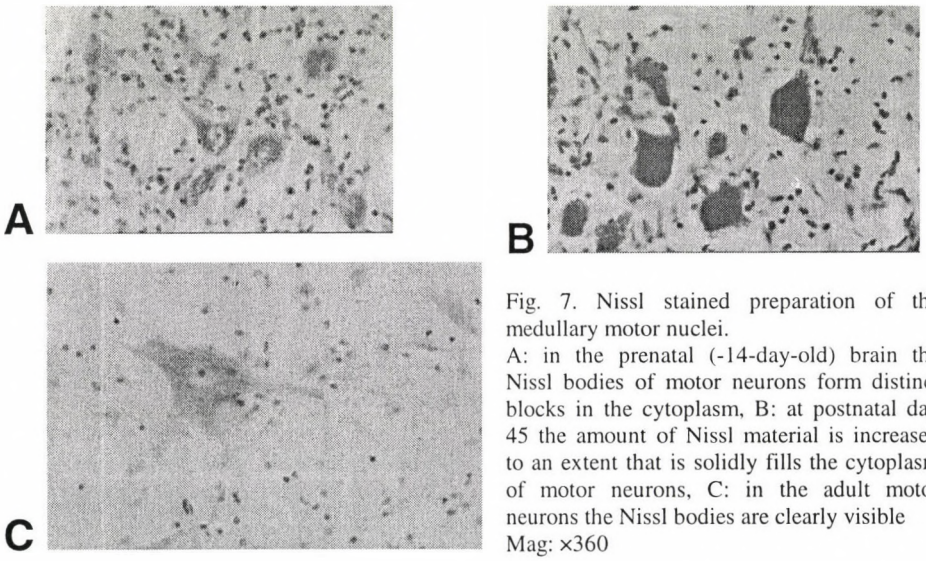

Fig. 7. Nissl stained preparation of the medullary motor nuclei.
A: in the prenatal (-14-day-old) brain the Nissl bodies of motor neurons form distinct blocks in the cytoplasm, B: at postnatal day 45 the amount of Nissl material is increased to an extent that is solidly fills the cytoplasm of motor neurons, C: in the adult motor neurons the Nissl bodies are clearly visible
Mag: ×360

Cut at the level of the optic chiasma (Fig. 9), the septum and the corpus callosum could be observed bordering the rostral horn of the lateral ventricle from medial and above, respectively. While in the prenatal brain (Fig. 9A) the corpus callosum was a thin strip of transverse white matter, in the early postnatal brain (Fig. 9B) it appeared as a conspicuous cross-bundle, and in the adult (Fig. 9C) it was a thick, powerful commissure of myelinated fibres connecting the two hemispheres. It should be noted that in this series of preparations the development of the septum from a prenatal thin membrane to a thick, myelinated pathway-containing wall is evident. At this level, the growth of the striatum could particularly well be documented.

In the midbrain (Fig. 10), a peak accumulation of myelinated fibres was observed at postnatal day 45 (Fig. 10B) such that except for the periaqueductal central grey matter and the substantia nigra no internal detail were discernible. This was not the case in the prenatal and adult midbrains, where details such as the layers of colliculi, raphe nuclei, geniculate body, etc., could be percieved.

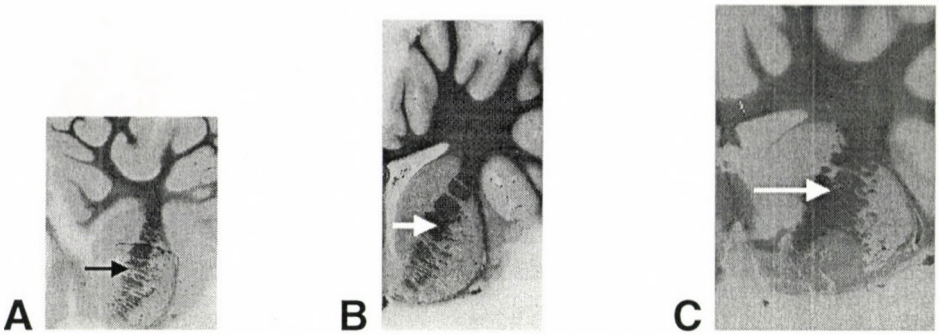

Fig. 8. Computer plots of myelin-stained sections showing the internal capsule (arrow).
A: prenatal, B: early postnatal, C: adult. Mag: ×2

A gradual increase of myelinated area was observed in the cerebellar white matter (Fig. 11). In the myelin-stained medulla only the prenatal preparation allowed to distinguish details. At later stages myelinated fibre bundles became overwhelming and masked non-myelinated elements (Fig. 11B). With the growth of the medulla in the adult, the myelin staining became less compact but it still covered evenly the entire section (Fig. 11C).

The white matter/grey matter ratio was followed within the developmental period studied (Fig. 12). Values are summarized in Fig. 19. From the comparison of ratios it appears that the bulk of myelin formation occurred in the period between prenatal day 14 and postnatal day 45. Myelinated territories became wider and more ramified. After this period there is an overall growth of the brain but apparently not followed by a further increase in myelinated fibres. This is reflected in a less intense myelin staining, because myelinated fibres are spread over a larger territory in the adult as compared to the perinatal brains.

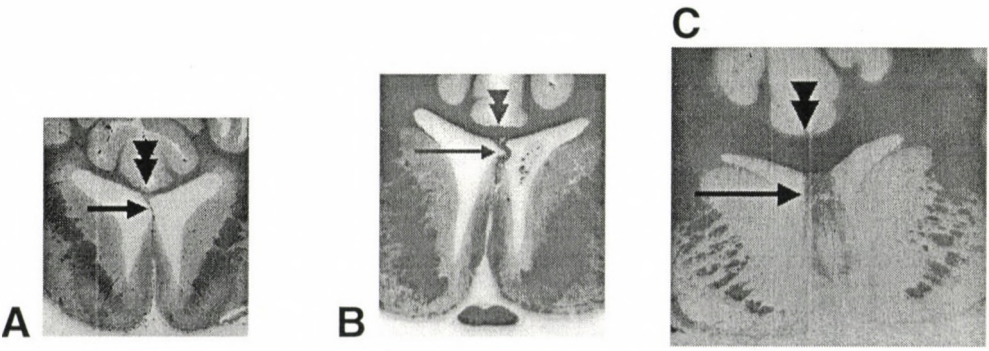

Fig. 9. Computer plots of myelin-stained sections at the level of the septum (arrow) showing the trunk of the corpus callosum (arrowheads). A: prenatal, B: early postnatal, C: adult. Mag: ×2

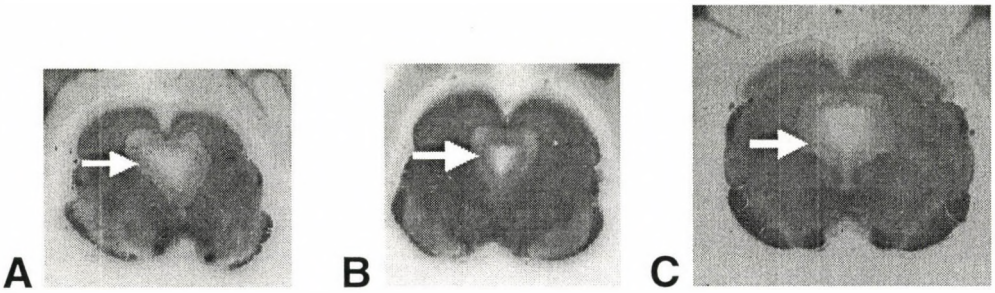

Fig. 10. Computer plots of myelin-stained sections of the midbrain. Arrow points at the periaqueductal grey matter.
A: prenatal, B: early postnatal, C: adult
Mag: ×2

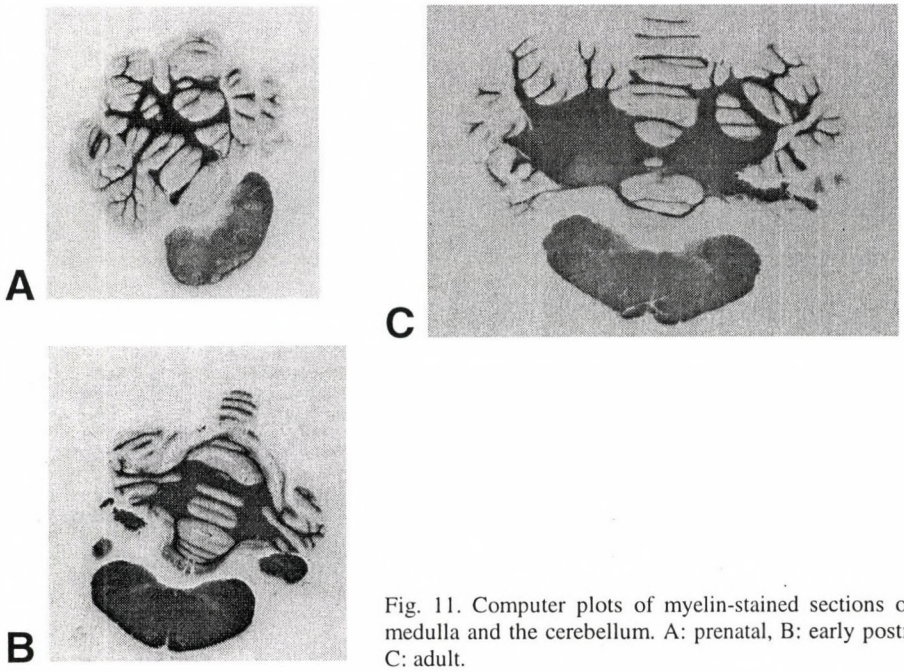

Fig. 11. Computer plots of myelin-stained sections of the medulla and the cerebellum. A: prenatal, B: early postnatal, C: adult.
Mag: ×2

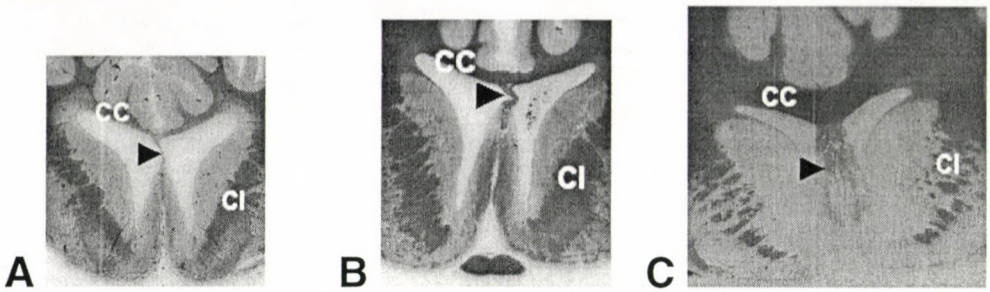

Fig. 12. Ratio of the white and grey matter of various developmental stages as seen in computer plots of myelin-stained sections. Observe the growth of corpus callosum (CC), capsula interna (CI) and septum (arrowhead). A: prenatal, B: early postnatal, C: adult.
Mag: ×2

Contour and area measurements and related calculations

Dimensions of brains

The length of the entire brain and its units showed a doubtless increase with age (Fig. 13). The most prominent difference appears between the perinatal (pre- and early postnatal) and adult brains in all parameters measured. The data of the brain length in prenatal (D-14 – 14 days, D-4 – 4 days, D0 – 0 day before expected birth) and early postnatal ages (D4 – 4 days and D45 – 45 days after birth) showed a small degree of oscillation but this deviation did not exceed 6 mm. After one and half month this parameter reached its maximum. This oscillation reappeared in each data line: the hemispheres and the brainstem ranged between 74 – 94 mm and 61 – 72 mm, respectively in the prenatal and early postnatal ages. In the cerebellum the difference between minimum and the peak was only 12 mm.

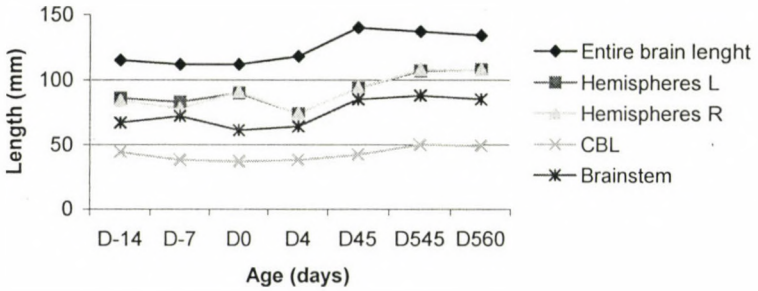

Fig. 13. Length of the brain and its parts in different ages
Abbreviation: CBL - cerebellum

The marked volume increase between early postnatal and adult periods was due to the elongation of the entire brain was more pronounced in the hemispheres than in the cerebellum and brainstem. The sizes of the left and right hemispheral pairs are more or less identical.

In order to clarify the exact reason of volume increase we examined the area and the perimeter of the hemispheres, the cerebellum and the brainstem in each section (Fig. 14).

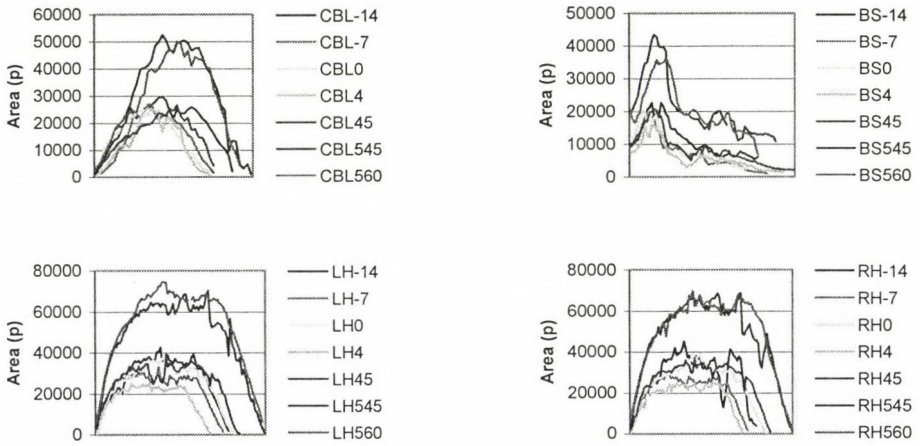

Fig. 14. Rostro-caudal areal measurements of major parts of the brain of different ages.
Abbreviations: CBL - cerebellum, BS - brainstem, LH - left hemisphere, RH - right hemisphere

Similarly to longitudinal parameters the most obvious changes appeared between the perinatal age and adulthood. Within the time period between days 14 prior to and 45 following birth, alterations did not exceed the level of individual differences. Data of each major part of the fully developed adult brains were also similar but the areal alteration from perinatal brains to the adults displayed remarkable increases. For comparison of the two groups averages of major parts were calculated

In Fig. 15 averaged perinatal and adult brains are compared. None of the differences within the perinatal period showed such a deviation which can be seen between the perinatal and adult groups.

Maximum diameter of the hemispheres

Beside longitudinal parameters the maximum of transverse diameter was also analysed in the series of coronal sections (Fig. 16). This was measured across the caudal end of the tuber cinereum as it is illustrated in Fig. 17. Its values were similar within the prenatal and adult groups with a small variance but the difference between them was already significant. This profile was similar to the data of longitudinal measurements, which suggests the proprortional growth of the entrire brain.

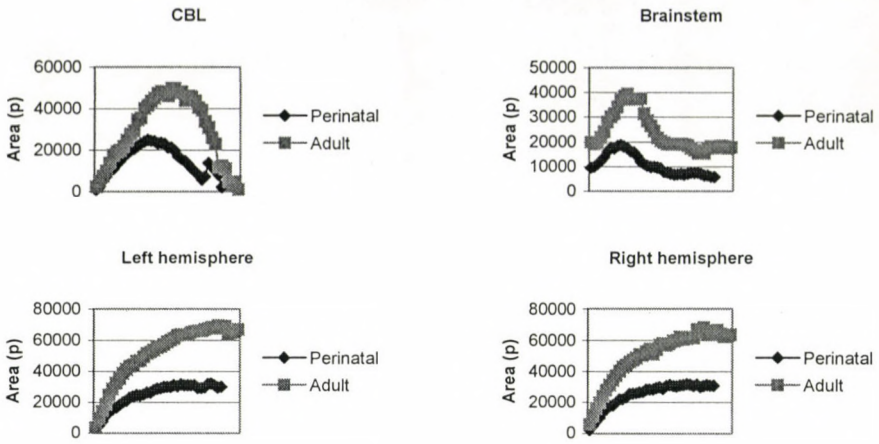

Fig. 15. Rostro-caudal areal measurements of major parts of the averaged perinatal and adult brains

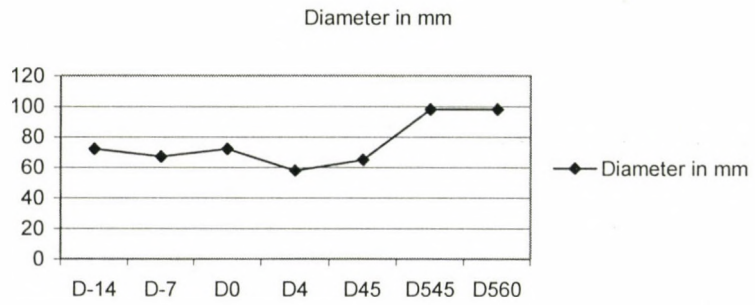

Fig. 16. Diagram of maxima of transverse diameters in different ages
Abbreviations: D-14 = 14 days before birth, D-7 = 7 days before birth, D0 = at birth, D4 = 4 days after birth, D45 = 45 days after birth, D545 = 545 days after birth, D560 = 560 days after birth

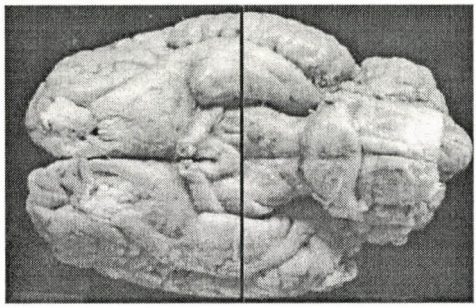

Fig. 17. The maximum of transverse diameter was measured at the level of the optic decussation indicated by a black line

Gyrifacation index (GI)

The difference - more exactly the quotient - between the free and the convoluted surfaces of the brain reflects degree of convolution of the cortex. Depending on the depth of sulci this value, the GI value, varied between 0 – 1. At 1 there was no difference between the two contours at all, in other words: the cortex is flat. In our diagrams, however, there were some values exceeding the theoretical maximum of 1 because of some technical reasons. At some extreme areas (rostral and caudal poles) the convoluted contour crossed the way of the free contour, this way it decreased the value of the free countour which may result in values higher than 1 in our calculations.

As it is indicated in Table 1 the average GI value ranged between 0.64 – 0.766. This showed that there was no signifcant difference in the GI of the brains. In Fig. 18. GI of couples of hemispheres are shown. There is no common chararacteristic feature in the diagram. All data are oscillating around 0.6 – 0.7 forming an irregular curve.

Table 1. Calculated average of gyrification index of both hemispheres

	L. H.		R. H.		AVG of both
Age	**GI**	**SEM**	**GI**	**SEM**	
-14	0.671	0.074	0.608	0.104	**0.640**
-7	0.662	0.075	0.674	0.105	**0.668**
0	0.765	0.095	0.689	0.094	**0.727**
4	0.757	0.100	0.775	0.123	**0.766**
45	0.723	0.127	0.698	0.132	**0.711**
545	0.654	0.099	0.684	0.101	**0.669**
560	0.685	0.071	0.704	0.069	**0.694**

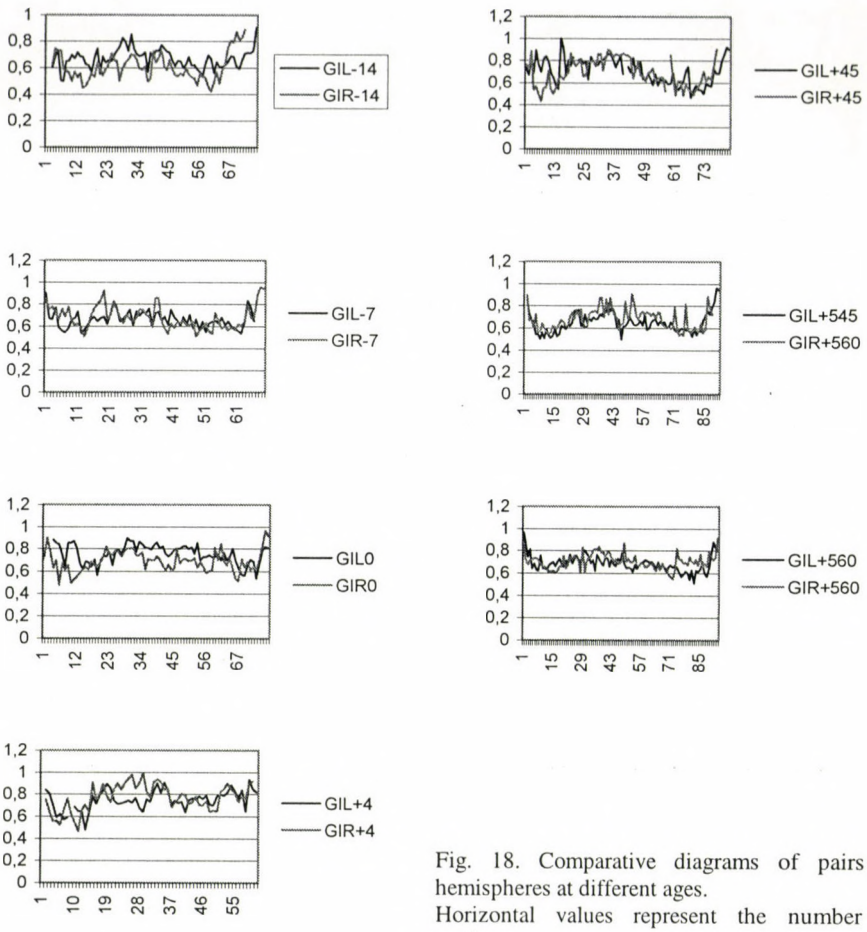

Fig. 18. Comparative diagrams of pairs of hemispheres at different ages.
Horizontal values represent the number of sections

Total amount of myelin

Total amount of myelin was measured on the basis of segementation of digital images. The higher density of the myelinated tracts in the myelin sections stained specially for myelin made the segmentation obvious. The territory occupied by the darker (myelin) area was expressed as a percentage of the total area (Fig. 19).

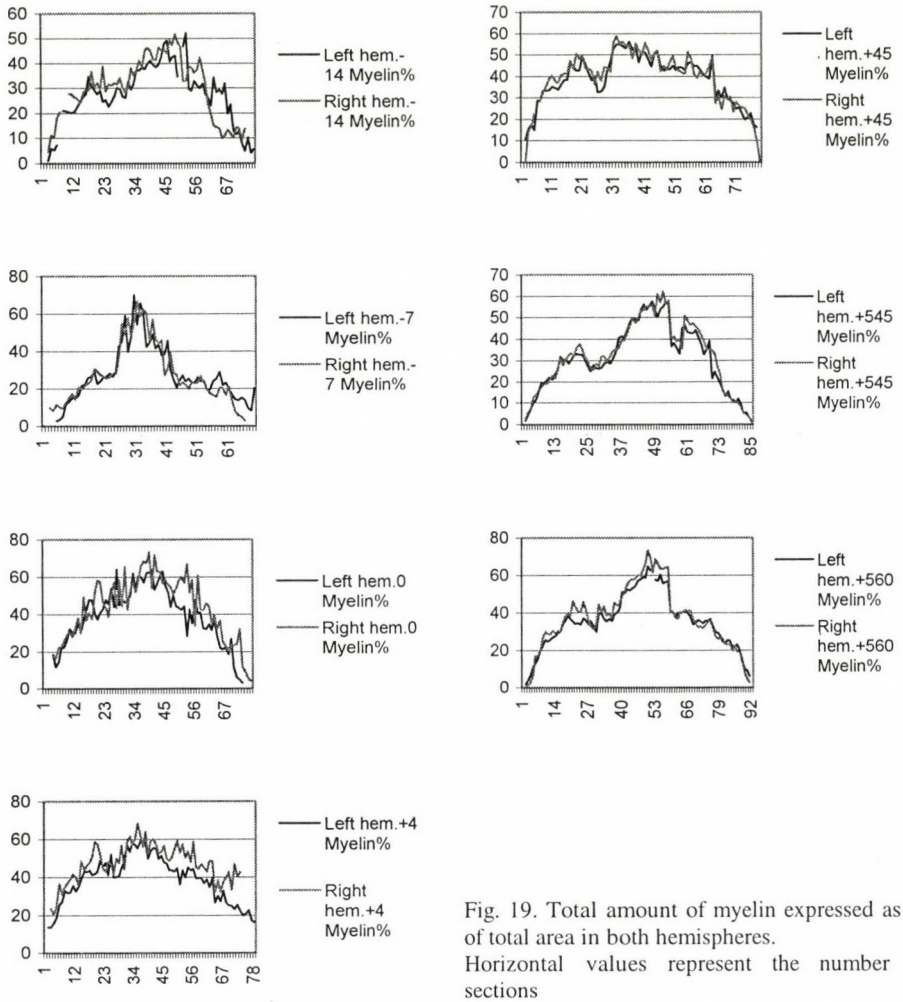

Fig. 19. Total amount of myelin expressed as % of total area in both hemispheres.
Horizontal values represent the number of sections

In the first rostral series of coronal sections until the level of corpus callosum, hemispheres were separated from each other by the interhemispheric fissure in the median line. At the occipital lobe sections contained the two hemispheres and the mesecephalic section. In the portion between them, the hemispheres were arbitrarily cut from basal ganglia and the thalamus by a line drawn from the top of corpus callosum to the lateral rhinal sulcus. This sulcus separates the neopallium from the rhinencephalon rostrally and the temporal cortex and the piriform lobe caudally.

The profile of these curves contains one major peak in the mid-region where the optic and acoustic radiations are found in the continuation of the basal ganglia. The notch in front is due to the artificial separation of the hemispheres where the lateral part of the basal ganglia was also included. This technical error, however, is equally present in each brain, therefore they remain comaprable.

In the cerebellum a more or less similar regular distribution of the myelin was observed. The curve profile of these distributions slightly changes from a hillock to a bell shape. This curve actually follows the areal profile of the cerebellum (Fig. 20). In the middle of the cerebellum the medullary body and the cerebellar peduncules give the majority of the tissue which seemed fully myelinated.

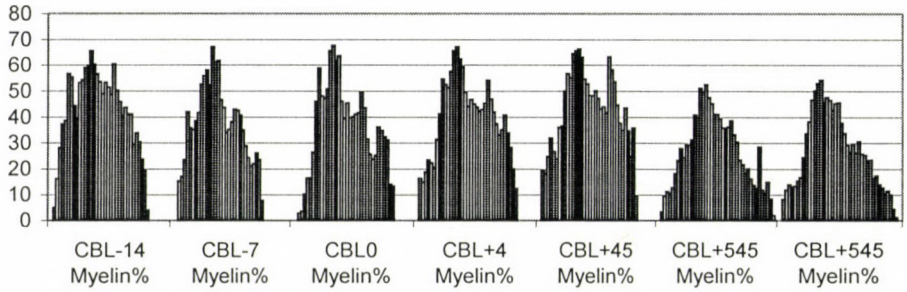

Fig. 20. Rostro-caudal profile curves of myelin amount expressed as % of total area in each cerebellum

Circumscribed motor pathways and areas

As the total amount of myelin showed a significant increase between prenatal and early postnatal brains, in the following we focussed on certain pathways. Among the motor pathways which play an important role in the execution of different types of movements the internal capsule, the pyramidal tract, some parts of the extrapyramidal tract (rubrospinal, cerebellospinal tracts) and the corpus callosum were measured. In the myelin-stained section each of these pathways were evenly stained. Their myelination seemed to be normal. Detailed observations on their myelin sheath could not be performed because of the unusual thickness of the sections (20 μm).

For quantitative description constant sections of motor pathways were measured for an average. The internal capsule was measured at the level of the basal ganglia in a V shape from the corona radiata to the mesencephalic flexion. Its size varied within a narrow range in the young brains (from 14 prenatal days to 45 postnatal days) but substantially increased in the adults.

Data of the pyramidal tract were collected along the medulla oblongata on the ventral surface. In order to avoid addition of the height of the arcuate nucleus only the tract was measured at the horizontal diameter. A moderate increase can be seen in the young brains and an approximate duplication can be identified in the adults in the diameter charaterizing the thickness of the pathway (Table 2).

Table 2. Summarizing table of the diameter of circumsribed myelinated motor pathways

Age (days)	CI (mm)	P (mm)	EP (mm)	CC (mm)
-14	22.30	2.06	1.68	0.96
-7	21.51	2.33	1.89	0.96
0	23.01	2.57	2.17	1.20
4	22.89	2.47	2.27	1.23
45	23.04	2.76	2.47	1.66
545	33.19	4.67	3.34	3.38
560	34.17	4.93	3.10	3.47

Abbreviations: CI = capsula interna, P = pyramydal tract, EP = extrapyramidal tract, CC = corpus callosum

The increase of the diameter of the extrapyramidal tracts is more even then in the previous two cases.

The most significant rise was measured in the corpus callosum. For reduction of technical errors only the trunk of the corpus callosum was measured. The growth of this pathway showed a continuous increase parallel with age.

Cellular parameters of the cerebella

Since brains were cut coronally, we took the advantage of MRI in reconstructing a virtual series of sagittal sections. In both adult horse brains MRI image sequences were taken resulting a 256 cubic voxel image matrix. This matrix was used in a three dimensional reconstruction work in order to create a virtual longitudinal section along the median plane of the brains (Fig. 21). This longitudinal section assisted us in identifing the folium of the vermis in our preliminary studies. The most prominent lobe of the vermis of the cerebellum in cross sections was the folium vermis.

Fig. 21. Virtual longitudinal section of the cerebellum of an adult horse. The image was reconstructed from MRI cross-sections

Each folium of the folium vermis was investigated and Purkinje cells were counted in each microscopic field measuring 200 μm^2. In the young animals the cell number varied between 36 – 64 while the Purkinje cells of the adults showed an evenly packed distribution with a more or less constant average. These data are collected in Table 3. It is worthy to note that the deviation of the cell counts are twice or even three times higher in the young brain than in the adults.

Table 3. *N = average number of Purkinje cells per territorial unit

Age	N* (Purkinje cells)	S.E.M.
-14	36	± 8
-7	60	± 11
0	55	± 14
4	54	± 6
45	64	± 5
545	40	± 3
560	42	± 5

In the late prenatal and even in the early postnatal age an intense cellular migration can be observed in the cerebellar molecular layer. Cells of a former external granular layer are migrating through the stratum moleculare presumably along the glial processes (Altman and Bayer, 1985). This phenomenon is known in the most frequently studied species such as rodents and cat, and to some extent in man, but is less investigated in the horse.

The molecular layer contained migratory cells, however, no external granular layer could be demarkated in our specimens. A 200 μm^2 territoreal unit was observed and digitally segmented. The area of the dark, isolated particles corresponding to the cells were compared to the total microscopic field and expressed in percentage. This method, however, adds the number of "local" cells of the molecular layer such as basket and stellate cells to the total cell count, but this was considered as a constant in our measurements.

As it is summarized in Table 4 the number of migratory cells in the molecular layer is remarkably high in the prenatal brain, somewhat lower in the early postnatal foals and definitely low in adults. This decrease, however, is not linear due to an increase at 4 and 45 postnatal days.

Table 4. Cell counts expressed in ‰ of territorial units of the cerebellar molecular layer

Age	N*	S.E.M.
-14	1.42	± 0.2
-7	1.83	± 0.34
0	0.65	± 0.07
4	0.9	± 0.07
45	1.5	± 0.26
545	0.48	± 0.05
560	0.45	± 0.04

N*= average number of cells

According to the active cell migration from the molecular to the internal granular layer in the young population a change in thickness of these layers and their proportions is expected. The quotient of the thickness of the molecular and the internal granular layers is introduced in Table 5. Since the thickness data strongly deviate, 100 measurements were taken within the entire lobe in one section.

Table 5. A quotient was calculated from the thickness of molecular (Mol) and internal granular layer (IGL)

Age	Mol/IGL
-14	1.43
-7	1.03
0	1.65
4	1.19
45	0.98
545	1.02
560	0.93

Cellular measurements in the cerebral primary motor cortex

Unlike in the most frequently studied species such as cat, laboratory rodents and dog the primary motor cortex of the horse is not around the cruciate sulcus. According to Breazile's works based on electrical stimulations (Breazile et al., 1966) this area is situated along the dorsal margin of the rostral half of the hemispheres (Fig. 22). In his opinion the entire rostral half medial to the suprasylvian sulcus corresponds to the primary motor area. The contralateral somatotopic representation of the limbs matches the medial surface aproximately at the level of the rosrum and the genu of corpus callosum.

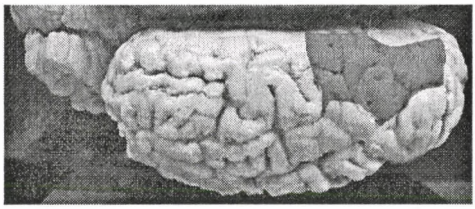

Fig. 22. The dorsal margin of the right hemisphere. The primary motor cortex is indicated by the shaded area

The cross-section cortical thickness varied within a wide range depending on the plane of the section, therefore a well-defined area was measured situated immediately at the rostral tip of the genu of corpus callosum in the cingulate gyrus.

The difference in cortical thickness from the youngest to the adult brains is demostrated in Table 6. The linear increase of thickening can be observed.

Table 6. The thickness of the cortical primary motor cortex in mm

Age	MC thickness (mm)	S.E.M.
-14	1.32	± 0.4
-7	1.23	± 0.41
0	1.31	± 0.5
4	1.62	± 0.39
45	1.93	± 0.35
545	2.17	± 0.2
560	2.29	± 0.21

DISCUSSION

Qualitative analysis

Owing to the fact that hardly any knowledge is available on the cytoarchitectonics and its development in the equine brain, one must rely on analogous events in better investigated species. Even this is rather uncertain since it is well established that there are great interspecies variations in cellular and pathway maturation of the brain (Noetzel and Rox, 1964; Korr, 1980). In the foal, the almost immediate standing up and hopping after birth make it obvious that this species is born with a fairly developed motor system which, however, lacks the economy of function and target-oriented coordination. Our findings relevant to cellular maturation and the development of myelinated areas of the central motor system support this assumption: the main motor centres and pathways are present already prenatally. Nevertheless, we could observe a spectacular maturation of these areas regarding both cytology and fibre quality up to postnatal day 45 from where growth rather than maturation became the leading phenomenon. This statement is based on a staining representative of the granular (rough) endoplasmic reticulum, an organelle responsible for massive protein synthesis on one hand, and on the gross histologic demonstration of myelination as a measure of impulse conducting capability on the other.

The sequence of neural cell maturation is proliferation, migration and differentiation (Jacobson, 1978). The precursors of nerve cells are generated in the subventricular layer of the developing brain vesicles. They undergo repeated mitoses and then start to migrate. Positioning of neurons to the final locations is guided by the processes of the radial glia (Hajós and Bascó, 1984) spanning the entire width of the neural tube and its early derivatives. The differentiation of motor neurons begins at different time intervals after they have finished their migration and occupied a final position in the brain (Altman, 1963; Atsumi, 1971). This comprises, as a next step the outgrowth of neuronal processes as indicated by ther multitude of growth cones and other cytologic and biochemical signs of axonal pathfinding observed in this period (Landis, 1983; Pfenninger, 1983). Axonal and dendritic growth require an intensive protein synthesis for which only the cytoplasm of the cell body possesses the proper synthetic apparatus.

A second wave of protein synthetic activity is coupled to synapse formation (Balázs, 1971; Kornguth et al., 1968; Larramendi, 1969; Buchs et al., 1993). Our findings support the view that in the equine, the outgrowth of neuronal processes, particularly in the motor system takes place prenatally. Whether these processes are already capable to establish specific contacts, remains to be elucidated. Nevertheless, as it can be implicated from the incoordinated

movements immediately after birth, there seems to be a rather diffuse flow of impulses along the motor pathways.

Looking at our findings at the cellular level, it is evident that the cytoplasmic organelle primarily associated with protein synthesis, i.e. the granular endoplasmic reticulum, hypertrophizes greatly at postnatal day 45. This is reflected by a significant increase in the intensity of Nissl staining within the cytoplasm of motor nerve cell perikarya to such an extent that it masques all other cytoplasmic details which cannot be distinguished under the massive Nissl substance staining. In the previous and following periods studied, the Nissl substance of the motor neurons is present in the cytoplasm in the form of scattered large granules (Nissl bodies) showing the typical stacks of cresyl violet-stained endoplasmic reticular cisterns. This suggests a burst-like up-regulation of in protein synthesis around postnatal day 45.

If viewed on a larger scale, all the motor areas showed a similar time course of changes in the amount of Nissl substance. This is particularly conspicuous in areas such as the striatum where digitized images of Nissl stained serial sections could readily be identified even at low magnifications on the basis of their cellular structure. The Nissl pattern was prenatally poor, showing hardly any cells, whereas in the early postnatal period the cellular architecture of the striatum became evident: the caudate nucleus was distinguishable from the putamen and the typical stripes crossing the internal capsule were also revealed. A similar trend was observed in other motor regions as well.

The cerebellum deserves special attention since it is thought to be an important centre of motor coordination. It should be therefore emphasised that in the prenatal equine cerebellum an external granular layer was hardly present indicative of an advanced cerebellar development alrerady in the late fetal period. This is particularly notable if we consider the persistence of this layer in other species over long postnatal periods (Markstrahler, 1947; Larsell, 1954; Kaufmann, 1959; Halmos, 1961; Lange, 1978).

On the basis of the present overall findings we can only speculate on the significance of the above peak in the amount of granular endoplasmic reticulum throughout the motor system. Supposed that this increase takes place up to a time (postnatal day 45) when neuronal processes have reached or at least approached their targets, we suggest that increased protein synthesis may reflect massive synaptogenesis, as a next phase of pathway maturation. Comparison with other species is not really relevant since there are extreme variations in the maturity of the motor system at birth. If the protein synthetic peak is assumed to correspond to synaptogenesis, the coincidence between this peak and the increase in the level of gait coordination observed in this postnatal period can be explained. This assumption, however, requires further cytochemical and electron microscopic support.

The decrease of Nissl staining between early postnatal age and adulthood seems to be due to an expansion of the brain volume within this period not followed by an additional increase in Nissl substance. As shown at higher power, cell bodies, but particularly the area of motor nuclei grow substantially. This may result in an apparent reduction of staining which is getting scattered over a larger territory.

The functional significance of the myelin sheath is that by its internodal structure it enables the fastest velocity impulse conduction, the so-called saltatory conduction (Lillie, 1925; Tasaki and Takeuchi, 1941; Huxley and Stämpfli, 1949). At the nodes of Ranvier the axon is practically exposed to the extracellular space so that ionic changes take place in a sequence of 'bouncing' from one Ranvier node to the other skipping thereby the distance of the internodal segment of the axon.

It has been realized in classical strudies (see for references: Jacobson, 1978) that the development of the myelin sheath is a final step in the maturation of the neuron. With regard to what was said above it is concievable that in the motor pathways normal velocity, target-oriented impulse conduction can happen only after the formation of a myelin sheath along the full length of the axon. In several developmental or neurological disorders myelination is either delayed or a demyelination occurs (Szalay et al., 2001). It is also well documented that the primary target of the so-called neurodegenerative diseases is the myelin sheath (Toews, 1999)

The time of myelination shows wide interspecies variations but in all cases it is marked by events such as an increase of protein synthesis in the parent cell body of the axon, and by a proliferation of oligodendroglia responsible for the formation of the myelin sheath (Río Hortega, 1930). In the present work the maturation of the neuronal cytoplasm, particularly of its main protein synthetic organelle was followed (see above), while subsequent sections were stained for myelin. Data on oligodendroglia proliferation could not be obtained from our material due to technical reasons arising from a long post mortem period elapsing prior to fixation of the brains.

Our results obtained with myelin staining show that the amount of myelin increases parallelly with the intensity of Nissl staining, i.e. it is the highest at postnatal day 45. Here it should be noted that as a measure of myelin, its staining density over unit area was looked at. Therefore, it has to be mentioned that not only the staining density of myelinated pathways but also the area occupied by them increased substantially between the prenatal and early postnatal periods studied.

It also appeared that, at least with the method used, no regional differences could be detected in the advancement of mylination. Of course there were regions and structures such as the corpus callosum, septum, ansa lenticularis which underwent a spectacular growth between prenatal and early postnatal periods. This growth was apparently due to an increase in myelinated fibres. However, by postnatal day 45 the motor areas and tracts of the brain showed an equally advanced myelination. It is noteworthy that the area occupied by myelinated fibres also increased substantially, in other words, the white matter areas that contain the motor pathways expanded. This could be particularly well documented in the white matter of the primary motor cortex and the internal capsule but the increase in other areas was also obvious.

Measurements of brain size in the different age groups clearly shows that if outer parameters are regarded, there is no net increase in size between th brains of the prenatal (-14-day-old) fetus and the 45-day-old foal. It is remarkable that this is the period when the bulk of internal changes occur either in cellular and cytoarchitectonic maturation or in myelin sheath formation. This discrepancy between overall growth and internal maturation is a novel finding.

Our observations suggest that the period between postnatal day 45 and adulthood brings about a significant growth of the overall size of the brain but adds little to the already formed myelin. It is most likely that the growth subsequent to the early postnatal period is due to the further ramification of dendritic trees, the development of the astroglial system and the increase in vascularization.

It is also suggested that the most important events of cytoplasmic maturation in the motor cell bodies are concluded by postnatal day 45.

All these stress the unusually early maturation of the equine motor system which appears to have a critical period of development up to the third months after birth. This means that future developmental, morphological, and functional analyses should concentrate on this period to understand more about the mechanisms of emergence of a coordinated gait in the foal.

The values of brain mass at various developmental stages suggests that the growth of the brain is not parallel to the growth of the entire body mass. While there is no increase in brain mass in the period between prenatal day 14 and postnatal day 45, the entire body mass of the foal increases 3-fold during the same time. In the following period up to adulthood, the increase in the mass of the brain is 1.8-fold, whereas that of the body 10-fold.

This again shows that foals are born with a relatively well-developed brain which, after an intensive perinatal internal maturational process, grows moderately to reach the appr. 600 g mass typical for the adult. This growth is not comparable to the growth of the entire body mass which greatly supersedes the growth of the brain.

Quantitative image analysis

Beside general growth of the brain, length, coronal section areas and diameters were measured and analysed. Results obtained from the qualitative study of histological preparations and of computer plots of large series of coronal sections were substantiated by the image analysis of digitized specimens.

Among major parts of the brain such as the hemispheres, cerebellum and the brainstem the longitudinal growth of the hemispheres was the most prominent. There was no difference between the left and right side as revealed in parallel sections. Within the perinatal age the change of this dimension follows the growth of the head but grows to a lesser extent than the head. When the coronal area measurements are regarded the cerebellum appears to be the fastest developing part which means that the cerebellum develops rather in the transverse direction by the development of its hemispheres. Since differences within the perinatal age were definitely smaller than the differences between data of perinatal and adult, mean of the perinatal and the adult groups could be created. It is known (Latshaw, 1987) that the vermis starts its development earlier than the hemispheres. In the perinatal age the histological maturation of the cerebellar cortex takes place and probably this causes the bulk of cerebellar growth. When the maxima of the transverse diameters were examined, a similar temporal change could be observed, as in growth of longitudinal dimensions. These data suggest that the growth of the equine cerebral hemispheres from the perinatal age to the adulthood is most pronounced longitudinally, while the cerebellum grows mainly in the transverse direction.

The amount of the cortical convolution numerically expressed by gyrifiaction index (Wosinski et al., 1996) seems to be high already in the late prenatal age. The change of GI is small within the range of examined ages. This also supports the hypothesis that the equine brain is well developed by birth. An increase can be observed, however, at the day of birth which indicates the dynamism of the cortical structures at this time. This phenomenon is underlined by the data of thickening of the cortex, too. From the youngest to the eldest (2 years period) the cortical thickness increases 1.8 times while from the youngest to 45-day-old (2 months period) 1.46 times. This disproportional distribution of thickening shows an intense cellular development found in the primary motor cortex.

The proportion of myelin to the entire area of the sections (white/gray matter ratio) is outstanding in the first two months. From the last period of the prenatal age to postnatal day 45 an intense myelination was observed in the hemispheres and the cerebellum. This is in a good accordance with the development of the coordinated locomotion during this period of life. In section from the adult brain, the myelin occupies a relatively smaller territory in addition to the cellular elements. This is also supported by the values of cortical thickness.

Based on our findings territorial and temporal distribution of myelination among the major motor pathways and their temporal distribution it can be concluded that the internal capsule

does not develop significantly while the pyramidal and extrapyramidal tracts grow 1.5-fold within the perinatal age. The necessary amount of myelinated motor pathways for basic locomotion during the weeks following birth are already present along the entire length of the pyramidal system. The thickening of the pyramidal tract at the medullary portion indicates that additional bundles join this tract deriving from the mesencephalon and the cerebellum. Similar developmental course was seen in the extrapyramidal system. By the end of the investigated period the internal capsule increase 1.5-fold, the pyramidal tract to twice, the extrapyramidal tract 2.5-fold and the corpus callosum 3.5-fold. In the perinatal period the development of corpus callosum, the major interhemispherial commissure is 1.7-fold. These data indicate that the commissural relationships are not of primary significance around the day of birth but intensely develop afterwards. The function associated with this commissural pathway is coupled to that of similar areas between the hemispheres synchronizing this way the concurrent actions of the represented muscles.

In our histological inspections the number of Purkinje cells was high from the 7th day before birth to the 45th day following birth. Though these cells develop among the first ones in the cerebellum, their position in the piriform cell layer is not finalized by birth (Altman and Bayer, 1985). Due to this postembryonic migration the Purkinje cell count varies in the perinatal period. In the adult cerebellum, however, the cell count appears lower and its variability is definitely less which reflects the settlement of Purkinje cells by adulthood.

The presumed migration of postmitotic cells from the external germinative layer through the piriform cells to the final internal granular layer was hardly recognized in the early postnatal stage. The higher number of cells in a migratory phase in the molecular layer was encountered only in prenatal cerebella. In the 45-day-old foal the cell-count was also high but the reason of high cell number is obscure. One possible explanation is an increase in the number of "native" cell types (stellate and basket cells) of the molecular layer. Due to the cell migration emptying of the molecular layer and increase of the internal granular layer appears concurrently as we observed it at the perinatal age. The highest value of coefficient of molecular/IGL was found at the day of birth but it decreased in later stages.

For observations of cortical thickening of the motor cortex the cingulate gyrus was selected because the represenatation of the limbs in the primary motor cortex corresponds to this territory (Breazile et al., 1966). Results of this sampling cannot be applied to the entire brain but are in a tight correlation with the development of the control of locomotor apparatus. By the end of the 2nd month of perinatal term the majority of thickening (1.45-fold) is already completed. The further thickening (0.2-fold) up to adulthood lasts for almost 2 years which indicates a disproportional growth of the motor cortex. We can state that the overwhelming share of development in the primary motor cortex takes place in the perinatal period.

In conclusion, our findings obtained by qualitative and quantitative investigations suggest that the overall brain size does not increase between 14 days prior to expected birth to postnatal day 45, meanwhile significant internal changes take place: (i) there is an increase in the amount of Nissl substance, even if viewed at the level of single neurons; (ii) the amount of myelin increases involving the intensification of myelin staining and the appearance of newly formed myelin. This results in the enlargement of the areas of myelinated motor tracts. Between postanatal day 45 and adulthood the overall size of the brain, as indicated by external parameters is almost doubled. This is not followed by an equal degree of myelin and Nissl substance formation showing that the main events of motor pathway maturation occur between prenatal and early postnatal periods, whereas growth in size of the brain follows only after this maturation process.

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ERRATUM

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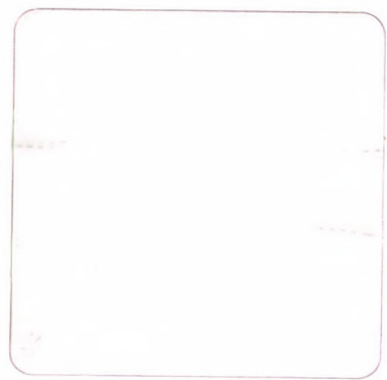

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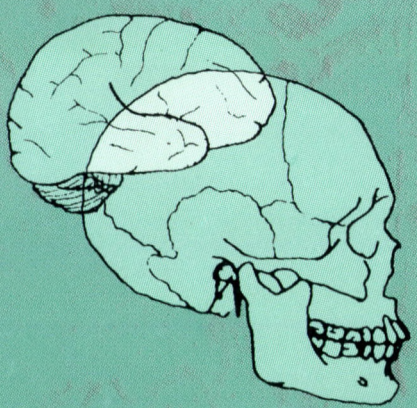

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RESEARCH REPORT

APPEARANCE OF ANNEXIN II IMMUNOPOSITIVITY IN REACTIVE ASTROCYTES BUT NOT IN MICROGLIA

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According to some *in vitro* data of other authors, annexin II may be involved in the formation of the glial fibrillary acidic protein (GFAP) filaments from their subunits. To check this *in situ*, the present study investigated the annexin II immunopositivity in the reactive glia around stab wounds in rats. The annexin II immunopositive astrocytes were less in number and less widely distributed than the GFAP immunopositive cells. In double-labeling studies applying fluorescent dye-labeled antibodies, GFAP was detected in the annexin II immunopositive cells, whereas a number of GFAP immunopositive cells were negative to annexin II. In the territories of annexin II immunopositive astrocytes no *Griffonia*-labeled microglia were found. The results suggest a role of annexin II in the post-lesional glial reactions, probably in the post-inflammatory stage, but not in the appearance of the GFAP immunopositivity.
Key words: calpactin-I, glial reaction, lipocortin-2, microglia, reactive glia

INTRODUCTION

The annexins are cell membrane glycoproteins binding calcium ions and phospholipoproteins. They participate in a wide range of basic cell functions, e.g. the cytoskeleton organization, endo- and exocytosis, regulation of proliferation, connection to the extracellular matrix, and intercellular signalling, and due to these latter functions they have cytoprotective and anti-inflammatory effects (Burgoyne and Geisow, 1989; Donato and Russo-Marie, 1999). Annexin II is identical with the lipocortin 2 and the "heavy" unit of calpactin I (Crumpton and Dedman, 1990), and it has a role in the control of inflammatory processes and wound healing through different mechanisms, e.g. plasminogen activation, or TGFβ1 effect mediation (Munz et al., 1997; Kang et al., 1999).

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Annexin II, together with other annexins, has been demonstrated in the brain tissue (Woolgar et al., 1990; Regnouf et al., 1991). At the early stage of neurohistogenesis, annexin II appeared in the radial glia (Reeves et al., 1992), whereas it was found only in the Bergmann glia in the mature brain (Burgoyne et al., 1989). In human glial tumors, the less mature they were, the more annexin II was found in them (Reeves et al., 1992; Roseman et al., 1994). Annexin II was also detected in human neurodegenerative diseases (Elderfield et al., 1992; Eberhard et al., 1994). In the astrocytes of intact spinal cord (Naciff et al., 1996) mentioned the presence of annexin II. Annexin II could be a tenascin receptor (Chung and Ericson, 1994; Chung et al., 1996) which suggests the possibility that it participates in the control of axon growth during development and after lesions, since tenascin is a major inhibitor of axon growth (Taylor et al., 1993).

According to some *in vitro* data, annexin II may be involved in the formation of the glial fibrillary acidic protein (GFAP) filaments from their subunits (Garbuglia et al., 1995; Bianchi et al., 1996). Annexin I, V and VI were not effective on this process. The GFAP content and immunopositivity of the astrocytes varies according to the functional state, e.g. increases in the reactive gliosis (see e.g. Bignami et al., 1980; Fernaud-Espinosa et al., 1993). The data, however, are poor on the factors which control the formation of the GFAP filaments and the balance between the free and filament-integrated GFAP subunits. The aforementioned data suggest that annexin II may be one of these factors. The rapid and extensive increase of GFAP expression after lesions provides a good model to check the correlation between the appearance of annexin II and GFAP *in situ*. The present study therefore compared the immunopositivities to annexin II and GFAP in the reactive glia around stab wounds in adult rats. The possible co-localization of the microglia marker *Griffonia* lectin (GSAI-B4, Kaur and Ling, 1991; McKanna, 1993; Savchenko et al., 2000) was also investigated.

MATERIALS AND METHODS

Adult albino rats (3 of our breeding) were deeply anaesthetized with ketamine and xylazine (20 and 80 mg/kg body weight, respectively). After opening the skin and making a burr hole on the skull, a deep brain lesion was performed with a sterile disposable needle in the parietal cortex and the underlying hippocampus and diencephalon, then the skin was sutured. After operation the animals were under control until recovery. Before and after the operation the animals were feeded and supplied with water *ad libidum*. The animals survived for 7 days, in accordance with our former experience and the data published (see e.g. Bignami et al., 1980; Fernaud-Espinosa et al., 1993) on the time-course of glial reaction. Then the animals were overanaesthetized with ether and perfused transcardially with a fixative containing 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4).

Serial coronal sections (thickness 50 μm, 5-6 of every animals) were cut by a Vibratome. Parallel sections were processed for immunohistochemical staining against either annexin II or GFAP. Floating sections were pretreated with 3% H_2O_2 (for 5 min), and then 20% normal goat serum (for 1.5 hours, at room temperature). Monoclonal mouse antibodies raised against annexin II (Transduction Laboratories, Lexington, Ken.) or GFAP (Boehringer, Mannheim) were diluted 1:50 or 1:100, respectively, in PBS containing 0.5% Triton X-100, and were applied for 40 hours at 4°C. After that biotinylated rabbit anti-mouse immunoglobulin and

streptavidin-biotinylated horseradish peroxidase complex were applied subsequently, and the immunocomplex was visualized by the diaminobenzidine reaction.

The colocalization of annexin II with either GFAP or the microglia marker *Griffonia* lectin was investigated in double-labeling studies with fluorescent dye-conjugated reagents. The number of animals operation, fixation, and sectioning were similar to described previously. In these experiments the anti-annexin II was followed by an anti-mouse immunoglobulin conjugated with Cy™-3 dye (Jackson ImmunoResearch Lab. Inc.), in a dilution 1:300, at room temperature for 3 hours. The sections were processed further in two different ways. On the odd sections a polyclonal anti-GFAP antiserum (rabbit, Sigma, dilution 1:100) was applied at 4°C, for 40 hours, followed by fluorescein-isothiocyanate (FITC) conjugated anti-rabbit immunoglobulin (Jackson ImmunoResearch Lab., Inc). The other sections were incubated with biotinylated *Griffonia simplicifolia* lectin I (isolectin B4; Vector) at 4°C, for 40 hours, followed by fluorescein-isothiocyanate (FITC) conjugated streptavidin (Jackson ImmunoResearch Lab., Inc.). In both cases the fluorescent reagents were used in a dilution 1:300, at room temperature for 3 hours. The Cy™-3 dye fluoresces in red when excited by green light, whereas in the case of FITC the excitation light is blue, and the emitted light is green. The photomicrographs were taken by a digital camera in both green and blue lights, subsequently, on the same areas. The colored pictures were converted into black-and-white and their contrast was slightly enhanced.

RESULTS

After immunostaining by the annexin II antibody, a number of immunopositive cells were observed in same areas around the lesion. The number of the cells decreased in a short distance from the wound, although in the white matter (e.g. in the corpus callosum) some distant immunopositive cells also appeared (Fig. 1). The annexin II immunopositive cells resembled astrocytes (Fig. 2). No immunopositive cells were found in the intact brain areas. The same lesion evoked the appearance of GFAP immunopositive reactive astrocytes in the full length of the lesion track. These cells were numerous even in a considerable distance from the lesion (Fig. 3). It is to be emphasized that, in contrast to GFAP, the annexin II immunopositive cells were not found everywhere along the lesion track. They occurred only in some areas, although without preference to any brain regions (i.e. cortex, hippocampus or diencephalon). When double-labeling was applied, all the annexin II immunopositive cells proved to be immunopositive to GFAP as well (Figs 4 and 5). A number of the GFAP immunopositive cells (i.e. astrocytes), however, were negative to annexin II. No colocalization occurred between annexin II and the *Griffonia* lectin. In the areas, where the annexin II immunopositive cells were found, no *Griffonia*-labeled microglia was visible. The labeling with *Griffonia* lectin, however, indicated a number of microglial cells in some areas in which no annexin II immunopositive cells were detected (Fig. 6).

DISCUSSION

The results suggest a role of annexin II in the post-lesional glial reactions but not in the appearance of the GFAP immunopositivity. Microglia is characteristic of the inflammatory phase of the post-lesional glial reaction (see e.g. Fernaud-Espinosa et al., 1993). It may be supposed that the inflammatory phase does not end synchronously in the whole territory of the lesion, therefore both microglia-colonized and microglia-free areas can be found at the same post-operative time. The anti-coincidence of microglia and annexin II immunopositive astrocytes suggests that annexin II gains importance in the post-inflammatory processes of glial reaction, in contrast to annexin I (lipocortin 1, calpactin II, Crumpton and Dedman, 1990), which has been demonstrated in both microglia and astrocytes (McKanna, 1993; Savchenko et al., 2000), as well as in the reactive glia after lesions (Johnson et al., 1989; Elderfield et al., 1992, 1993; Eberhard et al., 1994; Mullens et al., 1994). In a recent paper Young et al. (1999) reported that microglial expression of lipocortin 1 (i.e. annexin I), however, faded in 4 or 5 days after the kainic acid lesion of the cerebellum. Therefore, following the present preliminary results, further studies are in process to analyse the time-course of annexin II immunoreactivity after lesion. The importance of these data is enhanced by the findings that microglial activity would be a determinant of the outcome of the brain injuries (see e.g. Balasingam et al., 1996).

Fig. 1. Annexin II immunostaining in the hippocampus, diaminobenzidine labeling. The lesion is at the right edge of the figure. Numerous immunopositive cells are visible, a few of them are pointed by arrows. The astrocytic shape of the cells is demonstrated in the next figure. Bar: 250 μm

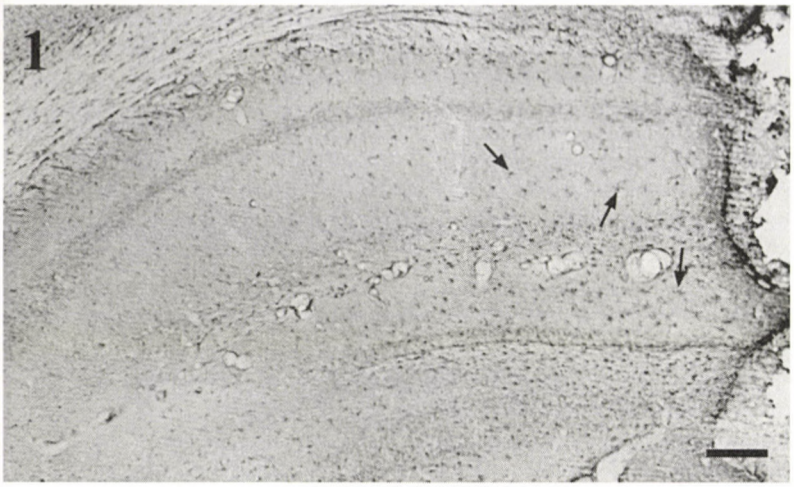
Fig. 2. Enlarged part of the area shown in Fig. 1. Arrows point to stellate-shaped astrocyte-like cells. Bar: 100 μm

Fig. 3. GFAP immunostaining in the hippocampus, in a parallel section to that seen in Fig. 1, diaminobenzidine labeling. The lesion is at the right edge of the figure. Note that the population of the labeled cells (few of them are pointed by arrows) is denser and more extended than in Fig. 1. Bar: 250 μm

Fig. 4. Fluorescent dye ($Cy^{TM}3$)-labeled immunostaining against annexin II (excited by green light). Astrocyte-like cells (arrows) are visible near the lesion, which is in the right lower corner of the figure. Bar: 100 μm. Inset in the lower left corner (arrowhead): an immunoreactive cell enlarged

Fig. 5. Fluorescent dye (FITC)-labeled immunostaining against GFAP, the same area as in Fig. 3 (but the inducing light was now blue). Arrows mark the same cells as in the previous figure. Bar: 100 μm. Inset in the lower left corner (arrowhead): an immunoreactive cell enlarged

Fig. 6. Staining with fluorescent dye (FITC)-labeled *Griffonia* lectin in an area different from Fig. 5. A number of microglial cells are stained, two of them are pointed by arrows. No annexin II labeling was found in this area. Bar: 60 μm. Inset in the upper right corner (arrowhead): an immunoreactive cell enlarged →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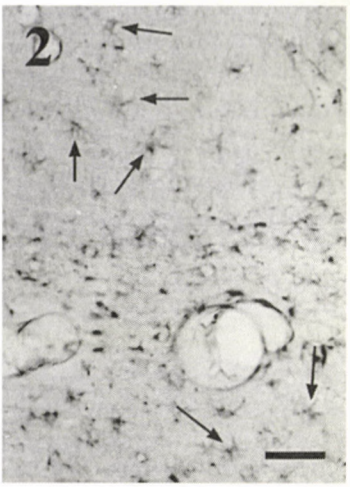
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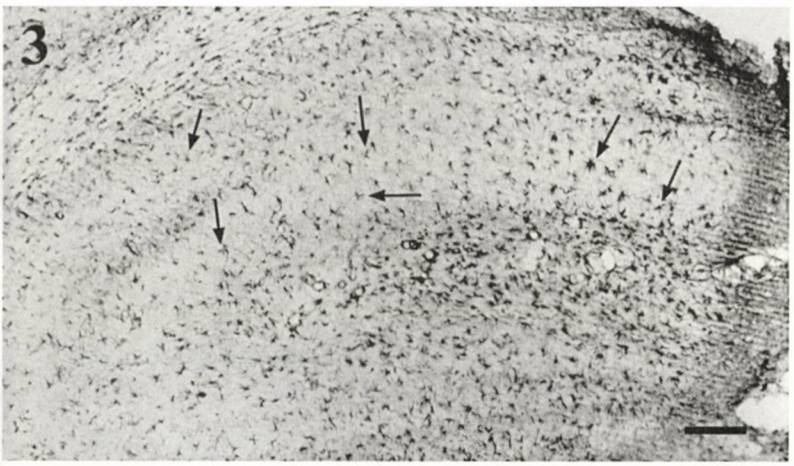
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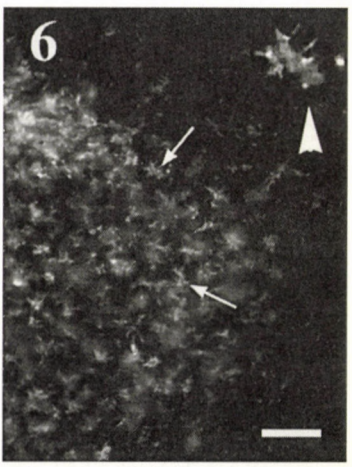
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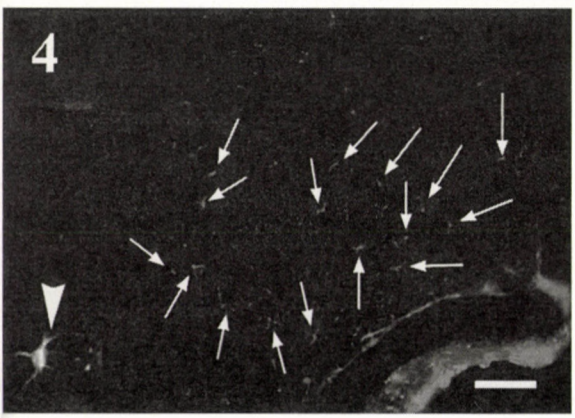
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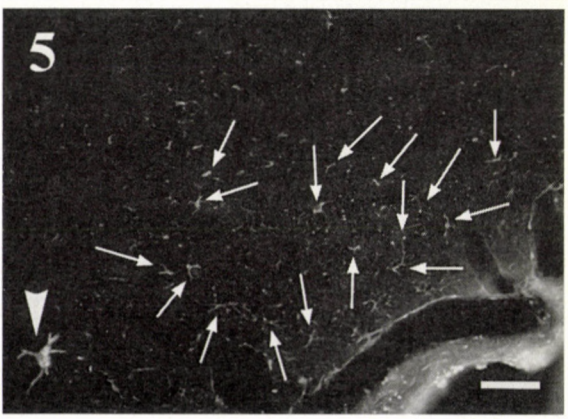
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RESEARCH REPORT

ADENOSINE RECEPTORS DO NOT MEDIATE THE MELATONIN PROTECTION AGAINST PENTYLENETETRAZOLE INDUCED SEIZURES IN RATS

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Presence of adenosinergic receptors in pineal gland suggests an interaction between the two-neuroactive substances adenosine and melatonin at a functional level. Since melatonin and adenosine both demonstrate anticonvulsant activity in various models of seizures, therefore present study was undertaken to determine the effect of adenosinergic agents, on the anticonvulsant effect of melatonin, in pentylenetetrazole PTZ-induced seizures in rats. Wistar rats were administered melatonin (100-400 mg/kg, i.p.), 30 min before PTZ challenge. A complete protection against seizures was observed with 400-mg/kg dose of melatonin. Selective adenosine A_1 and A_2 receptor antagonists, DPCPX and DMPX in doses of 1 mg/kg, i.p. given 5 min prior to melatonin, could not reverse the protection in PTZ seizures. However, pretreatment with a non-specific adenosine receptor antagonist theophylline, reversed the protection of melatonin, but only at higher dose (100 mg/kg, i.p.). Combination of sub-anticonvulsant doses of melatonin (200 mg/kg) and adenosine (500 mg/kg), on the other hand, offered a synergistic protection. These findings suggest that neither A1 nor A_2 adenosinergic receptors, has a modulatory role on anticonvulsant effect of melatonin against PTZ seizure.
Key words: melatonin, adenosine, pentylenetetrazole, seizures, rat

INTRODUCTION

The role of adenosine in the synthesis of the pineal hormone melatonin has been suggested in several species (Falcon et al., 1991, Falcon et al., 1997, Vacas, 1989). Multiple receptor subtypes of adenosine has been described i.e. A_1, A_{2a}, A_{2b} and A_3. Of these primarily the adenosine A_2 or A_{2b} receptor have been implicated in the facilitatory role of adenosine on melatonin secretion (Nicholls et al., 1997, Falcon et al., 1995). However, presence of adenosine A_1 receptors has also been demonstrated on the pineal gland though their function remain

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obscure (Falcon et al., 1997). Adenosine is known to exert anticonvulsant effect *in vitro* as well as *in vivo* (During and Spencer, 1992). The anticonvulsant effect is mediated primarily by adenosine A_1 receptor (Malhotra and Gupta, 1997). Melatonin also has an anticonvulsant action in animal models, however the receptor subtype involvement is not known. The fact that both these endogenous substances exert anticonvulsant effects and, the presence of adenosine receptors on pineal gland modulating its activity raises the possibility of an interaction between these two neuroactive substances at a functional level.

Therefore in the present study we determined the effect of adenosinergic agents on the anticonvulsant effect of melatonin in pentylenetetrazole (PTZ) induced seizures in rats.

MATERIALS AND METHODS

Male Wistar rats weighing 150-200 g were used. Six animals per cage were group housed in polypropylene cages and maintained under standard laboratory conditions with a natural light dark cycle. The rats were acclimatized to the environment for a week. They were fed standard laboratory rat diet (Golden feeds, India) and water *ad libitum*. Each treatment group consisted of 8-10 animals. The animals were used only once in the study.

Pentylenetetrazole (PTZ) seizures

PTZ (Sigma, U.S.A.) was dissolved in normal saline (0.9% sodium chloride) and administered in a dose of 60 mg/kg, intraperitoneally. This dose of PTZ has been standardized in our laboratory as 100% convulsant with minimal mortality in rats (Malhotra and Gupta, 1997). The animals were observed for 30 min after PTZ challenge. The percent incidence of generalized clonic seizures with loss of righting reflex was noted.

Drugs and treatment schedules

All drugs were prepared freshly and administered intraperitoneally (i.p.) in a volume not exceeding 1 ml/100 g, using a 26-gauge needle.

Dose response for melatonin

Experiment with different graded doses of melatonin were carried out to find out the 100% anticonvulsant and the subanticonvulsant doses for further experimentation with adenosinergic agents.

Melatonin (courtesy, Dabur India Ltd., India) was dissolved in propylene glycol and administered in graded doses of 100, 200 and 400 mg/kg, i.p., 30 min before the convulsant dose of PTZ. Melatonin (400 mg/kg) abolished seizure activities in all (100%) animals and was selected for interaction experiments involving pretreatment with adenosine antagonists. Whereas 200 mg/kg was taken as subanticonvulsant dose (showing 50% protection) for experiments with adenosine.

Experiments with adenosine receptor antagonists and melatonin

The non-specific adenosine receptor antagonist theophylline (courtesy, Sun Pharma, India) was dissolved in warm saline and injected at doses of 50 and 100 mg/kg, i.p., 5 min before the 100% anticonvulsant dose of melatonin. The selective adenosine A_1 receptor antagonist 8-

cyclopentyl-1,3-dipropylxanthine (DPCPX; RBI, USA) and A_2 antagonist -3,7-dimethyl-1-propargyl xanthine (DMPX; RBI, USA) were dissolved in DMSO and distilled water, respectively and administered 5 min prior to the 100% anticonvulsant dose of melatonin. Subsequently, 30 min later these animals were challenged with the 100% convulsant dose of PTZ (60 mg/kg).

The doses and the pretreatment times of the various adenosine antagonists were selected on the basis of our earlier studies (Malhotra and Gupta, 1997).

Experiments with combination of subanticonvulsant doses of adenosine and melatonin

Adenosine (Sigma USA) was suspended in 8% Tween 20 and administered in a subanticonvulsant dose (500 mg/kg) in combination with the subanticonvulsant dose of melatonin (200 mg/kg). Control experiments were performed with the vehicles for each drug tested i.e. propylene glycol, 8% tween 20, DMSO, saline and distilled water.

Statistical Analysis

The results were analyzed statistically by using Microsoft computer program, "Microstat" Ecosoft Inc. U.S.A. Fisher's exact test was applied for incidence of seizures.

RESULTS

In control experiments, PTZ 60-mg/kg challenge produced myoclonic jerks and generalized clonic seizures in 100% animals. Pretreatment with different vehicles used (distilled water, saline, DMSO, 8% tween 20 and propylene glycol), had no effect on either incidence of seizures, as compared to PTZ alone (Table 1).

Effect of melatonin on PTZ induced seizures

Melatonin showed a dose dependent anticonvulsant effect against PTZ induced seizures, the 400-mg/kg dose being 100% effective. However, this dose was associated with marked sedation though in none of the rats there was loss of righting reflex. At the lower doses as well i.e. 100 & 200 mg/kg, mild sedation could be discerned.

Effect of adenosine antagonists on melatonin protection in PTZ seizures

Pretreatment with both the specific adenosine A_1 and A_2 receptor antagonists DPCPX 1 mg/kg and DMPX 1 mg/kg, respectively, failed to modify the anticonvulsant effect of melatonin 400 mg/kg. We have previously reported that 1 mg/kg dose of DPCPX is sufficient to completely reverse the protection of both adenosine and adenosine A_1 receptor agonist N^6-cyclopentyl adenosine (CPA) against PTZ induced seizure. Interestingly pretreatment with theophylline 100 mg/kg prior to melatonin, significantly reduced melatonin protection. Neither DPCPX nor DMPX even up to 5 mg/kg doses, *per se* caused convulsions or alter the incidence of PTZ induced seizures (Malhotra and Gupta, 1997). Similarly, theophylline 50 and 100 mg/kg, i.p. *per se* neither caused convulsions nor aggravated PTZ seizures. But pretreatment with theophylline 100 mg/kg prior to melatonin, significantly reversed the melatonin protection.

Table 1. Effect of adenosine receptor antagonists' theophylline, DPCPX, DMPX on melatonin protection against pentylenetetrazole induced seizures in rats

Pretreatment drug	Dose in mg/kg	Animals showing clonus/ animals tested	Percent incidence of clonus
Control*		8/8	100
Melatonin	100	8/10	80
	200	4/8	50
	400	0/8	0
Adenosine	500	7/8	87.5
	1000	1/8	12.5
Adenosine+melatonin	500+200	0/8	0
Theophylline+Melatonin	50+400	0/8	0
Theophylline+Melatonin	100+400	6/9	66.6
DMPX+Melatonin	1+400	0/8	0
DPCPX+Melatonin	1+400	0/8	0

Control group refers to animals treated with respective vehicle used viz normal saline, propylene glycol, 8% tween 20 and DMSO in 8 animals each. All vehicle treated rats showed convulsions were clubbed.

Effect of combination treatment with subanticonvulsant doses of melatonin and adenosine

When a combination pretreatment with melatonin (200 mg/kg) and adenosine (500 mg/kg) was carried out, before PTZ challenge, a significantly greater protection as compared to either drug alone was observed. However, the combination did not present any advantage as far as the sedative side-effect was concerned.

DISCUSSION

In the present study, melatonin showed a dose dependent protection against PTZ-induced seizures in rats. This anticonvulsant effect of melatonin is in agreement with earlier studies in experimental animals (Pilo and Reiter, 1978, Albertson et al., 1981, Browicz, 1999). Both melatonin and adenosine have been proposed to be candidate endogenous anticonvulsant substance along with many other neurotransmitters (Dragunow, 1986). Adenosine has been shown to exert a modulatory influence primarily on the release process of many neurotransmitters in the brain (Malhotra et al., 2000).

Though many lines of evidences suggest a modulatory role of adenosine in melatonin secretion (Nicholls et al., 1997, Gharib et al., 1989, Falcon et al., 1997) to date the interaction of adenosinergic system and melatonin at a functional level has not been studied. Therefore in the present study, presence or absence of adenosinergic component in the anticonvulsant effect of melatonin was evaluated.

The fact that both adenosine A_1 and A_2 receptor antagonists DPCPX and DMPX failed to reverse the melatonin protection of PTZ seizures suggest the absence of any adenosinergic component in the anticonvulsant effect of melatonin. Interestingly a synergistic effect was observed when the subanticonvulsant doses of melatonin and adenosine were used in

combination. The mechanism for this enhanced protection when used together is not clear. But an augmented release of melatonin is a possibility.

Another interesting finding in this study was the reversal of anticonvulsant effect of melatonin with the higher dose theophylline (100 mg/kg). A similar reversal of melatonin protection in maximal electroshock seizures in mice on aminophylline pretreatment has been reported recently by Browicz but, in view of our results with specific adenosine A_1 and A_2 receptor antagonists, the reversal by higher dose theophylline cannot be ascribed to adenosine receptor antagonism (Browicz et al., 1999). It is very well known that methylxanthine theophylline is a non specific adenosine receptor antagonist, but this adenosine receptor antagonism does not completely account for its various actions in the body. Rather adenosine antagonism is seen at lower doses, while on increasing the dose, other activities like phosphodiesterase inhibition, calcium mobilization, etc. may manifest (Serafin 1996, Gupta and Malhotra, 1998). Thus though adenosinergic system is involved in modulating melatonin release, modulatory role at functional level appears unlikely. Furthermore, though adenosine and melatonin show a synergistic effect against PTZ-induced seizures, the additivity as far as the sedative side-effect is concerned, makes the combination a poor candidate from therapy point of view.

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ABSTRACTS

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Organizer:

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SUBCELLULAR DISTRIBUTION OF m2 MUSCARINIC RECEPTORS IN THALAMIC NUCLEI

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Cholinergic transmission exerts a powerful control on behavior dependent thalamic relay functions via synaptic and possibly also via non-synaptic mechanisms, but the distribution of cholinergic receptors is poorly investigated in thalamic nuclei.

In this study the precise localization of m2 muscarinic receptors has been investigated by pre-embedding gold immunostaining in the rat thalamus. At light microscopic level dense punctuate staining was observed in the anteroventral, anterodorsal, anteromedial and reticular nuclei. Immunoreactivity was less pronounced in midline nuclei and was weak in sensory relay nuclei. At the electron microscopic level gold particles were present mainly on the membranes of medium and thin dendrites in the anteroventral and reticular nuclei. Axon terminals were largely free of m2-immunoreactivity in these nuclei but large m2-immunoreactive terminals forming symmetrical synapses were observed in the posterior nuclei. In sections double immunostained for m2 muscarinic receptor and choline acetyltransferase (CHAT) m2 receptors were occasionally found at perisynaptic localization adjacent to CHAT-positive terminals. Using computer assisted 3D reconstruction of m2 receptor-immunoreactive dendrites the precise spatial distribution of the receptors was determined relative to the neighboring cholinergic terminals. Our data suggest that in certain dendrites the density of m2 receptors increases in the vicinity of cholinergic terminals resulting in a patchy distribution of the receptor.

Our data provide anatomical basis of the strong muscarinic hyperpolarizing action of acetylcholine in the reticular nucleus and suggest that limited diffusion of this transmitter may take place to activate muscarinic "hot spots" on relay cell dendrites of the anterior nuclei. The exact role of m2 muscarinic receptors in these limbic nuclei remains to be determined.

ISATIN (2, 3-DIOXO-INDOLE) MODIFIES THE EFFECTS OF PACAP-38 ON OPEN-FIELD ACTIVITY

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Previous studies in our department indicated that pituitary adenylate cyclase-activating polypeptide-38 (PACAP-38) had behavioural actions in the active avoidance paradigm, in the elevated plus maze and in the open-field test. Other studies demonstrated that thermoregulatory actions of PACAP-38 might be inhibited by the endogenous indole isatin. Present experiments

were carried out to clarify whether isatin is able to inhibit the actions of PACAP-38 on open-field activity in adult, male Wistar rats.

PACAP-38 (0.5-1 μg) increased locomotion and grooming 0.5 h after intracerebroventricular (icv.) administration, but decreased locomotion and rearing 3 and 6 h after injection in a dose-related manner, whereas it had no effect on grooming. PACAP-3 8-antiserum completely abolished the actions of PACAP-38 on open-field activity. Isatin (50 mg/kg, ip.) inhibited the behavioural actions of PACAP-38 at 0.5 and 3 h.

Our results indicate that different mechanisms participate in the mediations of PACAP-38-actions at 0.5 hand at 3 h in the open-field test.

SEARCH FOR ASTROGLIA IN THE GFAP-FREE AREAS OF THE BRAINS OF CARTILAGINEOUS AND BONY FISHES APPLYING IMMUNOHISTOCHEMICAL STAINING OF GLUTAMINE SYNTHETASE AND S-100 PROTEIN

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Among amniotes, in the mammalian and avian brains the free astrocytes are the predominant astroglial elements, and large brain areas are poor or nearly devoid of GFAP immunopositivity. In the reptiles, in which radial ependymoglia predominate, every brain area is rich in GFAP. In both mammals and birds the GFAP-free areas also contain astrocytes, which can be visualized by the immunostaining of glutamine synthetase or S-100 protein. Our former GFAP studies suggested that a similar evolution tendency exists in Chondrichthyes. The squalomorph shark brain had ependymoglia, but no very astrocytes (free stellate shaped cells), and an approximately even distribution of GFAP immunopositivity. In the skate brain, however, numerous astrocytes were found with only scarce ependymoglial fibers, but large areas displayed a paucity, almost a lack of GFAP-immunopositivity. In the teleost (carp) brain ependymoglia were predominant, but confined brain areas, e.g. in the vagal lobe, seemed to be free of GFAP. The present study therefore compares the immunopositivities against GFAP, glutamine synthetase and S-100 protein in the brains of skates *Dasyatis pasinaca* and *D. pastinaca,* presented kindly by Campona Tropicarium Budapest), *Cyprinus carpio* (a teleost fish), and *Acipenser ruthernis* (a kind of sturgeon, a chondrostean fish, as a representative of the non-teleost bony fishes). After an immersive fixation in 4% paraformaldehyde dissolved in phosphate buffer (0.1 M, pH 7.4), and 10% sucrose for the skates, the immunohistochemical stainings was performed on floating Vibratome sections. The primary antibodies were mouse monoclonal anti-GFAP (Boehringer, Mannheim), anti-glutamine-synthetase (Translab), and rabbit monoclonal anti-S-100 (Sigma), the immunohistochemical procedure was carried out according to the avidin-biotin method. In skates, both S-100 and glutamine synthetase immunostainings detected the same glial elements as immunostaining to GFAP, e.g. perivascular astrocytes and radial fibers in the telencephalon. Several non-perivascular astrocytes were, however, also detected, which proved to be immunonegative to GFAP. In both carp and sturgeon both S-100 and glutamine synthetase immunostainings detected the same

glial elements as immunostaining to GFAP, but no solid proof was obtained for the existence of glial elements in the GFAP-free areas of carp brain. In the sturgeon, the corresponding areas were poor but not free of GFAP, which suggest that in bony fishes during evolution the GFAP-expression withdraws, similarly to that observed in the other vertebrate stocks.

PLASTIC ELECTROPHYSIOLOGICAL AND HISTOLOGICAL CHANGES INDUCED BY CHRONIC EPILEPTIFORM ACTIVITY

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In addition to its normal function as a co-transmitter and neuromodulator, synaptically released zinc plays an important role in pathology, like epilepsy. The role of zinc in both normal and pathological function is intensively investigated in the hippocampus. However not much information is available about the specific structural and/or functional chronic changes in the neocortex, such as have been shown for epilepsy models in the hippocampus. Therefore in the present study we have chosen the 4-aminipyridine (4-Ap)-induced seizure activity as an *in vivo* model of epileptogenesis to investigate the possible changes in zinc-sensitivity, zinc content axon sprouting and cell death in the neocortex after preceding chronic epileptic activity.

In one group of adult rats epileptic seizure was induced daily during 2 weeks by i.m. injection of 4-Ap, while control group were injected with saline. In acute electrophysiological experiments, zinc sensitivity of the cortex of both groups was tested in the present of zinc solution (200 μM) on the somatosensory evoked potentials as well as the manifestation and propagation of 4-Ap-induced epileptiform activity. After experiments the neocortical tissue and the hippocampus of some epileptic and control animals were used to determine and to compare the content of the zinc (by ICP-AES technique) of these neuronal tissues. The brains of the rest of the animals were fixed and used for immuno- or immunohistochemical (TUNEL) staining to detect the possible cell death or axon sprouting, respectively.

The expression and propagation of epileptiform activity of animals experiencing chronic epileptiform activity was depressed, while the somatosensory evoked responses increased in the presence of exogenous zinc in comparison to controls. The zinc content of the hippocampus was about double of the control value in the epileptic animals, while it did not differ significantly in the neocortex in the two groups. This is in a good correlation with the structural changes: axonal sprouting was obvious in the hippocampus but in the neocortex; similarly, cell death was detected in the hilar region of the fascia dentate, and no cell death was observed in the neocortex of the epileptic rats.

These observations suggest plastic changes induced by chronic epileptiform activity: sensitivity to modulation by zinc in the neocortex; while structural alterations and an increase in the zinc content appear mainly in the hippocampus in the 4-Ap-epilepsy model.

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DYE-COUPLED SECONDARY SENSORY NEURONS IN THE CENTRAL NERVOUS SYSTEM OF THE FROG

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Previous experiments have demonstrated that a part of the primary afferent vestibulocochlear fibers establish mixed, electrotonic and chemical synapses on the Mauthner cells and on the second order vestibular neurons of lower vertebrates. In a series of experiments we wanted to study whether the gap junctional coupling exist between the other primary afferent fibers and the secondary sensory neurons. The Neurobiotin, as an indicator of the gap junctional connection, was applied on the primary afferent fibers to demonstrate the dye-coupled neurons. When the tracer was applied on the vestibular nerve the granule cells were intensely labeled in the auricular lobe of the cerebellum. In the lateral vestibular nucleus the large neurons were labeled and the Mauthner cell also exhibited weak labeling. The primary trigeminal afferent fibers were in dye-coupled connection mainly with the neurons located in the termination area of the descending fibers of the mesencephalic trigeminal nucleus, called as Probst tract. The cervical and lumbar dorsal root fibers proved to be dye-coupled with the granule cells of the corpus cerebelli, with the neurons of dorsal column nuclei, and with the neurons of Probst tract termination area. In the spinal cord a large number of labeled neurons were detected in the dorsal horn, the smaller part of labeled neurons were found in the ventral horn. The application of the Lucifer yellow also labeled the neurons in the same position. In the control experiments, the cobalt lysine and the biotinylated dextrane amine labeled only the primary afferent terminals; no postsynaptic neurons were detected. When the glycyrrhetinic acid, known to block the gap junctions, was injected into the cerebellum or applied on the primary afferent fibers the dye-coupled neurons almost completely disappeared. The functional significance of the dye-coupling may be to synchronize the afferent input and amplify the synaptic activation of the postsynaptic neurons by increasing the number of active fibers.

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INTRAAMYGDALAR MICROINJECTIONS OF ANGIOTENSINS MODULATE DRINKING BEHAVIOR AND MEMORY FUNCTIONS IN RATS

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The brain renin-angiotensin-system has a pivotal role in the regulation and modulation of losses of body fluids and is critical for reestablishing homeostasis. In our experiments the effects of angiotensin II (A II), its fragments — the angiotensin III (A III) and the angiotensin

IV (A IV)- and two receptor antagonists, losartan and the A III antipeptide (both of them are AT 1 receptor antagonists) were injected into the central and basolateral nuclei of the amygdala (ACE, ABL, respectively). Water intake and learning and memory functions in Morris Water Maze were tested on the effects of these substances. One hundred ng A II and 90 ng losartan, 100 and 200 ng A II and 200 ng A III antipeptide, 200 and 400 ng A IV or vehicle microinjections were applied in male CFY rats after 23 h water deprivation. Liquid intake was registered in every 5 mm for 30 mm and in the 60th mm as well when drugs were injected before drinking. In another paradigm rats were tested in Morris-Water-Maze during which A I and A IV were applied after swimming. The A II was effective in increasing water intake in both structures, and also the A III was in the ACE, but not in the ABL. Furthermore, losartan eliminated the A II induced drinking response in the ACE. The A III antipeptide inhibited the facilitory function of the A II and the A III. During the water maze experiment A II significantly and radically decreased the latency of finding the platform in all cases. A IV did not modify drinking injected into the ABL in rats, and also had no effect in the memory test. There has been opposing data demonstrated earlier in the amygdaloid body (AMY) considering A II and A IV. The effects of A II, III and IV – in these paradigms have not been tested in the ACE and ABL, the finding that water intake increased in water – deprived animals suggests that AT receptors in these nuclei play an important role in the regulation of water intake, as well as in learning and memory functions in the ABL.

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EFFECT OF LOCAL LIDOCAIN INFUSION AND SPREADING DEPRESSION ON THE ACTIVITY OF BASAL FOREBRAIN NEURONS

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Identification of transmitter systems, sending monosynaptic projections to the cortex, confirmed the role of the brainstem in cortical activation. However, application of immunohistochemical methods also revealed that the source of ACh released in the cortex during activation, is not the brainstem, but almost exclusively the large-celled basal forebrain (BF) cholinergic system. Up to now, very little is known about the cooperation between the brainstem and BF activating structures.

In our previous experiments we described the existence of two forms of phasic, rhythmically recurring cortical activation in urethane-anesthetized rats. Pattern I consisted of the alternation of few-second long periods with fast and slow waves, respectively. In Pattern II, at a deeper level of anesthesia, deep-negative shifts lasting for a few hundred milliseconds interrupted rhythmically an almost isopotential EEG curve. In some F-cells (higher firing rate during fast cortical waves) recorded in the BF, both patterns were observed. Neuronal activation preceded cortical changes during pattern I, while followed them during pattern II. This observation raised the possibility that cortical activation is achieved through different pathways in the two patterns.

To test this possibility, 2 microliters of 2% lidocain was unilaterally infused into the substantia innominate of urethane-anesthetized rats, while cortical EEG was monitored on both sides through transcortical electrodes at Br -4; L 2.0. Following infusion, cortical activation in pattern II persisted, while it was abolished on the injection side during pattern I induced by a noxious stimulus (tail pinch). In a subsequent series of experiments in a similar preparation, F-cells showing good correlation with the deep-negative shifts during pattern II were recorded in the BF. Frontal EEG (Br 2.0; L 2.0) was monitored on both sides. Spreading depression was induced unilaterally by dropping a small amount of 1 M KCl solution on the pial surface at the vertex (Br -4.5; L 2.0). Following a short period of activation, ipsilateral induction of spreading depression abolished neuronal firing almost completely. With the recovery of cortical activity, rhythmic firing in correlation with the deep-negative shifts also reappeared.

These results clearly show that activation ascends through the BF to the cortex during pattern I, while it descends to the BF from the cortex during pattern II. As these findings were achieved in anesthetized animals, some precaution should be exercised. Nevertheless, patterns I and II can be related to EEG phenomena occurring in unanesthetized animals and the pathways of activation described above should be also present in intact animals.

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UNILATERAL MODELS OF PARKINSON'S DISEASE EVOKED BY 6-OHDA IN RAT

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Parkinson's disease (PD) is one of the most devastating neurodegenerative diseases affecting the aging population in the welfare societies. The clinical symptoms (resting tremor, muscular rigidity, postural abnormalities, bradykinesia, block of movement initiation) are due to the progressive and selective loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc). In the rodent model of PD this nigrostriatal destruction can be evoked by injection of the selective neurotoxin 6-OHDA into the striatum.

Considering the fact that the underlying cellular pathobiochemical events are very similar in different chronic neurodegenerative disorders (Alzheimer's, Parkinson's, Huntington's), therefore similar pharmacological intervention may arise. Thus, a molecule effective in a neurodegenerative model can be promising in models of other neurodegenerations.

In the current experiments we aimed to install the acute and chronic model of PD evoked by unilateral 6-OHDA administration. The loss of dopaminergic neurons were followed by the measurement of dopamine (DA) and metabolite concentration in the striatum, various behavioural tests (rotation in open field, rotarod, catalepsy, rod-suspension, elevated body swing test, neurological score). The histopathological evaluation based on tyrosine-hidroxylase immunostaining is under process.

Stereotaxic injection of neurotoxin in the acute model elicited an almost total loss of DAergic neurons, the DA concentration in the affected hemisphere diminished to 3%

compared to the contralateral striatum leading to a marked ipsilateral circling behaviour. Although the clinically used MAO-B inhibitor L-deprenyl (Selegiline) was effective in blocking the DA metabolism in the contralateral side, proved to be unable to alleviate the DA imbalance between the two hemispheres due to the minimal number of surviving neurons.

In the chronic model evoked by constant, slow infusion of the same dose of 6-OHDA by Alzet osmotic minipumps for two weeks the DAergic neuron loss was partial. The DA level was reduced to 38% compared to the contralateral striatum. This moderate DAergic depletion manifested in a tess marked rotational behaviour. Biochemical measurements revealed significant positive effect of L-deprenyl.

Both the acute and chronic unilateral 6-OHDA PD model proved to be applicable as animal model of PD but the latter provides more opportunity to select promising neuroprotective agents since it mimics better the progressive feature of the human pathology.

NEUROCHEMISTRY OF THE ENTERIC NERVOUS SYSTEM IN EARTHWORMS AND ACTION OF SOME NEUROTRANSMITTERS

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The chemical neuroanatomy of the stomatogastric nervous system of the earthworm, *Eisenia fetida,* has been examined using antibodies raised against serotonin, tyrosine-hydroxylase, octopanine, gamma-amino butyric acid, FMRFamide, proctolin, *Eisenia* tetradecapeptide and neuropeptide Y. Neurons immunoreactive to these antibodies could be observed throughout the stomatogastric ganglia. The labelled cells comprised altogether 95.4% of the total number of neurons in a stomatogastric ganglion. Immunoreactive projections from one to other stomatogastric ganglia as well as towards the enteric plexus were demonstrated. In the entire length of the enteric plexus intrinsic neurons containing the examined signal molecules were present. Serotonin immunopositive perikarya, however, were observed only in the hindgut. The density of the different immunoreactive neurons was the highest in the pharyngeal plexus. The number of labelled neurons decreased along the alimentary canal towards the hindgut.

The action of some putative neurotransmitters has been tested by pharmacological methods on foregut preparations. Dopamine and octopamine had an excitatory effect on the musculature whereas the effect of serotonin depended on both the concentration and the actual muscle tension. Following precontraction evoked by acetylcholine, serotonin in low concentrations (10^{-7} and 10^{-6} M) caused relaxation, while in higher (10^{-4} M) concentration it evoked slight contractions. Atropine proved to be ineffective when applied together with serotonin, dopamine or octopamine. Similarly, the Na-channel blocker tetrodotoxin failed to influence the contractile effect of dopamine, octopamine and serotonin. These results suggest that the examined signal molecules act directly on the muscle cells of the alimentary tract.

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DIFFERENT EXPRESSION OF K^+/CL-COTRANSPORTER (KCC2) IN RELAY AND RETICULAR NUCLEI OF THE RAT THALAMUS

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In the thalamus, chloride homeostasis of neurons in the reticular and relay nuclei shows characteristic differences. Active chloride extrusion mechanisms set the reversal potential of GABA-induced anion currents (EGABA-A) 10 mV more hyperpolarized in neurons of relay nuclei than in reticular cells (1). Chloride extrusion mechanisms mediated by KCC2 have been shown to play an important role in the regulation of GABA-A receptor-mediated responses (2). In the present immunogold study the neuropil of all relay nuclei was found to display intense KCC2 immunostaining, among them the ventrobasal complex showing the strongest immunoreactivity. In sharp contrast, the majority of reticular neurons were negative for KCC2. In the anterior and dorsal part of the reticular nucleus, however, KCC2 immunostaining was comparable to the relay nuclei. At the ultrastructural level, KCC2 immunoreactivity was located mainly in the membrane of thick and thin dendrites of relay cells, many times in close association with asymmetrical synapses formed by primary as well as cortical afferents. Quantitative evaluation of KCC2 distribution at the EM level demonstrated that the density of KCC2 did not correlate with dendritic diameter or synaptic coverage but is three times higher on perisynaptic membrane surfaces than on extrasynaptic membranes.

Our results suggest that the presence of KCC2 in relay cells may be an important factor that sets EGABA-A to produce hyperpolarizing fast IPSPs in these cells, whereas the lack of this major chloride extrusion protein in reticular cells may result in shunting IPSPs. The presence of KCC2 near asymmetrical synapses in relay cells suggests that K-Cl cotransport also plays a role in neuronal volume regulation in response to ionic shifts mediated by excitatory postsynaptic receptor channels.

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EFFECT OF CHANNEL NOISE ON THE FIRING BEHAVIOUR OF NEURON

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We studied the behaviour of Hodgkin-Huxley equations in case when additive noise appears in the differential equations determining the dynamics of gating variables. The equations were considered to be of Stratonovich type. We found periodical solutions, even in cases when the dispersion of noise was very small (0.025 or less). We studied the behaviour of the deterministic system in the vicinity of the asymptotical fixedpoint and found it unstable. That could explain the observed phenomenon.

CHARACTERISATION OF NOCICEPTIN BINDING SITES IN MAMMALIAN AND NON-MAMMALIAN BRAIN MEMBRANES

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The endogenous heptadecapeptide, nociceptin/orphanin-FQ (Phe-Gly-Gly-Phe-Thr-Gly-Ala-Arg-Lys-Ser-Ala-Arg-Lys-Leu-Ala-Asn-Gln) shares some homology with the selective κ-opioid peptide dimorphin-A-1-17, whereas it binds specifically to a distinct receptor-type, termed nociceptin or ORL1 receptor. Nociceptin receptor is closely related to the multiple opioid receptors and composed of seven hydrophobic transmembrane segments. In order to characterise nociceptin binding sites in the CNS, two radioligand, [^{3}H]Tyr^1-nociceptin-OH (40 Ci/mmol) and p[^{3}H]Phe^1-nociceptin-NH_2 (25 Ci/mmol) were prepared by catalytic dehalotritiation of their respective diiodinated or monoiodinated precursor peptides. In rat brain membranes, specific binding of the radioligands was reversible, saturable and of high affinity (Kd≈1 nM). Nociceptin derivatives potently displaced the binding of the labelled peptides, however neither opioid agonists nor naloxone were effective in inhibiting the nociceptin binding sites. Nociceptin receptors were also detected in frog *(Rana esculenta)* brain. In the amphibian CNS, a single, high affinity set of binding sites with about 180 fmol/mg protein maximal binding capacity was obtained in saturation and homologous competition experiments. Equilibrium binding could be inhibited in the presence of sodium ions at physiological concentrations, or by the addition of STP analogues, e.g. 100 μM 5'-guanylylimidodiphosphate. Nociceptin dose-dependently stimulated the binding of the non-hydrolysable, radiolabelled GTP-analogue, guanosine-5'-O-(3-thio)triphosphate ([^{35}S]$GTP_\gamma S$) to S-proteins in frog brain membranes and this process was not reverted by the addition of the

opiate antagonist naloxone. These data together strongly suggest the existence of S-protein coupled, specific nociceptin receptors in frog brain membrane fraction.

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PHYSIOLOGICAL ROLE OF ZINC IN SPINAL INHIBITORY CONTROL

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Whilst several physiological studies have demonstrated that zinc affects GABA and glycine induced synaptic responses, the presence of zinc in inhibitory neurons or terminals in the brain has not yet been demonstrated. In the present study we investigated the co-localization of zinc with inhibitory amino acid transmitters in the lamprey spinal cord by using Timm's method, TSQ-staining and postembedding immunocytochemistry. The physiological effects of zinc in the spinal cord have also been examined by intracellular recordings from motoneurons and network interneurons.

Zinc-containing terminals were found throughout the spinal cord with the exception of the medial dorsal columns and lateral plexus. At the ultrastructural level the zinc was accumulated over vesicles of terminals containing both GABA and glycine but no zinc staining was found in identified glutamatergic spinal synapses. Paired intracellular recordings were used to examine the effects of zinc on glycinergic and glutamatergic inputs. Zinc increased the amplitude of monosynaptic glycinergic inhibitory postsynaptic potentials in motoneurons and unidentified network interneurons. Zinc reduced the amplitude of monosynaptic glutamatergic EPSPs evoked by stimulation of reticulospinal axons. It did not affect responses to GABA in motoneurons and locomotor network interneurons, but potentiated the GABA-evoked responses in sensory interneurons. These effects of zinc were associated with modulation of the output of the locomotor network. Zinc reduced the frequency and regulatory of NMDA-evoked locomotor bursts, and the amplitude of locomotor-related depolarizations in motoneurons and network interneurons.

In this study, we showed the first time the presence of zinc in inhibitory synaptic terminals. Co-released zinc from the terminals potentiates inhibitory synaptic inputs and in parallel reduced the glutamatergic responses.

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BIOCHEMICAL AND FUNCTIONAL CHARACTERIZATION OF HIGHLY POTENT ETORPHINE DERIVED OPIOID COMPOUNDS

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Etorphine is a synthetic narcotic compound chemically related to morphine. Etorphine exerts its actions through opioid receptors, which belong to the family of G protein-coupled receptors. Etorphine is known to cause strong analgesia, catatonia and blockade of conditioned reflexes in laboratory animals (rodents, cats, dogs, monkeys) and is widely used for immobilization of many species of game animals. Several novel derivatives of etorphine were synthesized, including its 3-O-methylated form and 7β stereoisomers. Here we present the results of characterization of these new compounds in radioligand binding and functional [^{35}S]GTPγS binding assays in rat brain membranes. Our data suggest that 3-O-methylation decreases the affinities of the compounds towards opioid binding sites and 7α configuration of the compounds is more favourable for G proteins activation.

ORIGIN OF THE GLUTAMATERGIC/ASPARTATERGIC AFFERENT FIBRES TO THE SUPRAMAMMILLARY NUCLEUS OF THE FAT AS REVEALED BY [^{3}H]D-ASPARTATE AUTORADIOGRAPHY AND IMMUNOCYTOCHEMISTRY

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The distribution of presumptive glutamatergic/aspartatergic (glu/aspergic) neurons, retrogradely labeled following injections of [^{3}H]D-aspartate (^{3}HDA) into the supramammillary nucleus (SUM) of the rat was investigated. The major sources of afferents to the SUM that could be labeled by retrogradely transported ^{3}HDA were in the border area between the medial and the lateral septum, subnuclei of the lateral septum, the medial preoptic area, the ventral premammillary nucleus, the lateral habenular nucleus, the apical subnucleus of the interpeduncular nucleus (IP-A), the dorsal and the median raphe nuclei. On sections processed for ^{3}HDA-autoradiography and immunocytochemistry, radiolabeling was found over a small number of both calbindin- and calretinin-immunostained cells in the septum. In the raphe nuclei and the WA which were immunostained for serotonin (5-HT), radiolabeled cells were scattered among 5-HT-immunopositive neurons. These presumably glu/aspergic cells never exhibited 5-HT immunoreactivity.

It is concluded that the SUM receives glu/aspergic afferents from significant number of brain regions. These excitatory amino acid inputs may play important role in theta-related activity of supramammillary neurons.

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ENDOMORPHINS: NEW ENDOGENOUS LIGANDS FOR THE OPIOID RECEPTORS?

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Since the discovery of three endogenous opioid peptide families (endorphins, enkephalins and dynorphins) extensive studies have been undertaken for investigating their localisation and characterisation as well as identifying their binding sites. The existence of three types of opioid receptors, μ, δ, and κ was also proved. Although selectivity of the enkephalins for the δ receptor and of dynorphins for the κ receptor was demonstrated, no endogenous ligand has been attributed to the μ receptor (MOR) until very recently, when Zadina and colleagues isolated two peptides, named endomorphin 1 (Tyr-Pro-Trp-Phe-NH_2) and endomorphin 2 (Tyr-Pro-Phe-Phe-NH_2). The very high affinity and excellent selectivity of these peptides for MOR were indirectly shown and raised the possibility that they might serve as endogenous ligands. For obtaining direct evidence, we tritiated both peptides, showed their binding characteristics and proved receptor type specificity. We have assessed the functional selectivity of these ligands for MOR by using CHO cells stably transfected with the μ or the δ opioid receptor and brain preparations of wild type or MOR knock-out mice, which completely lack μ opioid binding sites (Matthes et al. 1996). By functional assays (GTPγS binding and adenylyl cyclase activity measurements) and direct *in vitro* radioligand binding assays using tritiated endomorphin 2 demonstrated that endomorphins are inactive on cell and tissue preparations lacking MOR specifically. Although MOR specificity of endomorphins is unquestionable, the currently investigated uncommon binding properties and the lack of precursor(s?) indicate that the final proofs are still missing for designating endomorphins as classical endogenous ligands.

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EFFECTS OF ENDOMORPHIN-2 ON OPEN-FIELD BEHAVIOUR AND HYPOTHALAMO-PITUITARY-ADRENAL SYSTEM

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The effects of intracerebroventricularly administered endomorphin-2 (EM2) on open-field activity and the hypothalamo-pituitary-adrenal (HPA) system were investigated. EM2 (0.25-1 μg) significantly increased both the locomotor and the rearing activity, resulting in a bell-shaped dose-response curve. EM2 also enhanced corticosterone release, with an even more profound downturn phase at higher concentrations. The corticotropin-releasing hormone (CRH) antagonist α-helical $CR14_{9-41}$ completely abolished the EM2-evoked endocrine and behavioural responses. These findings reinforce the hypothesis that the endomorphins may play a significant role in the regulation of locomotion, rearing activity and the HPA system through the release of CRH.

SIMPLE PCB MICRO-DRIVE FOR MULTIUNIT RECORDINGS ON BEHAVING ANIMALS

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A simple multi-channel micro-drive ("hyperdrive") is described. The micro-drive uses printed circuit board (PCB) technology and flexible fused silica capillaries. The modular design allows for the fabrication of 4 to 32 independently movable electrodes or "tetrodes". The drives are reusable and reloading the drive with electrodes is simple. The PCB design also allows the application of silicone interrubber connectors for multi-channel electrophysiological recordings. The micropositioner described is not only simple but has the flexibility needed in most electrophysiological laboratories.

CEREBROSPINAL FLUID-CONTACTING NEURONS IN THE BAT, THEIR SUPPOSED ROLE IN NONSVNAPTIC COMMUNICATION

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Cerebrospinal fluid (CSF)-contacting neurons are located periventricularly or inside the brain ventricles and central canal. They directly contact the CSF via their dendrites, perikarya and/or axons.

Intraventricular axons terminate freely in the CSF or form synapses on CSF contacting dendrites and neuronal pericarya. Axons also form synaptic contacts with ependymal cells on its free ventricular surface. Synapses on the ependyma suggest a neural control of the activity of these cells.

The free axonal endings in the CSF are supposed to release synaptic-like mediator substances into the ventricular cavity and act by diffusion on other periventricular structures ("diffusional transmission"). The axons running to the basal lamina of the outer surface of the brain form endings of neurohormonal-type. Bioactive materials released from these terminals seem to be mediated by the outer CSF-space.

Hypothalamic CSF-contacting neurons resemble chemoreceptors. Their dendrites protruding into the third ventricle bear a 9+0-type receptory cilium. In the present work, we investigated the periventricular area of micro- and megachiropteran bat species. We found intraventricularly and subependymally located neurons in the infundibular and preoptic region of all investigated bat species (*Myotis blythi oxygnathus, Rhynolophus ferrum equinum, Taphozous longimanus, Scotophylus hethei, Scotophylus temmencki, Cynopterus sphinx* and *Rousettus niloticus*).

In the preoptic region near the rostral end of the third ventricle neuronal pericarya and nerve processes are sitting in the CSF on the free ventricular surface of the ependyma. Only a thin ependymal layer separates these hypendymal neurons from the CSF of the third ventricle. There are no tight junctions between the ependymal cells, therefore we think, that these subependymal neurons — like CSF-contacting nerve cells of submammalians — are closely related to CSF. Axonal processes of the hypendymal and intraventricular neurons were traced to hypothalamic synaptic zones and to the outer brain surface.

The close contact of these neurons to the ventricle suggest their participation in the nonsynaptic neuronal communication mediated by the CSF and the intercellular space. Axons of the CSF-related neurons that run to hypothalamic synaptic zones may convert CSF-derived nonsynaptic information to synaptic one.

EFFECTS OF SUBACUTE 3-NITROPROPIONIC ACID TREATMENT ON THE IMMUNOCYTOCHEMICAL STRUCTUTRE OF THE RAT STRIATUM

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Problematics

In the pathogenesis of neurodegenerative brain diseases, endogenous excitotoxins such as glutamate, aspartate and quinolinate exert their action on cerebral excitatory amino-acid (EAA) receptors. One of the consequences of the activation of the EAA receptors, present in highes concentrations in the striatum, the hippocampal region, and the temporal lobe, is neuronal necrosis. Another mechanism by which excitotoxicity could occur is a consequence of a progressive impairment of energy metabolism, which may lead to secondary slow excitotoxic neuronal death (Beal et al., 1993). We examined whether subacute administration of the mitochondrial toxin, 3-Nitropropionic acid (3-NP), an irreversible inhibitor of succinic dehydrogenase, which is part of both the Krebs cycle and complex II of the mitochondrial electron transport chain, produces alterations in the immunohistological pattern in the striatum, the hippocampus and the temporal lobe.

Results

3-NP affects both neuronal and astroglial elements, in a temporal cortex→hippocampus→striatum gradient, the most severe (almost selective) alterations characterizing the striatum. KAT-1 immunoreactivity, the marker of kynurenic acid, the only endogenous NIMDA receptor antagonist, is concentrated mainly in shrunken glial cells after 3-NP treatment. Consequently, KYNA may become unable to prevent alterations in the CNS areas investigated. The effect of 3-NP treatment on neurons in various areas of the CNS is diversed the number of PV IR cells decreases in the striatum and in the hippocampus, while the less affected are NOS IR cells in all areas investigated. In the temporal cortex the number of Ca^{2+} binding PV-IR cells is increased, probably due to the increased influx of Ca^{2+} resulting from secondary excitatoxicity.

Conclusions

The pattern of severe striatal lesions is strikingly reminiscent of some of pathological changes in Huntington's neurodegenerative disease (Beal et al., 1993, Brouillet et al., 1993). 3-NP treatment with different doses and ages of animals might serve as a model of Huntington's disease, probably useful in detecting the biochemical changes by which the HG gene, located on the short arm of chromosome 4 (Gusella, 1987), might lead to neuronal degeneration.

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GLIAL ELEMENTS OF THE NERVOUS SYSTEM OF *EISENIA FETIDA*

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Glial cells are present in the central and peripheral nervous system with various morphology and functions. Their function is better known in vertebrates. Beside their supporting role in the nervous tissue (mechanical function), the glial elements can form myelin sheets around axons (isolation). They also provide nutrition for nerve cells (material transport) and take part in the ontogenesis and regeneration of the nervous system. Among invertebrates most information on glial cells has been collected in insects so far. Several types of glial cells can be distinguished according to their functions and their locations in the ganglions.

The aim of our examination was to classify and describe glial cells of the intact nervous system of the earthworm *(Eisenia fetida),* therefore to provide a basis for examination of the role of these cells during regeneration.

The glial elements of the nervous system of *Esenia fetida* were observed by light microscope with toluidine blue and immunocytological (GFAP) staining as well as by electron microscope.

The existence of glial cells has been identified both in the central and the peripheral nervous system. We have determined the following categories:

1. Neurolemmal glial cells can be found in the capsule covering the ganglions and on its inner and outer surface.
2. Glial cells which wrap their processes around the nerve cell bodies and induce invagination in the membranes of the nerve cells.
3. The glial cells forming myelin like configuration around the giant axon.
4. Glial cells around axonal processes in the neuropile.

Glial elements can be observed in the enteric plexus of the gut and the inner surface of the body wall musculature.

It may be supposed that there is a continuous connection between the glial cells of the central nervous system and the body cavity by certain coelomocytes. Due to unknown stimuli these cells migrate into the ganglia and may be able to transform themselves into glial cells. This process may have some significance in ganglion regeneration. Additional experiments are necessary to certify our hypothesis.

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AGE-DEPENDENT DECLINE OF THE SEXUAL ACTIVITY AND ITS CORRECTION BY (-)DEPRENYL IN MALE RATS

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Sexually active male rats display toward the receptive female mounting; intromission and ejaculation. However there are males which fail to ejaculate for several weeks retaining mount and intromission as well (sexually "sluggish" males; Tagtiamonte et al., Dalló et al.). Previously we found that chronic treatment with (-)deprenyl effectively improved the ejaculatory activity in these males (Knoll et al., 1983).

In the present series of experiments twenty-two sexually active males aged 6 months, weighing 350 g each were observed during a forty weeks' period. Males weigh failed to show ejaculation for six consecutive weeks were considered as sexually sluggish. At the end of twenty weeks eight males met this criterion: These 8 males were treated with (-)deprenyl in a dose of 0.25 mg/kg sc. three times a week for further twenty-two weeks.

The remaining fourteen males were left without treatment. In the 14 untreated males sexual sluggishness completely developped afterwards while in the deprenyl treated males ejaculation recovered. Mounting and intromission still persisted in both groups. These data suggest that (-)deprenyl treatment effectively antagonized the age-dependent loss of ejaculatory activity. Since (-)deprenyl has a complex pharmacological spectrum, it is an open question which component of the drug is responsible for the observed effect.

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COMPARISON OF THE IMMUNOCYTOCHEMICAL LOCALIZATION OF CRYPTOCHROME (CRY2) IN THE PINEAL ORGAN AND RETINA

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Environmental light entrains circadian and circannual rhythms by regulating several endocrine and metabolic functions. In submammalians the photoreceptive pineal organs seem to entrain the genetically fixed endogenous rhythmicity. In mammals, pineal photoreceptor structures are reduced. Retinal impulses reach the pineal via a multisynaptic brainstem pathway and by autonomic nerves originating from the superior cervical ganglion. As we could not yet find any

autonomic fibers that innervate mammalian pinealocytes mediating the supposed retinal light information, we looked for persisting photoreceptor structures and compounds present in the retina and light-sensitive submammalian pineal organs.

Continuing to test the possibility of a direct pineal photosensitivity, we examined the localization of cryptochromes. It was already known more than 100 years ago, that plant development actively influences by blue light. A blue light-absorbing compound, named cryptochrome, was supposed to mediate this effect. Cryptochromes contain pterin and flavin prostetic groups, earlier only retinal containing proteins were shown in animals, as photoreceptors. Localized in the cell nucleus, cry2 was found to entrain circadian rhythmicity. There is a significant amount of cry2 in the ganglion cell layer and inner nuclear layer of the retina.

In the present work, we compared the immunocytochemical localization of cry2 in the pineal organ and retina of the mouse and guinea fowl. In the mouse, nuclei of the pinealocytes showed cry2 immunoreactivity. In the mouse retina nuclei of the bipolar cell-layer and the ganglion cells nuclei were immunoreactive. Pineal cell nuclei of the guinea fowl and nuclei of the retinal bipolar as well as ganglionic cells showed strong immunoreactivity. Absorption spectrum of cryptochromes overlaps the wavelength influencing the release of melatonine, therefore, cry2 may be a candidate of photoreceptor compound in pinealocytes. Experiments are in progress to test the supposition whether cry2 may allow pinealocytes to entrain the circadian clock according to environmental light conditions.

LOCALIZATIONS AND EFFECTS OF SEROTONINERGIC STRUCTURES IN RABBIT ILEUM

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Although serotonin is one of the best known neurotransmitter in the mammalian gastrointestinal tract, repeated experiments to identify and localize this substance in the myenteric plexus of rabbit failed. However entero-endocrin cells in the ephitelial layer and mast cells of the mucosal and submucosal layers contained this substance. After preincubation in serotonin (10^{-5} M) dissolved in Krebs solution, an extensive fibre system could be detected with immunocytochemical methods. Application of reserpin (10^{-5} M) prevented serotonin uptake.

The immunopositive fibre network was strong and the individual processes bore numerous varicosities. Stained fibres left the myentericus plexus and run to the muscles layers. Labelled cell bodies could not be found. Tryptophan-hydroxylase antisera revealed a rich immunopositive fibre system, but labelled neurons could not be again detected. These processes were able to accumulate serotonin as was evidenced by double immunofluorescence.

Parallel to morphological studies, pharmacological experiments evidenced that serotonin caused concentration-related contraction in longitudinal muscles layer of rabbit ileum. Treatment by tetrodotoxin strongly reduced the effect of serotonin. The preparations were

pretreated with cholinergic muscarinic receptor blocker atropine, slight decrease of response evoked by serotonin. Combined administration of tetrodotoxin and atropine significantly reduced the response to 5-HT, but not completely. A 5-HT_2 receptor antagonist methysergide and a 5-HT_3 receptor antagonist tropisetron were used to identified receptortypes. Tropisetron had no effect, but methysergide caused a small reduction in the motility. The combined blockade of methysergide and tetrodotoxin completely prevent the response to serotonin. These immunocytochemical studies show that intrinsic serotonin is absolutely absent from the myenteric plexus of rabbit ileum. Although intestine is supplied with a weakly innervation from mesenteric ganglia. Furthermore an extensive uptake system works in this plexus and it might release this substance as a transmitter, which is likely derived from the mast cells and entero-endocrin cells of mucosa and from the mesenteric nerves. The released substance evokes a serious contraction in indirect and direct ways throughout 5-HT_2 receptors and till unknown receptors. The pharmacological experiments indicate that 5-HT_2 receptors could be found on the muscle fibres and the unknown receptors is on the nerves elements of myenteric plexus.

CHRONIC EFFECTS OF PILOCARPINE INDUCED STATUS EPILEPTICUS ON CORTICAL EEG AND SINGLE UNIT ACTIVITY

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Pilocarpine, a muscarinic cholinergic agonist applied systematically produces behavioral automatisms and motor limbic seizures in 15-20 min. Discharges essentially originate from the hippocampus and often progress to limbic status epilepticus (SE) in an hour. Status epilepticus lasts for several hours and accompanies with ictal and interictal epileptiform discharges on EEG recordings. After a mean latency of 14-15 days recurrent spontaneous seizures appear in 40-50% of the surviving rats. The extensive interconnectivity between the limbic structures (hippocampus, entorhinal cortex) and other cortical regions might be responsible for the propagation of seizures to other brain areas, i.e. the temporal lobe and sensorimotor cortex. Short-term electrophysiological and morphological effects of acute pilocarpine treatment are extensively studied both *in vivo* and *in vitro*, especially in limbic structures. But very few data are concerning the long-term effect of pilocarpine induced SE in the developing brain.

Our aim was to characterize the long-term effects of pilocarpine induced SE on single cell activity in the sensorimotor cortex of rats during development.

Status epilepticus was induced with 40 mg/kg pilocarpine i.p. in 12 (12D) and 25 (25D) days old male rats (Wistar). After 60 days the surviving animals were anesthetized with urethane (1.2 g/kg) and placed to a stereotaxic instrument. EEG and extracellular unit recordings were made from the left sensorimotor cortex.

Total of 27 animals were investigated ($n_{control}$=11, n_{12D}=5, n_{25D}=11). Recurrent spontaneous epileptic activity was never seen in the EEG recordings, neither in the 12D group, nor in the 25D group. On the basis of the autocorrelogram and interval histogram analysis the distribution of the firing characteristics of the recorded cells was (burst vs. irregular firing): – control (total:

46 cells): 45 vs. 55 %, – 12D group (total: 19 cells): 58 vs. 42 %, – 25D group (total: 59 cells): 66 vs. 34 %. Significant increase in the ratio of bursting cells and a parallel decrease of the firing frequency of these cells were observed in both treated groups. On the basis of the cross-correlogram analysis with EEG positive correlation was detected in 95% of the recorded cells in all groups.

In conclusion, the present data are generally in agreement with recent hypotheses that slight changes in single cell parameters caused by application of a convulsant can contribute the permanent pathophysiological activity of the neural network.

ROLE OF H_1- AND H_2-RECEPTORS IN MENINGEAL BLOOD FLOW REGULATION

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Vasodilatation in the dura mater is thought to play an important role in the pathophysiology of vascular headaches. Histamine has been suggested to contribute to the vascular changes. To test the vasoregulatory potency of histamine we used a model of meningeal perfusion in the rat. The parietal dura mater was exposed by trepanizing the skull. Blood flow in the medial meningeal artery was monitored by laser Doppler flowmetry. Topical application of histamine (10^{-4} M) caused increases in meningeal blood flow which were reduced by pre-application of the H_2-receptor antagonist cimetidine (0.01, 0.1, 1 mg/kg). Local pretreatment with the H_1-receptor antagonist cetirizine (1, 10, 20 μg/ml) further increased the histamine-induced vasodilatation. Increases in flow caused by either topical application, or i.v. injection of histamine (10 μg/kg) were attenuated by i.v. cetirizine (50 μg/kg) but not by i.v. cimetidine (5 mg/kg) pre-administration. The results indicate that histamine-induced relaxation of dural blood vessels is mediated by H_2-receptors, most likely located on smooth muscles and by endothelial H_1-receptors. H_1-receptors of vascular smooth muscles are responsible for histamine-induced vasoconstriction.

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THE SUMMATION OF SYNAPTIC POTENTIALS IN MULTI-COMPARTMENT MODELS OF THREE CA1 INTERNEURON SUBPOPULATIONS

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Neurons receive thousands of excitatory and inhibitory inputs that are widely spread across their dendritic arbor. The way of their integration can vary to a great degree depending on spatial arrangement synapses. To study the effect of synaptic distributions on the summation of

synaptic potentials multi-compartment models of three functionally different subpopulations of hippocampal CA1 interneurons were constructed. The selected subpopulations were the calbindin (CB)-, calretinin (CR)- and parvalbumin (PV)-containing interneurons responsible for perisomatic and dendritic inhibition, or the control of other interneurons, respectively. The density of excitatory synapses is the highest, while the morphotonic distance between them is the lowest on PV cells. On CB and CR cells the density of excitatory synapses is similar, but on CB cells it is balanced by larger density of inhibitory synapses than on CR or PV cells. Within a cell neither the number of synapses per unit dendrite length nor the morphotonic distance of neighbour synapses or the amplitude of local synaptic potentials show uniform distribution. The activation of neighbour synapses results in smaller somatic EPSPs than the activation of synapses on different dendrite segments. The effectiveness of EPSP summation decreases towards the distal dendrites in case of neighbour synapses, but remains the same when synapses are activated on different dendrite segments. The summation of EPSPs is the most effective in PV cells. Coactivation of inhibitory and excitatory synapses results in the largest depolarisation in PV cells and the smallest in CB cells. In PV cells a small percent of activated excitatory synapses can induce somatic depolarisation large enough to initiate action potential.

CHARACTERIZATION OF G-PROTEINS INTERACTING WITH KAPPA OPIOID RECEPTORS IN FROG BRAIN MEMBRANES

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Kappa opioid receptors are promising targets of new analgetic drugs. They are devoid of the two main side-effects of mu opioid receptors, respiratory depression and tolerance-dependence. Frog (*Rana esculenta*) brain is a rich source of kappa opioid receptors (1 and references cited therein). The aim of the present work was to identify the heterotrimeric G-proteins that are present and interact with the endogenously expressed kappa opioid receptors in frog brain membranes. Western-blotting with specific antisera raised against synthetic peptide fragments of known sequences of mammalian G-protein types revealed that 2 $G\alpha_s$, 2 $G\alpha_i$ and 1 $G\alpha_o$ forms were present, but the bands corresponding to the known molecular weights of α_{i1} and α_{o2} were not labeled. The β_{common} and β_1 antibodies both immunoreacted with a doublet of bands of about 35 kDa suggesting the luck of the β_2 subunit. Etylketocyclazocine (EKC), a prototypic kappa opioid ligand dose-dependently stimulated [^{35}S]GTPγS binding achieving 60% stimulation over basal values. Two other kappa ligands, U-50,488 and bremazocine also caused 20-30% stimulation over basal binding. EKC, U-50,488 and morphine (mu opioid agonist) stimulated the photoincorporation of [α-^{32}P]GTP azidoanilide into four proteins with molecular weights of 39-43 kDa. Two of these bands were identified by means of photoaffinity labeling and subsequent immunoblotting and classified as $G\alpha_{i2}$ and $G\alpha_{o1}$ of 39 and 40 kDa molecular weights, respectively. The other two bands were also stained by the $G\alpha_{common}$ antibody, but were not further identified.

Our results suggest that native kappa opioid receptors interact with multiple G-proteins *in situ*, and that the structure of most G-protein subtypes is strongly conserved during phylogenesis.

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SPONTANEOUS ACTIVITY OF SUPRACHIASMATIC NUCLEUS IN *IN VITRO* SLICE EXPERIMENTS

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Suprachiasmatic nuclei (SCN) located above the chiasma opticum serve as the biological clock organizing daily rhythms in mammals. Photic information, ensuring synchronization of the clock with environmental cycles, reaches the clock through direct fibers running upward from the chiasma (retinohypothalamic tract) and through indirect pathways originating in the intergeniculate leaflet (geniculohypothalamic tract). While light is the main Zeitgeber for the clock, feedback from behavior can also influence the phase of the internally generated rhythms in constant conditions. The exact mechanism of this feedback is not completely understood, but it seems that serotoninergic and cholinergic fibers play an important role in this process. Possible interactions of these systems were analyzed in the present experiments.

Coronal slices were prepared from the ventral hypothalamic area. Spontaneous unit activity was recorded from SCN neurons using NaCl filled extracellular electrodes. Cell types were classified by electrophysiological characteristics. To investigate the co-effect of cholinergic and serotonergic transmitters, carbachol (50 μM) was bath applied either alone or together with serotonin in two different concentrations (25 and 50 μM).

In accordance with the relatively few studies available in the literature, recorded neurons (n=19) fell into one of the following four main categories on the basis of their spontaneous activity: - regular spiking cells (n=6), irregular-continuously spiking cells (n=9), irregular-intermittently spiking cells (n=1) and bursting cells (n=3).

By the end of the 30-min bath application, carbachol caused, on average, an 88±8% inhibition in spontaneous activity. Co-application of carbachol and serotonin in a ratio of 1:1 resulted in a comparable inhibition of 83±14%. In contrast, a smaller amount of serotonin applied in a ratio of 0.5:1 compared to carbachol decreased the inhibitory effect to 48±21%.

On the basis of these results we can conclude that the effect of the two drugs is not additive. Carbachol effectively inhibits the spontaneous activity; the effect is reversible and does not modulate the type of activity. Higher concentration of serotonin did not influence this inhibitory effect, however, given in a lower dose, strongly decreased the carbachol effect. There is no trivial explanation for this interaction, but it can be supposed that serotonin exerts

its effects on different receptors or cell types. The different role of pre- or postsynaptically located receptors in these regulatory processes has also to be taken into consideration.

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THE PREVENTION OF AGE-RELATED CEREBROVASCULAR CHANGES WITH CALCIUM CHANNEL ANTAGONISTS AND DIETARY SUPPLEMENTS

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Based on our previous investigation and literary data, we have promoted a hypothesis on the involvement of cerebrovascular risk factors in the development of cognitive disturbances. Our hypothesis has suggested that compromised vasoregulation contributes to a decreased cerebral blood flow, which in turn can cause pathological ultrastructural changes in the cerebral capillary wall. Such morphological vascular damage can have functionally detrimental implications in nutrient transport through the blood-brain barrier, meaning a hindered glucose transport from the blood to the neural tissue. The resulting low cellular metabolism in neurons can thus lead to the loss of their plasticity, and a consequent cognitive decline.

In the here presented studies we were aiming at preventing either the cerebrovascular or the cognitive disturbances imposed by vascular risk factors.

In our first set of experiments we wanted to determine whether calcium channel antagonists could prevent the degeneration of the cerebral capillary wall in spontaneously hypertensive stroke-prone rats (SHR-SP). The morphology of capillaries in the cerebral cortex of the following experimental groups was compared with the use of an electron microscope: 40-and 60-week-old normotensive (WKY) and hypertensive (SHR-SP) rats to visualize a potential age effect on vascular pathology in the brain, and to identify hypertension-induced cerebromicrovascular damage. Furthermore we investigated the cerebral capillary ultrastructure of 60-week-old SHR-SP rats treated for 20 weeks with either of the two potent calcium channel antagonists nimodipine or nifedipine, and placebo controls, to visualize drug effect on capillary ultrastructure in the brain. The results can be summarized as follows.

A significantly increased degeneration of the capillary basement membrane was seen as the consequence of aging and hypertension in the SHR-SP groups. The application of nimodipine and nifedipine could prevent the age- and hypertension-related, enhanced microvascular pathology. Considering that the here used calcium channel antagonists can penetrate the blood-brain barrier, and that their targets, the L-type channels are present on neural elements but presumably not on endothelial cells, we suggest the following preventive mechanism. Nimodipine and nifedipine could probably maintain the integrity of the cerebral capillary network by preventing exaggerated calcium influx to neuronal and astrocytic cell bodies involved in vasoregulation.

In the second set of experiments we have set up a paradigm to investigate the potentially beneficial working of dietary supplements on learning in a model of cerebral hypoperfusion. The cerebral blood flow of Wistar rats was chronically reduced to 70-80% by a surgical, bilateral occlusion of the common carotid arteries (2VO). Both the 2VO rats and the controls were split into three dietary groups. The first diet contained a high amount of n-3 type

polyunsaturated free fatty acids (PUFA's) (Suppl-1), the second experimental diet was enriched with neurotransmitter precursors in addition to the PUFA's present in the first diet (Suppl-2) and the third diet served as Placebo control (Plac). Here we are selectively presenting the spatial learning data obtained in the Morris water maze 3 months following 2VO, and the results of the GFAP (glial fibrillary acidic protein) immunocytochemistry in the hippocampus CA1 region.

The 2VO condition caused a setback in spatial learning, which could be prevented by Suppl-2. GFAP immunoreactivity in the hippocampus CA1 appeared to be more pronounced due to 2VO, and was further increased in the Suppl-2 diet groups. We concluded that the combination of n-3 PUFA's and neurotransmitter precursors effectively improved spatial learning, to which the proliferating astrocytes (which potentially counteract neuronal damage) contributed. The protective effect of the combination of n-3 PUFA's and neurotransmitter precursors could be achieved by the structural incorporation of PUFA's into endothelial or neuronal cell membranes or by improving the efficacy of neurotransmission.

We can conclude that deficiencies of the central nervous system caused by vascular risk factors may be moderated by drugs or dietary supplements targeting functional proteins or structural lipids of the neuronal and the endothelial cell membrane.

THE EFFECT OF PERIPHERAL NERVE INJURY ON MOTOR CORTICAL RESPONSES ELICITED BY SOMATOSENSORY STIMULATION. AN ELECTROPHYSIOLOGICAL AND AUTORADIOGRAPHIC STUDY

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The primary motor cortex (MI) of the rat is in close functional connections with the primary somatosensory cortex (SI). Stimulation of the peripheral vibrissae elicits evoked activity in about one-third of the neurons in the vibrissal field of the MI, due to the strong associational connections between the SI and the MI. For many years, it has been known that peripheral nerve injury (denervation) allows the rapid appearance of new receptive fields through a reduction of cortical GABAergic inhibition. This leads to the unmasking of existing, but normally silent connections. For instance, deefferentation of the MI (unilateral facial nerve denervation, n7X) extinguished contralateral vibrissal movements evoked by intracortical microstimulation, while ipsilateral vibrissae began to respond within 4 mm. This suggests the denervation-induced disinhibition of callosal connections between the MIs on both sides.

In our experiments, the questions considered were, how the amplitude and cortical extent of somatosensory evoked potentials differ in response to n7X, and whether the autoradiographic changes in the activity of the MIs on both sides follow the n7X. Before n7X, somatosensory evoked potentials could be recorded in the MI contralateral to stimulation. However, after n7X, a strong facilitation was observed in the contralateral MI. Furthermore, 4-9 min after n7X, evoked potentials appeared and facilitated in the ipsilateral MI. The results of autoradiography are in good agreement with the electrophysiological observations. ^{3}H-glycine labelling was

strong in the MIs of both hemispheres after n7X as compared to the control. In the contralateral MI cortical layers I-V, while in the ipsilateral MI layers I-II were labelled most strongly.

All these results suggest the unmasking of latent cortical associational and callosal connections between the SI-MI and the MIs on both sides through the denervation-induced cortical disinhibition.

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ASSOCIATION OF CART-IMMUNOREACTIVE ELEMENTS WITH THYROTROPIN-RELEASING HORMONE-SYNTHESIZING NEURONS IN THE HYPOTHALAMIC PARAVENTRICULAR NUCLEUS AND ITS ROLE IN THE REGULATION OF THE HYPOTHALAMIC-PITUITARY-THYROID AXIS DURING FASTING

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Since CART coexists with α-MSH in the arcuate nucleus neurons and we have recently demonstrated that α-MSH innervates TRH-synthesizing neurons in the hypothalamic paraventricular nucleus (PVN), we raised the possibility that CART may also be contained in fibers that innervate hypophysiotropic TRH neurons and modulate TRH gene expression. Triple-labeling fluorescent *in situ* hybridization and immunofluorescence was performed to reveal the morphological relationships between proTRH mRNA-containing neurons and CART- and α-MSH-immunoreactive (IR) axons. CART-IR axons densely innervated the majority of proTRH mRNA-containing neurons in all parvocellular subdivisions of the PVN and established asymmetric synaptic specializations with proTRH neurons. CART prevented fasting-induced suppression of proTRH in the PVN when administered icv. These studies establish an anatomical association between CART and proTRH producing neurons in the PVN and demonstrate that CART has a stimulatory effect on hypophysiotropic TRH neurons by increasing proTRH gene expression.

INTRAAMYGDALAR MICROINJECTION OF BOMBESIN LIKE PEPTIDES INFLUENCE FEEDING BEHAVIOR AND SERUM BLOOD GLUCOSE LEVEL IN RATS

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It has been shown that the amphybian neuropeptide bombesin (BN) is involved in the mechanisms of satiety. Gastrin-releasing peptide (GRP) and neuromedin B (NMB) belong to the mammalian BN-like peptide family because they share similar C terminal peptide sequence with BN. Binding sites of GRP and NMB have been found in the central part of amygdala (ACE). Pharmacological evidence strongly support the existence of two receptor subtypes present in the ACE, namely the GRP- and NMB-preferring receptors. In the present experiments GRP and NN4B were microinjected into the ACE and liquid food intake and blood glucose level were studied. GRP (25, 50, 100, 150 or 300 ng) or NMB (15, 30 or 60 ng, dissolved in 0.5 μl of 0.15 M NaCl) or vehicle were microinjected into the ACE and liquid food intake was registered in the home cage, every 5 mm for 30 mm and at the 40^{th} and 60^{th} mm following bilateral peptide or vehicle injection. Fifty, 100, 150 ng GRP significantly suppressed consumption for 5 mm. Twenty-five ng and 300 ng GRP were not effective on food intake. Fifteen, 30 and 60 ng NMB decreased food intake for 5 mm. After 30 and 60 ng NMB treatment a compensatory overeating was observed. Effects of GRP and NMB were prevented by prior application of BN antagonist (50 or 100 ng). The effects of GRP or NMB microinjection into the ACE on serum glutcose levels were also investigated. After 150 ng GRP or 30 ng NMB or vehicle treatments blood samples were taken from the tail vein at the 5^{th}, 10^{th}, 20^{th}, 30^{th} and the 60^{th} mm. Blood glocose level was determined by enzymatic detection (Glucometer Elite, Bayer). After 150 ng GRP treatment blood glucose level significantly increased 10 and 20 min after GRP injection. NMB treatment was not effective on serum glucose level. On the basis of present results it can be supposed that GRP and NMB-preferring receptor subtypes in the ACE may play partially independent roles in mediating satiety effects.

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SOME NEUROSTEROIDS INHIBIT THE GLYCINE RECEPTOR

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Strychnine sensitive glycine receptors are chloride permeable ion-channels most abundant in the spinal cord where glycine is the main inhibitory neurotransmitter. Neurosteroids exert their action in the nervous system mainly via ion-channels, associated with glutamate, and $GABA_A$

receptors. Such allosteric modulations of glycine receptors by neurosteroids have barely been examined. We studied the steroidal modification of the glycine-activated channels in cultured spinal cord neurons prepared from 17-day-old rat embryos, using whole-cell patch clamp method, and measured the chloride currents evoked by 50 μM glycine (≈EC5O). The neurosteroids pregnenolone-sulphate (PS) and dehydroepiandrosterone-sulphate (DHEAS) reduced the peak amplitude of the current with IC50 values of 19 and 46 μM, respectively. We also measured the sensitivity of the glycine receptor ionophores in spinal cord cell cultures of different *in vitro* ages to the subtype-specific channel blockers picrotoxin, strychnine and cyanotriphenyl-borate. Since it is known that the low affinity to picrotoxin indicate the presence of the ß subunit, while the high affinity to strychnine and cyanotriphenyl-borate signify that the channels contain the α1 subunit, the results can be reconciled with dominant presence of heterooligomeric α1ß (adult type) glycine receptors after 8 days *in vitro*. In conclusion, PS and DHEAS inhibit the ionophore function of α1ß glycine receptors at micromolar concentrations.

CA-BINDING PROTEINS AND CA-CURRENTS IN CULTURES OF SPINAL GANGLION NEURONS

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Cultures of spinal and trigeminal ganglia were prepared from newborn rats. Ganglia were dissected and mechanically triturated. The cells were placed in culture medium on polylysine coated plates and incubated in O_2/CO_2 thermostat at 37^0C. The development, chemical and physiological maturation of these cultured ganglion cells were followed by immunocytochemistry aid Ca-imaging from day 3 to day 7.

By day 3 most of the ganglion cells were fully matured, as was evidenced by indirect immunofluorescence labeling of neurofilament-H protein. A well-developed neurofilament network was seen all over the cell body and long, slender processes were often seen. Only some cells showed pseudounipolar morphology, other neurons were bipolar or multipolar.

Buffer-type Ca-binding proteins (parvalbumin, calretinin and 28 kDa Ca-binding protein (calbindin)) were already present in the somata from day 3. Interestingly, the strongest staining was seen in the nuclei of the cells at this timepoint. By day 4 parvalbumin-immunopositivity in the cells disappeared. At this point we do not know if the cells which contained parvalbumin died or the protein expression was downregulated in some of the ganglion cells. Calretinin and calbindin were then seen in the processes of the nerve cells. The intensity of immunolabelling gradually increased until day 6 and declined slightly by day 7. Calretinin-labeled cells were larger than calbindin-positive cells. Both calcium-binding proteins were expressed in bipolar and multipolar cells but the morphology of the immunopositive nerve cells was clearly distinct. Pseudounipolar cells were labeled with anti-calretinin but not with anti-calbindin antibodies.

Ca-transients were evoked by high extracellular K concentrations and were measured with the application FURA-2AM Ca-sensitive dye. The dye excitation was performed at 340/380

nm while the fluorescence emission was monitored at 530 nm. From day 3 the cells were responsive to depolarizing extracellular concentrations of K^+. They responded with fast increase in intracellular Ca^{++}. The Ca-transient behaved two different ways. In one group of the cells a fast decline was observed after an initial Ca^{++}-peak, while in other cells the initial peak was followed by a plateau phase. In both cases the Ca-concentration returned to normal within a few seconds. The best measurements could be made on day 5 and day 6.

Our findings show that morphological and physiological maturation precedes chemical maturation in cultured spinal and trigeminal ganglion cells. Cultured cells may exhibit morphological characteristics not seen in intact ganglia. The calcium-binding proteins are similar in cultured and intact ganglion cells so is the capability of neurons to produce large Ca-transients on depolarizing stimuli. Since the cultured ganglion cells can be easily examined with electrophysiological methods, they may therefore be useful in determining neurochemical and physiological characteristics and responses to drugs.

INTERACTION OF CHEMICAL AND ELECTRICAL SYNAPSES AND THE ROLE OF NEUROMODULATION IN OUTER RETINAL INFORMATION PROCESSING

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The outer retina is a particularly useful model in studying interactions of chemical and electrical synapses and role of neuromodulation in information processing. This is due to the fact that there are no spike-generating neurons here and therefore all processes can be monitored directly on cells which operate with graded depolarization of their membranes.

Neuromodulation in the retina offers several options through which second-messenger systems can influence neural function. Neuromodulatory processes contribute to (i) control of transmitter release, (ii) influence the conductance state of the ligand-gated ion channels, (iii) modify the activation function of voltage-gated channels, and (iv) control the permeability of some but not all gap junctions.

Two major outer retinal neuromodulators are dopamine and rnelatonin, the second being the dark signal of the circadian cycle and is produced by the photoreceptors. This topic is touched only very briefly in the presentation. The firstly mentioned substance, dopamine is the master controller of the short-term light adaptation. The synthesis and release of dopamine is influenced by several factors which will be briefly discussed in the presentation. Dopaminergic amacrine cells are present in the retina with a fairly uniform distribution. Processes of these cells form a dense network all over the retina, individual processes may reach across 1/4 of the entire retinal diameter. Dopaminergic cells are activated by light stimuli to which they release dopamine and maintain a concentration of about 1 μM all over the entire retina. Although they do not form synapses with any of the nerve cells in the outer retina, they are still able to influence them profoundly

Both D1 and D2 receptors can be found on different cellular elements of the outer retina. D2 receptors regulate the permeability of gap junctions between rods and cones, while homologous rod-rod and cone-cone gap junctions are not regulated. Furthermore they also

inhibit the delayed I_h K^+ current of photoreceptors thus contributing to a faster rebound to normal dark membrane potential after light stimulation. The D1 receptors are found on horizontal cells. The light responses of these neurons become faster and the conductance of their non-NMDA type ionotropic glutamate receptors substantially increases. Intracellular injection of cAMP into dark-adapted horizontal cells changes the response properties of these cells; they become more transient and resemble those of the light-adapted horizontal cells. Their ability to follow high frequency flickers at high light intensities also increases substantially.

Both D1 and D2 receptors exert their effects through the cAMP signaling system. They activate or inhibit a number of processes that occur parallelly. The presence of dopamine in the system activates both receptor types. Although the receptors activate concurrent intracellular processes the overall systemic effect is manifested as increased spatial and temporal resolution, that is, light adaptation. Thus two receptor types mediating opposite intracellular effects, if located on different cell types, may still be synergistic at the system level.

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INVOLVEMENT OF ELECTRICAL SYNAPSES IN THE EPILEPTIFORM ACTIVITY INDUCED *IN VIVO* BY 4-AMINOPYRIDINE

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Although chemical synaptic interactions clearly play an important role in neuronal synchronization during seizures, a variety of studies have shown that nonsynaptic mechanisms like gap junction communication can synchronize neurons in the absence of chemical synaptic transmission. In the present study the assumption that neuronal interconnection through electrical synapses could be a mechanism underlying epileptiform discharges was examined with carbenoxolone, a gap junction blocker in an acute *in vivo* epilepsy model.

The effect of carbenoxolone on the induction, manifestation and propagation of 4-aminopyridine induced cortical epileptiform activity was tested in electrophysiology experiments in anaesthetized rats. In different groups of the animals the surface of the somatosensory cortex was locally treated with carbenoxolone either before the induction-, or during the already established epileptiform activity, respectively.

The electrocorticographic manifestation of ictal discharges was quickly reduced in the already active epileptic focus after the local application of carbenoxolone, indicated by significant decrease in the summated ictal activity and the lack of the propagation to the contralateral hemisphere of cortical epileptiform discharges.

On the other hand pretreatment of the cortex with carbenoxolone had a weak. Influence on the induction and maintenance of epileptiform activity and apparently did not influence the basic cortical electric activity or the configuration of somatosensory evoked responses.

In physiological conditions the coupling ratio between cells is supposed to be low in the adult neocortex, therefore the contribution of gap junctions to the induction of epileptic activity

is mostly modulatory. The observation that seizure discharges of high frequencies (10-15 Hz) were almost missing in the pretreated animals suggests that gap junctions are probably involved in the generation of these frequencies even at their base line level. On the other hand the elevated level of electronic coupling in the already active epileptic focus may significantly amplify the synchronous interconnections among the cells and the expression of seizure discharges.

Our observations suggest that electronic coupling probably contribute in an activity dependent manner to generation of paroxysmal discharges in the 4-aminopyridine epilepsy model.

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EFFECT OF GYKI-16739 AND GYKI-16227 ON EVOKED RESPONSES AND LTP IN THE HIPPOCAMPUS OF THE RAT

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As preliminary studies suggest GYKI-16739 and GYKI-16227, recently synthesized in Drug Research Institute, Hungary, are new candidates to modulate the kinetics of glutamate receptors in the brain. Moreover, behavioral investigations indicated memory potentiation effect of these drugs.

Our recent experiments were performed on hippocampal slices of rats. The effect of the drugs on the field-responses evoked in the CA1 region were studied to indicate the efficacy of excitatory amino acid transmission. During the experiments we stimulated the Schaffer-collaterals with a test stimulus (0.05 Hz). Drugs were bath-applied in 20 and 100 μM concentration. Both NMDA and non-NMDA glutamate receptor dependent component of the evoked responses were determined by blocking NMDA receptors with 25 μM APV. Inducing LTP we investigated the role of these drugs in the basic learning and memory processes. We applied week tetanization stimuli, which enhances mainly the non-NMDA components of the evoked response.

GYKI-16739 in both concentration increased the area of both the NMDA and non-NMDA-receptor dependent components of the evoked field potentials in the CA1 region. We observed similar result in the case of 100 μM GYKI-16227, however, the increase of the area of the NMDA-receptor mediated component was more significant. 20 μM GYKI-16227could not alter the area of the responses compared to control response. Aniracetam, a positive modulator of AMPA receptor, in 1 mM concentration increased primary the area of the non-NMDA-receptor dependent component but only slightly enhanced the NMDA receptor mediated component.

Tetanizing stimuli induce LTP in each of the cases, but the amount of LTP was different depending on the drug treatment. In control slices LTP only occurred in the area of the non-NMDA-component in accordance with the previous finding that tetanization with relatively low stimulation intensity causes LTP only in the case of AMPA receptor mediated events.

However, in GYKI-16739 treated slices (20 and 100 μM) a robust LTP developed which associated with primary the NMDA component. In the GYKI-16227 containing medium no significant LTP developed. Aniracetam containing medium not only the non-NMDA but also the NMDA components were slightly potentiated following tetanization.

These findings indicate that GYKI-16739 and GYKI-16227 have significant effect on the excitatory synaptic transmission probably by a complex interaction with distinct glutamate receptor subunit.

LOCOMOTOR ACTIVITY OF MORPHINE-DEPENDENT RATS FOLLOWING WITHDRAWAL

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Objective. Different opioid receptor types affect the mesolimbic dopamine (DA) transmission and the DA-related behavioural activity in different ways. μ and δ receptor agonists enhance dose-dependent manner the DA release, increase or decrease the motility and elicit conditioned place preference, while activation of the κ receptor inhibits the DA release, decreases the motility and elicits conditioned place aversion (1). Relatively less data can be found in the literature about the behavioural consequences of the chronic, high dose morphine (Mo) treatment and the drug withdrawal. Aim of the present experiments was to check how the motility of the Mo tolerant and dependent rats change following spontaneous or antagonist-induced withdrawal. Naloxone (NX) a pure antagonist and n-cyclopropylnorazidomorphine (CAM) a mixed κ agonist/ μ antagonist were used for precipitation of withdrawal.

Methods. Mo-dependence was developed in male Wistar rats according to the method described by Buckett (2). Gradually increasing dose of Mo was given for 11 days in two daily portions, starting with a daily dose of 40 mg/kg and ending with that of 345 mg/kg. Motor activity (both horisontal locomotion and vertical rearing) of the animals was measured in an "Animal Activity Measuring System".

Results. 1. Chronic Mo treatment resulted in strong tolerance to the motility inhibiting effect of Mo 2. There was no change in the activity of chronically Mo-treated animals after withdrawal of the drug. 3. NX (10 mg/kg) induced much more marked decrease in the activities of Mo-treated animals, than in Mo-naïve ones. 4. Different doses of CAM significantly inhibited the motor activity in Mo-naïve rats, while in Mo-treated ones only the smallest dose used (0.1 mg/kg) decreased it, the higher doses, showing stronger κ agonist effect titled to produce any change. These results indicate that tolerance to the κ receptors-mediated behavioural effects develops during chronic Mo-treatment.

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GLUTAMATE-ACTIVATED CATIONIC CHANNELS IN *LYMNAEA*

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We have identified a group of neurosecretory light yellow cells of *Lymnaea,* which express a very high sensitivity to L-Glu mediated by cationic NMDA-like receptors. Here, we characterize the glutamate-gated membrane currents in isolated light yellow cells under voltage clamp. We found that L-Glu selectively activates a population of Na^+/Ca^{2+} ion channels in a concentration dependent manner in the range of 1-250 μM. The current was very sensitive to L-Glu with half maximal activated with 27 μM glutamate. Ihus, glutamate can affect neuronal receptors that participate in synaptic transmission. The Hill-coefficient was 1.74 suggesting that at least two L-Glu-molecules are necessary to open the channel. This inward Glu-gated current was found to be voltage dependent with a very slow inactivation kinetic and evidence of unusually slow desensitization. The calculated E_{rev} (15 mV) suggests that cationic channels were involved in the process. The rise time of the current was strongly dependent on the L-Glu concentration. At higher concentrations (>150 μM) the activation was concentration independent. Based on our both very recent and earlier data, we concluded, that molluscan NMDA receptors may show similarity to vertebrate NR1/NR2D subunits.

MORPHOLOGY AND PHARMACOLOGICAL PROPERTIES OF SEPTALLY PROJECTING NON-PRINCIPAL CELLS IN THE RAT HIPPOCAMPUS

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We aimed to study the morphology and pharmacology of non-principal cells that give rise to the hippocampo-septal GABAergic projection. Fluorescent microbeads were injected into the medial septal area of young rats (PD 16-17) in order to retrogradely label the hippocampo-septal projecting cells (HS cells). After 2 days of survival, *in vitro* hippocampal slices were prepared. The HS cells were identified in the CAl area str. oriens with a fluorescent microscope using a CCD camera. Cell-attached and subsequent whole-cell recordings were performed to investigate the response properties of HS cells to different drugs. The effect of several receptor agonists (Ach, 1 mM; ACPD, 10 μM; 5HT 10 μM) has been tested on the cells using the U-tube application system (1-3 sec application of the drugs) in the presence of ionotropic glutamate and GABA receptor blockers. In the cell-attached mode, the pharmacological modulation of the cells' firing was tested. In the whole-cell mode (Vh = -60 mV), the underlying agonist-induced currents were measured. During the recordings the cells were filled

with biocytin through the recording pipette. They were then visualized using the ABC method and reconstructed using camera lucida.

In the vast majority of HS cells all 3 agonists increased the firing of the cells and induced an inward current. The HS cells had local axon collaterals in strata oriens and radiatum, three of them even projected across the hippocampal fissure into the dentate hilus. Therefore, they are most probably distinct form the well-characterized O-LM (oriens/lacunosum-moleculare) cells, and likely correspond to the backprojection neurons. Using double fluorescent retrograde labeling we could also prove that non-pyramidal cells in the CA1 area str. oriens project both tho the medial septum and to remote regions (>3 mm) of the hippocampal complex.

BASAL FOREBRAIN CHOLINERGIC NEURONS RECEIVE ADRENERGIC INPUT IN THE RAT

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Previous studies described a significant dopaminergic and noradrenergic input to basal forebrain neurons including cholinergic corticopetal cells. Regarding the adrenaline, it has been widely accepted that due to the tow levels of adrenaline in basal forebrain tissues, the physiological influence of this third cathecholamine on the cholinergic corticopetal system is presumably negligible. During the last decade, several immunohistochemical and tract tracing studies repeatedly highlighted the existence of adrenergic axons in basal forebrain areas originating from the C1-C2 adrenergic cell groups in the medulla oblongata. Since the vast majority of functional studies focused on the noradrenergic input due to the hypothesis mentioned above, the physiological effect of adrenaline on the basal forebrain corticopetal system is still a question of debate. To address the morphological aspect of this issue, we performed an immunohistochemical double-labeling study in the rat using antibodies against phenylethanolamine N-methyltransferase (PNMT) and choline acetyltransferase (ChAT), the synthetic enzymes for adrenaline and acetylcholine, respectively. ChAT-immunoreactive neural structures were found intermingled with loose networks of adrenergic axons and terminals in basal forebrain territories such as the medial septum, diagonal band, ventral pallidum, peripallidal regions and substantia innominata (SI). Several axosomatic and axodendritic close contacts, suggestive of synaptic input, between adrenergic boutons and cholinergic structures were encountered. Our semiquantitative light microscopic analysis showed a peak frequency of putative contacts in the SI and BST. Furthermore, the axodendritic type contacts outnumbered the axosomatic type in almost each area studied. The correlated light- and electronmicroscopic analysis revealed both axosomatic symmetric and axodendritic asymmetric synapses between adrenergic boutons and ChAT-immunoreactive neural structures. Our results suggest that the adrenergic system may modulate basal forebrain cholinergic functions, such as arousal, sensory processing, attention, learning and memory.

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GLUCOCORTICOIDS AND AGGRESSION. TONIC AND PHASIC EFFECTS

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The acute inhibition of glucocorticoid synthesis by methyrapone decreased aggressive behavior markedly. An acute corticosterone treatment abolished this effect. Adrenalectomy (ADX) did not affect the level of aggresiveness, but ADX animals directed attacks to more vulnerable body parts of the opponent, and attack signalling by threats was reduced. Again, acute treatment with corticosterone abolished these changes. ADX animals submitted to aggressive encounters showed a strong c-fos activation in the central amygdala. Acute treatment with corticosterone abolished this effect. It is concluded that (i) an acute HPA-axis activation promotes aggressive behavior; (ii) normal variation in plasma glucocorticoids are responsible for maintaining normal attack patterns; (iii) the long-term effects of glucocorticoids on the central amygdala may at least partly be involved in maintaining normal patterns of attack, possibly via an anxiety-related mechanism. Low levels and low variability in glucocorticoid secretion are correlated with pathological aggression in humans. It appears that a similar phenomenon occurs in rats. Our data suggest that the central amygdala (which has an important role in anxiety) plays a role in this process.

DISCOMFORT INDUCED BY INTAKE: A PUTATIVE MECHANISM FOR SAFETY

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We had earlier hypothesized that most of the intestinal stimuli inhibiting intake behavior were unpleasant. In this experiment we tested the idea that satiety itself had an unpleasant character which was probably necessary for the adequate control of intake.

In the first experiment, rats were deprived of water and were let to drink freely from a calibrated tube. Amount consumed and behavioral elements were recorded continuously. Intake was divided into three periods: intensive drinking, intermittent drinking, occasional drinking, hypothetically corresponding to thirst, balance and satiety, respectively. In the third, satiety phase a new taste was gradually presented in each conditioning session. When subsequently tested, rats consumed significantly less of the 'satiety taste' than of the 'initial taste' showing taste aversion. This was accompanied by decreased appetitive and increased aversive behavioral scores, again supporting the presence of taste aversion.

In the second experiment taste-reactivity measurements were taken before the intensive, during the intermittent, and after the satiety phase. The number of appetitive responses showed a clear decreasing tendency whereas appetitive scores increased alongwise. The hedonic shift

refers to an increasing unpleasantness, which, on the other hand, proved to be mild since the most characteristic aversive response was passive dripping.

Based on the above data we feel that our initial hypothesis has gained strong support i.e. satiety really has a clear but mild aversive character. We suggest that this mild aversiveness, termed as discomfort, is an inherent component of the satiety.

ß-SHEET BREAKERS AND FUNCTIONAL ANTI-ß-AMYLOID ANTAGONIST OLIGOPEPTIDES IN THE PREVENTION OF ß-AMYLOID NEUROTOXICITY

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During the past decade compelling experimental and neuropathological data accumulated indicating a cardinal role of full-length and truncated ß-amyloid peptides (Aß) in the pathogenesis of Alzheimer's disease (AD). Besides the generally accepted phenomenon that Aß forms extracellular deposits termed senile plaques in AD, both *in vitro* and *in vivo* results support the hypothesis that non-aggregated or partially aggregated, non-fibrillar Aß oligomers may exert significant neurotoxicity. Thus, overt production of Aß species and/or pathological changes in the processing of their precursor, also referred to as the amyloid precursor protein, may lead to the formation of highly neurotoxic molecular forms of Aß well before the formation of senile plaques and manifestation of AD [1]. Taking the above considerations together, it became obvious that a specific 'anti-Aß strategy' and concomitant design of drugs which either inhibit the formation of certain molecular forms of Aß, or antagonize its action on particular cell-surface recognition (receptor) sites would be a feasible approach to fight Aß-induced neuronal damage in AD.

Besides experimental approaches aimed at blocking ß- and γ-secretase processing of the amyloid precursor protein, or immunological clearance of Aß from the brain [2], the design of ß-sheet breaker (BSB) and functional anti-Aß antagonist oligopeptides (fAAP) as a novel strategy emerged. By definition, BSBs act via chemical interaction with Aß protofilaments, or preformed Aß-aggregates and thereby inhibit the extension of ß-pleated Aß fibrils, whereas fAAPs may be chemically inert, but counteract Aß toxicity by ligand-like displacement of Aß from presumed cell-surface recognition sites and ameliorate pathological intracellular signaling cascades [1].

In the present account we provide correlative *in vitro* and *in vivo* evidence on the efficacy of a derivative of the ß[31-34] sequence of Aß, namely the Propionyl-Ile-Ile-Gly-Leu (Pr-IIGL) tetrapeptide, in attenuating Aß toxicity. This tetrapeptide spans the 'active center' of Aß and thus, is itself also a biologically active, but non-toxic compound. Recent studies of Laskay and his colleagues indicated that Pr-IIGL significantly attenuates Aß-induced membrane depolarization and subsequent intracellular Ca^{2+} accumulation in astroglial cells [3]. Microdialysis experiments in freely moving rats demonstrated that Pr-IIGL significantly prevented Aß-induced increase in extracellular glutamate and aspartate concentrations in the

rat magnocellular nucleus basalis (MBN), attenuated the Aß-induced learning deficit and the loss of cholinergic MBN neurons [4]. These data together with those of others [5] indicate that BSBs and fAAPs may be relevant in the prevention of Aß neurotoxicity in AD.

In spite of the promising experimental data several critical issues have to be considered to pave the way to the human application of BSB and fAAP oligopeptides. Infusion of peptidic compounds in the general circulation may lead to abrupt immune responses as well as to the rapid proteolytic degradation of oligopeptides. These problems may be solved by synthesis of oligopeptides in all-D-conformation. In addition, transport of peptidic compounds through the blood-brain-barrier is relatively limited that implicates the use of effective drug-delivery systems to target Aß in the central nervous system. Moreover, reliable early diagnosis of the disorder could only justify the use of such therapy in AD patients.

In conclusion, application of BSB and fAAP oligopeptides may be a fruitful way to attack Aß aggregation and neurotoxicity. Future design of peptidomimetic compounds – based on recently available experimental data with pharmacologically active oligopeptides – might be a successful approach to design drug candidates that may halt or hamper the progression of AD.

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AGED ANIMALS WITH CORTICAL ß-AMYLOID DEPOSITS, ABNORMALLY PHOSPHORYLATED PROTEIN TAU AND NEUROFIBRILLARY TANGLES

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The deposition of ß-amyloid (Aß)-containing senile plaques and neurofibrillary tangles resulting from pathologically altered neurofilaments are classical hallmarks of Alzheimer's disease (AD). Both neuropathological alterations have also been found in aged individuals of several animal species. These mammals may serve as valuable natural models in addition to recently developed transgenic mice which overexpress the human ß-amyloid precursor protein and show Aß-deposits during aging. One main goal of the present study, therefore, was a detailed analysis of AD-like alterations by applying sensitive immunocytochemical techniques to the brains of different mammalian species.

A first set of experiments was focused on the screening of senile plaques in species in which the amino acid sequence of the ß-amyloid peptide is identical or very similar to human Aß [Johnstone et al. 1991, Mol. Brain Res., 10:299-305]. These experiments were primarily performed with frozen sections from paraformaldehyde-fixed perfused brains from six 28-year-

old rhesus monkeys *(Macaca mulatta)*. Five of six aged macaques displayed Aß-deposits in the prefrontal cortex which was previously shown to be highly susceptible to ß-amyloidosis. Senile plaques were revealed by sensitive immunoperoxidase staining and immunofluorescence labelling as well as by thioflavine S indicating a ß-sheet structure of the amyloid peptide in matured plaques.

A more detailed characterization of Aß-deposits in the prefrontal cortex of macaques [Härtig et al. 1997, Brain Res., 751: 315-322] revealed also some plaques immunopositive for Ap_{1742} (p3-fragment) which were obviously devoid of $Aß_{17-42}$-immunoreactivity (ir). Parallel carbocyanine double labelling demonstrated the association of apolipoprotein E, glial and vascular markers with Aß-ir.

In additional screening experiments with immersion-fixed brains of other aged non-human primates, carnivores, herbivores and guinea pigs Aß-deposits were detected in the cortices of several animal species. They were found in hamadryas baboon *(Papio hamadryas)*, Campbell's guenon *(Cercopithecus mona campbelli)*, snow leopard *(Uncia uncia)*, Indian bear *(Melursus ursinus)*, polar bear *(Thalarctos maritimus)*, dog *(Canis lupus f. familiaris)*, cat *(Felis silvestris f. catus)* and guanaco *(Lama guanicoë)*.

The screening of abnormally phosphorylated protein tau was carried out using brain tissue of more than twenty aged individuals from fourteen mammalian species. Investigated brain regions were the prefrontal, motor and entorhinal cortices and hippocampi of these cases. The main tool for the demonstration of abnormally phosphorylated protein tau was the well-characterized monoclonal antibody AT8 (Innogenetics, Belgium) recognizing the phosphorylated amino acid residues serine 202 and threonine 205 of tau. AT8-ir was detected in brown lemur *(Lemur fulvus mayottensis)*, rhesus monkey *(Macaca mulatta)*, hamadryas baboon *(Papio hamadryas)*, rabbit *(Oryctolagus cuniculus)*, spectacled bear *(Tremarctos ornatus)*, guanaco *(Lama guanicoë)*, reindeer *(Rangifer tarandus)* and American bison *(Bison bison athabascae)* [Härtig et al. 2000, Acta Neuropathol., 100: 305-312].

The further examination of cortical tissue from rhesus monkey and bison also revealed immunoreactivities for the monoclonal antibodies AT 100 and TG-3 indicating conformation-dependent hyperphosphorylation. Sparsely scattered tangles and neuropil threads in the bison cortex could be shown by Gallyas silver staining [Härtig et al. 2001, Neurobiol. Aging, 22: 25-33]. These findings agree with previous results regarding tangles in bears and in domestic animals like sheep and goat. Our data also complement the study of Schultz et al. [2000, 1. Neuropathol. Exp. Neurol., 59: 39-52] on filamentous tau pathology in nerve cells, astrocytes and oligodendrocytes in aged baboons.

The present work was extended by the elucidation of spatial relationships between the revealed alterations in aged animals and extracellular matrix components as proteoglycans presumably involved in such processes. Several classes of extracellular occurring proteoglycans were previously shown to be associated with lesions of Alzheimer's disease. Our experiments were focused on a specialized form of extracellular matrix known as perineuronal nets characterized by immunoreactivity for chondroitin sulphate proteoglycans (CSPGs) and binding sites for the N-acetylgalactosamine attaching lectin *Wisteria floribunda* agglutinin (WPA). However, the applied markers for perineuronal nets only occasionally stained Aß-containing plaques in aged macaques.

In contrast, abnormally phosphorylated protein tau was detectable only in neurons devoid of perineuronal nets in the cortices of two aged bisons as revealed by dual-peroxidase staining [Härtig et at. 2001, Neurobiol. Aging, 22: 25-33]. These data parallel the results of our previous quantitative study on *post mortem* cortical tissue of cases with Alzheirner's disease

[Brückner et al. 1999, Neuroscience, 92: 791-805]. Human cortical areas abundant in extracellular matrix CSPGs were found to be significantly less affected by neuropathological cytoskeletal alterations. Both in human and bison brain, abnormally phosphorylated protein tau was predominantly observed in regions such as the entorhinal cortex that contain less perineuronal nets than other areas. It might be concluded that CSPGs of perineuronal nets cause a reduced vulnerability of associated neurons

A HISTAMINERGIC SYSTEM IN THE CNS OF GASTROPODS *(HELIX, LYMNAEA)*

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The chemical-neuroanatomy, distribution and biochemical properties of histaminergic neurons in the CNS of the pulmonate snails, *Helix pomatia* and *Lymnaea stagnalis,* have been studied, applying antibodies raised against histamine (HA) and analyzing the uptake and release of ^{3}H-HA. In the CNS of both species, the distribution of HA-immunoreactive (IR) neurons is similar, however, their number is significantly different. In *Helix,* ca 400 HA-IR neurons could be visualized, whereas in *Lymnaea* their number is only ca 120. The buccal, cerebral and pedal ganglia contained the overwhelming majority of the labeled cells, whereas much less IR neurons were seen in the subesophageal ganglion complex occurring individually or forming small clusters. About 30% of the I-IA-IR neurons were always found in three large groups in the pedal ganglia. The labelled neurons were almost exclusively of small or medium size, except the metacerebral giant serotonergic cell (MGC) in *Helix.* The neuropil of all ganglia were densely innervated by varicose fibers, and the connectives, commissures and all peripheral nerve also contained numerous labeled processes, indicating the involvement of HA in both intra- and interganglionic, and peripheral regulatory processes. In the statocysts, attached to the pedal ganglia, 7 to 8 HA-IR sensory cells were found, projecting with a bundle of sensory axons to a small region of the cerebral ganglion neuropil. Following *in vitro* incubation with ^{3}H-HA, a high affinity, single component uptake system (affinity 37.6 μM) has been demonstrated in the *Lymnaea* CNS, capable of removing released HA from the synaptic cleft. The uptake of HA was found to be inhibited by imipramine, desipramin, benztropin, phenoxybenzamine and oubaine. The rank order of ^{3}H-HA incorporated by the different ganglia of the CNS was the following: viscero-parietal ganglion complex (36%), cerebral ganglia (34.2%), pedal ganglia (18.1%) and buccal ganglia (11.3%). The release of ^{3}H-HA incorporated previously could be evoked by either electrical stimulation or application of 100 mM K^{+}. On the other hand, K^{+}-induced release could, be prevented in Ca^{2+}-free physiological solution. Analyzing the binding properties of ^{3}H-HA to membrane preparations isolated from the *Lymnaea* CNS, the presence of a low affinity HA-receptor has been demonstrated, similarly to other monoamine receptors in gastropods. It is suggested that HA plays a significant transmitter role in the CNS of pulmonate gastropods.

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YOUNG AND OLD CELLS IN YOUNG AND OLD BRAINS - CHANGES IN HIPPOCAMPAL APOPTOSIS AND NEUROGENESIS WITH AGING IN THE RAT DENTATE GYRUS

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During early central nervous system development, both cell birth and cell death occur in large numbers throughout the brain, where they eventually contribute to the formation of functional networks. In adulthood, both processes have, however, virtually disappeared. Although apoptosis can easily be reintroduced following e.g. specific neurotoxic insults, neurogenesis was traditionally considered to be absent in adult brain.

Recently however, it has become clear that cell birth still persists well into adulthood, although in relatively small numbers and only in two areas: 1) the subventricular zone (SVZ) of the lateral ventricles, and 2) in the hippocampal dentate gyrus (DG), although, however, also in the cortex, rare cell birth has recently been described (Gould et al., 1999). The DG is one of the rare exceptions in the brain where cell birth and cell death appear tigthly associated, as a continuous cell turnover takes place in this area in many rodent species (Gould et al., 2000) and primates including man (Erickson et al., 1998). In spite of the many factors involved in neurogenesis control, its functional relevance and underlying mechanisms remain however, obscure.

Granule cells of the DG are derived from progenitor cells located in the hilar-DG border (Gage, 2000) and can be identified using Bromo-deoxy-uridine (BrdU) injection that is taken up by dividing cells and their progeny. After division, these newborn cells proliferate and migrate upwards into the granule cell layer, where they eventually grow axons to the cornu amonis (CA) 3 area. After approximately 10 days, the newborn cells have become part of the functional trisynaptic circuit of the hippocampus. Approximately 2000 new cells are born per rat hippocampus per day, which implies that, since DG volume remains largely unchanged, approximately equal numbers must die in the same period, most likely through apoptosis.

Adult cell birth is modulated by many factors, like e.g. estrogens, growth factors, glucocorticoid hormones (GC), but also by environmental factors like early life events, learning, stress, physical activity, enriched environmental housing, or deleterious conditions like ischaemia (Lemaire et al., 2000; Gould et al., 2000; Van Praag et al., 1999; Kempermann et al., 1998). Most of these factors increase neurogenesis, whereas stress and the associated rise in GCs rather reduce neurogenesis (Gould et al., 2000). Although certainly not consistently found, aging has generally been associated with increased GC levels (for a recent review see Lucassen and DeKloet, 2001). In senescent mice and rats, a reduced incidence of neurogenesis was found (Kempermann et al., 1998), that was proposed, but not actually shown, to relate to increased GC levels (Cameron and McKay, 1999). Furthermore, neurogenesis and apoptosis have, so far, hardly been investigated together, nor has survival and aging of particularly the individual new born cell been characterized in animals of different ages.

Therefore, we studied both neurogenesis and apoptosis in the DG of young (2 weeks of age), adult (6 weeks of age), middle aged (12 months of age) and old (24 months of age) rats after different survival times following BrdU injection. We assessed numbers of newborn,

remaining as well as dying DG cells (using ISEL/TUNEL (Lucassen et al., 2000)) in order to have a good impression of the net change in neuron number in the DG, which will be related to GC plasma levels and the stress response of the different agegroups.

Our results show extensive presence of newborn cells at 2 weeks of age, and considerable reductions in DG neurogenesis with age that started already at 6 weeks of age, and yielded very low numbers at 12 and 24 months of age. Other hippocampal subareas revealed considerably less but also prominent presence of newborn cells. Aging of the individual newborn cell revealed clear migration up into the DG granular cell layer at 1 week survival after BrdU injection, that was associated with a further increased cell division, i.e. an approximately two-fold increase in number as compared to 24 h after BrdU injection. At 1 month of survival after BrdU injection, considerably less cells seem to reach the DG granular layer and no further doubling in cell number is observed.

Apoptotic cells were present in considerable numbers only in the 2 weeks age group and were strongly reduced already from 6 weeks of age onwards when they occurred only at a very low frequency in the DG, but were also found in other hippocampal subareas. Very little changes in this low apoptotic frequency occurred in the older age groups.

In conclusion, both apoptosis and neurogenesis were reduced dramatically already in early adulthood but continued to occur in later ages, albeit with different frequencies for the two processes, and notably, also in areas outside the DG. The low apoptotic numbers is likely to be related to the very rapid clearance of dead cells. This implies that the chances to detect changes in ongoing apoptosis in tissue sections are very low (Perry et al., 1998; Lucassen, 2000; Lucassen et al., 2001). Further analysis will focus on completion of these data and on subsequent unbiased assessment of total DG cell numbers and its relation with GC plasma levels in aging.

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THE EFFECT OF GSM MOBILE PHONE IRRADIATION ON SPONTANEOUS SINGLE UNIT ACTIVITY AND MEMORY IN THE RAT

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It has been broadly discussed so far that electromagnetic fields may be hazardous to health. It is also proposed that the proximity of cellular mobile phones to the user's head leads to the absorption radiofrequency irradiation (RF). We created a simplified animal model to investigate the effects of RF waves on brain functioning. Our previous examinations were expanded to assess 1) spontaneous firing activity of cortical neurons in the rat; 2) motor reaction times (RTs) and learning capabilities of freely moving animals in a discrimination learning task. According to the results of our animal model experiments, long lasting (up to 2 h), reversible increase of spontaneous firing rates of single/multiple units was observed in extended cortical areas of the rat. In RT studies, decreases of response latencies with decreases of task accuracy were observed. Our present results indicate that RF irradiation caused by GSM cellular phones elicits short-term alterations in spontaneous brain activity, coupled with impaired cognitive and/or memory functions. As a consequence of the RF irradiation, the affected neurons become unable to attend to their previous functions - resulting in executive problems in particular tasks where high cognitive demands are present. In addition, the dynamics of recovery of basal neural firing rates, together with changes in task accuracy may both reflect on the time course of the underlying transient intracellular biochemical mechanisms.

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CHANGES OF MONOAMINE LEVELS DURING NONSPECIFIC AND SPECIFIC BEHAVIORAL AROUSAL IN THE SNAIL *HELIX POMATIA*

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In this study, we applied HPLC assay to monitor changes of 5HT and DA contents during different behavioral arousal in the organs playing role in: 1. the generation and execution of cyclic feeding movements (CNS, buccal ganglia, buccal mass), 2. the ingestion and digestion (esophagus, crop), 3. the external appearance of behavioral arousal (tentacles, lips, foot), the neurohormonal remote control (hemolymph).

During the environmental stimuli (shower) evoked behavioral arousal both DA and 5HT contents decreased in the CNS but increased in the hemolymph but DA remained dominant in the hemolymph. These results suggest that during behavioral arousal the environmental stimuli increased the monoamine transport and liberation without increasing the rate of synthesis.

Food induced feeding arousal increased both the 5HT and DA contents in the CNS, and the gastrointestinal tract. However in both the buccal ganglia and the hemolymph only 5HT content increased but DA content decreased resulting a significant 5HT dominance in both the buccal system and the hemolymph. These observation suggest that food induced feeding arousal as a motivated behavior increases both the transport and liberation and additionally increases the rate of synthesis of monoamines. However, the site of liberation is regulated centrally or locally during food intake. At the termination of food intake both 5HT and DA significantly decreased in the buccal ganglia, whereas they remained elevated in the CNS. In satiated state following the termination of feeding, both 5HT and DA decreased in both the CNS and the buccal ganglia, crop, esophagus, tentacles. In the hemolymph both 5HT and DA concentrations increased, but 5HT remained dominant. These observations suggest that satiating stimuli decrease monoamine transport and liberation in the peripheral organs and at the same time they significantly decrease the rate of central 5HT and DA synthesis. The present results indicate that during behavioral arousal and motivated behaviors as feeding, animals regulate or fit the level of activity of monoaminergic neurons and the concentrations of monoamines as neurohormones to the actual state of behavior or motivation which help the homeostatic effort of the organism.

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SEROTONERGIC INNERVATION OF THE SALIVARY GLAND IN GASTROPODS (HELIX, LYMNAEA): IMMUNOCYTOCHEMISTRY, BIOCHEMISTRY AND PHYSIOLOGY

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The salivary gland, an important organ of the feeding system of gastropods, is symmetrically located on both sides of the pharynx. Its continuous secretion as well as activity dependence on the actual state of feeding behavior of the animal need two types of regulation: an autonomic motor program forwarding the saliva towards the alimentary tract, and a higher order regulatory process connected to the neural network underlying feeding behavior. As a first step to analyze this complex regulatory system, we have investigated the immunocytochemical, biochemical and physiological characteristics of the aminergic innervation, with emphasis on the serotonergic (5HTergic) innervation of the salivary gland in the pulmonate snails, *Helix pomatia* and *Lymnaea stagnalis*. At light microscopic immunocytochemical level, a rich innervation pattern of 5HT-IR elements has been visualized. As a part of the salivary nerve, a 5HT-IR axon bundle enters the gland. Labeled varicose fibers arborizing first from the bundle establish an extremely rich innervation of the muscle wall of the primary salivary duct, then axon processes partly project towards salivary ducts of lower order, and partly form a network of varicose fibers within the gland. This latter elements both surround individual gland cells and form *en passant* contacts. According to HPLC assays, the salivary gland of both species contains 0.8-1.57 pmol/mg 5HT and 0.2-0.4 pmol/mg tissue dopamine (DA). Applying ^{3}H-5HF, a single component uptake system and a K^{+}-evoked release can be demonstrated in the *lymnaea* salivary gland. Analyzing the contractile (muscular) elements of the isolated *Helix* salivary duct by *in vitro* sometric technique, 5HT has been shown to cause a transient (phasic) contraction in high concentrations (10^{-4}-10^{-6}< M), whereas it evokes a tonic relaxation in low concentrations (10^{-7}-10^{-9} M). In contrast, DA had a dose-dependent effect, evoking sustained contractions. Myanserin, a general 5HT-receptor antagonist had no effect on the 5HT-induced phasic contraction. However, 10^{-5} M 5HT effectively blocked the DA-induced contraction. It is suggested that 5HT plays a modulatory role in the regulation of the function of the salivary duct in gastropods.

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THERAPEUTIC APPROACHES TO VASOGENIC BRAIN EDEMA: THE COLD LESION MODEL IN RODENTS

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Background and Purpose

Brain edema development following disruption of the blood-brain barrier (BBB) is a potentially life-threatening complication of a wide range of cerebral diseases, such as ischemia, trauma and tumor. The resulting brain edema leads to secondary damage, thus further increasing the lesion volume due to the primary insult. A classical model of vasogenic edema is cold lesion of the exposed cerebral cortex, originally developed by Klatzo in the cat. Our experiments employed an adaptation of this method in the rat and mouse. This model also mimics the main pathophysiological characteristics of mild human brain contusion trauma. In our study we investigated the role of 3 putative mediators of secondary brain damage, endothelin (ET), bradykinin (BK) and nitric oxide (NO). ET induces constriction of large cerebral arteries via endothelin-A receptor (ET_A-R), BK enhances vasogenic edema via bradykinin-B_2 receptor (BK_2-R)-mediated increase of BBB permeability, whereas the large amount of NO produced by activated neuronal (type I) and induced immunological (type II) NO-synthase (NOS) after injury is potentially neurotoxic. NO and its reaction product peroxynitrite induce DNA damage, which activates the nuclear repair enzyme poly(ADP-ribose) polymerase (PARP); overactivation of this enzyme, however, leads to cell demise through energy depletion. The aim of our study was thus to investigate the effects of specific inhibitors of the abovementioned receptor subtypes and enzymes on lesion volume in rats and the effect of BK_2-R inhibition on edema development in mice, respectively. Given the conflicting results in the literature regarding the antiedematous potential of dexamethasone (Dxm), we also investigated the effect of this glucocorticoid type II receptor agonist on brain swelling in the cold lesion model, adapted to the mouse.

Materials and Methods

Cold lesion was induced by impressing a metal probe (diameter 3 mm, -78 °C) into the parietotemporal cortex for 6 s (lesion volume) or 30 s (edema), respectively, under brief anesthesia. After 24 h brains were removed. Lesion volume was determined morphometrically in serial slices after triphenyltetrazolium-chloride (TTC) reaction; brain swelling was estimated using the wet/dry weight method. The ET_A-R antagonist RO 61-1790 was applied intracerebroventricularly (icv) 0.5h before lesion (833 μg/kg); the BK_2-R antagonist Hoechst 140 in subcutaneous osmotic minipumps over 25 h starting 1 h before lesion (300 ng/kg/min); the NOS I inhibitor 3-Br-7-NI (Br-7NI) intraperitoneally (ip) 1h before lesion (25 mg/kg); the NOS II inhibitor aminoguanidine (AG) 7.5 h after lesion (100 mg/kg); the PARP inhibitor aminobenzamide (AB) icv 0.5 h before lesion (10 mg/kg), and Dxm ip 24, 12, 1 h before and

12 h after lesion (6.25 mg/kg), respectively. Sham-operated animals were treated identically but only vehicle solution was administered.

Results and Discussion

Lesion volume was reduced significantly ($p>0.05$) in all treatments: by 23% (RO 61-1790), 19% (Hoe 140), 20% (AG), 21% (Br-7NI), 17% (AG+Br-7NI) and 18% (AB).

The *in vivo* neuroprotective effect RO 61-1790 – a very potent competitive antagonist of the ET_A-R, able to reach the cerebral arteries after icv application and abolish the ET-1 evoked constrictory response of the middle cerebral artery *in vitro* – indicates that ET is involved in the secondary brain damage. Our finding that selective blocking of NOS I and II reduces lesion size implies that NO from these enzyme isoforms is a harmful mediator after cryogenic injury. However, the effects of a NOS I and NOS II inhibitor, given together, were not additive, suggesting interaction between the enzyme isoforms. Furthermore, our data indicate that PARP is an important effector of NO-mediated brain injury also in this model.

Brain swelling was attenuated significantly by the BK_2-R antagonist Hoe (14%), implying that the beneficial effect on lesion volume was related to decreased edema development. Dxm also attenuated brain swelling (15.5%). Other Dxm application protocols and doses, however, elicited no significant antiedematous effects, thus emphasizing the importance of its optimal concentration at the target site.

Conclusion

ET, BK and NO are important mediators of brain damage in the 24 h after parietotemporal cold injury. The selective inhibition of ET_A-R, BK_2-R receptor subtypes, the NOS I and II enzyme isoforms and of PARS significantly reduced lesion volume and/or brain swelling, indicating their involvement in cold-lesion induced secondary brain damage. Finally, Dxm significantly reduces brain swelling in this model of brain injury.

SYNERGISTIC ANTINOCICEPTIVE INTERACTION OF ENDOMORPHIN 1 WITH DEXMEDETOMIDINE AFTER INTRATHECAL ADMINISTRATION IN RATS

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The spinal cord is an important neuronal structure for pain transmission and it is the pharmacologic site of action of agents such as opioids, α_2-adrenoceptor agonists. The purpose of the present study was to investigate the antinociceptive interaction of the recently discovered endogenous μ-opioid ligand, endomorphin-1 with the selective α_2-adrenergic agonist dexmedetomidine at spinal level in awake rats.

Methods: Intrathecal catheters were implanted into male Wistar rats weighing 250-350 g. Acute nociceptive sensitivity was assessed by using the tail-flick test. Dose-response and time-

course curves were determined for endomorphin-1 (0.6-50 μg), dexmedetomidine (0.1-10 μg). Dose-effect curves were generated for various fixed dose ratios (4:1, 6:1 and 20:1) of endomorphin-1 and dexmedetomidine. Analgesic latencies were converted to % maximum possible effect (%MPE). Isobolographic analysis of the interactions between dexmedetomidine and endomorphin-1 was also conducted.

Results: Endomorphin-1 resulted in a dose-dependent increase in thermal withdrawal latency, with the peak effect occurring at 10 mm. The highest dose caused a close to 100% MPE, and also brought a temporary motor impairment. Dexmedetomidine at the lower doses produced a slight and short-lasting, but significant increase in MPE; only the highest dose caused a very effective, long-lasting antinociception, but this was associated with substantial diuresis and sedation. Co-administration of dexmedetomidine and endomorphin-1 resulted in a significant increase in the tail-flick latency in a dose-dependent fashion. The time-course curves revealed that dexmedetomidine in higher doses not only potentiated, but also prolonged the antinociceptive effect of endomorphin-1. Isohолographic analysis showed that this interaction was synergistic at every ratio. Animals receiving the combinations exhibited no severe side-effects or unusual behavior.

Discussion: The synergistic interaction between these drugs may be of therapeutic significance in the future by allowing a decrease of the dose of either drug required to achieve an acceptable level of analgesia without side-effects.

LONG-TERM EFFECT OF MATERNAL SEPARATION ON EXCITOTOXIC DAMAGE TO CHOLINERGIC BASAL FOREBRAIN NEURONS IN ADULTHOOD

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More and more experimental results show that early life experiences have a strong impact on the development of the stress response system, namely on the sympathico-adrenal and hypothalamo-pituitary-adrenal (HPA)-axis. They can induce long-lasting changes in individual stress reactivity, characterized mainly by either enhanced or blunted glucocorticoid response upon stress stimuli depending on the nature of the early life events. In addition, it was shown that they could affect aging-related cognitive decline as well. Glucocorticoids (such as corticosterone in rat and cortisol in human) received during the last decade increasing attention due to their neuromodulator nature. It is well established that in the central nervous system, next to their negative feedback control on the HPA-axis, they play an important role in the regulation of the excitability and activity of both neuronal and glial cells. As the consequence of that glucocorticoids can modulate the viability of the central nervous system and influence neurodegenerative processes. These informations led us to ask, whether can early life

experience – via modulation of the HPA-axis – have an effect on the vulnerability of the brain against excitotoxic stimuli.

In our laboratory we established a postnatal manipulation model, so-called postnatal handling, which includes daily separation of the pups from the mother for 15 min, from postnatal day 1-21 (handled animals) whereas pups from other nests are left undisturbed (control animals). First we performed detail study on the effect of this specific manipulation on behavioral and neuroendocrine stress reactivity during adulthood. It revealed, in line with the international literature, that postnatally handled animals have decreased behavioral stress reactivity (increased exploration in the open field test, decreased anxiety in the elevated plus maze test) and attenuated HPA-axis reactivity (blunted corticosterone, prolactin response after both mild and severe stress stimuli).

Following that, with a new group of rats, we tested the effect of postnatal handling on the sensitivity of cholinergic nucleus basalis magnocellularis (MBN) to excitotoxic lesion-induced neuronal damage. Earlier studies from our laboratory demonstrated that different plasma levels of corticosterone have a strong impact on N-methyl-D-aspartate (NMDA)-induced cell death in the MBN. When adult handled and control rats (Wistar) reached an adult age of 11 months they were subjected to unilateral NMDA infusion into the MBN (60 nmol in 1l 0.01M PBS). In the week thereafter, first we measured the effect of postnatal manipulation on cholinergic damage-induced cognitive impairment in the passive avoidance test. Then 12 days after lesion, animals were perfused and brain sections were subjected to different histochemical stainings, to measure the effect of the lesion on the cholinergic system. In the passive avoidance learning test, handled animals had significantly better performance (retention) than control animals. Analysis of acetylcholinesterase positive fiber loss in the somatosensory cortex revealed significantly increased degeneration in handled animals and determination of choline-acetyltransferase-positive cells in the nucleus basalis showed increased cell loss in handled animals compared to controls. In summary, postnatal handling attenuated excitotoxic lesion-induced cognitive impairment during adulthood, but the detected enhancement of cholinergic cell death may suggest an increased susceptibility to acute neuronal damage. Further analysis is necessary to elucidate this contradictory result. However, our data clearly show that early environmental influences can contribute substantially not only to the development of the HPA-axis responsiveness to stressful stimuli, but they can result in stable individual differences in the sensitivity toward acute neurotoxic challenges. The long-lasting effects of a specific postnatal influence can result in both beneficial and harmful consequences, depending on the context in which they are investigated.

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DETECTION OF ESTROGEN RECEPTOR-ß MESSENGER RIBONUCLEIC ACID AND IMMUNOREACTIVITY IN ASTROCYTES AND OLIGODENDROCYTES OF THE RODENT BRAIN

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Estrogen influences a variety of glial functions in the central nervous system, including plasticity of astrocytes and myelinization by oligodendrocytes. Conflictingly, studies using *in situ* estrogen receptor (ER) detection provided either no or only week evidence for the presence of the classical α isoform of ER in glial elements of the rodent brain. The present studies were undertaken to address the presence of the recently-cloned ß isoform of ER (ER-ß) in astrocytes and oligodendrocytes of the rat and guinea pig brains. Studies applying *in situ* hybridization histochemistry (ISHH) provided evidence for expression of ER-ß messenger ribonucleic acid (mRNA) in glutamin synthase mRNA-positive astrocytes. In addition, ER-ß mRNA was detected in oligodendrocytes of the optic chiasma as well as other white matter structures. Light and electron microscopic immunocytochemical studies using several affinity-purified antibodies verified the presence of ER-ß in glial cells and demonstrated a predominantly nuclear localization of ER-ß. The observation of ER-ß mRNA and immunoreactivity in astrocytes and oligodendrocytes indicates the involvement of the recently-discovered ER-ß in the modulation of various glial functions.

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PERIPHERALLY INJURED C-FIBRE PRIMARY AFFERENTS TRANSPORT CHOLERAGENOID-HORSERADISH PEROXIDASE INTO THE SUBSTANTIA GELATINOSA OF THE SPINAL CORD

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Peripheral nerve lesions result in an increased transganglionic transport of choleragenoid-horseradish peroxidase conjugate (CTX-HRP) to the substantia gelatinosa of the spinal dorsal horn. These findings were interpreted in terms of an A-fibre sprouting response to peripheral nerve injury, since CTX-HRP is regarded as a marker of myelinated afferents and these do not terminate normally in this area. The aim of the present study was to explore the role of capsaicin-sensitive primary afferent neurons in this neuroplastic phenomenon.

Experiments were performed on adult male Wistar rats. The transganglionic transport of intraneurally injected CTX-HRP was studied two weeks after the transection of the sciatic nerve. Capsaicin-sensitive afferents were eliminated either by pretreatment of the nerve with

capsaicin or by systemic injection of capsaicin (100 mg/kg) 2 weeks to 2 months prior to nerve transection.

In intact animals intraneural injection of CTX-HRP resulted in a distinct labelling of the deep dorsal horn. Peripheral nerve transection resulted in a marked labelling also in the marginal zone and the substantia gelatinosa. Selective elimination of C-fibre capsaicin-sensitive primary afferents prior to nerve section prevented the transganglionic labelling of the substantia gelatinosa but not the deep dorsal horn.

These observations indicate that peripheral nerve lesion-induced transganglionic labelling of the substantia gelatinosa may be accounted for by the uptake and transport of CTX-HRP by capsaicin-sensitive C-fibres. The findings also suggest that injury-induced transganglionic transport of CTX-HRP to the superficial dorsal horn may be connected with a phenotypic change of C-fibres rather than a sprouting response of the Aβ-fibres.

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THE ROLE OF NPY IN THE MEDIATION OF OREXIN-INDUCED HYPOTHERMIA

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The effect of the recently identified neuropeptide orexin-A and the mediation of its action on body temperature was investigated. Different doses of orexin-A (140-560 pmol) were administered intracerebroventricularly (i.c.v.) to adult male rats, and colon temperature was used as an index of their thermoregulatory action. Orexin-A decreased colon temperature 60 mm after administration and exhibited a bell-shaped dose-response curve. I.c.v. pretreatment with NPY antiserum 24 h before orexin administration completely abolished the orexin-induced hypothermia. These data suggest that this appetite-regulating peptide might play a role also in thermoregulation and its hypothermic effect seems to be mediated by NPY.

VERTICAL BIAS IN DENDRITIC TREES OF MULTIPOLAR CORTICAL NEURONS EXPRESSING GAD67-GFP *IN VITRO*

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The goal of this study was to develop a reliable method to visualize GABAergic neurons in living brain slices, for electrophysiological and morphological studies. For this purpose, organotypic brain slice cultures were transfected with enhanced green fluorescent protein (GFP) gene driven by the glutamic acid decarboxylase (GAD67) promoter.

In transsected cortical slices, with almost no exceptions, GFP expressing neurons were non-pyramidal. These putative GABAergic interneurons were brightly fluorescent, and could often be visualized with their entire dendritic and axonal arbors.

A sample of 150 medium and large multipolar GFP-expressing neurons were digitally traced from confocal image stacks, and the degree of vertical bias in their dendritic trees estimated from the weighted average of the cosine of the dendritic growth angles relative to the vertical, a measure which should cluster around 0.64 for randomly oriented dendrites. We found that over 70% of the neurons in our sample had dendritic trees with a highly significant vertical bias. We conclude that GABAergic neurons make an important contribution to the vertical neocortical neuropil, and are likely to participate in neuronal computations within their own module.

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REACTIVE GLIA PROMOTES AXON GROWTH: A SHARPLY TIMED TRANSITION OF PERMISSIVE STAGE TO NON-PERMISSIVE STAGE

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The permissive or non-permissive nature of the reactive glia to the growing axons has been a topic of discussion for a long time. The present experiments offer a system to study, how the former permissive nature of the reactive gia turns to non-permissive in a short-time interval. The glial reaction and the presence of neural fibers were detected by the immunohistochemical staining of glial fibrillary acidic protein (GFAP) and neurofilament protein (NFP), respectively.

The lesions were deep incisions in postnatal rats, behind the coronary suture, under ether anaesthesia. After a survival period the animals were overdosed by ether and perfused transcardially with 4% paraformaldehyde in phosphate buffer (0.1 M, pH 7.4). Parallel sections were processed for the immunohistochemical staining of GFAP and NFP. The applied reagents were mouse monoclonal antibodies (Boehringer, Mannheim) to GFAP or NFP, in a dilution of 1:100, and biotinylated anti-mouse immunoglobulin and streptavidin-biotinylated horseradish peroxidase complex (Amersham). In the animals lesioned between P2 and P5 the thalamus was penetrated by an intensely NFP immunopositive strip corresponding to the lesion track. Inside this strip, high-power objective revealed an irregular system of fibers, which proved to be axons under electron microscope. When animals were lesioned after P6 or later, the lesion track was conspicuously negative to NFP immunostaining. GFAP immunostaining detected reactive glia in every case, independently from the age, and the territories of GFAP and NFP immunopositivities matched each oilier. The NFP-immunopositive strip appeared earliest on the 3rd post-lesional day, after the appearance of the glial reaction, and was found still in two month. As post-lesional fusions between the thalarnus and hippocampus were usually observed, it suggests that the ingrowing fibers are hippocampal axons seeking their path to the mamillary body. To understand better the background of the different permissivity of the reactive glia, we have started to investigate several factors in diencephalic lesions between P2 and P9. According to our preliminary data, reactive glia of every case were immunopositive to nestin and vimentin, markers of immature glia, which had been observed by other authors in adult animals. Components of the extracellular matrix, laminin, heparine-hinding protein, tenascin and chondroitin sulphate, which are known to influence axon growth, and factors of cell-to-cell communications as N-CAM and L1, displayed no difference in their distribution, in correlation of the permissive or non-permissive stage of the reactive glia.

EFFECTS OF AMPA RECEPTOR MODULATORS IN THE CHICKEN RETINA

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AMPA/kainate receptors have major role in learning and memory processes. Derangement of AMPA receptor mediated synaptic transmission may be a contributing factor in neurological and neurodegenerative diseases. Drugs that positively modulate AMPA receptors can facilitate the AMPA receptor mediated processes and may have therapeutic benefits in treating memory deficits in dementia or after brain insults.

Chicken retinal spreading depression (RSD) was described as a suitable and relatively specific *in vitro* model for testing glutamate receptor antagonists. Effects of AMPA receptor blockers in the retina model are in good correlation with patch clamp experiments.

We elicited RSD in the posterior eyecups of day-old chicks by 5 μM S-AMPA (for antagonists) and 2 μM RS-AMPA (for positive modulators). We tested various AMPA positive and negative modulators. AMPA antagonists (NBQX, GYKI-52466, GYKI-53405, GYKJ-53655, EGIS-8332) concentration-dependently blocked the initiation of RSD (IC_{50} are 0.2, 16.6, 7.0, 1.4 and 5.3 μM, respectively) while positive modulators (cyclothiazide, IDRA-21

and aniracetam) accelerated the initiation and propagation of this process. This potentiatory effect reflected in a decrease of the latency of the RSD (EC_{50}-s are 8.5 μM, 126 μM and 1.43 mM). These drugs also shifted the concentration response-curve of GYKI-52466 to the right in a near parallel fashion indicating a strong interaction between allosteric sites. When drugs have been added in equiactive concentrations potencies of positive modulators were different regard to the reversal of GYKI 52466-induced inhibition.

We suppose that AMPA positive modulators can act at multiple allosteric sites and they allosterically interact with the negative modulatory site. When positive modulators were co-administered, aniracetam increased the effect of cyclothiazide, however, we found significant additive effects at the lower concentrations. This may confirm the existance of multiple positive modulatory binding sites on the AMPA receptor. Additivity can be also explained by the heterogeneity of the AMPA receptor i.e. positive modulators differently act on the various receptor subunits and splice variants. We conclude that retinal spreading depression is a suitable pharmacological test to predict the efficacy of AMPA positive modulators.

HOMEOSTATIC ALTERATIONS AFTER METHYLGLYOXAL MICROINJECTION INTO THE VENTROMEDIAL HYPOTHALAMIC NUCLEUS OF THE RAT

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The ventromedial hypothalamic nucleus (VMH) and its glucose-monitoring (GM) neurons have already been shown to play important roles in the central regulation of feeding and metabolism. Furthermore, selective destruction of these GM cells was found to lead to the development of various diabetes-like alterations. To further test the hypothesized role of forebrain GM neurons in the homeostatic control - and to examine the possible central action of diabetes or metabolic X syndrome inducing carbonyl-stress mechanisms -, acute and chronic homeostatic-metabolic consequences of methylglyoxal (MOO) administration into the VMH was studied in complex, behavioral-biochemical experiments in the rat. A single bilateral MGO microinjection into the VMH elicited pathological elevation of blood glucose concentrations both in acute or chronic (10 mm or 3 wks after MOO administration, respectively) glucose tolerance tests. MGO microinjections also led to deficits of food intake in response to physiological challenges (i.p. insulin or 2-DG), and caused alterations of some plasma metabolite (uric acid, triglycerides and cholesterol) concentrations as well. Preliminary histology of the pancreas showed normal islet structure, whereas that of the kidneys glomerular and vascular malformations in all animals examined. Our findings provide evidence for that MOO microinjection destroyed, at least partially, the ventromedial hypothalamic GM neural network and thus, caused diabetes-like disturbances of feeding and metabolism. The results, along with our previous data, further substantiate the validity of a new model of central

homeostatic control for a better understanding of eating disorders and metabolic illnesses as well.

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PRESENILIN-1 IS PRESENT IN THE AXONS, IN THE AXON TERMINALS AT THE NEUROMUSCULAR JUNCTIONS AND BIDIRECTIONALLY TRANSPORTED IN THE SCIATIC NERVE OF ADULT RAT

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder which affects the human central nervous system. Genetic studies of familial AD revealed that presenilin-1 (PS-1) may play an important role in the pathogenesis of this devasting disease. Presenilin-1 (PS-1) has been localized in the perikarya of the different neurons, but could not be found beyond the trans-Golgi network. Recently, PS-1 has been found in neuritic processes and in growth cones during neuronal differentiation in the hippocampal neurons of rat. Immunohistochemically, we studied the axonal presence and the transport of PS-1 and compared it with the localization of amyloid precursor protein (APP), synaptophysin (SYN) and vesicular acetylcholine transporter (VAChT) in the motoric neuronal cell body, and in the sciatic nerve and its axon terminals in adult rat. For study of the anterograde and retrograde movement of PS-1 and APP within the axons, the sciatic nerve was double-ligated for 6, 12 or 24 hours. The immunohistochemical results demonstrated that PS-1, APP, SYN and VAChT can be found in colocalization not only in the neuronal perikarya, but also in the axons of the sciatic nerves and in their axon terminals at the neuromuscular junctions. In the double-ligated and double-stained sciatic nerves, PS-1 and APP staining was present above the upper ligature and below the distal ligature. The immunoreactivity of PS-1 disappeared from the middle segment after a 6-h ligation. This result was confirmed by Western-blot studies. The immunohistochemieal results lead us to suggest that PS-i may be present in the axons, where it is transported bidirectionally.

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THE EFFECT OF ANALGETICS WITH DIFFERENT MECHANISM OF ACTION IN AN EXPERIMENTAL MODEL OF NEUROPATHIC PAIN

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Neuropathic pain represents heterogeneous conditions with different etiology and various clinical symptoms. In some cases, patients afflicted with pain, which is evoked by stimuli that are normally innocuous such as light touch. This phenomenon is called allodynia. These are

often poorly managed by conventional analgesic such as opioid analgesics and non-anti-inflammatory drugs. Activation of NMDA receptors is a key step in abnormal pain processing. NR2B subtype selective compounds are a new class of NMDA antagonists, apparently free from the classical NMDA side-effects. Some members of this class of compounds were shown to provide significant relief in animal models of pain states. Therefore, we studied the effects of NR2B antagonists in an experimental model of neuropathic pain in comparison to some other relevant pharmacological agents.

In our experiments neuropathy was performed by placing a section of split polyethylene tubing on the right sciatic nerve of SPRD rats. The cuff-induced sciatic nerve constriction produced long-lasting ipsilateral mechanical allodynia. Withdrawal threshold to the touch of the ipsilateral hind paw was measured with a set of Von Frey hairs ranging from 0.4 to 20 g. The paw withdrawal thresholds remained below 6 g for 3 months.

The opiatergic analgesic, morphine dose-dependently reversed the mechanical allodynia (1-10 mg/kg i.p.), however the effective dose was very high and induced severe side-effects. Gabapentin is a structural analog of GABA, which penetrates the blood brain-barrier. It is commonly used as an anticonvulsant and antinociceptive drug against postherapetic neuralgia. In accordance with its clinical activity, it proved to be effective in our nerve injury model (30-100 mg/kg i.p.). Lamotrigine is a new anticonvulsant, which acts by stabilizing the slow inactivated conformation of a subtype of sodium channels. The compound also reversed the mechanical allodynia in our experiments (10-30 mg/kg i.p.). The selective NR2B antagonists Co 101244 (PD-174494) and Cl-1041 (PD 196860) produced a dose-dependent reversal of mechanical allodynia without causing side-effects at the dose of 3-10 and 10-30 mg/kg i.p., respectively.

Our results have shown that selective NR2B/NMDA receptor antagonists without the induction of side-effects can effectively reduce touch-evoked pain. This finding predicts that this type of molecules may be efficient in the treatment of neuropathic pain in man.

THE EFFECTS OF SUBSTANCE P INJECTED INTO THE RAT AMYGDALA IN THE ELEVATED PLUS MAZE AND IN MORRIS WATER MAZE TESTS

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The substance P (SP) belonging to the tachykinin peptide family has been implicated in processes of learning and memory. This peptide facilitated learning in various tasks when injected into the medial septum or ventral striatum. It also has anixiolytic-like effects when administered in the periphery or into the basal nucleus of the rat ventral pallidum. By means of immunohistochemical or radioimmunoassay methods it was shown that the amygdaloid body (AMY) is rich in SP receptors. The amygdala plays an important role in learning and serious memory deficits develop after lesions of amygdaloid nuclei. This limbic structure has long been thought to be involved in emotional behaviour, and its role in anxiety and conditioned fear has been suggested. The aim of our study was to examine the possible anxiolytic effects of

SP injected into the central nucleus of amygdala (ACE) in the rat elevated plus maze model of anxiety and to investigate its effect on learning in a Morris water maze paradigm.

Microinjections of 100 ng, but not long / 0.5 μl dose of SF significantly increased the amount of time the rats spent on the open arms and on the end of the open arms of the elevated maze. It also increased the number of excursions into the end arms, indicating an anxiolytic-like profil. In water maze test rats receiving immediate post-trial injection of 100 ng, but not 10 ng SF exhibited improved performance over control rats in acquisition trial. Our results show that SF injected into the ACE has anxiolytic-like effects in the elevated plus maze model, it facilitates learning in Morris water maze task, and these effects are dose-dependent.

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DESCENDING FIBERS IN THE BRAINSTEM AUDITORY PATHWAY

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The specific afferents of various order, ascending in the lateral lemniscus (LL) terminate and make synaptic contact with the relay cells and interneurons in the Central Nucleus of the Inferior Colliculus (CNIC). The aim of the present study was to localize the termination of the descending fiber components of the LL originating from the CNIC, where they may influence the incoming information. In the experiments BDA (dextran biotin, 10 000 MW lysine, Molecular Probes) was administered with iontophoretic microinjections and HRP (Peroxidase from horseradish 550 U/mg, Serva) by pressure injection with Hamilton syringe in rat and double labeling was carried out. In deep anesthesia, the animals received first the anterograde tracer into the CNIC, and five days later in a second step the retrograde tracer was injected in the same animal into the contralateral CNIC. Following the optimal survival period the animals were perfused and the brainstem sections were processed according to immunocytochemical procedures and examined with light microscope.

In the experiments the appearance of HRP labelled neurons ipsilaterally in the LL, superior olivary nuclei and in the contralateral dorsal LL, lateral superior olivary and cochlear nuclei confirmed the result of previous studies.

BDA labelled terminals were found in the dorsal and ventral LL, medial and lateral superior olivary and cochlear nuclei ipsilaterally. In all periolivary areas labelled terminals were seen on the side of the BDA injection and the ventral periolivary areas were the only sites on the opposite side where labelled terminals could be observed. In the cochlear nuclei, the labelled fibers - giving off fine collaterals terminating in knob-like fashion - were followed into the cochlear nerve.

On the basis of these findings the descending terminals may play a role in the forwarding processes in the ipsilateral relay nuclei and the cochlear receptor and via the ventral periolivary neurons in the contralateral receptor organ, respectively. The difference in the distribution of the descending fibers compared with the ascending pathways may call attention to the functional significance of the commissural and crossed connections between the brainstem auditory nuclei at olivary, lemniscal and collicular levels.

LOCALIZATION AND NEUROCHEMICAL SPECIFICATION OF GLUTAMATERGIC/ASPARTATERGIC NEURONS IN THE SEPTAL-DB COMPLEX OF THE RAT. A STUDY USING THE [^{3}H]D-ASPARTATE AUTORADIOGRAPHY AND IMMUNOCYTOCHEMISTRY

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The occurrence and topographic distribution of glutamatergic/aspartatergic (glu/aspergic) neurons was studied in the septal-diagonal band (SOB) complex of the rat, in relation to the localization of other neurochemically identified cell groups containing choline acetyltransferase (ChAT), parvalbumin (PV), calretinin (CR) or calbindin D28k (CB). Following microinjections of [^{3}HID-aspartate (^{3}HDA) into the septum itself or into brain regions known to be target areas of septal projection, the occurrence and localization of retrogradely radiolabeled neurons in the SDB were investigated. ^{3}HDA labeled cells in various divisions of the SDB-complex were revealed. Injections into the border area between the medial and the lateral septum resulted in radiolabeled neurons in the medial area of the septum and in the vertical (vDB) and horizontal (hDB) limbs of the diagonal band. The radiolabeled cells do not exhibit ChAT or PV immunoreactivity, while some of them are immunostained for CR or CB. After hippocampal injections a small part of ChAT-immunopositive neurons in the medial septum (MS) and the vDB contained retrogradely transported ^{3}HDA. Radioactive tracer injections into various hypothalamic nuclei resulted in radiolabeled cells exclusively in the lateral septal (LS) subdivisions, while after injections into the supramammillary nucleus, in addition to LS subdivisions, labeled CR- and CB-immunostained neurons were detected in the dorsal part of the MS, the border area between the MS and LS and in the vDB.

These results provide direct morphological evidence on (I) the occurrence of glu/aspergic neurons in the SDB, (2) the topographic distribution and (3) the projections of the septal glu/aspergic neurons. In addition, they suggest the presence of glu/aspergic interneurons in the SDB-complex. The results support the view that glu/aspergic neurons in the SDB, with intra- and extraseptal projections, may play a role in cortical and subcortical functions.

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MECHANISM OF 4-AMINOPYRIDINE BLOCK OF A-CURRENT IN SNAIL NEURONES

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Mechanisms of 4-aminopyridine (4-AP) block of the A-current was investigated in identified RPa3 and LPa3 neurones of *Helix pamatia* L. using two microelectrode voltage-clamp technique. Experiments were performed in Na^{2+} free low Ca^{2+} solution in order to eliminate

currents out of interest. Earlier Thompson (1982) has found that 4-AP produced two kinds of effect on A-currents of *Tritonia* and *Anisodoris:* the first involved the block of open channels, the other was a steady-state block. He concluded that closed channels with open inactivation gates are more susceptible to block than open channels. Thompson developed a model for 4-AP block assuming that there were two independent binding sites for 4-AP. Recently, we have studied the mechanism of 4-AP block in *Helix* and it was observed that 4-AP inhibited A-current in a voltage- and dose-dependent manner. For blocking action binding of two 4-AP molecules were required. Our results suggest that 4-AP block of the A-current also involves block of both open and closed states of the channel, however the open channel block was more effective. To test whether the channel needs to open before block can occur two types of experiments were made: First, the test pulse was preceded by 1000 ms conditioning pulse to –100 mV. Then 4-AP was applied and the cell was depolarized to 0 mV in every 10 s until the block saturated. A 2.5 mm long exposure to 2 mM 4-AP caused 50% block of the peak current. In the second type of experiments, cell membrane potential was held at –100 mV for 4-5 mm in the presence of 2 mM 4-AP before the first depolarizing test pulse was delivered.

As the channel must open before block can take place, one would not expect any block at the beginning of test pulses. However, 10% block was observed already at the first test pulse. Evidences support the hypothesis that open channel block occurs due to the binding within the pore itself. However, the open channel block does not occur through 'trapping' the 4-AP molecule. It is suggested that the second 4-AP molecule binds allosterically causing the steady-state block of the A-channel. Experiments with intracellular protease have suggested the presence of a 'ball-and-chain' inactivation mechanism. Furthermore it was found that inactivating region contains cysteine that renders the inactivating ball domain sensitive to oxidation. Experiments with chloramine-T have suggested that A-channels in *Helix* are RCK4-type rather than Shaker-type.

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THE MODULATORY EFFECT OF ESTROGEN ON THE EVOKED NEURONAL ACTIVITY OF THE SOMATOSENSORY CORTEX IN RAT

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For many years, it has been kown that steroids play an important role in plastic changes of the central nervous system. In an earlier study we demonstrated that systemically administered 17ß-estradiol affected both the spontaneous activity and the somatosensorily evoked activity of the neurons in the arcuate nucleus. Previous studies have shown the presence of estrogen receptors (ERs) in several brain areas, especially in the hypothalamus, but less is kown about

the role of estrogen in the somatosensory cortex. Since the somatosensory cortex has a characteristic structure (barrel), it is extremely suitable for the study of functional plasticity.

The aim of the present study was to examine the influence of iontophoretically applied 17ß-estradiol hemisuccinate on the barrel cortex neurons. Responses were evoked in the barrel cortex by vibrissal deflection and unit discharges were recorded with a carbon fiber electrode surrounded by 6 barrels. Since glutamate has general excitatory effects on neurons, it was used to check on the correct functioning of the recording system. During the monitoring of evoked neuronal activity, 17ß-estradiol hemisuccinate (0.1 mM, pH 7.2) was ejected at —100 nA for 60 seconds.

The results clearly revealed that iontophoretically applied 17ß-estradiol is able to increase the evoked neuronal activity of the somatosensory cortex within 17-40 minutes. This latency suggests that the effect of 17ß-estradiol is on the genomic mechanism rather than on the membrane.

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PLEIOTROPY OF CHANDELLER CELL CARTRIDGES

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Introduction and Problematics. The vertical arrays of terminal boutons of chandelier cells were originally supposed to surround apical dendrites (Szentágothai, 1975); the cartridges were identified later as inhibitory structures attached to pyramidal cell axons (Arbib et al., 1998). Cartridges were shown to contain parvalbumin (PV), GABA (van Brederode et al., 1990), corticotropin-releasing hormone (Lewis and Lund, 1990), Reelin (Pesold et al., 1999), glutamate decarboxylase (Yan et al., 1998), tyrosin hydroxylase (Sesack et al., 1999), calbindin D-28k (Del Rio and DeFelipe, 1997) and the high affinity GABA transporter GAT-1 (DeFelipe and Gonzalez-Albo, 1998). The question arises whether topography of cartridges and analogous structures support their pyramidal selectivity and whether their integrity is dependent on dopamine metabolism.

Results. Immunocytochemical analysis proves that only ~50% of the cartridges are arranged around pyramidal cell axons in area 46 of the primate prefrontal cortex. The rest of the cartridges were localized either around apical dendrites or around axons of interneurons. PV was found to be colocalized with GABA, as demonstrated by post-embedding technique. Pairs of PV-IR axons often proceeded in a parallel array, closely attached to the surfaces of axons and/or dendrites. NPY-IR axons were arranged in a similar strategic localization. In the temporal cortex, analogous structures exert CGRP-IR. Inhibition of tyrosine hydroxylase (with L-α-methyltyrosine) induced degenerative alterations of cartridges.

Discussion and Conclusions. Cartridges seem to represent inhibitory devices in general, not only of pyramidal cells but also of local circuit neurons. NPY may subserve similar function in parallel axonal pairs. A causal correlation is suggested between catecholamine metabolism and

cartridge cytochemistry, probably related to abnormal brain functions characterizing schizophrenia (Lewis and Akil, 1998).

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NR2B SUBUNIT CONTAINING NMDA RECEPTORS' CONTRIBUTION TO THE SPINAL SEGMENTAL REFLEX; AN *IN VITRO* STUDY

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NMDA antagonists, including the NR2B subunit specific ones, have been reported to reduce spinal hyperalgesia and allodynia, phenomena associated with some forms of chronic pain. Wind-up, a frequency dependent potentiation of spinal responses, is considered as an electrophysiological model of the development of spinal hyperalgesia. Typical NMDA receptor antagonists block wind-up in various models. Evoked potentials were recorded from the L ventral root following electrical stimulation of the corresponding dorsal roots (DR-VRP) of hemisected spinal cords isolated from 6-day-old rat pups. Single DR-VRP (evoked by low frequency, i.e. 0.01-0.03 Hz stimulation) is composed of an initial sharp peak, which corresponds to a monosynaptic population action potential (MSR), followed by a long-lasting tonic potential generated mainly by motoneuronal EPSPs. Our previous studies with selective antagonists indicated that MSR is predominantly mediated by AMPA-type glutamate receptors, while both AMPA and NMDA receptors are involved in generating the EPSP. In our present experiments we found that the NR2B selective NMDA antagonist CP 101,606 (1 μM) did not affect MSR. On the other hand it depressed the EPSP partially. The inhibition confined to the post stimulus time window of 10-300 ins, with a maximal effect at around 100 ins. In contrast, APV (40 μM) blocked the whole course of EPSP. Increasing the stimulation frequency to 1 Hz caused a summation of the tonic potential (wind-up). This wind-up phenomenon was significantly depressed by APV but not by CP 101,606. Our findings suggest that NR2B receptors are not involved in the formation of wind-up, since they only play a role in some

early components of the EPSP, and not in the late parts that are relevant to the summation of the tonic potential. The discrepancy between our results and the reported wind-up inhibition by CF 101,606, *in vivo* (Boyce et al., 1999) prompts further investigations.

ASTROGLIAL CELLS SUPPORT THE NEURONAL DIFFERENTIATION OF IMMORTALIZED NEUROECTODERMAL PROGENITOR CELLS

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Neuroectodermal cells of the NE-4C cell line, derived from the forebrain vesicles of 9 days old, p53 deficient mouse embryos, give rise to neurons and astroglia upon induction with all trans retinoic acid (RA) (Schlett and Madarász, 1991). Such multipotential progenitor cells if implanted into the injured brain can participate in regeneration and cell replacement. The fate of the implanted progenitors, however, highly depends on their cellular environment.

To examine the influence of astroglial cells on the fate of uncomitted neuroectodermal progenitors, we performed *in vitro* co-culture experiments. To label the immortalized neuroepithelial progenitors we used vectors encoding modified green fluorescent protein (GFP) or placental alkaline phosphatase enzyme (PLAP). Labeled cells were planted onto pure astroglial cultures, derived from perinatal rat forebrains. A set of the 4C cells, originally characterized by an epithel-like morphology in their non-induced state, displayed branching processes on the surface of glial monolayer from the 4-5th day of co-culture. Detection of co-expression of neuron specific class III-type ß-tubulin and the marker protein GFP or the substrates of PLAP indicated that a portion of the GFP-4C or PLAP-4C cells became neurons. The neuron formation by 4C cells happened in the lack of exogenous retinoic acid. The rate of neuronal induction, however, remained below the rate of neuron production caused by all trans RA. The environment of astroglial cells did not restrain the proliferation of undifferentiated progenitors. The intensively growing clusters of GFP-4C or PLAP-4C cells were usually surrounded by astrocytic processes. Our data indicate that the success of neuronal induction of neuroectodermal progenitor cells is rather due to a close cell to cell contact than soluble factors released into the medium by astroglial cells.

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QUANTITATIVE DIFFERENCES IN THE DISTRIBUTION OF MYENTERIC NITRERG NEURONS ALONG THE LONGITUDINAL AXIS OF THE DEVELOPING HUMAN FETAL INTESTINE

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The aim of the present study was to investigate the quantitative differences in the distribution of the myenteric nitrerg neurons of the small intestine and the colon in the developing human fetal intestine.

Intestinal segments of the week 15 and week 19 old fetuses were obtained immediately after legally approved or spontanieous abortions and were processed for NOS immunocytochemistry or NADPH-diaphorase histochemistry. Camera lucida drawings were used to count nitrerg neurons of randomly selected myenteric ganglia. The neuronal density were calculated as the numbers of stained cells per mm^2. The data obtained from each area and each age group were subjected to two-way analysis of variance (ANOVA) and the Student-Newman-Keuls test.

Between the week 15 and week 19 of gestation the originally narrow-meshed myenteric plexus with its high ganglionic density progressively became wide-meshed and the ganglionic density decreased. The quantitative analysis revealed a significant ($P < 0.001$) decrease in the nitrerg nerve cell density with age. On week 15 gestation the density of the nitrerg neurons were significantly lower in the colon than in the small intestine. By week 19 of gestation this difference was equalized between this two intestinal segments. Camera lucida drawings of the nitrerg cells revealed the non-random distribution of this cell population within myenteric ganglia. The quantitative evaluation of this regional distribution is in progress.

The result of the present investigations indicate that the decrease in the density of the innervation may be due to the marked increase in the musculature, which causes the originally densely packed network to expand and leads to a lower cell density with increasing age.

FUNCTIONAL NEUROANATOMY OF IMMUNE-TO-BRAIN SIGNALING

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There is a bi-directional communication between the immune and central nervous system. Infectious, inflammatory and allergic challenges provoke series of centrally mediated reactions that include adaptive changes in the neuroendocrine, autonomic and behavioral regulation, while central reactions have a great impact on immune responses. In the past decade much has been revealed on the immune mediators and afferent pathways that convey relevant immune-related information from periphery to the hypothalamic centers involved in regulation of stress

response. Stimulation of cellular as well as humoral aspects of immune system results in activation of corticotropin-releasing hormone (CRH)-secreting neurons in the hypothalamic paraventricular nucleus that initiate the stress response and in neurons involved in central autonomic regulation. In addition, various cell types in the circumventricular organs also display immediate-early gene markers of cellular activation in response to cytokine injections. However, the primary interface, where immune mediators gain access to the brain, remains to be explored. Recently, special attention has been focused on the barrier structures as well as on immune cells associated with sensory nerves. Macrophages, dendritic cells and mast cells have been found in intimate connection with vagal afferents and these cells release various mediators that may act in paracrine fashion to stimulate neurons in the nucleus of the solitary tract. Moreover, immune cells are present in the CNS, such as mast cells in the thalamus. Recent findings suggest that these mature cells may enter the normal adult CNS via a vascular route through the blood-brain barrier and their number is changing rapidly depending on the physiological status. In addition, the very recent report that immature bone marrow cells not only entered the brain, but these cells had, upon reaching the brain, become neurons, may change our view of immune-brain communication and may lead to new perspectives in therapy.

THE EFFECT OF Li^+-ION ON THE MEDIAL PREFRONTAL CORTEX OF THE RAT

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For more than 50 years Li^+ has been one of the most important agent in the therapy of manic depression (MD), but the mechanism of its effects remain still unknown. An important factor of the illness is the fluctuance of norepinephrine (NE) levels in certain brain areas. Experiments carried out in the 70s dealt mainly with the effect of Li^+ on NE-receptors. By means of iontoforesis, they found that Li^+ inhibits NE-elicited single-cell supression on cerebellar Purkinje-cells (Siggins et al., 1979). The same effect was also recorded on hippocampal cells (Segal et al., 1974). The experiments of the oncoming years dealt mostly with the effect of Li^+ on second-messenger systems, *in vitro* (Avissar et al., 1988), so there is no evidence of action of Li^+ in forebrain regions proved to be important in MD, such as medial prefrontal cortex (mPFC) or striatum. In our experiments we examined *A)* the effects of intravenous Li^+ injection on single unit activity of mPFC neurons; *B)* the effects of Li^+ on the NE-elicited neuronal activity when extracellularly injected directly to mPFC neurons.

According to our present results, *A)* Li^+ decreases the cell activity of most mPFC cells, *B)* Li^+ suppresses NE-elicited reduction of neural activity, and even inhibits the excitation induced by smaller concentrations of NE. Recordings showed the similar action of Li^+ in the mPFC like in other regions of the brain, which were previously proved to be parts of the catecholaminergic system. In contrast, Li^+ had no obvious effect on neural activity in areas suggested to be indifferent for MD. Our present study indicates that Li^+ does not only suppresses NE-elicited reduction of cellular activity, but also inhibits the excitation caused by smaller concentrations of NE. Therefore, the action of Li^+ may be to set a relatively steady-

state activity level of neurons, independently from the oscillations of endogenous NE levels. Our results, in line with observations carried out in the 70s, may give more details to clarify the problem of the 50 years unsolved cellular actions of Li^+.

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ESTIMATION OF THE *IN VIVO* NUCLEOSIDE CONCENTRATIONS IN THE HUMAN BRAIN BASED ON ANIMAL MODEL EXPERIMENTS

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Nucleoside derivatives are widely used as immunosuppressants, anti-tumour and anti-viral agents which often induce neurological side effects. For this reason, there is an increasing need to understand the role of nucleosides in the central nervous system.

One of the essential steps to understand the role of nucleosides in normal and pathological human brain is the mapping of nucleoside distribution in different human brain structures. After this, noteworthy step would become possible to find correlation between the anatomical changes and nucleoside concentrations in different brain areas.

The analysis of *post mortem* tissue samples from human brain is the only possibility for the mapping brain tissues for nucleosides. Our human brain samples were dissected from cerebral and cerebellar cortex and white matter within 2 hours after death. These samples are the most reliable *post mortem* brain samples actually available. However, because of the rapid metabolism the concentration of nucleosides and its metabolites differ from that in living tissue.

To make a reliable estimation of the real *in vivo* tissue concentration of nucleosides we developed a model experiment on rat. Based on this experiment we determined back-extrapolation coefficients for each compound calculated from the *in vivo* and the *post mortem* rat brain tissue concentrations.

We found that our rat model experiment is valid to estimate the *in vivo* nucleoside concentrations in human brain. The average *in vivo* concentration of nucleosides in human brain are as follows: adenosine 19.65 pmol/mg, inosine 97.81 pmol/mg, hypoxanthine 103.64 pmol/mg, xanthine 37.92 pmol/mg, guanosine 18.20 pmol/mg, uridine 90.56 pmol/mg, uracil 3.94 pmol/mg.

THE ACTIVATION OF UROCORTIN IMMUNOREACTIVE NEURONS IN THE EDINGER-WESTPHAL NUCLEUS FOLLOWING ACUTE PAIN STRESS

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Urocortin (UCN), a member of the corticotropin releasing factor (CRF) peptide family, has a 45% sequence identity to CRF. Urocortin is ten-times more potent than CRF in increasing cAMP in cells expressing the CRF_2 receptor, therefore it was postulated to be an endogenous ligand for this receptor. Urocortin possesses the biological activity of CRF, and by activating the CRF_2 receptors, it can directly affect autonomic functions and play an important role in modifying the efferent components of endocrine, immune and behavioral responses to stress.

Although UCNs distribution in the rat brain has been demonstrated, with the most abundant urocortin-ir perikarya present in Edinger-Westphal nucleus (EW), little is known about the physiological significance of brain urocortin. Since immediate early gene expressions were seen in several midbrain regions, such as in the EW, following acute stress, we hypothesized that acute pain stress can result in the activation of the urocortinergic neurons in the EW.

Immunoreactivity of the protein product Fos, of the immediate early gene *c-los,* was used as a marker of cellular activity. Double-label immunohistochemical and double label immunofluorescence techniques were used in an acute pain stress (APS) model to reveal the colocalization of Fos-immunopositivity with UCN-immunoreactivity (ir) within the EW.

Our results showed that acute pain stress resulted in the activation of UCN-ir neurons in the EW peaking at 4 h after APS, based on the colocalization of Fos-ir with UCN-ir, and the upregulation of the urocortin transcripts in the EW. Based on these results, we suggest that the EW belongs to brain areas, which are chronically activated upon by painful stimuli, rather than immediately respond to stress.

CODES, OPERATIONS, MEASUREMENTS AND NEURAL NETWORKS

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More problems than solutions emerged in studies of either neural codes or neural operations. The classical, influential findings came first from Weber, Fechner later from Hartline or Boole and McCulloch and Pitts, respectively.

Pulse frequency coding for periphery and "immanent" logical operations concerning central parts of brain became accepted as realities, albeit demanding further details and supplements.

In 1967 Perkel and Bullock listed already more than 30 candidate mechanisms for coding. As to operations, beyond logical ones, arithmetical operations also appeared on the scene.

Early efforts followed the few relevant experiments, but later analogies of technical and mathematical origin became dominant.

This presentation accents *automatic measurements* as a plausible neural mechanism, which means that neurons and their networks can be regarded as measuring devices in strict terms of mathematical theory of measure. Such a general framework may treat both logical and arithmetical operations and can be applied also to a broad range of possible codes. Such a way of coding has already been proposed many years ago by the author without detailed elaboration.

Simulation methods were used. Equations for neurons are piecewise linear interval maps. The units are special measuring devices or counters with dead time. Nearly 20 different logical and arithmetical operations were demonstrated with matrix like mathematical objects.

The strict conditions at which spike record counts behave precisely as mathematical measures were described. Here *special subadditivity* and *refractoriness* are the critical properties or condition, respectively.

Among others, the role of single spikes and bursts, various transitional logical gates, approximate operations, phenomena arising in huge networks, limits of measure-property, interplay of excitation and inhibition, different feedback, operations which are between arithmetical and logical ones as well as conditions and role of supra-additivity of spikes in converging neuronal lines were discussed.

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ROLE OF GROUP I AND GROUP II METABOTROPIC GLUTAMATE RECEPTORS (mGluRs) IN THE 4-AMINOPYRIDIN-INDUCED CORTICAL EPILEPTIFORM ACTIVITY IN RAT

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The mGluRs, include at least eight receptor subtypes grouped into three subclasses (I-III), have diverse effect on the cellular and synaptic properties of neurons. Several studies suggest that mGluRs may play a role in neuronal plasticity and epileptogenesis. Group I mGluRs proved to be proconvulsant in many *in vitro* experimental model of epilepsy as opposed to group II and III mGluRs, which have been shown to be rather anticonvulsant.

In the present study, our aim was to investigate the function of group I and II mGluRs in the 4-Ap-induced cortical epileptiform activity *in vivo,* in anaesthetized rats. In one group of the animals somatosensory cortical surface was locally treated with (1S,3R)-ACPD ((1S,3R)-1 - amino-1,3-cyclopentanedicarboxylate) an agonist of both group I and H mGluRs, in other group with DCG-W ((2S, 1R,2R,3R)-2-(2,3-dicarboxycyclopropyl)glycine) a selective agonist of group II mGluRs for 60 mm before the induction of seizure. In the third group the already established epileptic focus was treated with ACPD.

Pre-treatment with ACPD provoked rhythmic discharges by itself and highly enhanced the induction of seizure activity and propagation of discharges. On the other hand, application of

ACPD in the already established epileptic focus significantly inhibited the expression of ongoing seizure activity. Pre-treatment with DCG suppressed the induction of seizures, significantly reduced the amplitudes of epileptiform discharges and almost completely prevented their propagation to the contralateral hemisphere.

Based on these results and information available in the literature, we suppose that in the case of ACPD pre-treatment the repetitive discharges and the following high seizure susceptibility can be the consequence of the predominant overactivation of group I mGluRs. The proconvulsant effect of group I mGluRs is probably mediated through promoting ionotropic glutamate receptor-dependent excitatory synaptic process. On the other hand, the reduced epileptiform activity, observed both after ACPD treatment in the already established focus and in the case of DCC pre-treatment, might be explained by the state-dependent activation of group II mGluRs. It is supposed that group II mGluRs diminish the induction, maintenance and propagation of highly synchronized epileptiform discharges probably by blocking presynaptic transmitter release.

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EFFECT OF STIMULUS RATE ON THE AUDITORY SSR IN CAT AND MACAQUE MONKEY

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High frequency repetitive auditory stimuli elicit steady-state response (SSR) in the gamma-band both in humans as well as in mammals. The purpose of the present study was to investigate whether there is an optimal stimulation frequency which elicits high amplitude auditory SSR in cat and in macaque monkey.

We carried out our experiments on three cats and two monkeys. An epidural electrode matrix was chronically implanted above the auditory areas of the neocortex in cats. Electrodes were placed epidurally in the monkey similar to the 10-20 standard in human. Tone bursts (1 ms, 2000 Hz) served as auditory stimuli. The tone bursts were delivered in a train, where ISI remained constant for 2 s than decreased in 1 ms steps from 44 ms to 22 ms (21/s – 46/s). One session lasted about half an hour and during this time we delivered 20 trains with half a minute brake between each train. During the experiments the cat was resting in the home-cage, and the monkey was sitting in a monkey chair.

The power of the SSR for each stimulus rate was calculated by FFT analysis after averaging single trials in the time domain. Topographic distribution of the transient auditory evoked potential components and the SSR were displayed on amplitude distribution maps.

We found that:

- The amplitude of the SSR was largest when the stimuli were presented at a rate of 27-29/s in cat, and 29-33/s in monkey.
- The "optimal stimulation frequency" differed among individual animals.
- SSR was much more prominent in the first half of the session, than in the second and the waveform of the SSR was also different. This was caused probably by the decrease

of arousal. If we "activated the animal" (e.g. gone into the cage or gave her some food) the SSR increased again.

- Topographic distribution of the transient auditory evoked potential components and the SSR was different in both cat and macaque monkey.

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NEUROENDOCRINE MECHANISMS FOR THE ACTION OF LEPTIN ON THE HYPOTHALAMIC-PITUITARY-THYROID (HPT) AXIS

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The biosynthesis and secretion of thyrotropin-releasing hormone (TRH) in hypophysiotropic neurons of the paraventricular nucleus (PVN) are regulated by a negative feedback control mechanism that depends on circulating levels of thyroid hormone. During fasting, however, when thyroid hormone levels fall, a seemingly paradoxical reduction in hypophysiotropic TRH and inappropriately normal or low plasma TSI-I are observed, consistent with central hypothyroidism. These alterations are part of an important homeostatic control system necessary to allow conservation of energy during fasting and can be completely reversed by the systemic administration of leptin. Thus, leptin maybe the single most important peripheral signal to the hypothalamus that allows resetting of the set point for feedback regulation of hypophysiotropic TRH by thyroid hormone.

Since ablation of the hypothalamic arcuate nucleus abolishes the homeostatic response of fasting to reduce thyroid hormone levels and the ability of exogenous leptin to restore the thyroid axis, the central action of leptin on hypophysiotropic TRH may be exerted indirectly, primarily via the arcuate nucleus. Several arcuate nucleus-derived peptides have been identified that may mediate the actions of leptin. Neuropeptide Y (NPY), a peptide with potent orixogenic activity, is present in axon terminals that establish numerous symmetric synapses with TRH-producing neurons in the PVN, suggesting an inhibitory function. Indeed, intracerebroventricularly administered NPY to *ad lib* fed animals or animals pair fed to artificial CSI-infused controls, results in marked suppression of proTRH mRNA in the PVN and suppressed circulating levels of thyroid hormone and TSH, despite an increase in circulating leptin levels. Similar inhibitory effects on TRH secretion are observed with agouti related protein (AGRP), an orixogenic peptide that coexists with NPY in the same arcuate nucleus neurons.

Ablation of the arcuate nucleus, however, is associated with a significant fall in circulating levels of thyroid hormone and TSH, and does not result in an increase in TRH gene expression the PVN as might be expected if NPY and AGRP were the only arcuate nucleus-derived peptides regulating hypophysiotropic TRH. In addition, the binding of AGRP to the melanocortin receptor would suggest the possibility of a stimulatory role of α-MSH on regulation of the TRH gene. Along these lines, axons containing α-MSH that arise exclusively in the arcuate nucleus, do establish synaptic contacts with TRH neurons in the PVN,

particularly in the periventricular parvocellular subdivision. In addition the intracerebroventricular administration of α-MSH to fasting animals can completely reverse the effect of fasting on TRH gene expression but is not capable of fully restoring suppressed thyroid hormone levels to normal. Similar stimulatory effects are observed with cocaine- and amphetamine-regulated transcript (CART), a protein that coexists with α-MSH in arcuate nucleus neurons.

The arcuate nucleus, therefore, has an important role in the modulation of hypophysiotropic TRH neurons through direct inhibitory and stimulatory pathways, mediating the central actions of leptin on the HPT axis. NPY, AGRP, α-MSH and CART, however, are likely only a few of other potential candidates that may affect TRH neurons in the PVN.

EFFECT OF VINPOCETINE ON THE VERATRIDINE-EVOKED [CA^{2+}] RESPONSES IN HIPPOCAMPAL SLICES

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The alkaloid derivative vinpocetine has a well-known beneficial effect on the brain in hypoxic or ischemic conditions. It increases DNS blood flow, improves cell metabolism, but relatively little is known about the underlying cellular mechanisms on the single cell level.

Apoptotic or necrotic cell damage is always preceded by an increase in $[Ca^{2+}]_i$. We used a cooled CCD camera based ratio imaging system to follow the effect of vinpocetine on $[Ca^{2+}]_i$ changes in single pyramidal cells in the vulnerable CA1 region of rat hippocampal slices. Cells were loaded with fura 2/AM and the drugs were applied to the superfusion. The ischemic attack was stimulated with veratridine. This alkaloid activates Na^+ influx, causes depolarization, and $[Ca^{2+}]_i$ increases in the cells.

Preperfusion and continuous administration of vinpocetine (10 μM) inhibited the elevation of $[Ca^{2+}]_i$ induced by veratridine. When the drug was administered after the veratridine effect had already occurred, it could speed up the recovery of cellular calcium levels.

It is concluded that vinpocetine, in a pharmacologically relevant concentration, could inhibit pathologically high $[Ca^{2+}]$ elevations in individual pyramidal neurons and this effect might contribute to the neuroprotective effect of the drug.

AN UNUSUAL PHOSPHORYLATION OF Ca^{2+}/CALMODULIN-STIMULATED PROTEIN KINASE II: A POSSIBLE ROLE FOR ZINC IN SYNAPTIC TRANSMISSION

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Calcium and calmodulin-stimulated protein kinase II (CaMPK-II) is a key regulatory enzyme in living cells. Modulation of its activity, therefore, could have major impact on many cellular processes. We found that Zn^{2+} has multiple functional effects on CaMPK-II. Zn^{2+} i) generated a Ca^{2+}/CaM independent activity which correlated with the autophosphorylation of Thr^{286} ii) inhibited Ca^{2+}/CaM binding which correlated with the autophosphorylation of Thr^{306} and most likely a direct binding of Zn^{2+} to CaMPK-II; iii) inhibited CaMPK-II activity at high concentrations which correlated with the autophosphorylation of Ser^{279} The relative level of autophosphorylation of these three sites was dependent on the concentration of zinc used. Autophosphorylation of Thr^{306} did not require the prior phosphorylation of Thr^{286}, as occurs with Ca^{2+}/CaM. The simultaneous autophosphorylation of at least these three sites, together with Zn^{2+} binding generated an increased mobility form of CaMPK-II on SDS gels. Overall, autophosphorylation induced by Zn^{2+} converts CaMPK-II into a different form than the binding of Ca^{2+}/CaM. In certain nerve terminals, where it has been shown to play a neuromodulatory role and is present in high concentrations, Zn^{2+} may turn CaMPK-II into a form that would be unable to respond to calcium signals.

FEEDING CONSEQUENCES AND TASTE REACTIVITY OF VENTROMEDIAL HYPOTHALAMIC STREPTOZOTOCIN MICROINJECTION IN THE RAT

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Streptozotocin (STZ), originally used for the treatment of insulinomas, and also for producing the animal model of /type II/diabetes mellitus leads to the development of various diabetes-like alterations when injected directly into the ventromedial hypothalamic nucleus (VMH). Specific 'glucose-monitoring' (GM) neurons are known to be located - in addition to other forebrain and brainstem structures - in the VMH. Our previous experiments showed that STZ induced selective destruction of these GM cells caused alterations of feeding and metabolism. To

further study the hypothesized role of forebrain GM neurons in the homeostatic control - and to examine the possible contribution of gustatory mechanisms to development of the above symptoms -, acute and chronic effects of STZ was studied on feeding and metabolic functions of male Wistar rats. A single bilateral STZ microinjection into the VMH not only elicited alterations of plasma metabolite (uric acid, triglycerides and cholesterol) concentrations, and pathological elevation of blood glucose levels in acute as well as in chronic glucose tolerance tests, but the central application of STZ also led to deficits of food intake in response to physiological challenges (i.p. insulin or 2-DG). In addition, characteristic disturbances of taste reactivity to pleasant and unpleasant gustatory stimuli were observed as well. As histological analysis of the pancreas showed normal structure of Langerhans islets in all STZ treated animals tested, the present findings further substantiate the role of altered functioning of the ventromedial hypothalamic GM neural network in diabetes-like homeostatic disturbances. Furthermore, our electrophysiological and behavioral data indicate the involvement of altered gustatory functions in the above feeding deficits.

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CHOLINERGIC NEURONS RECEIVE NEUROPEPTIDE-Y-ERGIC AFFERENTS IN THE HUMAN BASAL FOREBRAIN

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Cholinergic neurons distributed in the basal forebrain (BE) have a crucial role in the orchestration of arousal and attention. The regulatory mechanisms of the human cholinergic system are mostly in obscurity. Neuropeptide-Y (NPY) has recently been found to participate in the regulation of cholinergic cells of the rat BF (Záborszky and Duque, 2000). The present study was undertaken to reveal the role of the central NPY-ergic system in the putative modulation of the cholinergic cell population of the human BF. For mapping the two distinct neuronal systems and exploring their communication, a sensitive immunocytochemical double labeling study was used that utilizes diaminobenzidine (DAB, brown) and silver-gold intensified DAB (black) chromogens (Liposits et al., 1986). The study was carried out on sections obtained from *post mortem* human brains (Ethics Board permission No: 372). The anti-NPY serum was raised in rabbit and used at 1:5000 dilution. For the detection of the central cholinergic system, its nerve growth factor receptor expression was traced by means of a highly specific monoclonal antibody (anti-p75, mouse; dilution 1:5000). NPY-ergic fibers formed a dense plexus in the BF. Both coarse and fine varicose axons were observed to innervate the region. In addition to the fibers, small-sized NPY-immunoreactive (IR) neurons also appeared in the BF, many of them intermingled with the large-sized cholinergic neurons. The cholinergic cell clusters were embedded into reach networks of NPY-IR axons. The NPY axons approached the neurons and established morphological connections with them. Frequently, multiple NPY axons targeted a single cholinergic neuron. The fibers formed

terminal button and *en passant* type connections. The cholinergic neurons received the NPY-ergic terminals on their cell bodies and dendrites. The NPY-ergic input seemed to arise from local neurons scattering in the BE and also from long projection systems. The present results indicate that neuropeptide-Y is involved in the neuronal regulation of cholinergic cells of the human forebrain and its role should be considered in physiological processes and pathological alterations of the BF.

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REGENERATION OF THE SEROTONERGIC ELEMENTS AFTER REMOVAL OF THE CEREBRAL GANGLION IN *EISENIA FETIDA*

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The annelid nervous system is a commonly used model in invertebrate neuroscience research. The capacity of the invertebrate nervous system to regenerate provides models for studying mechanisms of neural regeneration. Morphological and functional regeneration takes place not only if segments from the ventral cord are extirpated, but also after removal of the cerebral ganglion. Serotonin is one of the major neurotransmitters in the invertebrate nervous system, including annelids. The distribution of serotonergic elements in annelidas is well known. In the present study we give a detailed description of the time course of the regeneration process following the removal of the cerebral ganglion, using a highly specific serotonin immunohistochemical method.

The regeneration of the cerebral ganglion after removal was examined daily during the first 10 days, every two days between days 10-20, then every 5 days until day 45 and then every 10 days till the 70th postoperative day. Morphological regeneration of the cerebral ganglion takes place rapidly after the removal of the ganglion. Forty days after operation, the cerebral ganglion regains its original shape and structure. From the first days of regeneration, increasing number of serotonergic fibers grow from the circumpharyngeal connectives to the scar tissue, finally building up the neuronal network of the cerebral ganglion. Most of the fibers grow from the connectives, a smaller portion of the fibers seem to arise from the pharyngeal wall. During regeneration, serotonergic perikarya are found to appear from day 25, and the intact cell number is reached at the end of the examination period. In contrast, the number of serotonergic neurons in the subesophageal ganglion is less than in intact animals throughout the entire observation period. The changes found in the subesophageal (first intact) ganglion implies that the subesophageal ganglion plays a role in the regenerating process.

GUSTATORY DISTURBANCES AFTER STREPTOZOTOCIN MICROINJECTION INTO THE ORBITOFRONTAL CORTEX (OBF) IN THE RAT

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In addition to demonstrating involvement of OBF neurons in taste information processing, our previous studies also provided evidence for that intraorbitofrontal cortical microinjection of streptozotocin (STZ), originally used for the treatment of insulinomas, and also for producing the animal model of /type I/ diabetes mellitus, leads to the development of various diabetes-like homeostatic alterations. To further examine the hypothesized role of orbitofrontal - supposedly so-called 'glucose monitoring' (GM) - neurons in the central (behavioral) regulation of homeostatis, and, in more specific, to examine the possible contribution of gustatory mechanisms to development of the above symptoms, effects of STZ was studied on general taste reactivity and the acquisition of conditioned taste aversion (CTA) of male Wistar rats. A single bilateral STZ microinjection into the OBF, as shown previously, elicits alterations of oral glucose tolerance, also of some plasma metabolite concentrations, and leads to deficits of food intake in response to physiological challenges (i.p. insulin or 2-DG). In the present experiments, characteristic disturbances of taste reactivity to pleasant (e.g. sweet or very mild salty) and unpleasant (e.g. bitter or sour) gustatory stimuli were seen in STZ treated animals. In addition, differential neophobia to saccharin and citric acid was also observed. By contrast, acquisition of CTA remained undisturbed after STZ microinjection into the OBF. Our findings, along with previous electrophysiological and behavioral data, provided further evidence for involvement of the orbitofrontal cortical GM neural network in gustatory information processing. The contribution of altered taste perception to the STZ induced feeding deficits is indicated as well.

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FINE STRUCTURAL COMPARISON OF THE RETINA IN MICRO- AND MEGACHIROPTERAN BATS

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Due to their nocturnal lifestyle and echolocation, the retina of bats has a special role in orientation and timing circadian rhythm. As there are few data available on this issue; in the present paper, we compared the retinal fine structure and immunocytochemistry in species belonging to the *micro-* and *megachiroptean* suborders.

We found that all examined *mirochiroptean* species *(Myotis blythi oxignatus, Rhynolophus ferrum equinum, Taphozous longimanus; Scotophylus hethai, Scotophylus temmeckii)* have small sized eye and reduced retina, corresponding to the fact that these animals navigate with well-developed echolocating organs. The photoreceptors and neurons exhibit a weak-to-medium immunoreaction for excitatory amino acids, glutamate and aspartate. The synaptic zone contains ribbon-type synapses, with presynaptic glutamate accumulation.

In contrast to this, the retina of the fruit-eating bats - *Cynopterus sphinx* and *Rousettus niloticus,* both members of the megachiroptean suborder -, has an unusual structure by forming folds and crypts in its photoreceptor layer, significantly increasing the number of receptor cells available for light detection. Immunocytochemistry was also performed for glutamate and aspartate, and showed practically the same pattern of immunoreaction, as in other species. The use of peanut agglutinin lectine (PNA) and antibodies against different cone types revealed that the *Megachiroptean* retina contains a significant cone population, constituting about 1-5 percent in *Cynopterus sphinx,* while being present in about an order of magnitude lower numbers in *Rousettus niloticus.*

Since this unusual folded retina is not capable of decoding two-dimensional images, we suppose that this peculiar organisation is connected to a "pineal-like" photometer task of the eye of these night-time flyers. For this reason, we also compared the structure and immunocytochemistry of the pineal and the retina in bat species and found an unusual degree of resemblance.

Further examinations are planned to access the functional significance of the pineal-like organisation of the retina in these species and to decide wether the photoreceptor-like pinealocytes are capable of light detection, or receive light information from the retina via afferent nerve fibres as it has been proposed in other mannnals.

EFFECTS OF GALANIN ON ACUTE AND CHRONIC MORPHINE ACTIONS IN MICE

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Galanin is expressed in the central and peripheral nervous system where acts as a neurotransmitter/neuromodulator. Galanin-like immunoreactivity and receptors are found in dorsal root ganglion cells and in dorsal horn interneurons, suggesting that this neuropeptide may have a role in sensory transmission.

We tested the effects of the peptide treatment given intracerebroventricularly on pain sensitivity, morphine analgesia and on tolerance to and dependence on chronic morphine administration.

Heat-radiant tail-flick method was used to assess the nociceptive threshold in CFLP mice. Galanin given intracerebroventricularly itself had an analgesic effect, and it increased the analgesic effect of morphine. Galanin significantly increased the chronic tolerance to morphine.

Our results indicate that galanin has an analgesic effect, and also interacts with acute and chronic effects of morphine.

CHANGES OF CALCIUM BINDING PROTEIN-CONTAINING INTERNEURONS IN THE CA1 REGION OF THE EPILEPTIC HUMAN HIPPOCAMPUS

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The types and distribution of calcium binding protein-containing {calretinin (CR), calbindin (CB) and parvalbumin (PV)} interneurons were analyzed in the hippocampal tissue surgically removed from temporal lobe epileptic patients, and compared to the normal human hippocampus (2-4 h *post mortem* samples, immersly fixed or perfused). The CA1 region of the epileptic samples showed different degrees of hippocampal sclerosis. Changes in the number, distribution and morphology of the examined interneurons correlated well with the degree of sclerosis. The PV-positive interneurons disappeared from the CA1 region, only a small number of them survived in the stratum oriens. The number of CR-positive interneurons decreased unevenly in the epileptic tissue: part of the cells in the stratum lacunosum-moleculare survive, whereas others with radially oriented dendrites in the strata pyramidale and radiatum tend to disappear. Number of CB-immunoreactive interneurons is reduced in the CA1 region, although they are preserved in the dentate gyrus. In samples with severe sclerosis morphologically abnormal CB-positive interneurons can be observed with profusely arborizing dendrites, somatic and dendritic spines. Electron microscopy showed that the synaptic input of these cells was decreased, their somata and dendrites were partially covered with glial processes. CB-positive cells are the least sensitive in the CA1 region, although their morphology and input-output connections are altered, suggesting functional changes in the inhibitory network of the epileptic CA1 region.

ANTERO- AND RETROGRADE CONNECTIONS OF THE DESCENDING VESTIBULAR NUCLEUS IN THE RAT

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The primary afferent fibers originating in the vestibular sense organs terminate in the superior (SVN), medial (MVN), lateral or Deiters (LVN) and descending (DVN) vestibular nuclei of the brainstem. The functional differences of these nuclei can be related to the different innervation patterns by the primary afferent fibers originating in different labyrinthine organs, and to the different projections of these nuclei to brainstem and spinal cord structures. Despite the large number of electrophysiological and morphological experiments, the data are

contradictory about the organization of integrated function of these projections. In our previous experiments we have studied the brainstem and spinal cord projection of the MVN, SVN and LVN in the rat. The aim of this study is to investigate the central anterograde- and retrograde connections of the DVN.

The neurobiotin was injected into the descending vestibular nucleus (DVN) in order to study its ascending and descending projections. The following areas of termination could be discerned: (1) In the diencephalon the labeled fibers of DVN origin were less numerous than the fibers of MVN, LVN and SVN origin and were detected bilaterally. (2) At the level of the mesencephalon the terminals were found bilaterally in those areas which are involved in the eye movements. Labeled terminals were also detected in the red nucleus. (3) At the level of the pons the ipsilateral and contralateral vestibular nuclei received fibers from the DVN establishing the intrinsic and commissural connections, respectively. A large number of fibers terminated in the abducens nuleus, the nucleus of prepositus hypoglossi, nucleus of solitary tract and the dorsal part of the reticular formation. (4) In the medulla oblongata the ventral and dorsal medullary reticular field, spinal nucleus of trigeminal nerve, nucleus of the solitary tract, gracile and cuneate nuclei. (5) In the spinal cord the descending fibers could he followed mainly in the ipsilateral anterior funiculus. The terminals were distributed in the Rexed laminae V, VI, VII, VIII and IX. Some of the descending fibers were found in the lateral and posterior funiculi. The retrogradely labeled cells were found in the following areas: vestibular nuclei, parvocellular reticular nucleus, intermediate reticular nucleus, nucleus of prepositus hypoglossi, spinal nucleus of trigeminal nerve, ventral and dorsal medullary reticular field, spinal nucleus of trigeminal nerve, nucleus of the solitary tract, gracile and cuneate nuclei. Our findings are in good agreement with the results of the physiological experiments.

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PHARMACOLOGICAL INTERVENTION OF NEURONAL DEGENERATION UNDERLYING ACUTE STROKE AND TRAUMATIC BRAIN INJURY: NEUROPROTECTIVE EFFICACY OF 5-HT_{1A} RECEPTOR MEDIATED MECHANISMS

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Ischemic brain damage caused by stroke or traumatic brain injury is characterized by an immediate depletion of cellular energy levels. Massive ion fluxes across the plasma membrane and breakdown of the energy-driven membrane potential induces liberation of neurotransmitters into the extracellular space, in particular the excitotoxin glutamate [1, 12, 13]. Excess of glutamate leads to a continuous activation of NMDA, AMPA and metabotropic glutamate receptors, resulting in massive calcium influx and mobilization of intracellular calcium stores. An extensive and long-lasting rise in intracellular calcium levels represents a non-physiological stimulus, triggering various intracellular processes including activation of lipases and nitric oxide synthase, formation of oxygen free radicals and release of neurotransmitters as glutamate, dopamine, serotonin etc. [2, 6, 7]. As a result of this cascade

neurons finally die and represent new sources of neurotoxic glutamate [14]. Based on the prominent role of glutamate, strategies for development of pharmacological principles interfering with the cascade of ischemia mediated cell death have initially focused on receptors, which trigger the neurotoxic effects caused by glutamate. So far, most glutamate receptor antagonists investigated in clinical trials revealed no therapeutic efficacy due to unfavorable risk-benefit ratio or lack of efficacy [3]. Alternative strategies to attenuate glutamate mediated toxicity have been set up. These approaches include inhibition of second messenger cascades involved in glutamatergic signaling and blockade of ion channels which may counteract excessive ischemia-induced neuronal depolarization. 5-HT_{1A} receptor agonists have been tested in various models of ischemic damage and have revealed some neuroprotective efficacy *in vivo* and *in vitro* [11]. Recently, the aminomethylchroman derivative Repinotan hydrochloride (Repinotan HCl) was described as a novel highly potent 5-HT_{1A} receptor full agonist with pronounced neuroprotective properties in various animal models of ischemic brain injury [4, 5].

Repinotan HCl administered as infusion for 4 h immediately after occlusion displayed pronounced neuroprotective efficacy, by 65% infarct volume reduction after 7 days at 3 and 10 μg/kg/h in a rat stroke model (permanent occlusion of the artery media cerebri [pMCA-O]) [8, 9]. The wide U-shaped dose-response curve suggests no significant loss of efficacy in the dose range from 1 to 20 μg/kg/h. The infarct volume was significantly reduced even when the i.v. infusion of the drug (0.003 - 0.01 mg/kg/h) started 1 hour (63% reduction of the infarct volume) or 3 hours (48% reduction of the infarct volume) after the occlusion. Five hours delay of the treatment resulted in a moderate, not significant neuroprotection (26% reduction of the infarct volume). That the neuroprotective efficacy of Repinotan HCl is mainly mediated by activation of 5-HT_{1A} receptors could be proven using the 5-HT_{1A} receptor antagonist WAY 100635. The neuroprotective efficacy of Repinotan HCl could be blocked in the pMCA-O model when administered together with the specific antagonist. As expected from *in vitro* findings Repinotan HCl, administered as a bolus i.v. injection directly after MCA-O inhibited the ischemia induced endogenous glutamate release by approximately 50% at 1 and 10 μg/kg [10]. In a traumatic brain injury model of the rat (subdural hematoma) Repinotan HCl was as effective and potent as in the rat model of stroke, displaying strong infarct volume reduction by 76% at 1 μg/h/kg if administered immediately after trauma [8, 9]. Again, Repinotan HCl displayed a wide U-shaped dose response curve and showed significant neuroprotective efficacy after 5 h delay of treatment. Moreover, in behavioral tests subsequent to the induction of acute subdural haematoma pronounced sensorimotor deficits (grasping reflex, forelimb flexion, abnormal body twisting) were seen for at least one month. After a multiple post-injury i.v. bolus administration of 0.01 mg/kg Repinotan HCl (immediately, 2 hours and 4 hours after injury) these functional impairments were significantly improved

Taken together, these data demonstrated that Repinotan HCl is a promising candidate for treatment of acute ischemic stroke and traumatic brain injury.

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PARALLEL PROCESSING IN THE STRIATOTEGMENTAL SYSTEM OF THE CHICK *(GALLUS DOMESTICUS)* - A DOUBLE RETROGRADE PATHWAY TRACING STUDY

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The ventral tegmental area (AVT) and the substantia nigra (SN) are functionally similar regions involved in the initiation and co-ordination of movements, as well as in the regulation of movement-related limbic functions. The AVT, however, is mainly involved in limbic processes, whereas the SN is implicated both in limbic and motor functions. Striatate neurons projecting to the SN or the AVT are located in the same visceral/limbic striatal subregions and ventral pallidum. The striatonigral pathway also originates from the somatic striaturn (paleostriatum augmentatum, PA). We used a double fluorescent retrograde tracing technique (fast blue and Texas red labelled latex beads) to clarify whether the same visceral/limbic striatal neurons project to both the AVT and SN in a divergent fashion, or these neurons are distinct, albeit located in the same striatal subregions.

The position of neurons retrogradely labelled from the SN or AVT showed a considerable overlap in the the medial lobus parolfactorius, nu. accumbens, tuberculum olfactorium, bed nucleus of the stria terminalis and ventral paleostriatum. The PA was only labelled from the SN. A mere 0.22% of all labelled neurons in the striatum were double labelled.

According to these results, the AVT and the SN are innervated from the same visceral/limbic striatal subregions. At the cellular level, however, striatonigral and striatoventrotegmental neurons belong to almost completely separate neuronal populations. Consequently, information passing from the striatum to the AVT and SN is processed by parallel, rather than divergent, pathways. This anatomical situation implies that, in birds, distinct striatal regions with similar 'cortical' input participate in parallel locomotor (somatomotor) and limbic signal processing simultaneously.

RAPID NON-GENOMIC GLUCOCORTICOID EFFECTS ON AGGRESSIVE BEHAVIOUR

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In the present study we investigated the interaction between acute changes in plasma glycocorticoid levels and the aggressive response given to the occurrence of a potential opponent. Ultradian oscillations in the plasma concentration of glucocorticoids induced oscillations in the propensity to behave agressively in male Wistar rats. The increasing phase of the oscillations was characterized by higher aggressiveness than the decreasing phase of the oscillations. When such oscillations in plasma glucocorticoids were induced by combined treatments with the corticosterone synthesis inhibitor methyrapone and corticosterone there also was a positive correlation between corticosterone levels and aggressiveness (corticosterone is the main natural glucocorticoid of this species). In an attempt to establish the velocity of the effets corticosterone was administered just a few seconds before an agressive encounter to animals in which the endogenous secretion of corticosterone was inhibited by methyrapone. Offensive threats but not attack counts were increased in the following 5 min in the corticosterone-treated group as compared with the vehicle-treated groups. It occurs that glucocorticoids exert a very fast stimulatory effect on attack signaling (threats) but not on attacks (bites) themselves. This conclusion was also supported by a complex experiment involving combined methyrapone and corticosterone-treatments applied to fighting rats over several encounters. Interestingly, attacks and attack signaling appear to be controlled by different brain centers. Here we report that the effects of glucocorticoids on these two behaviours is also different.

In conclusion, glucocorticoids exert a major effect on agressiveness in male rats. The effect on offensive threats appears to be faster than the effects on attack behaviour. One can hypothetise that the former effects were mediated by a non-genomic mechanism of glucocorticoid action, which have raised increasing interest in the last few years.

ACUTE AND CHRONIC EFFECT OF CADMIUM ON GABA ACTIVATED ION CURRENTS OF GASTROPODA NEURONS

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The acute and chronic effects of cadmium chloride ($CdCl_2$) have been studied on GABA activated responses and ion-channels in identified neurons of the snail, *Lymnaea stagnalis* L. Current clamp and two microelectrode voltage clamp techniques were applied. Heavy metals were perfused directly by the external solution.

In the case of semi-intact preparation, using conventional current clamp technique, the effect of GABA was studied before and after the acute effect of the heavy metal. As a result the elimination of GABA effect was observed following the application of higher concentration (1 mM) of $CdCl_2$.

Using voltage clamp technique, the acute application of Cd^{2+} higher concentrations (>50 μM) proved to be an effective channel blocker. At lower concentrations (<50 μM), a concentration and time-dependent inhibition occurred. The lowest studied concentration of Cd^{2+} (1 μM) resulted in a slight potentiation after a proportionally lower inhibition of the ligand activated ion current. This type of experiment was also performed on chronic treated animals (1 mg/l $CdCl_2$) and the results showed an evolved resistance to the effect of GABA compared to the control.

It is suggested that the toxic metal Cd^{2+} modifies the effect of GABA by the specific inhibition or potentiation of ion permeability of the neuronal membrane. In the mechanism of the effect both the binding of the transmitter molecule and the channel gating (activation and inactivation processes) can play a role. These alterations may influence the activity of the neurons, the neuronal interactions, hence the control function of the central nervous system.

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EFFECTS OF MECHANICAL STIMULATION OF THE SMALL INTESTINE: *IN VITRO* MOTILITY COMPARED TO EARLIER *IN VIVO* BEHAVIORAL OBSERVATIONS

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Earlier we suggested that stimulation of the gastrointestinal mechanoreceptors was almost always associated with mild or stronger unpleasant feelings that seemed to be necessary for the consequent behavioral changes. These results were obtained in experiments in which, following a deprivation of water for 23 h, small intestinal Thiry-Vella loops of the rats were distended with rubber balloons and fluid intake was measured. The balloon was distended to one of the previously selected volumes: 0.05 ml (weak), 0.09 ml (mild), 0.12 ml (strong) and 0.28 ml (painful). Besides fluid intake, behavioral changes were also recorded. Though intake decreased and aversive indexes increased parallel to the increase of volume, the relationship was not linear. In case of mild stimuli, i.e. in the 0.09-0.12 ml range, fluid intake did not decrease as much as expected and indexes were also off the general trend. Since these differences were consequently obtained in many different tests, we hypothesised that physiological changes in this range were responsible for the irregularity. The aim of the present work was to study the effects of mechanical distension on the small intestine *in vitro* by measuring motility changes.

About 2 cm long pieces of the rat small intestine were isolated and put into an isolated organ bath filled with Thyrode-solution. Temperature was kept 39 ^{0}C and the fluid was permanently bubbled with air. Motility changes were measured by a mechano-electrical transducer and, after proper amplification, were fed into a computer. A rubber balloon, filled with water, placed into the middle of the isolated intestine, and fixed to prevent movements, was used to

deliver stimuli. Distension volumes were 0.05, 0.09, 0.12 and 0.25 ml, respectively. Measured variables were tone, contraction period and amplitude.

Results showed that, in accordance with the *in vivo* observations, motility and tone changed differentially in the mild intensity range: amplitude stayed steady and tone increased only stlightly, as opposed to an expected increase of both. On the contrary, strong distension (0.25 ml) produced extreme increase of the tone and an almost complete abolishment of contractions. To interpret these findings, we suggest that changing compliance may be responsible for this effect, i.e. intestinal wall — probably by a local reflex — relaxes in the mild distension range. Since negative subjective feelings associated with volumen changes of the gut are probably proportional to the stretch of the intestinal wall, changing in compliance was perhaps responsible for the decreased aversivity of the mild distension observed *in vivo*. These findings are coherent with many human observations, too.

CIS-AC6C-ENDOMORPHIN-2 AS AN OPIOID RECEPTOR AGONIST

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Endomorphin-1 (Tyr-Pro-Trp-Phe-NI-1_2) and endomorphin-2 (Tyr-Pro-Phe-Phe-NH_2) isolated from mammalian brain were shown to have the highest specificity and affinity for the mu opioid receptor of any endogenous substance so far described and postulated to be natural ligands for this receptor (1). We have studied 2 new conformationally constrained analogs of endomorphin-2 in order to delineate the structural features of their binding to the opioid receptors. The derivatives, cis[(1S,2R)Ac^6c]2-endomorphin-2 (I) and cis[(1R,2S)Ac^6c]2-endomorphin-2 (II), contain a cyclic-ß-amino acid (2-aminocyclohexane carboxylic acid, Ac^6c) instead of proline in the second position of the peptide chain. Specificity of the ligands was evaluated in radioligand binding assays performed with the μ-specific [^{3}H]endomorphin-2, the δ-specific [^{3}H]Ile5,6-deltorphin and the κ-selective [^{3}H]D-Phe-D-Phe-D-Nle-D-Arg-NE_2 in rat brain membranes. Ligand-stimulated [^{35}5]GTPγS functional assays were used to measure the activation of G-proteins. Results show that compound II has negligible affinity to bind to opioid receptors and activate G-proteins. In contrast, cis[(1 S,2R)Ac^6c]2-endomorphin-2 (I) has high affinity and selectivity for the KI opioid receptors (KI= 0.3 nM), lower affinity for 5 receptors (Ki=256 nM) and the lowest for the κ receptors (KI>10,000). The active conformer (I) showed high potency (EO50 = 154 nM) and efficacy in the ligand-stimulated [^{35}S]GTPγS functional assay. The potency and efficacy of compound I is somewhat higher than those of the parent compound. It is concluded that the selectivity and mu-opioid receptor affinity increased by decreasing the flexibility of endomorphin-2.

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GROWTH HORMONE AND PROLACTIN: NEW VISTAS IN THE REGULATION OF THEIR SECRETION

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The hypothalamic somatostatin and dopamine exercise inhibitory regulation of the secretion of growth hormone and prolactin, respectively. However, these two hypophysiotrophic systems share a number of similar functional characteristics, they are considerable different. In the case of growth hormone secretion, somatostatin has a reciprocal interaction with the stimulatory hypophysiotrophic factor, so-called growth hormone releasing hormone. In contrast, prolactin is dominantly and tonically inhibited by hypothalamic dopamine. In responding to these essentially different (intermittent and tonic) types of stimulus, target cells must adapt differently. In the case of somatotropes, they can reversibly adjust their sensitivity to changes in the concentration of the signaling ligands. However, rnammotropes see high concentration of dopamine for a prolonged period of time. By adapting to this high concentration of signaling ligand, mammotropes shall adjust their sensitivity to the constant level of stimulus. Therefore, the importance of receptor regulation in cellular responsiveness to dopamine as well as the molecular mechanisms by which these processes manifest can provide important informations. Recently, increasing number of studies have been indicating that continuous, "tonic" inhibition in secretory function of cells differs from the "intermittent" inhibition. Tonic inhibition is especially challenged when a high secretory rate of mammotropes is physiologically required (for example due to suckling stimulus in lactating mothers). Continuous inhibition, at least transiently, must be turned off without affecting ligand-receptor coupling. Our laboratory has previously shown that a brief (10 mm) suckling stimulus renders mammotropes less responsive to dopamine inhibition (desensitization) as well as withdrawal-induced elevation of prolactin release (disappearance of dependence) and more responsive to stimulatory influence of TRH or angiotensi II (sensitization). Recently, we have provided evidences that these changes in mammotrope responsiveness manifest at the level of intracellular phosphorylation. Furthermore, our data suggest that the dephosphorylating side of the balance in phosphorylation is critical. More specifically, a rapid phosphorylation due to the decrease in the activity of protein phosphates 2A (PP2A) is responsible for the selective disappearance of the tonic inhibitory responsiveness as well as the parallel development of sensitization to stimulatory secretagogues. Consequently, this line of thinking indicates that the responses of mammotropes to inhibitory and/or stimulatory factors are not independent from each other.

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CHRONIC ETHANOL EXPOSURE UP-REGULATES NR2B SUBUNIT EXPRESSION IN PRIMARY NEURONAL CULTURES

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Cellular mechanisms underlying neuronal adaptation to ethanol are only partially understood. Previously, development of alcohol-dependence was observed in primary cultures of cortical neurones. After chronic (3-day) repeated (once a day) ethanol (50-100 mM) treatment, neurones became more vulnerable to excitotoxic insults and withdrawal of ethanol caused serious neuronal damage (Nagy et al., 2001). The extent of the damage was reduced by re-addition of ethanol, as well as by administration of N-methyl-D-aspartate (NMDA) receptor antagonists (MK-801, threo-ifenprodil), but not by the γ-amino-butyric acid-A ($GABA_A$) receptor agonist muscimol. According to these observations NMDA receptors were supposed to be involved in the development of *in vitro* ethanol dependence and in the neurotoxic effect of alcohol-withdrawal. The objective of the present study was to elucidate the functional changes as well as the possible alterations in the subunit composition of the NMDA receptors in ethanol pretreated primary cortical and hippocampal cultures.

Chronic (3-day) ethanol pre-treatment potentiated the NMDA induced increase in cytosolic concentration of calcium ions ($[Ca^{24}]_i$. In the presence of ethanol - in the same concentration as it was used during the pre-treatment - the NMDA induced increase in $[Ca^{2+}]_i$ was similar to what was observed in control cultures, i.e. re-addition of ethanol could normalise the effect of NMDA on $[Ca^{2+}]_i$ in ethanol pre-treated cultures. While there was no change in the inhibitory activity of the glycine-site selective NMDA receptor antagonist 5,7-DCK, the non-competitive antagonist MK-801 and the NR2B selective antagonist threo-ifenprodil were slightly (non significantly), whereas ethanol was significantly more potent in inhibition of NMDA evoked elevation in $[Ca^{2+}]_i$ after chronic ethanol treatment.

The expression of NMDA receptor subunits was monitored using subunit specific primary antibodies visualised with a fluorescein (FITC) conjugated secondary antibody. Cells were also labelled with propidium iodide (PI), and the fluorescence intensity of both dye was measured in a plate-reader. The subunit-expression was estimated as the ratio of FITC and PI fluorescence. While the ratios of fluorescence in case of NR1, NR2A, NR2C and NR2D subunits did not change, a 25% increase in the expression of the NR2B subunit was observed in the ethanol pre-treated cultures. Similar results were obtained when the fluorescent labelling was analysed using flow cytometry.

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AMINERGIC REGULATION OF LOCUST SALIVARY GLAND

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The secretory activity of the locust salivary gland (SG) stands partly under neuronal control. The units of the locust SG are the acini that are composed of zymogen and parietal cells. These cells are innervated from the suboesophageal ganglion (SOC) via suboesophageal nerve 1 (SN1) and suboesophageal nerve 2 (SN2). The neurons projecting through SN1 to the parietal cells are dopaminergic. The axons arriving through SN2 and innervating zymogen cells are serotonergic. DA_1, DA_2 and $5HT_2$ receptors have been demonstrated in the gland cells, according to the effect of the transmitters on the cAMP second messenger system. The aim of our study was to analyse serotonergic innervation of the acinar cells and the possible role of 5HT and DA during the feeding and digestion of locusts.

Immunocytochemistry performed on the SOG revealed 5HT-immunoreactive axons with varicosities running along the salivary duct and innervating directly the acinar cells. According to HPLC analysis of the SOG and SG in the course of feeding and digestion, SOG and SG contain 5HT (6 pmol/ganglion, 0.2 pmol/mg gland tissue) and DA (3 pmol/ggl, 0.3 pmol/mg gland tissue), but NA could not be detected. The 5HT content of SOG was constant, during feeding but its level increased at the beginning of digestion. The DA concentration was constant, meanwhile N-acetyl-DA (NAcDA) increased during digestion. In SG, the DA concentration was constant whereas that of NAcDA peaked after finishing feeding. 5HT level did not change in the SOG during feeding but started to increase by the end of it. When locusts could only sense the food without contacting it the monoamine levels in the SOG and SG were similar to that found during feeding. The α-amylase activity in the SG was high at the beginning of feeding, and decreased continuously therafter until digestion started.

Our results suggest that the function of the acinar cells is under the control of 5HT and DA. DA synthesized in SOG and released in the SG can be detected only by measuring NAcDA content. 5HT may act on initiating proteinous saliva production parallel with digestion. The α-amylase activity assay is suitable to analyse the locust salivation.

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INPUT-DEPENDENT SYNAPTIC TARGETING OF N_2-SUBUNIT-CONTAINING $GABA_A$ RECEPTORS IN SYNAPSES OF HIPPOCAMPAL PYRAMIDAL CELLS OF THE RAT

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Pyramidal cells, expressing at least 14 subunits of the heteropentameric $GABA_A$ receptor, receive GABAergic input on their soma and proximal dendrites from basket cells, activating $GABA_A$ receptors and containing either parvalbumin or cholecystokinin and vasoactive intestinal polypeptide. The properties of $GABA_A$ receptors are determined by the subunit composition, and synaptic receptor content governs the effect of the presynaptic neuron. Using a quantitative electron microscopic immunogold technique, we tested whether the synapses formed by the two types of basket cell show a difference in the subunit composition of $GABA_A$ receptors. Terminals of one of the basket cells were identified by antibodies to parvalbumin. Synapses made by parvalbumin-negative terminals showed five times more immunoreactivity for the α-subunit than synapses made by parvalbumin-positive basket cells, whose synapses were frequently immunonegative. This difference is likely to be due to specific $GABA_A$ receptor a subunit composition, because neither synaptic size nor immunoreactivity for the $ß_{2,3}$-subunits, indicating total receptor content, was different in these two synapse populations. Synapses established by axo-axonic cells on axon initial segments showed an intermediate number of immunoparticles for the α_2-subunit compared to those made by basket cells but, due to their smaller size, the density of the α_2-subunit immunoreactivity was higher in synapses on the axon. Because the two basket cell types innervate the same domain of the pyramidal cell, the results indicate that pyramidal cells have mechanisms to target $GABA_A$ receptors, under presynaptic influence, preferentially to distinct synapses. The two basket cell types act via partially distinct $GABA_A$ receptor populations.

EFFECTS OF ELECTRICAL STIMULATION OF THE MIDBRAIN PERIAQUEDUCTAL GRAY MATTER ON INTRACELLULARLY RECORDED AND LABELLED NEURONS IN THE ROSTRAL VENTROMEDIAL MEDULLA

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It is now established that analgesia elicited by stimulation in the midbrain periaqueductal gray (PAG) operates through inhibition of nociceptive specific neurons in the spinal dorsal horn. It is also generally accepted that the PAG exerts this descending control on spinal nociceptive neurons through monosynaptic activation of raphe-spinal pathways that arise from the rostral ventromedial medulla (RVM). However, there is a growing body of evidence suggesting that in addition to the monosynaptic connection an indirect, di- or polysynaptic pathway may also exist between the PAG and RVM. Here we intended to test this hypothesis.

Experiments were performed on anaesthetised and artificially ventilated Sprague-Dawley rats. Responses, elicited by single pulse electrical stimulation of the ventrolateral cell column of the PAG and the contralateral sciatic nerve were recorded in RVM neurons using *in vivo* intracellular technique. The intracellular electrode contained 3% biocytin for the intracellular labelling of the recorded neurons.

Recordings were obtained from RVM neurons that responded to PAG electrical stimulation either with excitation (n=10) or inhibition (n=1). The onset latency of excitatory PAG responses was 3.6 $\pm$ 0.9 ms (mean $\pm$ S.E.M.) in five neurons, whereas five other neurons responded with four times longer onset latency (14.8 $\pm$ 3 ins; Mann-Whitney $p<0.05$) to PAG electric stimulation. The duration of this longer latency excitation was also longer than that of the shorter latency excitation (17.5 $\pm$ 3.4 ms and 6.2 $\pm$ 1.5 ms, respectively). RVM neurons that responded with a short onset latency excitation to PAG stimulation were also excited, whereas those that responded with a longer onset latency excitation to PAG stimulation were inhibited by electric stimulation of the sciatic nerve and pinching of the tail.

The results suggest that some neurons in the RVM might receive polysynaptic activation from the PAG, and these neurons might be different from those that can be monosynaptically excited by PAG electric stimulation. It is proposed that an indirect excitatory projection from the PAG to the RVM could be mediated via the intermediate subdivision of the ponto-bulbar reticular formation.

POST-ISCHEMIC DAMAGE IN TRANSIENT FOCAL CEREBRAL ISCHEMIA OF RAT BRAIN. A MAGNETIC RESONANCE IMAGING STUDY

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Recent studies on transient cerebral ischemia reported recovery of energy metabolism at the early reperfusion phase, followed by secondary energy failure. As the apparent diffusion coefficient of water (ADC) is sensitive to the energy metabolism of the brain tissue, the aim of the current study was to follow the dynamics of the ADC during 1 hour of middle cerebral artery occlusion (MCAO) and 10 hours of reperfusion. Experiments were carried out in accordance with animal protection guidelines. MCAO was performed in 5 male Wistar rats inside the magnet using a remotely controlled thread occlusion model. Diffusion-, perfusion-, and T2-weighted images were performed repetitively, and relative ADC, perfusion- and T2 maps were calculated and normalized to the respective pre-ischemic value. The hemispheric lesion volume (HLV) at each time point was defined by relative ADC < 80% of control. At the end of 1 hour MCAO the HLV was 22±9%, which decreased to 6±5% in the first 2 hours of reperfusion ($p<0.01$), but it increased secondarily, and at the end of 10 hours reperfusion reached 17±9%. The mean relative ADC in the endischemic HLV significantly improved within 2 hours of reperfusion (from 66±1% to 91±7% of control), but later it declined and decreased to 75±8% of control at the end of the experiment. Pixels with secondary deterioration of ADC showed an increase of T2 value during the first 2 hours of recirculation, in spite of ADC improvement at this time, indicating improving cytotoxic, but generation of vasogenic edema at the early reperfusion. Significant hypoperfusion was not observed during 10 hours of recirculation.

We conclude that the improvement of ADC in the early phase of reperfusion is followed by secondary deterioration, indicating secondary energy failure, which is probably not caused by delayed hypoperfusion.

EFFECTS OF ANTIOXIDANTS ON AMYLOID PRECURSOR PROTEIN METABOLISM *IN VITRO*

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One of the theories involved in the aetiology of Alzheimer's disease (AD) is the oxidative stress hypothesis. Since supplemental administration of antioxidants may be beneficial in delaying age-related degenerative conditions, it is necessary to study their effects on the processing of amyloid precursor protein (APP). In our experiments, the levels of APP were analysed in rat embryonic basal forebrain neuronal cultures by Western immunoblot of samples

from conditioned media and cell lysates. Two different antioxidants were tested: the pineal hormone melatonin and N-acetylcysteine (NAC), a known precursor of glutathione. The neuronal cultures were maintained for 2 h on day 10 after plating (DIV10), either in the absence or in the presence of different concentrations (10 μM, 100 μM or 500 μM) of melatonin or NAC. The normal levels of secretion of APP into the media were increased by treating the neuronal cultures with different concentrations of melatonin. NAC treatment caused an elevated APP secretion only at 100 μM. Both melatonin and NAC were ineffective on the APP content of cell lysates.

These findings suggest that antioxidants acting via different mechanisms affect the metabolism of APP in different ways. It would seem that melatonin, even at low concentration, has a beneficial influence on the metabolism of APP. Since the endogenous melatonin level falls in advanced age and in AD, it may be relevant to the design of preventive and therapeutic strategies in AD.

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CHANGES IN VIP-ACCUMULATION IN THE PERIKARYA OF OPHTHALMIC AND MAXILLARY TRIGEMINAL SENSORY NEURONS OF THE AGEING RAT FOLLOWING PERIPHERAL NERVE TRANSECTION

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The transection of supraorbital (V/I) and infraorbital (V/2) vibrissal nerves resulted in transient upregulation of VIP expression in axotomized trigeminal sensory neurons accompanied with the depletion of such neuropeptides like CGRP and substance P. The aim of this work was to analyse the age-dependency of VIP accumulation in both the perikarya and central processes of the trigeminal primary sensory neurons following peripheral nerve transection.

Perikarya of axotomized ophthalmic as well as maxillary trigeminal sensory neurons in animals operated on the first postnatal day (P1) displayed strong VIP-immunoreactivity on P10. Similarly, the central processes of P1 axotomized sensory neurons were strongly VIP-immunoreactive in the trigeminal brainstem nuclear complex. Somata of axotomized maxillary trigeminal ganglion cells of 35-day-old rats, operated on P25, displayed moderate VIP immunoreactivity, while the perikarya of axotomized ophthalmic primary sensory neurons were not immunoreactive for VIP, although, the central processes of both ophthalmic and maxillary sensory neurons were strongly VIP-immunoreactive. In spite of the VIP-immunoreactivity in ophthalmic and maxillary primary afferent fibers in the trigeminal brainstem nuclear complex of 65-day-old rats, perikarya of neither ophthalmic nor maxillary sensory neurons were immunoreactive for VIP following peripheral nerve transection on P55.

Our results suggest that trigeminal sensory neurons undergo a postnatal, interdivision-dependent (V/I, V/2) maturation and one feature of this process is the decrease of accumulation of VIP in the perikarya of axotomized neurons.

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NEUROTOXIC EFFECTS OF N- AND C-TERMINAL FRAGMENTS OF ß-AMYLOID PEPTIDE1-42 IN *IN VIVO* HUMAN BRAIN TISSUE

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During recent years, it has become evident that the beta-amyloid (ßAP) component of senile plaques may be the key molecule in the pathology of Alzheimer's disease (AD). The source and the site of neurotoxic action of ßAP, however, are still a matter of controversy. The aim of the present work was to investigate the neurotoxic effects of fragments of ßAP1-42 on cholinergic and cholinoceptive cells in human brain samples. Hippocampus samples from AD brains were immunostained for ßAP N- and C-terminal fragments, and stained for AChE.

The results of the morphological investigations revealed signs of axonal degeneration: dilatation of neuronal processes could be observed only in senile plaques. These changes were visible in C-terminal-positive senile plaques.

In the surroundings of the plaques, the axons were healthy. Experiments involving double staining of the C- and N-terminal fragments indicated the localization of the fragments: the N-terminal fragment was stained in the centre of the plaques, and the C-terminal fragment in the peripheral region of the plaques. Dilatation of the processes was observed in the peripheral region, too.

Overall, these studies revealed that morphological changes were causing the neurotoxic effect of ßAT, not physical damage. Demonstration of the C-terminal fragment and neuronal degeneration in the same area is indicative of the neurotoxic effect of the C-terminal. However, the morphological changes was not induced by extracellular accumulation of N-terminal fragments. The results suggest that the C-terminal of ßAP1-42 is more neurotoxic than the N-terminal fragment.

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ISATIN (INDOLE-2, 3-DIONE) ANTAGONIZES THE HYPERTHERMIC EFFECT OF NATRIURETIC PEPTIDES AND PACAP-38

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Previous studies carried out in our laboratory have demonstrated that natriuretic peptides and pituitary adenylate cyclase-activating polypeptide-38 (PACAP-38) have hyperthermic properties, all able to be abolished with a cyclo-oxygenase inhibitor NSAID. Isatin (indole-2, 3-dione) is an endogenous indole, supposed to have physiological effects. It is reported to be an

endogenous inhibitor of the atrial natriuretic peptide receptor, however the metabolism and physiological action(s) of this compound are not yet clarified.

Since isatin antagonizes the hyperthermia induced by all the members (ANP-28, BNP-32, CNP-22) of the natriuretic peptide family, it seems that the action of isatin is not restricted to the ANP-receptor, therefore we investigated the effects of isatin on PACAP-38-induced hyperthermia in rats, in this study.

One μg intracerebroventricular (icv.) injection of PACAP-38 had hyperthermic effect in male, Wistar rats, with an onset of the effect at 2 h and a decline by the 6^{th} h after administration. Intraperitoneal (ip.) injection of different doses of isatin (25-50 mg/kg) significantly decreased the hyperthermic effect of 1 μg PACAP-38 (icv.), whereas 12.5 mg/kg isatin (ip.) had no inhibiting effect. Isatin alone did not modify the body temperature of the animals.

The capability of isatin to antagonize the hyperthermia induced by all members of the natriuretic peptides *and* PACAP-38 makes isatin unlikely to act directly on the natriuretic peptide receptor in these hyperthermic processes, but rather on a yet uncharacterized target that links the hyperthermic effect of natriuretic peptides and PACAP-38 to each other. The linking mechanisms between the above-mentioned netiropeptides and body temperature regulation need to be investigated further.

DISSIMILAR EFFECTS OF VARIOUS ORGANOPHOSPHATES ON THE CORTICAL AND SUBCORTICAL EVOKED ACTIVITY IN RATS

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The use of organophosphates (OPs) as insecticide agents results in the worldwide emission of large quantities into the environment, affecting the pesticide applicators and, in indirect ways, on the whole population. Although OPs are primarily enzyme blockers, some functional damages caused by them in the nervous system have a different extent and/or time course than that of acetylcholinesterase inhibition - indicating the existence of effects not (or not directly) related to anticholinesterase action. Other brain enzymes can e.g. also be targets and the effect of different organophosphates on these can be much dissimilar. The localization of the effect within the central nervous system is a further point of question. In the present work, these questions were approached in an acute animal model using two OPs, dimethoate (DIM) and chlorphenvinphos (CVP), having a different chemical structure and general toxicity (as shown by the LD_{50}).

The head of adult mate Wistar rats was fixed in urethane anesthesia and the left hemisphere exposed. Sensory stimuli were electric pulses (5-10 mA, 0.05 ms, 1 Hz) delivered to the whiskery skin. Evoked activity was recorded from the primary somatosensory cortical area and from the thalamic relay nucleus (VPM). A series of 20 stimuli was delivered every 10 minutes. After at least 60 mm control period, 1/25 LD_{50} of the agent studied (25 mg/kg b.w. DIM or 1.2 mg/kg b.w. CVP) administered via a peritoneal cannula and the recording was continued for further ca. 2 hours.

DIM induced no significant alteration in any of the parameters of either the cortical or the thalamic (averaged) activity. There was merely a slight (<10%) decrease in the cortical evoked potential amplitude. CVP, on the contrary, significantly ($p<0.05$) reduced the amplitude of the cortical evoked potential. This effect developed over ca. 40 minutes and remained nearly constant. On latency and duration, the effect was minimal (<5%). The amplitude of the thalamic evoked potential was increased by CVP but this was a less strong and stable effect than that on the cortex.

Although the two organophosphates were administered in "isotoxic" doses, the effects caused by them were dissimilar in several aspects. This may be attributable to the different individual, non-cholinergic, effect of the two.

DIRECT (PERINEURAL) CAPSAICIN TREATMENT OF THE ABDOMINAL VAGUS MODIFIES EXPERIMENTAL FEVER

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In explanation of the pathogenesis of experimental fevers induced by endotoxins (bacterial lipopolysaccharides, LPSs) a humoral pathway is conventionally regarded to be of outstanding importance. According to this idea, LPS induces the production of various endogenous pyrogens (e.g., interleukin-1 and other cytokines), which pass the blood-brain-barrier (or act at this barrier) and cause activation of inducible cyclooxygenase enzymes in the hypothalamus. The produced prostaglandin E (PGE) shifts the thermoregulatory set-point to a higher level, and this finally causes coordinated alteration of thermoregulatory effector functions in order to elevate body temperature. Because the short latency of temperature rise upon LPS administration cannot be easily explained exclusively by such pathway, it has been assumed that faster (mainly neural) pathways may participate at least in the initiation of the febrile response.

LPS is detoxified mainly in the liver, therefore afferent fibers from the gastrointestinal tract, particularly from the liver, have been proposed to play a role in the initiation of the febrile response to LPS. Earlier reports have shown that subdiaphragrnatic vagotomy, in particular transection of the hepatic branch, attenuated the fever reaction. The decreased febrile responsiveness was most pronounced at the early phase of LPS-response or in fevers induced by low doses of LPS. Vagotomy may, however, affect efferent vagal fibers as well as the afferent ones.

In order to exclude the possible involvement of efferent fibers in this attenuation, we have used intraperitoneal (IP) capsaicin pretreatment (small capsaicin doses, in order to avoid systemic desensitization), and have found that the first phase of LPS fever was attenuated, resembling the results seen following vagotomy.

Since the damage induced by IP capsaicin may not be confined to vagal afferents but may reach splanchnic nerve endings as well, in the present studies the anterior and posterior trunks plus the hepatic branch of the abdominal vagus of rats were locally pretreated by capsaicin. Under ketaxnine-xylazine anesthesia, a 3-4 mm wide cotton wool strip was introduced that

wrapped these parts of the vagus. It was isolated from other tissues by a small sheet of polythene, was soaked with 1% capsaicin, and was kept in place for 20 min. In sham-operated rats the vehicle was used for wetting, without of capsaicin. One week after this operation, in a second operation a jugular cannula was implanted to the rats. Further three days later the LPS fevers were compared in the two groups.

Sham-operated rats exhibited a characteristic biphasic febrile response to intravenous injection of 10 μg/kg LPS, i.e., a fever course well known from earlier studies. As compared with sham-operated animals, in rats with perineural capsaicin pretreatment the first phase of LPS-fever was attenuated, but later body temperature still increased. The results were very similar to those observed following IP capsaicin desensitization. It is concluded that abdominal IP capsaicin desensitization may be used to damage afferent vagal fibers, similarly as perineural capsaicin pretreatment. Both procedures modify the first phase of LPS fever.

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SYNAPTOLOGICAL AND NEUROCHEMICAL INVESTIGATIONS OF COMMISSURAL CONNECTION OF THE SPINAL DORSAL HORN

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It has been established that there is a strong functional interconnection between sensory neural circuits on the two sides of the spinal cord. After mechanical or thermal noxious stimulations of the hind limb, short-latency excitatory and inhibitory responses were recorded in neurons of the contralateral spinal dorsal horn. Inflammation of distinct areas of the skin and monoarthritis induced bilateral chemical changes in the superficial laminae of the dorsal horn. However, the neuronal background of these commissural interactions is poorly defined. In a previous study we demonstrated the presence of a distinct commissural connection between the lateral areas of the lumbar spinal dorsal horns of rats using the highly sensitive anterograde and retrograde neural tracers *Phaseolus vulgaris* leucoagglutinin and biotinylated dextran amine (Petkó and Antal, J. Comp. Neurol., 422:312-325, 2000). In the present experiments, the morphological characteristics, synaptic relations, and neurochemical properties of the commissural connection were investigated. Most of the somata of commissural neurons were polygonal, and partly fusiform. Terminals of the commissural axons established mostly symmetric (73.6%), partly asymmetric (26.4%) synaptic contacts with dendritic shafts (86.8%) and dendritic spines (8.8%), as well as somata (4.4%) of the postsynaptic neurons. A minor proportion, 10.3% of the labelled terminals showed GABA-immunoreactivity and 2.0% of them were immunoreactive for glycine. None of the labelled terminals were found to be immunoreactive for methionin-enkephalin. In some cases, commissural axon terminals were impinged onto dendrites of labelled neurons. The results suggest that commissural fibres interconnecting the lateral areas of laminae III-IV may exert a complex effect on neural circuits underlying sensory information processing in the spinal dorsal horn of rats.

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LABELING OF ELECTROPHYSIOLOGICALLY CHARACTERISED NEURONS WITH NEUROBIOTINE IN THE RAT PREFRONTAL CORTEX

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This study was undertaken to identify the morphology and neural connections of single medial prefrontal cortex (mPFC) neurons using both electrophysiological and neuroanatomical approaches in urethane-anesthetised rats. Single unit activity was recorded extracellularly with glass micropipettes filled with 2% Neurobiotine dissolved in 0.5 Ni NaCl solution. The ventral tegmental area (VTA) and/or the basolateral nucleus of the amygdala (BLA) were electrically stimulated. After electrophysiological characterisation each neuron was filled with Neurobiotine using Pinault's method. Inhibitory responses were observed in 9 of 10 neurons recorded and successfully stained in the mPFC after electrical stimulation of the BLA. Eight of the 9 neurons were identified as pyramidal cells, and one cell was interneuron. One pyramidal cell exhibited excitatory responses. Three pyramidal cells responded to both VTA and BLA stimulation with inhibitory responses. One of the 3 neurons was identified as output neuron projecting to the VTA, since it was antidromically driven from the VTA. One pyramidal cell exhibited excitatory responses to VTA stimulation and inhibitory ones to BLA stimulation.

The present results suggest that: (1) the most common inhibitory response evoked by BLA stimulation whith a latency about 20 ms can be elicited in different types of pyramidal cells and interneurons; (2) mainly inhibitory inputs from the VTA and BLA converge on single mPFC pyramidal cells; (3) some of the last mentioned neurons project backward to the VTA.

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DISTRIBUTION OF BRAIN-DERIVED NEUROTROPHIC FACTOR AND TYROSINE KINASE-B RECEPTOR IN THE RAT LUMBAR SPINAL CORD

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A long line of experimental evidence has already been accumulated that brain-derived neurotrophic factor (BDNF), a member of the neurotrophic family, plays a significant role in pain related behaviours. Recent studies have unequivocally demonstrated that due to chronic inflammation of peripheral tissues BDNF is upregulated in somata and central axon terminals of nociceptive primary sensory neurons in a nerve growth factor dependent manner. The release of BDNF in the spinal dorsal horn then may cause increased excitability of spinal neurons, and thus may lead to pain hypersensitivity.

Although BDNF appears to be instrumental in pain related central sensitization of spinal neurons, the distribution of BDNF and its receptor (tyrosine kinase-B receptor, TrkB-R) has not been elucidated in the spinal dorsal horn. Accordingly, in the experiments presented here we investigated the distribution of BDNF and TrkB-R in laminae I-II of the rat lumbar spinal cord using immunocytochemical techniques at the light and electron microscopic levels.

Here we demonstrated that in addition to terminals of primary afferent fibers, dendrites of spinal neurons also display strong immunoreactivity for BDNF. Immunoprecipitates indicating the presence of BDNF in dendrites were primarily associated to microtubules and distinct areas of the surface membrane both at synaptic and extrasynaptic locations. For the first time in the literature, we also detected TrkB-R immunoreactivity in perikarya, dendrites and axons of a substantial number of spinal neurons. In somata, TrkB-R immunoreactivity was associated to cisternae of rough endoplasmic reticulum and Golgi apparatus, whereas in dendrites TrkB-R immunoprecipitates were attached to microtubules, patches of the surface membrane and postsynaptic membrane thickenings. TrkB-R immunoreactivity was also revealed in axons and axon terminals.

The findings suggest that BDNF might exert a complex effect on spinal neurons. BDNF released from primary afferents may potentiate the postsynaptic effect of simultaneously discharged glutamate and neuropeptides, and modify gene expression in TrkB-R positive neurons. In addition, BDNF synthesized and presumably released by spinal neurons may provide additional cellular mechanisms for modulating neural activities underlying sensory information processing in the spinal dorsal horn.

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INTRACELLULAR ANALYSIS OF RESERPINE-SENSITIVE CELLS IN THE TURTLE RETINA

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We have investigated the acute effects of reserpine (monoamine-depletor) on retinal cells of the turtle *(Pseudemys scripta elegans).* Intracellular recordings were obtained from a superfused, light-adapted eyecup preparation and the effects of bath applied 10 microM reserpine on the light responses of retinal neurons were monitored. Some of the cells were filled with Neurobiotin and identified morphologically.

The action of the drug was diverse on different cells. Recordings of amacrine and ganglion cells show that the membrane potential remained unchanged and light-evoked responses were reduced or abolished. In other cases the ON-components of the light-responses and the interstimulus spikes were eliminated or the ON-transient component was transformed into ON-sustained component with no change in the membrane potential. Moreover, combined effects, such as decrease in light response amplitude and membrane depolarization also occurred mostly in horizontal cells.

The principal findings of this study are that reserpine primarily, by raising the extracellular concentration of serotonin, can produce profound acute effects. Since light-adapted retinae were investigated, dopamine could not play an important role in the changes brought about by reserpine. It is highly possible that the drug exerts its effects via serotonin receptor containing neurons. The identify of these receptors are unknown at present. The results suggest that acute treatment with reserpine also changed light-evoked responses of retinal cells not only in the inner but also in the outer retina. We infer that cells which respond with changes in the light responses only bear serotonin receptors while those neurons which show change in both membrane potential and light-evoked response do not have serotonin receptors.

TIME COURSE OF THE DEVELOPMENT OF AUDITORY STEADY-STATE RESPONSE IN CAT: INTRACORTICAL STUDY

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Auditory steady-state response (SSR) is a sinusoidal electrical response in the auditory cortex to repetitive stimuli. It appears when the rate of the stimuli is sufficiently rapid to produce overlapping of the auditory evoked potentials (AEPs) to individual stimuli. In some of the studies short trains of clicks, given at about 40 Hz rate, are used; while in others the repetitive auditory stimuli are applied continuously. In the present study we used both types of

stimulation to compare the responses and to determine how long repetitive stimulation is necessary to develop stable SSR.

Click stimuli were delivered through a bone conductor at presentation rates between 1 and 60/s. AEPs and SSR were recorded by chronically implanted arrays of epidural electrodes above the auditory cortex; and chronically implanted 16-24 contact linear array multielectrodes were used to record intracortical field potentials and multiunit activity (MUA) in the behaving cat.

The first click of the train elicited a transient AEP even if the repetition rate was in the gamma range. The components of this AEP modulated the SSR in the first 200 ms. In the first second the early surface-positive components could be recognized but they were progressively suppressed by the negative waves of the oscillatory SSR, which developed gradually. In the deep layers of the auditory cortex the local negative field potentials, representing the thalamic input could be recognized even at long-term stimulation, independently of the arousal level of the animal. However, the SSR on the surface and in the superficial layers disappeared in slow wave sleep and in barbiturate anesthesia.

CSD analysis of the intracortical field patterns revealed that SSR became stabilized only after at least 10-20 seconds. This has to be considered, if trains of auditory stimuli are used in SSR studies.

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CHOLINESTERASE INHIBITORS IN ALZHEIMER'S DISEASE

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The cholinergic theory of Alzheimer's disease (AD) states that neurodegeneration causes a decrease in cholinergic transmission because of the loss of cholinergic neurons, resulting in cognitive impairment. Currently, several cholinesterase inhibitors, including tacrine (Cognex®), donepezil (Aricept®) and rivastigmine (Exelon®), are available and provide symptomatic therapy for patients with mild and to moderate AD. Many more drugs are awaiting approval such as eptastigminc, TAK-147 and bis-tacrine. Cholinesterase inhibitors presumable act to reduce acetylcholine breakdown, thus allowing enhanced the cholinergic activity and boosted cognitive function. However, these inhibitors comprise a group of structurally diverse compounds with a wide range of relative specificities for the various AChE molecular forms found the brain. There are two AChE molecular forms exist in brain: globular monomers (G_1) and tetramers (G_4) of catalytic subunits. The C_4 form is lost selectively in AD brain. Therefore, the G_1 form is regarded as the most likely target for AChE inhibitor therapy in AD. It is still not clear how inhibitors react with AChE and their molecular forms. The AChE molecular forms from normal and AD *post mortem* brain cortex were separated and isolated by sucrose density gradient ultracentrifugation. In enzyme inhibition studies, the IC_{50} values of G_1 and C_4 were calculated by the linear regression of the log concentration of

inhibitors versus the inhibition (range 20-80%). The following inhibitors were used: tacrine, bis-tacrine, donepezil, TAK-147 (reversible inhibitors) rivastigmine (carbamoylating inhibitor), and metrifonate (phosphorylating inhibitor). The various AChE inhibitors show differential sensitivities for the molecular forms of AChE. Among inhibitors rivastigmine displayed preferential inhibition for G_1 form for both AD and normal brain AChE. Our data suggest the use of rivastigmine as molecular form-selective inhibitor for therapeutic application.

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CB1 RECEPTOR AGONISTS REDUCE GABAergic INHIBITION IN THE RODENT AMYGDALA VIA PRESYNAPTIC CB1 RECEPTORS

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Using a specific antibody directed against the intracellular C-terminus of the CB1 cannabinoid receptor we found a dense labelling in the basolateral nucleus (ßLA), but not in the central nucleus (CA) of the amygdala. Immunofluorescent double labeling showed that the CB1 receptor is expressed by the large cholecystokinin (CCK)-containing interneurons, but not by the small CCK-immunoreactive neurons. The parvalbumin-containing interneurons were never immunoreactive for CB1 receptor. At the electron microscopic level the immunogold labeling in the cell bodies was always localised in the membranes of intracellular organics, such as RER or the Golgi apparatus, but never in the plasma membrane. In contrast, the receptors were found in the cell membrane only in axon terminals that formed symmetric (presumably GABAergic) synapses. The specificity of the immunolabeling was further proved using wild type (WT) and CR1 -/- knockout (KO) mice. The WT mice shoved a similar pattern of CB1 receptor staining compared to the rats, whereas no immunolabeling was found in the BLA of the KO mice.

Using whole-cell patch-clamp technique, we examined the effect of CB1 receptor activation on the GABAergic synaptic transmission. We recorded $GABA_A$ receptor-mediated inhibitory currents from principal cells evoked by electric microstimulation in the BLA and CA. The CR1 receptor agonists (WIN55,212-2 and CP55,940, 1 μM each) reduced the amplitude of evoked IPSCs in the BLA by 39% and 41%, respectively. The effect could be fully reversed by the specific antagonist SR141716A. The agonist had no effect on the IPSCs recorded in the CA, in accordance with the anatomical observations. We also tested the effect of WIN in the BLA of KO mice, where no significant decrease was observed in the evoked IPSCs. With recording of spontaneous and miniature (in TTX and Cd^2) IPSCs from the BLA we determined the nature of the presynaptic receptor-mediated effect. WIN decreased the conductance and frequency of spontaneous IPSCs by 12% and 58%, respectively. The action potential and Ca^{2+} influx independent miniature IPSCs were not affected by WIN.

Thus, we can conclude that CB1 receptor activation may suppress GABAergic inhibition in the BLA, but not in the central nucleus, via reduction of extracellular Ca^{2+} influx into the axon terminals.

HIGH CONCENTRATIONS OF PACAP ARE PRESENT IN THE ANOXIA-TOLERANT TURTLE BRAIN

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Among vertebrates, only a few species can withstand severe hypoxia. Anoxia tolerance is most highly developed among some species of turtles, surviving up to 48 h of anoxia at room temperature, and several months submerged in anoxic water at 3 °C. Due to this remarkable anoxia tolerance, the turtle is a favorite model animal to study the possible mechanisms of anoxia survival.

Pituitary adenylate cyclase activating polypeptide (PACAP) occurs in two amidated forms, with 38 and 27 amino acid residues. The primary structure of PACAP is identical among all mammalian species, and differs in only 1-4 amino acids in other vertebrates. Since its discovery, numerous *in vitro* and *in vivo* evidences have been accumulated on the neurotrophic and neuroprotective effects of PACAP. One of the most intriguing effects of PACAP is its neuroprotective action under hypoxic/ischemic conditions. It was of interest to examine whether PACAP may be added to the list of numerous candidate factors which enable the turtle to withstand long-term anoxia. We investigated the tissue distribution of PACAP27 and 38 in the nervous system and peripheral organs of the turtle *(Pseudemys scripta elegans)* by means of radioimmunoassay (RIA) and compared the results to data obtained from corresponding rat samples.

The parallel displacement curve of serially diluted extracted tissues to synthetic PACAPs and RP-HPLC pattern suggests that the turtle PACAPs are very similar to the mammalian PACAPs. The content of PACAP27 was very low or undetectable. However, the longer form of the peptide, PACAP38 showed very high levels in the CNS and lower but significant levels in peripheral organs. When compared to concentrations measured in rat and human brains, the turtle brain contains strikingly high levels of PACAP38: 18-60, or 10-100-fold higher concentrations of PACAP is found in the turtle brain than in the rat or human brain, respectively. The strikingly high levels of PACAP38 in the turtle CNS suggest that PACAP might be an important endogenous factor involved in resistance against anoxia-induced neuronal damage.

AXON ORIGIN OF NEURONS IN THE RAT BRAIN AND SPINAL CORD

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It is generally assumed that the axons of the multipolar neurons in the central nervous system originate form the perikaryon. Even a superficial view on Golgi specimens, however, reveals numerous exceptions.

Golgi-Kopsch impregnated specimens prepared from the lumbar spinal cord and diencephalon of young adult rats (body weight 120-140 g) were studied. Neurones of characteristic shape and/or location with stained axons were selected for the analysis. The sample included large neurones in the ventral horn (presumably motoneurones; Class A); neurones in the intermediate zone of the spinal cord (Class B); neurones of the spinal dorsal horn with dorsally directed dendrites (Class C); thalamocortical projection neurones (Class D); and neurones in the medial-basal hypothalamus (Class E). In the first series of experiments the site of the axon origin (perikaryon, jointly with one of the main dendritic trunks or dendrite) was sampled. In the second series of experiments the size of the perikaryon, the distance of the axon origin from the perikaryon, the size of the axon hillock and the length of the impregnated portion of the axons were measured using a LUCIA image analysis system (Laboratory Imaging, Ltd). Dorsal horn neurones were not included into the second series due to small sample size. - Approximately two-thirds of the large size ventral horn neurones and the thalamocortical projection neurones had axons originating from the perikaryon. On the contrary, two-thirds of the zona intermedia neurones, dorsal horn neurones and neurones in the hypothalamus had axons originating either jointly with a main dendritic trunk or from one of the dendrites. Interestingly, the axons of the dorsal horn neurones never originated from a dorsally oriented dendrite. - Ventral horn neurones were much larger than the other types of neurones. The distance of the axon origin from the perikaryon was the longest in the zona intermedia neurones (17.64 ± 10.76 μm; n = 89) and the shortest in the ventral horn neurones (6.89 ± 1.65 μm; n = 60). The size of the axon hillock was proportional to the size of the neurones: the largest in the ventral horn neurones (7.00 ± 1.43 μm; n = 89) and the smallest in the neurones of the hypothalamus (2.64 ± 0.64 μm; n = 77). Also the length of the impregnated portion of the axon, indicating the initial unmyelinated portion, was proportional to the size of the perikaryon: longest in the ventral horn neurones (35.24 ± 1.43 μm; n = 89) and shortest in the thalamocortical neurones (20.48 ± 9.39 μm; n = 64). The axons of the hypothalamus neurones continued and showed varicosities. The present findings indicated that the perikaryon is often not the site of axon origin. This invites speculations about the development of the neuronal arborisation, the dendritic integration of the synaptic input, the routing of the axoplasmic transport and the validity of neuronal modelling.

REGIONAL CHANGES IN THE TOTAL NUMBER OF NEURONS IN THE MYENTERIC PLEXUS OF THE DEVELOPING CHICKEN GUT

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Up to now data concerning the total number of neurons in the enteric nervous system (ENS) of the developing chicken have not been published. In the present investigations Cuprolinic blue (quinolinic phthalocyanin) histochemical staining was applied to stain and count neurons in the developing myenteric plexus of the chicken gut. Cuprolinic blue binds selectively to single stranded RNA and thus acts as a ribosomal stain (Mendelson et al., 1983) and provides a remarkably specific demonstration of neurons. Cuprolinic blue staining marks more neurons than NADH-diaphorase histochemistry (Heinicke et al., 1987) and immunocytochemistry for constitutive nuclear oncoproteins like c-Fos and c-Myc (Karaosmanoglu et al., 1996) or for neurofilaments (unpublished observation).

The present experiments were performed on wholemounts prepared from intestinal segments of embryonic (day 19 of incubation) and hatched (1, 2, 4 and 7 days after hatching) chickens. Preparations from the proximal, middle and distal parts of the small intestine and also from the colon were stained with Cuprolinic blue according to Holst and Powley (1995). Ganglia and neurons were counted in randomly selected measured areas at 160-fold magnification. Data were analysed by using ANOVA and SNK test.

It was found that ganglionic density (ganglia/mm^2) and neuronal density (neurons/mm^2) decreased during the examined time-span. The decrease of the ganglionic and neuronal density changed with different dinamics in the different intestinal segments. Both ganglionic and neuronal densities were lower in the proximal segments of the small intestine and in the colon than in the middle and distal parts of the gut. The Cuprolinic blue positive cell number per ganglion rised in each intestinal segments also with different dinamics in the different segments of the gut. The number of neurons per ganglion doubled in the proximal segment of the small intestine arid rised in the middle and distal parts of the small intestine and in the colon. Double labelling with Cuprolinic blue and anti-neurofilament monoclonal antibody revealed that there are less neurofilament positive neurons than Cuprolinic blue positive neurons in the ENS of the chicken gut. The morphology of the neurofilament immunostained cells was highly variable.

The decline of the ganglionic and neuronal density refers to the growth of the musculature of the bowel. The lower ganglionic and neuronal densities in the proximal part of the small intestine and in the colon correspond to the advanced growth of these segments. The rising numbers of neurons per ganglion allude to a greater demand of innervation during the posthatching life. Since at the same time the ganglionic densities declines the rise of number of neurons per ganglion can be a compensation for needed innervation.

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ORGANIZATION OF NADPH-DIAPHORASE REACTIVE, FMRFamide- AND CARP-IMMUNOREACTIVE NETWORKS IN THE ENTERIC NERVOUS SYSTEM OF THE SNAIL, *HELIX POMATIA* L.

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The distribution of neural nitric oxide synthase (nNOS) and its histochemical marker, NADPH-diaphorase (NADPH-d) activity have been investigated extensively in the vertebrate alimentary canal. Nitrergic system can influence enteral peptidergic networks, hence plays a role in the control of gut motility.

The aim of our study was to demonstrate nitrergic elements in the snail digestive system, and by this way to furnish an anatomical basis for further functional analysis of NO transmission in invertebrates. The distribution of neural NADPH-d reactivity and two molluscan type neuropeptides, molluscan cardioexcitatory peptide (FMRFamide) and catch relaxing peptide (CARP) has been investigated in the enteric nervous system of the pulmonate snail *Helix pomatia* L. A histochemical technique was applied to detect NADPH-d activity, whereas FMRFamide and CARP were visualised by immunohistochemical methods.

Neural elements, showing NADPH-d activity, could be found in the caecum wall. Several NADPH-d positive unipolar neurons in the myenteric plexus and two rich axonal networks were seen in this area. Varicose NADPH-d reactive nerve fibers occurred in the circular layer of the caecum muscles. FMRFamide-immunoreactive (ir) and CARP-ir neurons were detected in all regions of the gastrointestinal tract. The majority of NADPH-d active neurons also expressed FMRFamide-ir. NADPH-d and CARP were coexpressed only in a few neurons. NADPH-d reactive fibers innervated several CARP-ir and some RMRFamide-ir neurons within the myenteric plexus of the caecum. NADPH-d active cells were also innervated by CARP-ir neurons.

These results indicate that putative nitrergic and different peptidergic elements are likely to be involved in the complex neural regulatory system of the alimentary tract in *Helix*. Neuronally generated NO may influence the release and/or signalling of neuropeptides, throughby affect the regulation of visceral muscle movements in the snail, *Helix pomatia* L.

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EFFECT OF DOPAMINE D3 LIGANDS ON MOTOR ACTIVITY AND BODY TEMPERATURE

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Since the identification of the dopamine D_3 receptor in rat its distribution and function have been extensively investigated, because it may represent a potential target of drugs employed in the treatment of neuropsychiatric disorders.

Until now a major problem in this field has been that truely selective ligands have been missing, therefore the observed behavioral responses cannot be unambiguously related to D_3 receptor function.

The aim of our study was to clarify the role of D_3 receptor in behaviors evoked by D_3-acting ligands such as locomotor activity and body temperature.

We have performed experiments with the compound SB-277011 as most selective (-500-fold) D_3 antagonist presently and U-99194A an earlier and less selective (>23-fold) D_3 antagonist. As D_3 agonist molecules we used PD-128907 (~600-fold selectivity) and a molecule of SmithKline Beecham (270-fold selectivity, in what follows SB agonist). We also used BP-897 (60-fold selectivity) as a partial D_3 agonist. SB-277011 was shown to evoke a non -dose-dependent increase in motor activity of previously habituated mice both after oral and subcutaneous administration. In rats the compound caused dose-dependent hyperactivity at doses of 13.5, 20 and 30 mg/kg p.o. This effect was similar to that of U-99194A, a less selective D_3 antagonist, given at doses of 5, 10 and 20 mg/kg sc. Neither SB-277011 at doses of 12 and 24 mg/kg sc., nor U-99194A at doses of 1, 3, 6 and 12 mg/kg sc., caused significant changes in the body temperature of mice.

The SB agonist caused non-dose-dependent hypomotility at doses of 16, 24 and 36 mg/kg sc. in mice. We tested the molecule on the body temperature of mice and found that in the dose-range of 3-12 mg/kg sc. it induced dose-dependent and long-lasting hypothermia.

The activity profile of PD-128907 was very similar to that of the SB agonist. The molecule caused non-dose-dependent hypomotility in the dose-range of 0.05-0.2 mg/kg sc. in rats. PD-128907 evoked significant hypothermia in mice, though its effect was transient.

BP-897 as a partial D_3 agonist elicited hypomotility both in habituated and non-habituated mice. Interestingly, its effect was dose-dependent in the former but not in the latter case. It induced dose-dependent hypothermia, too.

It can be seen that putative D_3 antagonists caused hypermotility and did not affect body temperature. Putative agonists evoked hypomotility and hypothermia. The effect of the partial agonist was like that of a full agonist. However, the effect of PD-128907 and U-99194A on motor activity and body temperature were shown to be mediated by D_2 and not D_3 receptors in experiments done with D_2 and D_3 KO mice. Since the SB agonist and antagonist showed uniform actions to their respective pharmacological analogues the above possibility cannot be excluded in their case, either. Experiments are under the way for more detailed characterization of the behavioral effects of these D_3 ligands.

INVESTIGATIONS ON THE LONG-TERM EXPRESSION OF THE NMDA RECEPTOR SUBUNIT NR2B IN THE CEREBELLUM OF TRANSGENIC NR2B/2C EXCHANGE ANIMALS

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NMDA receptors play important role during the development, plasticity, injury and regeneration of the central nervous system. NMDA receptors are heteromers, formed by NR1 and at least one type of NR2 (NR2A, NR2B, NR2C or NR2D) subunits. During development, NMDA receptor subunits show characteristic spatial and temporal distribution, and the properties of the receptor complex are greatly influenced by their subunit composition.

The developing cerebellum serves as an excellent model system to investigate the role of the different NR2 subunits in the NMDA receptor functioning, as NR2B, NR2A and NR2C subunits are expressed in a strictly regulated time-shift by cerebellar granule cells. During the early development and migration of the granule cells, NR2B subunits are expressed predominantly, but from the second postnatal week on, NR2B gets replaced by NR2A. By the end of the migrational period of granule cefls, NR2C subunits also appear and are expressed throughout adult ages.

To investigate the physiological consequences and importance of the NR2 subunit changes, a transgenic knock-in mouse line, NR2B/2C, was created by inserting NR2B cDNA into the NR2C gene, leading to long-term cerebellar expression of the NR2B, and a deletion of the NR2C subunits. RT-PCR, *in situ* hybridisation and Western-blotting techniques were used to show that the subunit exchange took place in transgenic animals. The functional involvement of the NR2B subunits into the NMDA receptor complex in transgenic animals was proven by patch clamp analyses. The effects of the exchange between NR2C and NR2B subunits on the migration of the granule cells were investigated by histological and time lapse videomicroscopic observations.

ULTRASTRUCTURAL CHARACTERISATION OF NADPH-DIAPHORASE POSITIVE NEURONS IN THE CAECUM OF THE SNAIL, *HELIX POMATIA* L.

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Recently, a unique NADPH-diaphorase (NAOPH-d) positive neuron population have been demonstrated in the wall of the caecum of the snail, *Helix pomatia.* Projections of the NADPH-d positive large solitary neurons were shown to form a myenteric and a submucosal plexus and

to terminate with varicose fibres on the perikarya of non-NADPH-d positive neurons of the plexus and of the intestinal nerve, as well as on the circular muscle fibres. In this study, we have analyzed the ultrastructural localisation of NADPH-d reaction.

It has been found that several intracellular components of the enteric neurons contained NADPH-d positive reaction product. Endoplasmatic reticulum elements near the nuclear envelope revealed intensive NADPH-d positivity in two (a slightly electron dense and an electron lucent) types of four ultrastructurally different large (d~100 μm) neutral perikarya in the wall of the caecum. Numerous axon bundles also contained NADPH-d active membrane fragments in the myenteric and submucosal plexus. Three types of axon varicosities, forming close membrane contacts with smooth muscle cells, could be distinguished, according to their vesicle content which showed NADPH-d positive reactivity along their membranes: i) varicosities with 80 nm spheroid, low electron dense vesicules; ii) varicosities with 80 pm spheroid homogeneous of medium electrondensity vesicules; iii) varicosities containing a mixed population of 20 nm spheroid NADPH-d positive clear and NADPH-d negative dense-core vesicules. In the submucosal plexus, NADPH-d positive type i) varicosities formed axosomatic contacts on the surface of two types, a NADPH-d positive and a NADPH-d negative of neurons. The NADPH-d positive ones contained clear vesicules and an electron dense material in the cytoplasm, whereas the others contained dense-core vesicules in a electron lucent cytoplasm.

Our results obtained on the *Helix* gut show similarities with the ultrastructural localisation of NADPH-d in the crustacean sensory axons and in different nuclei of the rat central nervous system (1, 2). On the basis of our ultrastructural findings, it seems that NO may participate in the modulation of the gut movement direct effect on the muscle fibres and through influencing the function of other enteric neurons in *Helix*.

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NOVEL SITES FOR ESTROGEN ACTION IN THE BRAIN

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For decades estrogen was thought of only as a "sex hormone", as it plays a critical role in regulating behavioral and physiological events essential for successful procreation. In recent years, estrogen has been shown to exert effects on the structure and function of the cortex, hippocampus and other brain regions that contained few estrogen receptors (ERs). The discovery of a new estrogen receptor, ER-ß, in 1996 and localization of ER-ß mRNA in many brain regions including the cortex and pyramidal cells of the hippocampus have provided new insight into how estrogen may directly modulate the morphology and function of these

neurons. Recent *in vivo* binding studies using a radiolabeled estrogen (^{125}T-estrogen) with an equal affinity for ER-α and ER-ß detected specific nuclear binding sites in regions of the ERßKO mouse brain where ER-ß is expressed. Subsequent ^{125}I-estrogen binding and immunocytochemical studies in rats have demonstrated the presence of ER-α throughout the brain. In the cortex, ER-ß is scattered throughout laminae II-VI of the isocortex, a pattern that matched ER-ßmRNA. ^{125}I-estrogen binding was also seen in the pyramidal cells throughout CA1-3 of the hippocampus, with the majority of cells in the ventral horn of CA2 and CA3 being labeled. The results of *in situ* hybridization studies revealed that both ERs are expressed in the hippocampal formation, although the intensity of the ER-α hybridization signal appeared to be stronger than ER-ß. The results of these studies have demonstrated the presence of biologically active ER-ß in many rodent brain regions including the cortex and hippocampus. Since an increasing body of work has suggested that estrogen can act as a neuroprotective agent, a gerbil model of global ischemia was utilized to investigate the neuroprotective role of estrogen in the hippocampus. Results from these investigations demonstrated that a variety of estrogens including 17ß-estradiol, estrone and DES, but not tamoxifen, protect the CAl pyramidal neurons from death after ischemic injury ovariectomized females. Together, these observations challenge our current thinking about steroid hormones and their mechanism(s) of action in the brain, in particular in regions associated with learning and memory and affected by the neurodegenerative conditions of aging.

STRUCTURAL PROOFS OF INDEPENDENT DENDRITIC INTEGRATING COMPARTMENTS IN THE CEREBELLAR PURKINJE CELL

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Introduction

This study aims to give an exact morphological base to assign functionally independent dendritic compartments in the cerebellar Purkinje cell, formerly revealed in physiological analysis of intradendritic records

A Visual C++ 6.0 software package has been elaborated for automatic processing of dendritic arborization. This set of programs is capable to be used for analogous tasks, as e.g. the morphometry of retinal arborization etc.

In earlier studies we demonstrated double (sometimes triple or quadriple) rhythmic prespike activity patterns in dendritic microelectrode records taken from cerebellar Pc. By their active and passive counteractions the actual input activity pattern will turn into the arrhythmic output spiking by a nonlinear and phase-sensitive integration. This curious compound spike-generating process can give rise to novel cerebellar functional models. The acting membrane dynamics rely upon both the ionic current machinery and the specific micromorphology of the dendritic tree, especially that of the branching areas. Although, statistical analyses on Pc dendritic tree generally followed graph-theory, and elaborated abstract parameters for complexity and hierarchy, but denied realistic geometry. Thus a novel, specific morphometric study should be carried out first relating the main branching sites.

Experimental procedure and preprocessing of microphotographs

a, HRP-labeled Purkinje cells (Histological samples were kindly provided by G. Bishop, J. King and also by J. Takács) were digitally photographed, then processed by an automatic program set (Dendron) for analysing the dendritic structure. The cascadic program blocks (developed inVisual C++) perform

a, the distinction of dendritic tree from the background,

b. gathering the pixels of the contour into a sequential file,

c, tracking the midline structure,

d, designing "jumping points" and trigones at large branching sites on the base of the diverging midline tracs. The configuration and the rotation/orientation and of the triangles can be considered as indicators of local asymmetry.

e, determining the relevant mean thickness as well as the length of all the three branching components,

f, specifying the orientation of axes of the mouth intersections, from which the three main angles of branching can be derived.

Survey of the statistical results:

Three main types of Purkinje cells have been classified, concerning their dendritic arborization:

Type I - symmetric cell, equipped with two separate dendritic shaft - an evidence of two obviously independent integrative fields.

Type II - symmetric cell with a common dendritic shaft - the first division results in branches of comparable diameter, carrying the separated dendritic fields.

Type III - asymmetric cell - the first division is unequal, followed by equal or unequal ones.

Interpretation of results

Type I symmetric Purkinje cell is definitely ready to give place to the formation of independent integrative fields, while

Type II is most probable to represent the same function, although it should be proved by two means:

a, The exact matching of physiological and morphometrical founds would need parallel studies on the same population of cells.

b, The conclusive proof could be obtained by multicompartmental functional modeling, directly based upon the new morphometric studies.

The same way it can be verified, that Type III cell can also be the substrate of independent dendritic integrative processes, giving rise to the more rarely observed triple prespike patterns.

RELATIONSHIPS BETWEEN SEIZURE ACTIVITY AND EXTRACELLULAR AMINO ACID AND NUCLEOSIDE CHANGES INDUCED BY 3-AMINOPRIDINE IN RAT HIPPOCAMPUS

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Our knowledge of the role of nucleosides in the CNS is very limited, however their transporters and receptors are well known. Depolarisation experiments suggest that some nucleosides can be released under pathological conditions like brain hypoxia and epilepsy, or ever under physiological conditions. Adenosine can be formed from the neurotransmitter ATP and is a well-established neuromodulator. Since UTP receptors have recently been identified, it is possible that uridine, being a degradation product of UTP may have similar roles as adenosine.

In an *in vivo* microdialysis study we examined the effect of 3-aminopyridine induced epilepsy on the extracellular changes of nucleoside- and amino acid concentrations and recorded EEG activity. Extracellular adenosine, inosine, uridine and glutamate levels significantly increased, while the extracellular concentration of glutamine decreased. 3-aminopyridine induced intense and frequent epileptic seizure after administration.

We concluded that uridine - similarly to adenosine - is a potent neuromodulator. It may play an important role in neuroprotection by protecting neurons from the damaging neurodegenerative effects of excitatory amino acids.

TOPOGRAPHIC DISTRIBUTION OF ESTROGEN RECEPTOR-ß IMMUNOREACTIVITY IN DISTINCT SUBDIVISIONS OF THE HYPOTHALAMIC MAGNOCELLULAR NEUROSECRETORY SYSTEM OF THE RAT

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Oxytocin (OT) and vasopressin (VP) neurons of the magnocellular neurosecretory system regulate reproductive events and water-electrolyte balance, respectively. Although estrogens influence many aspects of magnocellular neuronal functions, the issue of whether estrogen receptor is present in OT and VP neurons of the paraventricular and supraoptic nuclei (PVN; SON) is controversial. Recently, a novel subtype of estrogen receptor (ER-ß) has been discovered and its distribution in the rat brain mapped. Our experiments used a highly sensitive dual-label immunofluorescence detection method to examine the topographic distribution of estrogen receptor-ß (ER-ß)-immunoreactive OT and VP neurons in distinct subdivisions of the

PVN and the SON of the female rat. The results of dual-labeling assays showed the highest levels of ER-ß immunoreactivity in OT neurons of the caudal PVN which regulate autonomic functions via descending projections. In addition, somewhat lower levels of ER-ß immunoreactivity were present in the majority of magnocellular VP neurons in the PVN and the SON as well as many accessory magnocellular cell groups. In turn, ER-ß immunostaining was faint in magnocellular OT neurons of the SON which are implicated in the regulation of neurohypophysial functions. The use of a novel *in situ* hybridization triple-labeling technique to confirm these immunocytochemical findings revealed similar tendencies of ER-ß mRNA expression within hypothalamic OT and VP neurons. The distinct co-expression pattern of ER-ß with OT and VP immunoreactivities in individual subnuclei of the PVN indicates that estrogens can exert differential effects on topographic and functional subpopulations of OT and VP neurons in the PVN and the SON via ER-ß.

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SODIUM-AZIDE INDUCED CHRONIC ALZHEIMER'S MODEL IN RATS

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In neurological diseases affecting the brain and muscle selective abnormalities of mitochondrial enzyme activity play a crucial role. Partial inhibition of the mitochondrial respiratory chain causes reactive oxigen species generation, diminishes aerobic energy metabolism, interferes with microglial inflammatory responses, creating a deleterious spiral that may contribute to neurodegeneration [4]. Reduction the activity of cytochrome *c* oxidase (selective loss of complex IV ETC function) has been identified in mitochondria from blood platelets and *post mortem* brain tissue from Alzheimer's disease patients [1, 5]. Sodium-azide (NaN_3) at low doses causes a selective impairment in cytochrome *c* oxidase activity inducing chemical hypoxia (blocks iron in heme prosthetic group of A_3 in a reversible way reacting with the *ferric form* [Fe^{+3}] of iron). This inhibition is inducible by chronic (2-3 weeks) s.c. NaN_3 treatment [2], which evokes a long-lasting energy deficit, loss of neurons in hippocampal formation, in the mesencephalic reticular formation, in the central amygdala, as well as in the basal ganglia, and causing disturbances in the cholinerg system producing learning and memory deficits [3, 6]. These symptoms are also characteristic to Alzheimer's disease patients.

In recent study pilot investigations were executed before application of osmotic minipumps. Wistar rats (male, 280-300 g) were treated by four doses of NaN_3 (2-4-6-8 mg/kg, s.c.) three times a day during 11 days. Spatial orientation capability of animals (cognitive fanction) was tested in Morris water maze test (MWM) between the 8th-11th treatment-day. On the 12th day animals were sacrificed and the brains were removed for biochemical (ChAT and AChE activities in the cortical and striatal samples) and histological analysis.

The 3×2 and 3×4 mg/kg doses did not evoke any kind of significant changes in behaviour, cognition or biochemical data in treated animals, while the 3×6 mg/kg dose caused significant learning and memory deficit in MWM test, and the ChAT activity was significantly lower in

the striatum of these animals. However, the percentage of exit abruptly raised to 40% on the final day of experiment. The 3×8 mg/kg NaN_3 dose proved to be rather high, all animals died within 4 days.

The second schedule of treatment was expanded to 3 weeks, but two days were free from Na-azide after every fifth day. Three groups were investigated (a; NaN_3 3×6 mg/kg, b; NaN_3 3×7 mg/kg and c; NaN_3 3×6 mg/kg + BRX-218 2×10 mg/kg p.o.).

The Biorex's compound significantly prolonged the survival time of azide-treated animals ($p<0.05$, Fisher exact) and protected the learning and memory function of rats ($p=0.05^4$, Kruskal-Wallis) in MWM test. The 3×7 mg/kg NaN_3 dose, like to 3×8 mg/kg proved to be rather high.

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RETARDED MYELINATION IN THE LUMBAR SPINAL CORD OF PIGLETS BORN WITH SPREAD-LEG SYNDROME

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Piglets born with spread-leg syndrome, a congenital weakness of the hindlimb adductors, were investigated to determine the site of lesion leading to limb impairment.

Histological and immunohistochemical studies of the motor neuron unit showed no alterations but quantitative analysis revealed a reduction of axonal diameter and myelin sheath-thickness of the fibres innervating the adductors of the affected limbs. In the lumbar spinal cord a lack of myelination was observed in the tracts descending to the lower motor neurons.

Recovery from the syndrome was accompanied by a catching-up of myelination with that of the controls. The spread-leg syndrome is due to a nutritional deficiency in the sow; thus it is assumed that the deficient maternal substances, mainly choline and methionine, are essential for the normal myelin production by spinal white matter oligodendrocytes of the fetus.

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GABAergic INHIBITION IN THE FUNCTION OF SECONDARY EPILEPTIC FOCUS INDUCED BY 4-AMINOPYRIDINE

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GABA-mediated neurotransmission is involved in both the expression and control of epileptiform activity. The aim of this study was to investigate the role of (GABAs receptors in the function of secondary epileptic focus induced by 4-aminopyrodine (4-Ap) *in vivo*. The mirror focus (Mf) offers good possibilities to study epileptic events, because in this focus epileptiform activity is generated without any exogenous effects.

In one group of rats, a selective $GABA_B$ receptor antagonist, the CGP 35348 (1 mM) was applicated at the expected place of the Mf half an hour prior the induction of primary focus (Pf). In the other group of animals the antagonist was applied at the Mf with already ongoing seizure activity. The surface electrocorticogram (ECoG) was stored in computer memory for analysis of the different parameters of cortical seizure activity. Effects of the antagonist were investigated also on the basic ECoG and the somatosensory evoked potentials.

CGP 35348 increased the amplitudes of somatosensory evoked potentials. Pretreatment with CGP 35348 at the Mf significantly decreased or prevented epileptiform activity locally. On the other hand pretreatment with the antagonist in the Mf facilitated the maintenance of seizure activity in the Pf, indicated by an increase in the duration of ictal periods.

Treatment in the already active Mf resulted in a shift in the ratio of different discharges patterns in favour of higher frequencies in both foci.

In our previous study we have found that blockage of $GABA_B$ receptors at the site of the Pf facilitated both the induction and the maintenance of epileptiform discharges both in Pf and Mf.

From these observations we conclude that $GABA_B$ receptors are probably involved in the expression and propagation of epileptiform activity. The Mf might have a control feedback on the expression of seizure activity in the Pf. For better understanding of the role of $GABA_B$ receptors in the function of secondary epileptic focus additional experiments are needed.

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ESTROGEN-DEPENDENT NEURONAL PLASTICITY STUDIED BY PSEUDORABIES VIRUS TRACING

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In the past decade, a number of publications have demonstrated that estrogen induces plastic changes in synaptic connections in the central nervous system. These observations were based on evaluations of the density of dendritic spines or axodendritic synapses (1, 3, 5).

The method of transneuronal tracing with pseudorabies virus (PRV) is especially suitable for study of the changes in synaptic connections. In the present work, we studied the estrogen-dependent plasticity in connections between neurons of the arcuate nucleus (Arc) and the subfornical organ (SFO). In both of these, the neurons have estrogen receptors (2).

Female rats were ovariectomized (OVX), and 14 days later PRV was injected into the Arc (0.1 μl, 10^9 PFU). Four of the animals were treated with 17ß-estradiol (100 μg/100 g in sesame oil, i.p.) 12 hours before PRV injection (OVX+E-12 h), 4 others received the same dosage of estradiol 4 hours before virus injection (OVX+E-4 h), and the remaining 4 rats were treated with vehicle (OVX).

After a survival time of 72 hours, the animals were killed and the PRV immunoreactivity was tested in their SFO. In all the OVX±E-12 h animals, PRV-immunoreactive neurons were to be seen in the SFO. In the OVX+E-4 h and the OVX animals, no immunoreactivity was observed in their SFO.

These results demonstrate an estrogen-dependent immunoreactivity in the SFO, and suggest that estrogen plays a role in the regulation of synaptic remodeling in these brain structures.

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EFFECTS OF DEGRADATION PRODUCTS ON RECEPTOR BINDING PROPERTIES AND BIOLOGICAL ACTIVITY OF ENDOMORPHIN 1

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The recently isolated endogenous peptide endomorphin 1 (E1) has high affinity for the μ opioid receptor (MOR) and plays an important role in analgesia[1]. Several of its degradation

products have been isolated from the central nervous system[2]. Degradation products present structural similarities and may influence the receptor binding properties and biological activity of the parent compound. Therefore, we investigated how degradation products of E1 might influence the binding of the newly synthesised [^{3}H]E1 to the MOR, the consequent activation of S proteins and its antinociceptive effect. [^{3}H]E1 bound to a single binding site in the presence of naloxone (B_{max}=188 fmol/mg; K_d=1 nM) in rat brain membranes, which binding was selective to MOR. Both N- and C-terminal truncation of E1 resulted in peptides with low MOR binding potency. None of these peptides had an effect on GTP binding, nor was able to produce analgesia. However, due to their ability to displace an amount of the E1 binding, in high concentrations they might be competitors for E1 *in vivo*. Therefore, the decrease/loss of activity of E1 takes place not only because of degradation, but because of sterical inhibition of the binding to the MOR by the degradation peptides.

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EFFECTS OF CAPSAICIN OR CAPSAZEPINE ON FOOD INTAKE INDUCED BY FASTING OR NEUROPEPTIDE Y

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Neuropeptide Y (NPY) produced in the arcuate and released in the paraventricular nucleus is known to participate in the regulation of food intake: high NPY levels (of either exogenous or endogenous origin) are accompanied by increased, while low NPY levels by decreased feeding activity. Centrally applied NPY induces a coordinated energy conserving behavior: besides food intake, energy metabolism decreases. Hunger and satiety are influenced also by neural signals from the gastrointestinal tract. Intraperitoneal (IP) cholecystokinin (CCK) induces satiety, and gastric stretch has an additive action. Capsaicin desensitization of the abdominal nerve fibers prevents the CCK-induced satiety. No data are available on the possible interaction between the neural signals and central NPY activity.

In the present studies it was analyzed whether local capsaicin desensitization of abdominal vagal afferent fibers of cold-adapted (CA) or non-adapted (NA) rats can influence food intake induced by 24- or 48-h food deprivation (i.e., at different levels of endogenous NPY). Small IP capsaicin doses of 5 mg/kg were given one week prior to food intake measurements. Destroying these fibers was assumed to prevent satiety in the course of food intake and thus, to cause enhancement of food intake during a 3-h re-feeding period after fasting. It was also investigated whether or not such desensitization can influence food intake induced by exogenous NPY (0.5 to 10 μg injected into the lateral cerebral ventricle through a preimplanted cannula). The competitive capsaicin antagonist capsazepine, applied acutely (4 or 8 μg IP), was expected to exert actions similar to those of chronic capsaicin desensitization.

In capsaicin desensitized rats food intake upon re-feeding was found to be greater than in control rats (mainly the meal size of the first 30-mm feeding session was enhanced), but the NPY-induced food intake was unaltered. Accordingly, inhibitory (satiety) signals conveyed by the vagus appear to suppress central NPY release in the course of feeding, the effect depending on the level of endogenous NPY. However, once the NPY is present in a given amount (from exogenous sources), its effect cannot be influenced by these neural signals.

Contrary to expectations, capsazepine attenuated, and did not enhance, fasting-induced food intake, suggesting attenuation of vagally transmitted stimulatory (hunger) signals (stimulation of satiety signals, although possible, seems less likely). Capsazepine was also without effect on food intake induced by exogenous NPY.

In conclusion, capsaicin-sensitive afferent vagal fibers from the abdominal cavity may influence release of central NPY. Such fibers may convey not only satiety signals, but also hunger signals, suggesting that hunger signals of abdominal origin form a special entity, and hunger cannot be explained simply as lack of satiety. Physiological activation of vanilloid receptors is proposed to play a role in these hunger signals.

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SUPPRESSION OF PERITONEAL MACROPHAGE PHAGOCYTOSIS OF *CANDIDA ALBICANS* BY VV-HEMORPHIN 7

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Hemorphins are hemoglobin-derived oligopeptides with opioid activity, first reported by Brantl and coworkers in 1986 (1).

In order to study the biological effect of VV-hemorphin 7 (Val-Val-Tyr-ProTrp-Thr-Gln-Arg-Phe-OH) on macrophages, phagocytosis of live *Candida albicans* yeast cells by murine peritoneal macrophages was used as an experimental model. This method was described by Szabó and coworkers who found that the inhibition of phagocytosis by opioids is mediated via μ-opioid receptors (2). In the presence of submicromolar concentrations of VV-hemorphin 7, adherent macrophages ingested significantly less *Candida* cells compared to the control. The inhibition was concentration-dependent in the range of 10^{-10}-10^{-6} M and naloxone-reversible. Naloxone did not have a direct effect on *Candida* phagocytosis of macrophage cells when administered alone.

These results indicate that VV-hemorphin 7 may play a role in the regulation of macrophage functions in an opioid receptor-regulated manner.

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CALCIUM AND MITOCHONDRIUM

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Capsaicin, the pungent ingredient in peppers of the *Capsicum* family has became a lead molecule for the discovery of ion channels expressed selectively in noxious heat sensitive nociceptive primary afferent neurones. The cloned capsaicin receptor called VR1 is a nonselective cation channel with high Ca^{2+} permeability. The influx of Ca^{2+} can stimulate Ca^{2+}-sensitive enzymes, and so induce a secondary rise in the levels of intracellular mediators such as cGMP. The rise of calcium can modulate membrane channels and receptors. KCl also induces influx of Ca^{2+}, in this case Ca^{2+} enters the cell through the voltage-gated Ca^{2+}-channels. To explore intracellular consequences of calcium ion influx, one must examine roles of cellular calcium ion buffers such as mitochondria. Interactions between VR1 agonists or KCl and impaired mitochondrial calcium storage were analysed with the aid of a protonophore FCCP. In the presence of FCCP the inner mitochondrial membrane permeabilized to protons, the stored calcium ions get released to the cytosol. Primary cultures were made from Wistar rats and used 4-8 days after isolation. Dye loaded (1 μM fura-2 AM) cells were illuminated alternately at 340 and 380 nm light and the intensity of emitted light at 520 nm was measured.

Capsaicin (33 nM, 330 nM and 3.3 μM) and the capsaicin analogue resiniferatoxin (RTX, 1 nM) cause a transient increase in fluorescence of fura-2 loaded cultured trigeminal neurones as measured with ratiometric technique. The evoked calcium signals were compared to those evoked by 50 mM KCl. The protonophore FCCP (1 μM) induced rather small, less than 0.2 averaged calcium signals when applied first. The FCCP-evoked signal increased considerably when the test was repeated in the interval of 20 to 240 sec following previous stimulation with either KCl or capsaicin or RTX, Normalized means of FCCP signals were 10.8±1.77 with 33 nM; 2.72±2.04 with 330 nM and 7.23±3.46 with 3.3 μM capsaicin, while that with 50 mM KCI application was 3.66±2.68. There was no correlation between enhancement of FCCP-induced signal and the nature (capsaicin or RTX or KCl application) of the preceding calcium fluorescence change. The size and duration of the preceding signal affected slightly more consistently the change of FCCP-evoked signal than the nature of stimulation. Large and especially prolonged stimulus-evoked calcium signals tended to be associated with larger increment of FCCP-evoked signals.

When we used FCCP conditioning before capsaicin or KCl application, we expected an increase of the evoked calcium signals, because the protonophore should temporary block the mitochondria and eliminate their role in calcium buffering. To our surprise, FCCP application depressed both the capsaicin and RTX-evoked signals consistently to 30 or 40%. The mean response of control ratiometric calcium signals to capsaicin was 0.4+0.17 which went down to 0.19+0.1 after FCCP conditioning and recovered to 0.21+0.11 in 5 min wash. FCCP

conditioning depressed the RTX-evoked peak fluorescence to 33.5% followed by a recovery to 112%. Depression of KCl-induced fluorescence signals did not occur.

Our data are consistent with the widely accepted views concerning the role of mitochondria in buffering of intracellular calcium ion concentration. The cell distinguish the capsaicin-evoked Ca^{2+} influx from the voltage-gated Ca^{2+}-channel-coupled Ca^{2+} influx. We conclude, that the way which the Ca^{2+} enters the cell is important.

THE EFFECT OF BRAIN-DERIVED NEUROTROPHIC FACTOR ON THE DEVELOPMENT OF RAPID TOLERANCE TO MORPHINE

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Neurotrophins not only affect neronal development, but also influence neuronal function in the adult brain. Since brain-derived neurotrophic factor (BDNF) and its receptor TrkB are expressed in brain regions known to be involved in opiate addiction, the present experiments were designed to investigate the possible role played by this neurotrophic factor in the development and maintenance of rapid tolerance to the antinociceptive effect of morphine. Intracerebroventricular injections of BDNF (100 ng) were administered to intact mice either subchronically (i.e. for 3 or 7 consecutive days before tolerance induction) or concomitantly with the two days morphine treatment. For the induction of rapid tolerance mice were given 3 subcutaneous injections of morphine hydrochloride (5 mg/kg) and the development of rapid fractional tolerance was tested by measuring the tail-flick latemicy at 30, 60 mid 120 minutes after each morphine challenge. In experiments designed to investigate the effect of acute BDNF treatment on the maintenance of morphine tolerance, after a 3 days recovery period the animals were given a 5 mg/kg rechallenge dose of morphine. BDNF treatment resulted in a significant facilitation of tolerance development. After a subchronic neurotrophin treatment this effect was more evident. Three days after the last morphine challenge BDNF treated mice were still tolerant to the antinociceptive effect of the rechallenge dose of morphine. On the basis of these observations we conclude that BDNF influences both the development and maintenance of rapid analgesic tolerance to morphine.

COMPARATIVE ULTRASTRUCTURAL STUDY OF THE VOMERONASAL ORGAN

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Olfactory information is an important cue for animal behavior. This information is received and transferred through the main olfactory and the vomeronasat system. Former studies suggest that receptor cells undergo continuous replacement in both system and are functioning rather early in the perinatal and in the early postnatal period, too. However, the development of the vomeronasal system lags behind that of the main olfactory system. The precise role of the vomeronasal system is not known during development, but it seems plausible to suppose its contribution to direct neonatal animals to the mother (food) and nest which are essential for survival.

In the present work we examined the structure of the vomeronasal organ (VNO) at P12-14 postnatal days in order to compare its maturity in the guinea pig, rat and mouse. Light- and electronmicroscopic morphological features were similar in the rat and in the mouse. Well-branched microvilli of the receptor cells (RC) and numerous microvilli of the supporting cells (SC) were observed on the luminal surface of the sensory epithelium at about the mid-region of the vomeronasal duct (VND) characteristic of the ultrastructure of the mature VNO. In the cytoplasm of the receptor cells an accumulation of bizarre membraneous structures were frequently seen especially in the mouse. These membranes showed continuity with RER membranes, and could be the morphological signs of an enhanced metabolic activity of the receptor cells. In the nuclei of these cells two-three large nucleoli were present. However, less-developed areas - characterised by fewer and shorter microvilli on the luminal surface both on the receptor and on the supporting cells - were also observed at the terminal region of the organ. The lumen of the VND was smaller at this region and the sensory free epithelium (SFE) - localised at the lateral wall of the VND - showed a surprising complexity with the appearance of ciliated cells characteristic of the olfactory epithelium. These cells were present in the VNO of the guinea pig as well. The VNO of the guinea pig was somewhat larger, the shape of the supporting cells were more elongated and the receptor cells were covered with more branched microvilli. The VNO of the guinea pig is somewhat more differentiated and matured in comparison to that of the rat and the mouse. In conclusion, the maturity of the VNO is not complete at P 12-14 days in the investigated species. There are differences in the degree of differentiation even along the rostro-caudal extent of the VNO. At the caudal part of the organ morphological features of less developed VNO was also characteristic in all of the investigated animals.

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COMPARATIVE IMMUNOHISTOCHEMICAL ANALYSIS OF SURGICALLY REMOVED HIPPOCAMPI OF PATIENTS SUFFERING FROM TUMOR-ASSOCIATED VERSUS CRYPTOGENIC OR OTHER SYMPTOMATIC TEMPORAL LOBE EPILEPSY

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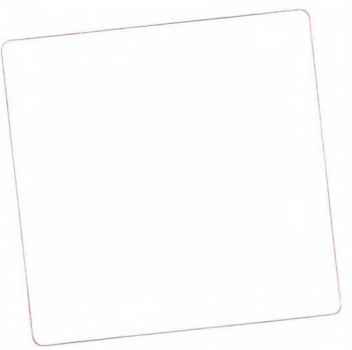
We performed an immunohistochemical study of different cell types in hippocampal tissue derived from patients with tumor-associated temporal lobe epilepsy. The results were then compared with hippocampi derived from patients suffering from therapy-resistent epilepsy, and with control *post mortem* tissue. The distribution and morphology of four neurochemically and functionally different hippocampal interneuron types was investigated: those containing calbindin (CB), calretinin (CR), parvalburnin (PV) or substance P receptor (SPR). We asked whether the epileptic seizures in these patients led to, or were caused by the same changes of these interneurons as seen in the cryptogenic cases with hippocampal sclerosis, or whether they were induced solely by the tumor without causing any permanent damage to the interneuronal networks. Hippocampi (removed 2-5 hours after death) of six control subjects with no sign of neurological disorders and 16 therapy-resistant patients with different types of extra-hippocampal tumor were immersion-fixed for immunohistochemistry. Immunohistochemical stainings were performed for CB, CR, PV, SPR, and the relative number, distribution, and morphology of the specific cells have been analysed. The tumor-associated epileptic patients showed very mild or no hippocampal sclerosis, while atrophy and sclerosis was present in over 90% of the non-tumorous group. The distribution and morphology of the interneurons were also different, strongly correlating with the degree of tissue sclerosis, but not with characteristics of the tumor. The density of most interneuron types decreased in the CA1 region. The number and distribution of SPR-positive cells was similar to the control, but their dendrites became strongly beaded and displayed more numerous short dendritic branches. The inhibitory interneurons of the hippocampus play a crucial role in the synchronous population discharge patterns and their impairment leads to epileptic seizures. We found that there is a loss of inhibitory neurons even in cases with negligible loss of principal cells. Changes of the interneurons are not linked to the presence of tumors, therefore the pathologic lesions in the tumor-associated cases are likely to represent consequences of the epileptic seizures.



THE EFFECT OF NEUROPEPTIDE Y ON OPERANT BEHAVIOR OF THE RAT

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Neuropeptide Y (NPY) is synthesized in the arcuate nucleus, and projects to regions important in the regulation of food-intake and energy-balance (e.g., paraventricular nucleus, dorsomedial nucleus and medial preoptic area). Exogenous NPY elicits food-intake, suppresses metabolic rate and body temperature. It is supposed that NPY also plays a role in the food rewarded operant behavior.

Methods

Male CFY rats were conditioned in Skinner-boxes. Their task was to press a lever in order to get a food-pellet. Then, a cannula was implanted in the lateral cerebral ventricle (LCV), through which 10 μg NPY and/or NPY-antagonist [D-Tyr^{27,36}, D-Thr^{32}-NPY(27-36)] was injected in 5 μl volume. Controls were given 0.9 % NaCl-solution. After injections, rats were placed back to the operant chamber, where the number of lever-presses were registered during the 60 minutes of the task.

Results

LCV NPY increased the number of lever-presses for the positive reinforcer (food-pellet). The NPY-antagonist significantly reduced the number of lever-presses, and the effect was immidiate. We also investigated the learning ability of rats: there was no significant difference between the NPY-treated and the control groups.

Conclusions

NPY does not only induce food-intake, but also affects the aim-drived operant behavior for food. These effects can be inhibited with NPY-antagonist. In addition, NPY does not have any effect on the learning ability of rats in the observed task.

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COLOCALIZATION OF GLUCOCORTICOID RECEPTOR AND VASOPRESSIN MESSENGER RNAs IN THE BED NUCLEUS OF THE STRIA TERMINALIS AND THE SUPRACHIASMATIC NUCLEUS OF THE RAT

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Glucocorticosteroids appear to influence the activity of vasopressin (AVP)-expressing cells from the bed nucleus of the stria terminalis (BST) and suprachiasmatic nucleus (SCN) differentially. Dexamethasone treatment increases AVP peptide levels in the SCN, but decreases AVP mRNA levels in the BST. The present studies used dual-label *in situ* hybridization histochemistry (ISHH) to test whether AVP neurons in the BST and SCN express

glucocorticoid receptor (GR). Hybridization was performed on 20 μm coronal sections of fresh-frozen brains. AVP mRNA-expressing neurons were detected using a digoxigenin-labeled complementary ribonucleic acid (cRNA) probe for AVP mRNA, whereas GR mRNA was targeted with a ^{33}P-labeled cRNA probe and visualized on emulsion autoradiographs. The mRNA encoding GR was ubiquitously expressed in the rat brain. In addition, subsets of AVP mRNA expressing cells (about 10-20%) in the BST and SCN contained hybridization signal for GR. Although these data suggest that glucocorticosteroids may directly regulate gene expression within AVP cells through GR, it appears that only subpopulations of AVP neurons in the BST and the SCN are directly responsive to circulating corticosteroids.

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THE NUMBER OF PURKINJE CELLS CHANGES DURING POSTNATAL DEVELOPMENT IN THE CEREBELLAR CORTEX OF CAT

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On the basis of our earlier observations, a prolonged migration of differentiating Purkinje cells (PCs) were seen in the white matter of the cerebellum using metabotropic glutamate type 1a (mGluR1a) immunohistochemistry for their visualisation. Integrating Purkinje neurons can also be seen in/or near to the internal granular layer (JGL) of the cerebellar cortex. The aim of this study was to clarify the relationship between the prolonged postnatal migration and integration, and the number of integrated Purkinje cells in the developing cerebellar cortex.

Four age groups were examined: P 0, P 42 and P 75 days old and adult cats. Disector method was applied in order to obtain unbiased estimates of the number of Purkinje neurons in developing cerebellum of kittens and cats. Purkinje cell nucleoli were used as particles of the counting method in semi-thin sections and the total number of PCs was estimated in each cerebellum of all age groups.

We have established that the number of Purkinje neurons increases from birth (~2.3 $\diamond 10^6$) gradually to the age of P75 (~3×10^6) then decreases to adult age (~1.7×10^6 /total cerebellum).

Our observations coincide with a known phenomenon of developing organism: the differentiating tissues including neural networks during development produce much more elements (cells) than it can be found in adults. In the case of developing cerebellum, this proceeds long after birth. The immigration of PCs results in a continuous numerical increase of this cell type until 75th postnatal day, followed by a period when programmed cell death (apoptosis) will be the dominant process leading to the final (adult) and probably permanent number of Purkinje neurons.

This work was supported by OTKA grants T026037 and by M028337.

BEHAVIOURAL AND NEUROTOXICOLOGICAL ALTERATIONS INDUCED BY SUBCHRONIC INORGANIC LEAD AND MERCURY TREATMENT IN RATS

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Lead and mercury diminish mental performance and cause behavioural and neurological disorders in children, and have similar effects in animal experiments. We studied the effect of 8 and 10 weeks relatively low-level mercury (0.5 or 2.0 mg/kg b.w. [$HgCl_2$]) and lead (5.0, 50.0 or 500.0 mg/kg b.w. [Pb-acetate]), *per os* by gavage, treatment on spontaneous exploration, acoustic startle response and short-term and/or long-term memory of male Wistar rats. Functional neurophysiological changes of the central nervous system were studied by recording cortical and hippocampal spontaneous as well as cortical evoked activity from anaesthetized rats. Behaviour was tested in an 8-arm radial maze from the 7 day of treatment. During acquisition using food search activity, the rats learned to enter certain arms. Then, in the short-term working memory (WM) test, 2 or 4 hour later, the remaining ones. In the reference memory (RM) test, they learned to enter only arm previously rewarded. Long-term performance was tested after a 2 weeks resting period. Open field activity (spontaneous horizontal and vertical exploration) and acoustic startle response of the rats was tested on the 5^{th} and 10^{th} week. The results were analyzed by means of ANOVA. No significant change in the body weight gain or other signs of general toxicity were observed, but there was a tendency to memory deficit from the acquisition phase on. In the 2 or 4 hours WM, significant, dose and time dependent performance decrease was seen. (Pb: 2 hours, $p<0.01$ for all doses vs. control; Hg: 2 and 4 hours, $p<0.1$ high dose vs. control.) In RM, Pb: $p<0.01$ high vs. control; Hg: n.s. Memory return after 2 weeks resting Pb: n.s.; Hg: $p<0.01$ low vs. control, $p<0.001$ high vs. control. WM: effects again significant in high vs. control. In the open field, horizontal and vertical exploration was diminished by all doses but the effect was marginally significant. High dosed rats were less active (and had less defecations). In the startle test, the number of responses was significantly and dose-dependently decreased by Hg ($p<0.01$, high vs. control). In the $HgCl_2$ treated rats, the ECoG activity was shifted to higher frequencies. The effect on evoked electrical activity (both central and peripheral) was below significance.

Lead and mercury seemed to affect emotion- and motivation-dependent activity and learning, and sensory-motor coordination. This underlines the risk arising from lengthy lead and mercury exposure of children.

CONTEXTUAL MODULATION OF ORIENTATION DISCRIMINATION IS INDEPENDENT OF STIMULUS PROCESSING TIME

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Processing of visual information from a given part of the visual field is strongly influenced by visual input from the surrounding region. Orientation specific neuronal responses in the primary visual cortex are inhibited and orientation discrimination threshold of human observers is elevated when the target stimulus is displayed in a dense array of similar elements. Comparison of electrophysiological and behavioral results suggests a close relationship between perceptual and neural effects of the contextual modulation. Contextual inhibition is most pronounced in the early transient response component of V1 neurons. This intriguing property of contextual modulation at the neural level allows us to make the following prediction concerning the perceptual effects of the context. Interruption of processing of the target with a high contrast mask should not effect the magnitude of the context induced orientation discrimination threshold elevation as far as the target-mask delay is longer than the duration of the early transient response component (~50 ms). To test this prediction we measured the orientation discrimination threshold of a target Gabor patch in the presence and absence of four similarly oriented flanking Gabor patches. We used backward masking to control processing time of the target. The ratio of thresholds with and without flanks is a measure for the effect of contextual inhibition. We found that this ratio remained essentially the same for all values of target-mask onset asynchrony. These results suggest that context induced elevation of the orientation discrimination threshold is closely related to contextual inhibition of the early transient response component of orientation selective neurons in the primary visual cortex.

POSTNATAL MIGRATION OF PURKINJE CELLS IN THE CEREBELLUM OF MOUSE

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The postnatal migration and differentiation of Purkinje cells (PCs) was studied during the postnatal period at P8/P9, P12 and P16 days in the mouse. In order to visualize juvenile Purkinje cells light- and electronmicroscopic metabotropic glutamate receptor type 1a (mGluR1a) immunohistochemistry was applied. This receptor is expressed in Purkinje cells

rather early. Genetically modified transgenic mouse strain (Ref. 1) was also used; in these mice the GAD67 synthetizing neurons are labelled with lacZ and can be visualized by beta-galactosidase cytochemistry in both adult and embryonic brains. Actively proliferating neuronal and/or glial cells were detected by BrdU immunohistochemistry following 80 mg/kg BrdU administration repeated daily at P0-P3 postnatal days.

The development of the cerebellar cortex is not completed by the time of birth. Granule cells are generated in the external granular layer (EGL) until P20/21 postnatal days in the mouse and migrate continuously to the internal granule cell lamina (IGL) via radial (Bergmann) glial cell-guided migration. Migration of interneurons to the molecular and granular layers was also observed to originate from the territory of the rhombic lip. As far as the PCs are concerned, this neuronal population was considered previously to be generated at about embryonic days E1 2/14 in the cerebellar anlage of the mouse. By the time of birth PCs were thought to occupy their final positions between the EGL and IGL laminae.

In the present study we have observed a prolonged migration of PCs during the peri- and postnatal period. Poorly differentiated cell forms were observed at P8/P9 days in the brainstem being attached to axonal bundles in the white matter of the cerebellar cortex near the cerebellar nuclei and also moving through the IGL in several vernal cerebellar lobules. The number of migrating PCs increased by P12 and especially by P16 days. At this time numerous migrating forms showing different levels of differentiation were frequently seen both in sagittal and in coronal serial sections targeting several neocerebellar and transitory areas lateral to the vermis. BrdU labelling in the nuclei of PCs was only occasionally seen. The overwhelming majority of this neuronal population, however, goes through their last mitosis before birth of the mice. We have detected at least two domain sites persisting in the brainstem beneath the dorsal surface, from which the PCs start their postnatal migration. One of these territories is the dorsal rhombencephalic neuroepithelium (DRN) described as an embryonic germinative zone supplying neurons which migrate via the pontine migratory stream (pms) at E14-E17 in the mouse (Ref. 2). From this zone, PCs seem to migrate either "directly" via the posterior cerebellar pedunculus or via the pms postnatally. Another source of PCs described for the first time in the present work is located beneath the dorsal surface of the pons near the mesencephalic border: the area of the dorsal pontine neuroepithelium (DPN). This neuroepithelium is most probably a remnant of the cerebellar anlage. PCs from this territory migrate "directly" to the cerebellar cortex via the anterior cerebellar pedunculus in surprisingly high number and during an extended postnatal period. The undifferentiated postmitotic precursors start their differentiation within the DPN after receiving axonal inputs of several types. The majority of axonal inputs to migrating PCs most probably derives from climbing fibers. Numerous climbing fiber-like axon terminals build up synapses on the somatic dendritic spines of the juvenile and migrating PCs, also on the dendritic shafts and dendritic spines occupying temporarily the place of the parallel fibers. Beside climbing fiber synapses other axonal endings containing larger round and/or pleoinorph synaptic vesicles are also seen in smaller numbers. During the axon guided neuronal migration PCs differentiate morphologically and by the time of their arrival to their final destination in the cerebellar lobules and sub-lobules they are morphologically similar to other PCs incorporated already in the Purkinje lamina. PCs were observed to migrate in groups made up of about 6-10 PCs. Oligodendrocytes are frequently seen in the neighbourhood of these migrating PCs supposing some role in the migration. There is a clear difference in the timing of the migration of PCs: late developing lobules lag behind the early developing ones. Migrating PCs occur strictly symmetrically in the two halves of the cerebellum, and the timing of their postnatal migration

seems to be strictly determined. We suggested that the observed prolonged peri- and postnatal migration of large population of PCs might be an important step contributing to the optimal morphological and functional maturation of the cerebellar cortex.

This work was supported by OTKA grants T 026037 and M028337.

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STUDY ON THE BASIC LEARNING AND BEHAVIOURAL CHANGES FOLLOWING TRANSSECTION OF INFRAORBITAL NERVE IN RATS

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The receptive field in the somatosensory cortex extends immediately after nerve transsection and recovery processes taking place in the two-four weeks following the surgery. In our previous study during this period we demonstrated an increased excitability of barrel cortex investigating some biophysical parameters of the affected neurones with intracellular recording technique.

The goal of the present study was to further analyse the altered excitability and sensitivity to LTP of barrel cortex neurones in *in vitro* slice experiments. Following the infraorbital nerve transsection of rats the slices were studied on the 1^{st}, 5^{th} and 14^{th} days. Changes of behaviour was parallel investigated in open filed experiments.

On the first day after the surgery the response evoked by stimulation of corpus callosum enhanced enormously compared to the control, the basically biphasic EPSPs become triphasic. This third component was NMDA receptor mediated as it was completely blocked by competitive NMDA receptor antagonist APV. Parallel with the increased excitability LTP was also enhanced. During the recovery period the excitability gradually decreased, although it was still higher on the 14^{th} day after the surgery.

The free behaviour of shame-operated animals follows the scheme of the normal behavioural trend: the orientation activity slightly decreased during the two-week experimental period, first of all the immobilisation time increased. The normal scheme, however, significantly altered as a consequence of the transsection of infraorbital nerve. Till the end of the experimental period immobilisation enormously increased. We suppose that fear and anxious level of the behaviour was enhanced.

PROJECTIONS OF PRIMARY AFFERENT FIBERS TO LAST-ORDER PREMOTOR INTERNEURONS IN THE LUMBAR SPINAL CORD OF RATS

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It is well established that neural circuits that generate rhythmic alternating activity of spinal motoneurones are located in the spinal cord. There is general agreement that neural activities generated by spinal neural mechanisms are transmitted to spinal motoneurones by a set of last-order premotor interneurons that establish monosynaptic excitatory and inhibitory connections with motoneurons. In addition to the strong drive from the spinal motor apparatus, last-order premotor interneurons also receive sensory inputs and descending commands from supraspinal motor centers. Thus, last-order premotor interneurons have crucial importance in the integration of activities generated by the spinal motor apparatus, sensory information and volleys arising from voluntary and non-voluntary higher motor centers, indicating that they play a substantial role in spinal motor functions.

Due to their contribution to spinal motor activities, last-order premotor interneurons have been extensively studied in recent years. Despite the extensive studies, however, there has been no attempt to make an accurate account concerning the morphological substantiation of the synaptic input systems of these neurons. Accordingly in the present experiments, we investigated the anatomical organization of one of the major synaptic inputs of last-order premotor interneurons. We have made an attempt to visualize and quantitatively analyze synaptic appositions between last-order premotor interneurons and primary afferents in the lumbar spinal cord of rats using double label neural tracing methods.

We have demonstrated that last-order premotor interneurons do really establish close appositions with terminals of primary afferents. However, we also found that labelled interneurons were contacted only by a few terminal of primary afferent fibers, and even this weak synaptic inputs were received only by a minor proportion (less than 10%) of last-order premotor interneurons.

The results indicate that last-order premotor interneurons presumably receive only a weak monosynaptic drive from primary afferents.

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PRO-GONADOTROPIN-RELEASING HORMONE PROCESSING

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GnRH is a major regulator of reproduction in mammals through its control of pituitary gonadotropin secretion. This decapeptide is first biosynthesized as a precursor molecule and is processed to a bioactive peptide. Studies in the immortalized LHRH neurons have revealed that

the pro-GnRH is converted to a GnRH intermediate and a peptide termed GnRH-associated peptide. The GnRH intermediate is next subjected to four additional modifications before bioactive GnRH is produced. Analysis of mRNA transcripts have shown that at least 4 different putative processing enzymes are expressed in the GT1 neurons. These enzymes consist of prohormone convertase 2 (PC2), carboxypeptidase (CPE), glutaminyl cyclase (QC), and peptidyl-glycine α-amidating monooxygenase (PAM). The first cleavage of the prohormone is by an endopeptidase. To determine whether PC2 or other members of the PC family of enzymes can process the pro-GnRH *in vivo,* heterologous cells were infected with vacinnia virus recombinants of GnRH and the different PC enzymes. These experiments demonstrated that PC2 could cleave pro-GnRH most efficiently. Expression of recombinant CPE together with recombinant pro-GnRH and PC2 revealed that the CPE could efficiently remove the Arg and Lys residues from the C-terminal of GnRH intermediate peptides. To determine whether the CPE could perform these cleavages in mammals, experiments were instituted with CPE^{fat} mice. These mice have been reported to be obese, diabetic and infertile. Analysis of pro-GnRH processing in hypothalamus demonstrated that the mutants are deficient in converting GnRH-$Gly^{11}Lys^{12}Arg^{13}$ or GnRH-Gly^{11} Lys^{12} to GnRH-Gly^{11}. Despite this fact, a small amount of GnRH is produced. Gonadotropin levels in serum are normal in both males and females. Serum estrogen levels are also normal in the female mutant, while progesterone concentrations are low. This latter effect appears to be due to the fact that the females were not ovulating and no corpus luteum was formed. Challenge with pregnant mare's serum gonadotropin and human chorionic gonadotropin is sufficient to produce ovulation. An examination of serum testosterone levels in male CPE mice demonstrated that these levels were normal. Sperm counts, however, were reduced in the mutants. Studies are currently underway to examine the basis of the male fertility in greater detail and to determine the roles of QC and PAM in the regulation of GnRH neuronal function.

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A LARGE-SCALE POPULATION MODEL OF THE SPINAL CPG FOR SWIMMING IN THE *XENOPUS* TADPOLE

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The basic behaviour of the *Xenopus* tadpole at stage 37/38 is that it can swim. This locomotion requires a neural network (Central Pattern Generator, CPG) that produces a co-ordinated alternating output to the muscles on the two sides of the body. The aim of this work was to create a population model for the tadpole's CPG, a computer model in which the number, distribution and synaptic connections of neurons are derived from anatomical evidences. We used hundreds of neurons of each type to match their real numbers in the tadpole's spinal cord

resulting in tens of thousands of synaptic connections. To reduce computational time these compact neurones were modelled as single compartments. GENESIS software was used and run on a Pentium III PC to build the population model. The details of the construction of the network and the patterns of neuronal activities will be presented.

We conclude that our population model of the tadpole's CPG may help us to understand the control mechanisms of swimming frequency, the importance of feedback chemical synapses from motoneurons onto premotor interneurons and the role of electrical synapses between motoneurons.

This work was supported by the Wellcome Trust and by the Hungarian National Academy of Sciences.

QUANTITATIVE COMPARISON OF DOPAMINERGIC AND DOPAMINO-CEPTIVE ELEMENTS IN THE STRIATUM AND TEGMENTUM OF DOMESTIC CHICK

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The striatonigral circuit in the domestic chick plays an important role in passive avoidance learning. The lobus parolfactorius (LPO, part of the avian striatum) projects to the brainstem dopaminergic nuclei: the area ventralis tegmentalis (AVT) and substantia nigra (SN). These projecting fibres presynaptically contain a D1 receptor related protein, the DARPP-32 (dopamine and adenosine related phosphoprotein, 32 kda). The SN and AVT also send dopaminergic fibres to the LPO, i.e. these regions have sophisticated reciprocal connections. We used confocal laser scanning microscopy combined with immunohistochemistry against DARPP-32 and tyrosine hydroxilase (TH) to quantify the juxtapositional connections between TH-positive and DARPP-32 containing structures both in the LPO and brainstem.

The TH+ fibre network is dense in the LPO and forms synaptic baskets around cells, whereas the DARPP-32+ fibers have fewer varicosities and do not form baskets in the SN and AVT. The number of juxtaposed TH+ boutons around the DARPP+ cells of LPO exceeded those boutons juxtaposed to TH+ cells of AVT and SN by a factor of two. In the LPO, only 40 percent of the TH+ synaptic baskets are occupied by DARPP+ cells.

The anatomical situation suggests that, whilst the observed TH+ baskets around DARPP+ cells in LPO make the existence of synaptic connections evident, both synaptic and non-synaptic (paracrine) connections between DARPP-32 fibres and TH+ cells of the brainstem are possible, including retrograde transmission from dopaminergic neurons. Such asymmetries between the striatonigral and nigrostriatal pathways suggest that the organization of these circuits involving dopaminergic transmission is more complex than simple reciprocity would imply.

AFFERENTATION, HORMONAL AND NEURAL EFFERENTATION OF THE AVIAN PINEAL

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The pineal organ (pineal gland, corpus pineale) that plays a significant role in regulating the light periods of vertebrates, synchronizes the genetically determined circadian and circannual rhythms with environmental light conditions. Pineal regulation is mediated by neural and hormonal effects, via nerve fibers running from the pineal to the brain stem, and by the secretion of melatonin. From cyclostomes to birds, the pineal organ receives light information necessary for its function from its own cone-like receptor cells. The number of light sensitive structures and molecules necessary for direct photoreception decrease in mammals, therefore, retinal light information is supposed to reach the organ via a hypothetical polysynaptic pathway and by autonomic nerve fibers.

In earlier works we demonstrated, that in mammals autonomic fibers do not terminate directly on pinealocytes but forming vasomotor endings on smooth muscle cells of pineal arterioles. We suppose that these fibers are regulating the blood flow of the organ according to changing metabolic needs during circannual and circadian light periods. Birds seem to be suitable subjects for further studies, because avian pineal - directly perceiving light - needs no retinal afferentation, therefore, we can easier estimate the role of the fibers innervating the organ.

In our present work, we compared the pineal ultrastructure of different bird species. We examined guinea fowl *(Numida meleagris)* being phylogenetically ancient species with more differentiated birds, like house sparrow *(Passer domesticus),* pigeon *(Columba livia*), zebra finch *(Taenophygia guttata)* and chicken *(Gallus domesticus).*

We found that all of the examined birds possess structures referring to direct light perception and neurohormonal efferentation. In contrast, a neural efferentation or a central innervation through the pineal stalk seem not to be well developed. The number of peripheral, autonomic fibers is significant in the organ. They may be considered as vasomotor fibers by reason of their endings around vessels. As it was already supposed concerning mammalian pineal, the role of these autonomic nerve fibers is probably to supply vasomotoric support of pineal metabolism changing with the photoperiods. No autonomic fibers were seen entering the pineal tissue, and innervating pinealocytes. As the pineal organ of birds has a direct photosensitivity, there is no need to suppose light information-carrying role for its vasomotor fibers as several authors supposed for similar fibers of the mammalian pineal.

THE INNER STRUCTURE OF THE BASAL OPTIC ROOT NUCLEUS (nBOR) IN THE CHICKEN

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The nBOR complex is an important member of the accessory optic system. According to the cell types three parts can be distinguished in the complex: the major middle part, which is the actual nBOR, the caudally positioned medic-dorsal pail, which forms a shell shaped strucure around it (nBORd), and the rostral latero-ventral part (nBORl) (Repérant 1973; Brecha et al. 1980). Because of its direct oculomotor and vestibulocerebellar connections it plays an important role in the optokinetic reflex (Brecha et al., 1980; Eke et al., 1977; Lázár 1973; Walley 1967; Simpson and Soodak 1978). Optic terminals in the nucleus were first reported by Ramon y Cajal. The projection of the optic fibres on the nucleus were first investigated with degeneration method by Cowan et al. (1961). Later, Karten et al. (1977) demonstrated that axons of extrapolated retinal ganglionic cells terminate on the cells of the nBOR complex.

The present study investigates the types and localisation of terminal ending of optic fibres using light and electron microscope. The optic fibres were traced with biotinilated dextran amine (BDA) anterograde tracer. The fibres ended both in the peripherial and central parts of the nucleus. The density of labelled terminals demonstrated rather the number of labelled fibres than actual terminal density. The branching of the terminal sections and the morphology of the terminals containing synaptic vesicles differs greatly from those in the optic tectum. In the nBOR complex the terminals establish asymmetrical synapses on dendrites and dendritic processes, but also on the soma of small neurons. Symmetrical synapses could also be observed on dendrites and dendritic processes, which were GABAergic.

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ABSTRACTS

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SUMMATION OF STIMULATED CORTICAL AND RETINAL EPSPS IN THALAMOCORTICAL NEURONES

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The effect of cortical feedback excitation on the efficacy of retinal transmission was studied. The role of the metabotropic glutamate (mGlu) component of the cortical EPSP, and of the distal dendritic Na^+ and A-type K^+ channels were investigated in a multi-compartment model of a cat dorsal LGN neurone. The somato-dendritic distribution of inward rectifying K^+ was uniform, the density of T-type Ca^{2+} and h-type channels were highest on proximal dendrites, while A-type K^+ channels were preferentially distributed on distal dendrites. Retinal synapses were coactivated on each dendrite and three spatial patterns of coactivated cortical synapses were used: *symmetric*) with synapses distributed among all dendrites, *single-dendrite*) with synapses distributed on all distal segments of a single dendrite, and *single-segment*) with a single distal synapse. The total maximal conductance of cortical synapses in the three patterns was kept constant by scaling the strength of individual synapses of the patterns.

First we tested the reproduction of somatically measured isolated cortical EPSPs evoked by single or repeated stimulation of corticothalamic fibers with and without blockade of NMDA and non-NMDA receptors. Cortical EPSPs below LTCP threshold were evoked and were followed by a retinal EPSP with 0 to 530 ms delay at different membrane potentials maintained by somatic current injection. Cortical EPSPs increased the number of APs evoked by retinal EPSPs arriving with short (2-200 ms) delay, for longer delays the number of APs evoked by the summated EPSP was even smaller than the number of APs evoked by the retinal EPSP alone. When the mGlu receptors were blocked, both the facilitory and the suppressive effect of the cortical EPSP decreased. The removal of the distal Na^+ channels reduced the number of action potentials without affecting the timing of facilitation-suppresion cycle. After removal of the distal A-type K^+ channels the number of APs evoked by the summated EPSPs or by the retinal EPSPs alone increased leaving the suppression of retinal EPSPs at longer delays unaffected.

In conclusion, distal dendritic currents shift the excitability of the neurone in an input-independent fashion, while the activation of the mGlu receptors change the time window where cortical feedback excitation facilitates the retinal input.

AFFERENT CONNECTIONS OF THE LATERAL VESTIBULAR NUCLEUS IN THE FROG AND RAT: A COMPARATIVE NEUROMORPHOLOGICAL STUDY

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The afferent fibers of the vestibular sense organs terminate in the vestibular nuclei of the brainstem. Earlier experiments suggest individual differences between the vestibular nuclei as far as their roles played in the maintenance of equilibrium and their involvement in the

vestibular compensation is concerned. The morphological background of the functional differences can be associated with different connections of the vestibular nuclei. In this experiment we have studied the afferent projections of the lateral vestibular nucleus (LVN) in the frog and rat. Following injection of neuronal tracer into the frog LVN the most rostral labeled cells were found in the thalamus. In the mesencephalon the nucleus of the medial longitudinal fascicle and a few cells in the tegmental were labeled. Most of the neurons were detected in the ipsilateral and contralateral vestibular nuclei and in different areas of the rhombencephalic reticular formation. Some of the cells may be equivalent to the mammalian inferior olive and the nucleus of prepositus hypoglossi. In the spinal cord the majority of labeled neurons were in the dorsal part of the gray matter representing the cells of origin of spinovestibular tract. In the rat the labeled neurons were detected only in the rhombencephalon and spinal cord. In the rhombencephalon, a large number of labeled neurons were located in the ipsilateral and contralateral vestibular nuclei. In the reticular formation the labeled neurons were detected bilaterally, the neurons of the inferior olive were labeled in the contralateral side. In the spinal cord, a few labeled neurons were in the cervical and lumbosacral segments. Our results indicate a significant reciprocal connection between the LVN and the corresponding areas of termination.

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EFFECTS OF ANGIOTENSIN II AND III MICROINJECTIONS INTO THE ZONA INCERTA ON THIRST MOTIVATED BEHAVIOR OF RATS

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Behavioral, endocrine and neural responses to losses of body fluids are critical for reestablishing homeostasis. The brain renin-angiotensin-system (RAS) has a pivotal role in the regulation and modulation of this action. Since, it does not readily cross the blood-brain-barrier (BBB), the circulating angiotensin II (AII), causes drinking by acting on the subfornical organ (SFO). All of the components of this regulatory system have been identified in the brain, and there is evidence for generation of endogenous brain angiotensins. The influences reaching the brain require integrative neural network including the structures of the lamina terminalis and the zona incerta (ZI). These regions receive afferent sensory input and process information related to hydromineral balance. It is supposed that AII acts on through these structures via its receptors (AT1 or AT2) to stimulate thirst under conditions of hypovolemia. The AT1 is prominently distributed in cardiovascular system and also in structures regulating or modulating the body fluid homeostasis and is especially sensitive to AII and also activated by angiotensin III (AIII). AT2 preferentially binds AIII, but also binds AII and may be involved in vascular growth and autoregulation of cerebral blood flow. These receptors have a prominent heterogenity in distribution, but both can be involved in dipsogenesis. The development and the use of selective subtype antagonists have resulted in the conclusion that the classic functions of the brain RAS are mediated by the AT_1 receptor. A non-peptide AT_1 receptor antagonist with increased potency was developed and is called losartan (Los, DuP753) and even up to 10^{-5} M blocked the responses to AI, AII

and AIII effectively. Another angiotensin receptor antagonist, namely the CGP 42112 (CGP), an AT_2 receptor blocker was synthetized, to enlighten the function of this receptor. Lesions in the rostromedial ZI produced persisting hypodipsia, transient hypophagia, a lack of responsivity to cellular thirst challenges and impaired responsivity to some extracellular thirst stimuli. Electrical stimulation of the ZI has been reported to elicit drinking in sated rats. There is anatomical evidence which suggests that the SFO has reciprocal connections with the ZI and it is possible that AII is the neurotransmitter involved in the projection from the SFO to the ZI. In our experiments the effects of AII and AIII microinjections into the ZI have been studied on drinking. Also, the influence of angiotensin receptor blockers on angiotensin induced drinking were tested. On each side of the brain, 90 ng Los or 200 ng CGP were injected prior to 100 ng AII or 200 ng AIII treatments, all dissolved in 0.3 µl vehicle (Veh), respectively. Rats were to drink 60 min a day, consequently. Measurements took place in every 5 min during 30 min and in the 60th min, and this way animals were completely undisturbed during water consumption. The following combinations of treatments were applied: AII or AIII injections for experimenting single agonist effects. Veh+AII, Los+AII or CGP+AII for testing antagonists effects on AII. Veh+AIII, Los+AIII or CGP+AIII for testing antagonists effects on AIII. Equal amounts of Veh were injected for rats served as control groups. The Los+Veh and the CGP+Veh injections were for testing the effects of separate receptorial blockade, without external angiotensin microinjections. AII, as well as, AIII significantly increased water consumption of rats. The effect of AII could be blocked by Los, but not by CGP. On the other hand, the effect of AIII could not be blocked by losartan, but by the CGP. When only the AT1 receptor was blocked, animals drank significantly less than after the blocking of the AT2. As the effects of AII, AIII, Los and CGP have not been tested in the ZI, the finding that water intake increased after AII or AIII injections and it could be blocked only by either of the antagonists suggests that AT1 and AT2 receptors play partially different roles in the regulation of water intake.

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IMPAIRED MUCIN SECRETION AND ABNORMAL EPITHELIAL GROWTH IN THE GASTROINTESTINAL TRACT OF GAD-67 TRANSGENIC MICE

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It is well established that cells of the gastrointestinal system with different embryonic origin express glutamate decarboxylase (GAD) and its product GABA.[1] It is proposed that GABA in the gut may function as a neurotransmitter[1], hormone[2] and paracrine agent[3]. The aim of the present study was to investigate the phenotypic changes in the gut of mice from the transgenic mouse line Tg(GAD67lacZ5)69, which carries 75 copies of a transgene containing 5kb of the flanking region of mouse GAD67 gene fused to the bacterial marker gene lacZ[4]. For this purpose, GAD67 transgenic mice were compared with wild type mice for ultrastructural

analysis and Mayer's mucicarmine staining[5]. The thickness of the different tissue layers around the intestinal lumen were also compared in the two groups of animals using the Image Pro Plus 3.0 morphometric program.

20-day-old mice homozygous for the transgene locus were compared with 20-day-old wild type mice. All GAD67 transgenic mice were markedly undernourished by this time and all exhibited an intestinal phenotype of varying severity. Mice were anaesthetized and dissected. The intestinal segments were fixed in a mixture of 2% glutaraldehyde and 4% paraformaldehyde. Selected segments were processed for electron microscopy, paraffin embedding and whole-mount preparations. The thickness of the tissue around the intestinal lumen were measured on Epon-embedded toluidine-blue stained semithin cross-sections taken from comparable regions of the small intestines and colons of control and transgenic animals. Image-Pro Plus 3.0 morphometric program was used for measurements. Statistical analysis was performed by using the one-way ANOVA on SPSS 9.0 software. A probability of $P<0.05$ was set as the level of significance in all analyses. Data were expressed as means ± SE. β-galactosidase histochemistry was performed on cryostat sections.[6] Ultrathin cross-sections were examined with Philips CM 10 electron microscope equipped with a MEGAVIEW II camera. Mucin containing cells were visualised with Mayer's mucicarmine staining on paraffin sections. GABA immunocytochemistry was applied on whole-mount preparations and on paraffin sections.

Postnatal growth of GAD67 transgenic mice was impaired relative to wild type counterparts. Morphometric analysis revealed that with the exception of the circular muscle, all tissue layers surrounding the intestinal lumen were significantly reduced in thickness compared to the wild type. Ultrastructural investigations revealed that transgenic mice displayed severe epithelial disintegration in the small intestine. Adjacent epithelial cells appeared to have lost their junctional integrity and the terminal web region was hardly recognisable. The nerve plexuses at the same time seemed to be ultrastructurally unaffected by the presence of the transgene. Mayer's mucicarmine staining revealed that the number of villar and intervillar goblet cells decreased 30-40% relative to wild type villi.

We examined GAD67 transgenic mice for gastrointestinal abnormalities as a possible explanation for the growth retardation. The most pronounced abnormality was the severe epithelial disintegration. It is known that impared mucin secretion is a factor that critically affects the epithelial integrity[5]. To evaluate mucin production, we compared Mayer s mucicarmine staining of wild type and transgenic mice and we concluded that diminished mucin level could account for the increased epithelial lesion.

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GABA CONSTRAINS VASOPRESSIN GENE EXPRESSION IN THE STRESS-RELATED PARVOCELLULAR NEURONS OF THE HYPOTHALAMIC PARAVENTRICULAR NUCLEUS

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The activity of hypothalamic stress-related parvocellular neurons depends on excitatory and inhibitory synaptic circuits. Intrahypothalamic GABAergic neurons may relay tonic inhibition from the limbic system to the hypothalamo-pituitary-adrenocortical (HPA) axis. To assess the impact of GABA in regulation the transcriptional and secretory activity of corticotropin-releasing hormone (CRH) synthesizing neurons, the effect of GABA-A antagonist bicuculline methiodide was compared *in vivo* and in organotypic hypothalamic slice cultures. Unilateral microinjection of the drug into the PVN region of freely-moving rats resulted in increased neuronal activity in subparaventricular and perifornical regions, but induced only weak-to-moderate cFos expression in the PVN itself. Although the blockade of GABA-A receptors increased vasopressin, but not CRH transcription in the parvocellular neurosecretory neurons and stimulated ACTH-release, our data suggest that disinhibition of CRH neurons in vivo is not sufficient to trigger full activity of stress-related neurons *in vivo*. In organotypic slice cultures, neurosecretory cells maintain their differentiated phenotype and local connections but loose all synaptic inputs from distant sources. In these preparations, bicuculline administration resulted in a robust cFos expression that was restricted exclusively to the parvocellular part of the nucleus. These data suggest that parvocellular CRH secreting neurons are under tonic GABAergic inhibition originating from local interneurons and this inhibitory tone may be modulated by extrinsic inputs *in vivo*.

CHANGES OF DIFFERENT NEUROTRANSMITTER CONCENTRATIONS IN RAT HIPPOCAMPUS TISSUE SLICES IN AN OXIDATIVE STRESS MODEL

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Changes in tissue levels of aspartate, glutamate, taurine, adenosine, inosine, diadenosine tetraphosphate and noradrenaline in an oxidative stress model (hydrogen peroxide treatment in perfused minislice model) were determined in rat hippocampus using an HPLC method. These results were compared with levels in preperfused slices fixed by liquid nitrogen.

The level of glutamate and aspartate were significantly decreased ($p<0.01$), in each concentration of H_2O_2 (0.5-5.0 mM) from 18.22±1.83, 5.82±0.79 nmol/mg protein to 11.81±1.27, 2.84±0.47 nmol/mg protein, respectively. The amount of taurine was decreased

from 4.89±0.6 to 0.55±0.05 nmol/mg protein, at extremely high concentration (500 mM) of H_2O_2.

The concentration of adenosine was four times higher than in control, 303.51±31.34-pmol/mg protein. The ratio of inosine to adenosine that indicate the rate of metabolic degradation of adenosine was highest at 500 mM H_2O_2. An increase in diadenosine tetraphosphate was also observed from 29.08±0.88 to 683.58±64.29 pmol/mg protein.

In the simultaneous presence of rotenone (10 μM) or oligomycine (10 μM) or rotenone plus oligomycine and peroxide in 0.25 mM induced a marked reduction in aspartate concentration to 2.19±0.31, 1.73±0.24 and 1.54±0.17 nmol/mg protein, respectively. The same treatments caused an increase in diadenosine tetraphosphate levels to 77.09±15.06, 96.71±2.85 and 72.73±7.21 pmol/mg protein, respectively. The increase in adenosine concentrations were not affected by rotenone plus oligomycine and peroxide treatments.

The residual concentration of noradrenaline changed from 88.41±5.30 to 7.09±2.41 pmol/mg protein.

These observations raise some problems of the experimental approach for study the changes of aspartate and diadenosine tetraphosphate.

GABAERGIC TERMINALS FROM THE ZONA INCERTA SELECTIVELY INNERVATE THE MATRIX COMPONENT OF THE RAT DORSAL THALAMIC NUCLEI

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Many thalamic relay cells in higher order nuclei are immunoreactive for calbindin, and form the thalamic "matrix". Calbindin-positive relay cells project to the supragranular layers of widespread cortical areas and may have an important role in the synchronization of cortical activity. In contrast, calbindin-negative relay cells (that are positive for parvalbumin in certain species) prevail in the first order nuclei and make up the topographically organized thalamic "core". Calbindin-negative relay cells innervate mainly the layer IV of restricted cortical areas and are responsible for simple sensory transmission. Up to now, the thalamic reticular nucleus (RT) has been regarded as the major source of the inhibitory input to both the matrix and the core. In this study, we describe the precise termination pattern of an inhibitory pathway to the dorsal thalamus that does not originate in the RT is specifically targets the thalamic "matrix".

During the course of mapping type 2 muscarinic acetylcholine receptors in the thalamus large, varicose, m2-immunoreactive terminals were observed restricted largely to higher order nuclei. Their arrangement closely matched the distribution of calbindin-positive relay cells. Subsequent electron microscopic studies indicated that these terminals establish symmetrical synapses. Postembedding GABA immunostaining revealed that m2-positive terminals are all GABAergic. Some of the terminals reached giant size (minor axis 1-2 μm, major up to 6-8 μm) and established multiple release sites. The major postsynaptic elements were thick proximal dendritic shafts of relay cells. In the higher order nucleus, incl. posterior, 49% of the

GABAergic terminals were m2-immunoreactive, whereas in first order nuclei this value varied between 1-3%. The distribution, ultrastructural characteristics and postsynaptic target selection of m2-immunoreactive terminals were incompatible with an origin from the RT.

Previous data, retrograde tracing and immunocytochemical results indicated that m2-positive terminals likely originate in the ventral thalamic derivative zona incerta (ZI). Following the injection of two different types of anterograde tracer (biotinylated dextrane amine, BDA and Phaseolus vulgaris leucoagglutinin, PHAL) into ZI revealed dense terminal staining restricted to higher order nuclei of the dorsal thalamus. The distribution of anterogradely labeled fibers from ZI was similar to m2-positive axons and matched the distribution of calbindin-positive relay cells. The afferent fibers formed multiple contacts with calbindin-positive relay cells. Using postembedding GABA immunocytochemistry the vast majority of the ZI terminals (90.4%; n=73) were found to be GABAergic in the posterior and centrolateral nuclei. They established symmetrical synapses, preferentially on the postsynaptic dendrites of relay cells. The distribution, ultrastructural characteristics and postsynaptic target selection of the anterogradely labeled fibers indicate that m2-immunoreactive fibers originate in ZI.

Our conclusion is that a major component of inhibitory terminals to the thalamic "matrix" originates outside the reticular nucleus. These extrareticular terminals establish multiple contacts preferentially on the strategically important proximal dendrites of relay cells in conjunction with large excitatory afferents. The known connectivity pattern of ZI suggests that they form a *conceptually new type of inhibitory circuit* with the thalamic matrix, that is qualitatively different from the GABAergic system of RT and makes it a perfect substrate for controlling (e.g. synchronizing) large populations of thalamic relay cells. ZI, as an inhibitory interface, integrated in the ascending and descending excitatory pathways can contribute to the unique sensory coding observed in higher order nuclei and can influence global cortical activity via relay nuclei with widespread cortical connections.

EFFECTS OF CHOLECYSTOKININ, MICROINJECTED INTO HIPPOCAMPAL CA1 AREA, ON RAT LOCOMOTION

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The octapeptide cholecystokinin (CCK-8) was one of the first gastrointestinal peptides to be detected in the brain of the mammals. We have demonstrated an asymmetry in the behavioral effects of the neuropeptide CCK-8 microinjected into nucleus accumbens and amygdala (Belcheva et al., 1994). These findings prompted us to study the possible role in the locomotion of CCK-8, microinjected into CA1 hippocampal area of rats.

The aim of the present study was to examine the locomotor response of rats to uni- or bilateral microinjections of CCK-8 into the left and/or right CA1 hippocampal area of male Wistar rats.

Materials and methods. After anesthesia (Ketamine 50 mg/kg i.p.) the rats were placed in a stereotaxic apparatus and guide cannulae (right and left) were implanted into CA1 hippocampal area according to the coordinates of the stereotaxic atlas of Pellegrino and Cushman (A=4.3; L=±2.0; h=-2.0). CCK-8 (Sigma) was dissolved ex tempore in saline and microinjected into left, right and/or both CA1 hippocampal area at a dose of 0.01 μg in 1 μl. Locomotor activity (number of horizontal and vertical movements) was recorded in an Opto-Varimex apparatus at the 5th, 20th and 60th min. The rats were placed in the apparatus immediately after the microinjection of CCK-8 and were left undisturbed for one hour.

ANOVA revealed marked effects for CCK-8 ($F_{1,90}$=10.261, P≤0.002), both for side of injection ($F_{2,90}$=37.285, P≤0.001) and for time ($F_{2,90}$=40.740, P≤0.001). There were significant interactions between CCK-8 X side ($F_{2,90}$=29.337, P≤0.001): CCK-8 X time ($F_{2,90}$=5.537, P≤0.05): time X side ($F_{4,90}$=10.402, P≤0.001) and CCK-8 X side X time ($F_{4,90}$=11.895, P≤0.001). *Post hoc* t-test comparisons demonstrated that CCK-8 significantly increased number of horizontal movements applied bilaterally at the 60th min (t=3.06, P≤0.01) and right at the 5th min (t=2.19, P≤0.05); 20th min (t=2.45, P≤0.01) and 60th min (t=4.3, P≤0.001) compared to the respective controls. Injections of CCK-8 into right hippocampal area produced a significantly greater number of horizontal movements as compared to the left at the 20th min (t=2.77, P≤0.01) and 60th min (t=3.43, P≤0.001) and compared to bilateral CCK-8 injections at the 60th min (t=2.59, P≤0.01). Neither unilateral nor bilateral hippocampal injections of CCK-8 induced changes in the number of vertical movements compared to saline injection during the whole period of observation. There was no significant effect for CCK-8 ($F_{1,90}$=1.223) and for side of injection ($F_{2,90}$=0.997).

These findings provide information on the locomotion-stimulating effect of CCK-8 when injected at a specific dose into right but not into the left CA1 hippocampal area. This suggests some asymmetric effect of CCK-8 on locomotion, depending on the microinjected hemisphere.

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DISTRIBUTION, MORPHOLOGY AND SYNAPTIC TARGETS OF COMMISSURAL INTERNEURONS IN THE LUMBAR SPINAL CORD OF NEONATAL RATS

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There is strong evidence that neurons with axons that extend to the contralateral side of the spinal cord-commissural interneurons (CINs) play an important role in the coordination of left/right alternation during locomotion. In this study we have investigated the localization and

projections of CINs to other CINs and motoneurons on the other side of the cord. We have injected biotinylated dextran amine (BDA) into the lateral motor column and into ventral gray matter on one side of the lumbar spinal cord in neonatal rats and labeled motoneurons on the opposite side by applying biocytin onto the ventral roots. Retrogradely labeled CINs contralateral to the site of BDA injections appeared in laminae VII, VIII and X along a three-segment-long section of the spinal cord. According to the shape of the cell bodies and orientation and branching pattern of the dendrites the labeled commissural interneurons could be diveded into three categories.

Anterogradely labeled axons that originated from CINs within the confines of BDA injections were observed crossing the midline and arborize intesively in the ventral horn and intermediate gray matter on the contralateral side of the spinal cord. Close appositions were found between these axon varicosities and somata and proximal dendrites of labeled motoneurons. Many of the axons terminated on the dendrites of retrogradely labeled CINs. These contacting axon varicosities were restricted to the proximal part of the dendritic trees of CINs. The results suggest that CINs make monosynaptic contacts with contralaterally located motoneurons. Our findings also indicate that direct reciprocal connections between CINs on the two sides of the spinal cord exist. The findings provide additional data to the understanding of neural connectivity in spinal networks that generate rhythmic motor activities including locomotion in mammals.

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IN VIVO RECORDING AND RECONSTRUCTION OF GABAergic MEDIAL SEPTAL NEURONS WITH THETA RELATED FIRING

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The importance of the medial septum in hippocampal theta generation is well established. Any damage in the medial septum disturbs or diminishes the normal hippocampal EEG. From the two major septo-hippocampal neuron populations the parvalbumin (PV) -containing GABAergic and the cholinergic the PV neurons heavily and selectively innervate hippocampal interneurons, thus taking a strong control over hippocampal network activity. Electrophysiological data also support the view that a subpopulation of the PV containing GABAergic neurons may serve as pacemakers of the hippocampal theta rhythm.

In vivo juxtacellular recording, and labeling combined with double fluorescent immunocytochemistry light and electron microscopy were applied to reveal the local connectivity and hippocampal EEG-related firing pattern of the PV containing GABAergic neurons in the medial-septum.

The appearance of theta activity in the hippocampal EEG was always accompanied by an increase of theta power in the autospectra of all PV neurons. Few PV positive neuron exhibited theta modulated firing even when the autospectrum of the hippocampal EEG indicated dominant non-theta activity.

The PV neurons are medium to large in size with dendrites arborizing in an elliptoid volume around the cell. Their principal axon usually originate from the ventral side of the soma, then

after few hundred micrometers it turns dorsally and leaves the septum dorso-caudally towards the hippocampus. The local axon collateral's arise from this main axon within the medial septum. These axons form irregularly spaced boutons in the medial septum, baskets were rarely seen. In the electron microscope they were found to form symmetrical synapses on thick proximal dendrites.

These results show that PV-positive medial septal GABAergic neurons are in an ideal position to synchronize the local, neuronal network as well as the hippocampus during theta generation, thus they may serve as pacemakers of the hippocampal theta rhythm.

THE ROLE OF κ AND μ MEDIATION IN THE ACTION OF MERF ON HYPOTHALAMO-PITUITARY-ADRENAL RESPONSE AND OPEN-FIELD BEHAVIOUR

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The effects of Met^5-Enkephalin-Arg^6-Phe^7 (MERF), on the hypothalamo-pituitary-adrenal system (HPA) and open-field behaviour were investigated in mice. Intracerebroventricular (icv.) injection of MERF produced dose-dependent increase in locomotion and rearing, which was measured in an "open field" apparatus thirty min after treatment. Administration of this heptapeptide also caused a significant increase in the corticosterone level. In order to reveal what type of opioid receptors are involved in these processes in a further set of experiments animals were pretreated with either the non-selective μ antagonist naloxone or nor-binaltorphimine (nor-BNI): the selective blocker of κ receptors. Both antagonists displayed a dose-dependent inhibition of the HPA activation elicited by MERF. On the other hand while naloxone inhibited the behavioural responses evoked by MERF in a statistically significant manner nor-BNI showed only a tendency of attenuation. These findings suggest that the natural derivative MERF acts as a non-selective activator of opioid mechanisms regulating behavioural and endocrine processes, and both and mediation might take part in the control of stress response, while in its action on such behavioural paradigms as locomotion, rearing μ–δ mediation appears to prevail.

RELATIONSHIP BETWEEN RECEPTIVE FIELD SCATTER AND ORIENTATION MAP IN THE CAT PRIMARY VISUAL CORTEX

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Introduction: Recent experimental findings indicate that the representation of visual field positions (retinotopic map) displays strong local non-homogeneities (Das and Gilbert, 1997). According to these data, RF positions change smoothly in orientation domains where orientation preferences change also smoothly between neighbouring sites, whereas a progressive separation of RF positions occurs in orientation centre regions where orientation preferences change rapidly between adjoining cells. A logical consequence of such an organisation of RF positions is a non-uniform cortical coverage of the visual space, even implying occasional "holes" in the representation. Here we analyse quantitatively how the structure of the retinotopic map correlates with regions possessing high (orientation centre) and low (orientation domain) orientation gradients.

Methods: An extracellular mapping technique was applied for determining preferred orientations, receptive field (RF) size and position of single and multi-units (n=380) in layers 2-4 of the primary visual cortex in anaesthetised, paralysed cats. RFs were determined with a hand-held visual stimulator and plotted on a tangent screen. Residual eye-movement was checked after every 2-6 recordings (about every 60 min) by plotting the retinal image. In some of the experiments, a test electrode was positioned in lamina A1 of the contralateral dLGN from which single unit activity was recorded regularly. RF position and preferred orientation obtained for each cortical cell were corrected in accordance to residual eye-movement. A quantitative analysis was made for all neuron pairs (n=5014) with smaller than 600 μm lateral separation. To this, RF centre distances between pairs were normalised to the mean RF diameters of each pair.

Results: Linear regression between the lateral separation of the recorded units and RF distances showed a smooth shift of RF positions. There was a weak, positive correlation between these parameters (Pearson's correlation, area 17: r=0.23, area 18: r=0.26). A similar trend was found between the difference in preferred orientations and RF separation (Pearson's correlation, area 17: r=0.04, area 18: r=0.15). In order to know whether the shift of RF position increases between units separated by zones of rapidly changing orientations, we plotted the rate of change (ROC) of RF positions as a function of ROC of preferred orientations. There was a positive but weak correlation (Pearson's correlation, area 17: r=0.46, area 18: r=0.43) implying that, on average, RF position does not change substantially within ~1/2 hypercolumn distance. Finally, the relationship between the scatter of preferred orientations and the scatter of RF positions was calculated. Again, positive correlations were found for the two areas studied (Pearson's correlation, area 17: r=0.45, area 18: r=0.16) suggesting only a moderate increase of RF scatter at locations of highly changing orientation preferences.

Conclusions: The results suggest that RF scatter increases moderately in regions of high orientation ROC (pinwheels and fractures). This, however, does not result in a systematic separation of RFs of units across such regions. Our findings speak for no visual "holes" at orientation centres. The larger RF scatter observed in area 17 than in area 18 is likely to compensate for the typically smaller RF sizes found in the former.

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FINE STRUCTURE OF THE PINEAL ORGAN IN YOUNG AND ADULT DJUNGARIAN HAMSTER *(PHODOPUS SUNGORUS)*

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The pineal organ plays a role in the seasonal timing of several functions like gonadal activity, moulting periods, pelage composition, etc. The pelage of the Djungarian dwarf hamster is grey and white during summer and it changes to white in winter. This is controlled by the environmental light circumstances because this change of color may be reproduced by artificial illumination characteristic for winter time. It is generally accepted that light information percepted by the retina reaches the mammalian pineal organ via its periferal sympathetic innervation from the superior cervical ganglia. In contrast submammalian pinealocytes are photoreceptors being directly sensitive to light. Since in some mammals like the gerbil and the ferret immunoreactive rhodopsin was demonstrated in pinealocytes some authors suppose the direct light sensitivity of mammalian pinealocytes. Due to the pelage color changes, the Djungarian dwarf hamster seems to be a good object for studying this problem. As the organization of the pineal of this species is not completely known, in the first step of our study we wanted to clearify the fine structure basis of the supposed neural and hormonal efferentation as well as the presence of structures needed for direct light sensitivity or an indirect light information coming from the retina.

The pineal organ of the dwarf hamster is attached to the inner surface of the skull through the confluence of sinuses and has a close contact with the vein of Galen. The conarian nerve containing some myelinated and several unmyelinated fibres runs around this vein and reaches the organ at its dorsocaudal tip. The fibers follow the vascular spaces of the organ. Similar nerves were found in the nervous tissue of the proximal part of the organ. These elements are already present in the first postnatal life period. Nerve fibers connecting the organ and the brainstem were not found in adult animals.

Histologically the pineal organ is composed by pinealocytes, glial cells and neurons. The pinealocytes are of light and dark-type, their cytoplasm contain some granular vesicles of various size. They bear cilia with 9×2+0 tubular arrangement and protruding into the intercellular space. We could not find any photoreceptor differentiations on these cilia. Pinealocytic processes do not form neurohormonal terminals on the basal lamina. The glial cells contain microfilaments and multiform dark nuclei. Intrapineal nerve cells have light cytoplasm containing rough-surfaced endoplasmic reticulum and Golgi areas.

Our results show that several peripheral afferent fibers reach the hamster's pineal organ and run in the connecting tissue spaces but the termination site of these nerves were not yet identified. Efferent nerve fibers are only present in young animals, therefore, we can suppose an exclusively hormonal efferentation in adults. As melatonin secretion do not need special release sites, the lack of neurohormonal terminals do not contradict this supposition.

Concerning the supposed direct light sensitivity of pinealocytes, we want to mention that no photoreceptor lamellae were found on the cilia of pinealocytes. As it is known in connection with deep encephalic photoreceptors sensory cells do not need any special membrane-structure

for being sensitive to light so in further histochemical studies we also want to test whether molecules of the phototransduction cascade are present or not in pinealocytes especially in young animals.

THE EFFECT OF PACAP ON RHYTHMIC MELATONIN RELEASE OF AVIAN PINEALS

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Pituitary adenylate cyclase activating polypeptide (PACAP) was isolated from ovine hypothalami on the basis of its adenylate cyclase activating effect. It belongs to the VIP/secretin/glucagon peptide family, and shows closest homology to VIP. PACAP is a pleiotropic peptide, having numerous actions in the central and peripheral nervous system. Beside its basic effect, as hypophysiotrophic hormone, it has been shown to act as neurotransmitter and neuromodulator, including neurotrophic and neuroprotective effects.

One of its recently discovered functions is the modulation of circadian activity. PACAP is abundant in the mammalian suprachiasmatic nucleus, in the retino-hypothalamic pathway and in the retinal ganglionic cells, suggesting that it is involved in the circadian pacemaker clock in mammals. Widespread distribution of PACAP-containing neurons and PACAP receptors has also been shown in the chicken. Recently, we have demonstrated that PACAP levels oscillate in a circadian manner in the chicken brain and retina. In birds, the pineal gland is considered as the conductor of the circadian rhythms. Unlike that of mammals, the pineal gland of birds possesses a high degree of autonomy in control of rhythmic functions. Avian pineals release melatonin (MT) in a circadian manner also *in vitro*. It has a direct sensitivity to light, and the *in vitro* rhythmic MT production can also be modified by various neurotransmitters, like norepinephrine (NE) and VIP and also by changes of environmental temperature and the magnetic field.

The aim of the present study was to investigate the direct effect of PACAP on the rhythmic MT release from avian pineals, and to examine the presence of PACAP in the chicken pineal gland by immunohistochemistry. In experiments pursuing this goal, explanted chicken pineal glands (ChPG) were studied in a perifusion system under continuous darkness for 5 days. The pineals were exposed to PACAP in various concentration between 1 nM and 200 nM for 1 hour in different phases of the circadian rhythm. From the effluent tissue culture medium MT and cAMP levels were determined.

In these experiments we found: (1) PACAP induces a transient (3-4 hour) increase in MT release from ChPG in a dose dependent manner. The response to cAMP is immediate, but there is a 1 h delay between the start of the PACAP exposure and the onset of the MT response indicating that a complex metabolic cascade is involved in this process. The timing of the responses suggests that cAMP can be an intermediate of the PACAP-induced MT response. (2) The MT releasing effect is independent of the phase of the MT cycle in which PACAP is

applied and does not shift the phase of the circadian clock of the ChPG. (3) PACAP immunoreactive fibers are present in the ChPG along blood vessels and pinealocytes, indicating a physiological role of PACAP in the ChPG. These data suggest a modulatory effect of PACAP on the circadian MT release from avian pineals.

SOMATO-DENDRITIC SYNAPSES IN THE RETICULAR NUCLEUS OF THE RAT THALAMUS

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In the reticular nucleus of the rat thalamus, about 30% of the synapses are brought about by the perikarya of parvalbumin-immunopositive neurons, which establish somato-dendritic synapses with large dendrites of nerve cells of specific thalamic nuclei. Somato-dendritic synapses were first described by Sétáló and Székely (1967) in the frog optic tectum; in the rodent thalamus, dendro-dendritic synapses were described by Lieberman (1973). Although the parvalbumin-immunopositive presynaptic structures bear resemblance to goblet-like or caliciform axonal endings, electron microscopic immunocytochemistry and *in situ* hybridization revealed that these structures are parts of the perykarial cytoplasm studded with synaptic vesicles. In about 15% of the somato-dendritic synapses, axons are seen to be in synaptic contact with the parvalbumin-immunoreactive parykarya. On the basis of *in situ* hybridization experiments it is evident that PV is being synthesized by the PV immunoreactive semilunar structures themselves rather than being transported there from some hitherto unknown places. It is assumed that the peculiar somato-dendritic synaptic complexes may subserve the goal of filtration of impulses arriving at the reticular nucleus from various thalamic nuclei, and processing them for further analysis.

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IMMUNOCYTOCHEMICAL LOCALISATION OF CRY1 AND CRY2 IN THE RETINA AND PINEAL ORGAN OF THE MOUSE AND GUINEA FOWL

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It has been known for more than 100 years that blue light actively influences plant development. A blue light-absorbing compound, named cryptohrome, was supposed to mediate this effect. Cryptochromes were chemically identified in 1980 (cryptochrome 1) and in 1996 (cryptochrome 2) in the plant *Arabidopsis thaliana* as vitamin B2-based photoactive pigments. Similar to plants, two cryptochromes called CRY1 and CRY2 have been identified in humans and mice.

Cryptochromes were found to participate not only in light perception and phototransduction, but also in clock functions of invertebrate and vertebrate circadian systems. In the mice cryptochromes are expressed in the hypothalamic suprachiasmatic nucleus, the main circadian oscillator of the brain. In the retina a significant amount of CRY1 and-2 has been found in the cell nuclei of the retinal bipolar and ganglionic layer. The nuclear localisation of cryptochromes was explained by their supposed role in the transcriptional feedback loop of circadian clock.

In our present work, using commercial (Autogen-Bioclear, Calne) cryptochrome antibodies we histologically localised cryptochromes comparing pineal organ and retina of mammalian (mice) and avian (guinea fowl) species. CRY1 and-2 showed a different localisation not only in the two organs but also in the same organ of the two species that suggests a different role for these two molecules in the tissue and species investigated. Cryptochrome immunoreaction was generally detected in the cell nuclei, and in some cells in the cytoplasm as well.

In the retina CRY1 and-2 was localised to the inner nuclear and ganglionic cell layer of the mouse, whereas, in the outer nuclear layer of the guinea fowl. The outer nuclear layer contains the perikarya of photoreceptor cells therefore in birds in contrast to mammals cryptochromes may be involved in the circadian photosensitivity of the retinal photoreceptor cells.

In the pineal organ CRY2 was localised to the nuclei of all pinealocytes of both species but CRY1 was selectively found in some pinealocytes of guinea fowl. Adult mammalian pinealocytes do not develop photoreceptor outer segments but in birds pinealocytes are photosensitive and have a similar cytologic organization as retinal cones. We found CRY1 only in photosensitive avian pineal organ and we were not able to find immunoractive CYR1 in adult mammalian pineal organ that supposed to be light insensitive. Therefore we suppose that the bird pinealocytes showing immunoreaction for cryptochromes represent photoreceptors similar to those of retina. As absorption spectrum of cryptochromes overlaps the wavelength influencing the release of melatonin, these cells may function as circadian photoreceptors. Our electronmicroscopic studies are in progress to identify the fine structure and type of these cryptochrome-immunoreacting cells.

CHRONIC ROTENONE-INFUSION INDUCED PARKINSON'S MODEL IN RAT

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Parkinson's disease (PD) is one of the most devastating form of chronic neurodegenerative diseases which will become more and more prevalent as the life expectancy in the industrialized states continues to increase. Although the etiology of PD is unknown, increasing body of evidence suggests that oxidative stress due to increased generation of reactive oxigen species related with mitochondrial dysfunction (impaired Complex I in respiratory chain and mutations in mtDNA) is playing a critical role. Abnormality in protein handling additionally leads either to misfolding of proteins or their incorrect disposal, similarly to other chronic neurodegenerative diseases with predominant sporadic cases where age is the main risk factor.

The marketed therapeutics are essentially symptomatic and are not able to block the degenerative process, so there is an urgent unmet need for neuroprotective strategy. A good experimental model has to meet the following criteria: produces parkinsonian symptoms progressively at neurochemical, neuropathological and behavioral level within a relative short period of time, and can be standardized for pharmacological testing.

In the current work we tried to adopt the PD model based on chronic partial and selective inhibition of mitochondrial respiratory chain induced by low-dose intravenous infusion of rotenone delivered by osmotic minipumps in Lewis and Sprague-Dawley rats, introduced by Betarbet et al. Rotenone is a potent inhibitor of mitochondrial Complex I, used as a common pesticide (Complex I activity is declined in brains of PD patients). The published optimal dose of rotenone for inducing the pathology of PD was 2-3 mg/kg/day in Lewis rats.

We faced several difficulties during introduction of the model for pharmaceutical purpose:

(A) In our experiments 3 mg/kg daily dose of rotenone prooved to be highly toxic in 4-week long application causing early, high mortality.

(B) Due to its extreme hydrophobic property rotenone forms precipitation in the blood-stream instantly. Thus, the available dose of the mitochondrial poison in different tissues cannot be controlled.

(C) Since osmotic minipumps deliver constant rate, with decreasing or increasing the body weight the delivered dose is uncontrollably changing.

Despite the difficulties and the variability of the rotenone-model, with the proper dosing and modification of rotenone application the model can be a useful pharmacological tool.

The animals showed:

- behavioral changes: decrease of locomotor activity in open field test ($p<0.01$), decreased motor coordination in rotarod test ($p<0.05$), increased rigidity in rod suspension test ($p<0.05$), cognitive defect in Morris water maze acquisition ($p<0.05$)
- histological changes: significant neuronal loss in substantia nigra pars compacta ($p<0.05$)
- biochemical alteration: in dopamine (DA) concentration

Close correlation was found between striatal DA level and nigral cell density ($r=0.77$ with $p<0.01$).

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EFFECTS OF ACUT FORMALIN EVOKED INFLAMMATION ON NK1R, GLuR1 AND GLUR2/3 IMMUNOREACTIVITIES IN THE LUMBAR SPINAL DORSAL HORN OF RATS

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Spinal neurons that express NK1R, GluR1and GluR2/3 are known to play crucial roles in spinal transmission of nerve signals evoked by noxious stimuli. It has also been suggested that acut, subacut and chronic inflammations of various areas of the body may evoke changes in the expression of glutamate and neuropeptide receptors in spinal neuron that may induce alterations in nociceptive information processing mechanisms in the spinal dorsal horn.Therefore, in the experiment presented here we studied the changes in the distribution of NK1R, GluR1 and GluR2/3 immunoreactivities in the lumbar spinal dorsal horn of rats in acut formalin evoked inflammation of the hindpaw. By using immunocytochemical techniques, we demonstrated that the numbers of immunoreactive perikarya and densities of immunostaining for the three investigated receptors show specific changes in lamina I-III of the lumbar spinal dorsal horn five minutes after formalin injection into the hindpaw. The number of NK1R immunoreactive perikarya were significantly increased in the medial, intermedial, and lateral areas of the dorsal horn ipsilateral to the inflammation. In addition to NK1R, the density of GluR1 and GluR2/3 immunoreactivities were also increased in the medial subdivision of the ipsilateral dorsal horn.

The results indicate that due to acut formalin evoked inflammation a population of spinal neuron in the superficial laminae of the dorsal horn increases the expression of substance-P and AMPA-type glutamate receptors, that might contribute to the development of central sensitization.

CHRONIC N-3 PUFA EFFECTS ON RECEPTOR DENSITIES IN A CEREBRAL HYPOPERFUSION MODEL

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Balanced intake of polyunsaturated fatty acids (PUFAs) of the n-3 and n-6 families is crucial for neuronal membrane functioning. PUFAs determine membrane fluidity and provide substrates for second messengers, both of which suffer a setback in aging.

In the present experiment we focused on hippocampal receptor densities after chronic administration of n-3 PUFA-rich diets in a model for accelerated cerebral aging. We hypothesized that n-3 PUFAs could modulate neuronal signaling at the receptor level.

Male Wistar rats were assigned to three dietary groups and received experimental food from weaning till the age of 7 months. The ratio of (n-6)/(n-3) PUFA additives was 0.2 in Supplement 1 and 0.3 in Supplement 2. Supplement 2 also contained additional phosphatidylserine and phosphatidylcholine. The third, Placebo diet served as control. At the age of 3 months, half of the rats in each group underwent a permanent, bilateral occlusion of the common carotid arteries, the other half served as SHAM controls. The experiment was terminated at the age of 7 months and hippocampal NMDA, serotonin1A (5HT1A) and muscarinic1 (M1) receptor expression determined by receptor autoradiography.

NMDA receptor density was unaffected by diet or cerebral hypoperfusion. However, Supplement 2 increased 5HT1A receptor density and both Supplements, 1 and 2 significantly elevated the density of M1.

The data provide evidence that n-3 PUFA-rich diets increase the density of G-protein coupled 5HT1A and M1 receptors but the precise mechanism and the specificity for G-protein coupled receptors need further clarification.

RESEARCH OF NR2B-NMDA RECEPTORS AS POTENTIAL THERAPEUTIC TARGETS

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NMDA receptors are known to play a crucial role in physiological and pathological processes including excitatory synaptic transmission, synaptic plasticity as well as neurodegeneration and excitotoxicity following ischaemic insults or injury of the central nervous system (CNS). Accordingly, NMDA antagonists have been proposed to have potential therapeutic utility in

stroke, epilepsy, Alzheimer's disease, Parkinson's disease, chronic- and intractable neurogenic pain symptoms. However, clinical experience with known NMDA antagonist compounds (e.g. dizocilpine, ketamine) have pointed to serious untoward side-effects, such as ataxia, confusion, sedation and psychotomimetic effects. Therefore, the incipient enthusiasm of several researchers and drug companies tended to reverse to disappointment. However, there is also a new stream of research for novel type NMDA antagonists without the typical side effects. One way to achieve this goal may be subtype selective agents. The NMDA receptors are tetrameric heteromeric assemblies comprising NR1 and NR2 subunits. The latter one has 4 known subtypes: NR2A-D. So far, subtype selective compounds (drug candidates) are restricted to those that can selectively down regulate NMDA ion channels containing NR2B subunit(s), briefly NR2B-NMDA antagonists. The prototype of these compounds is an old anti-hypertensive drug: ifenprodil, which is devoid of the typical NMDA antagonist side-effects both in animals and humans. Although ifenprodil selectively blocks NR2B-NMDA receptors vs. other NR2 containing ones, it has potent effects on alpha, 5-HT, sigma and several other receptors as well. A recent drug research race focuses on really selective NR2B-NMDA antagonists devoid of other receptorial effects. The frontrunner of this race is CP-101,606, which underwent Phase II/III trials as a neuroprotectant by intravenous administration. The clinical experience showed good tolerance and the lack of the typical NMDA antagonist related side-effects. However, owing to pharmacokinetic properties CP-101,606 is not optimised for oral use. Optimised orally active NR2B antagonists reaching clinical development have not been published yet. NR2B-NMDA antagonists have been shown to be potentially useful as analgesics[1,2,3,4]. Using some model compounds (CP-101,606, CI 1041, Ro-256981, Co 101,244) investigators found analgesic effects in several animal models of tonic, inflammatory and neuropathic pain. Moreover, in contrast with classical NMDA antagonists, there appeared to be a substantial separation between analgesia and appearance of side-effects.

In our studies, we used the above model compounds as well as our potent novel NR2B selective antagonists. We determined their NR2B selective NMDA antagonist potency and selectivity using patch-clamp, fluorescent calcium assays on rat cortical neurons and HEK-293 cells transfected with different rat and human NMDA receptor assemblies, [^{3}H]Ro-256981 binding and broad range receptor binding profiling. Then the most potent compounds were subjected to analgesic tests (e.g. formalin test, experimental neuropathies), *in vivo* CNS side-effect profiling in rodents, preliminary cardiovascular side effect tests and pharmacokinetic characterisation. It was concluded that producing highly potent (low nanomolar) and selective NR2B-NMDA antagonists with clean receptor profile, analgesic activity, good oral bioavailability and low side-effect liability is a realistic target.

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CORTICAL DISINHIBITION INDUCED BY PERIPHERAL NERVE INJURY AND FOCAL ISCHAEMIA

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Photothrombotic lesions were produced in rat motor cortex, and brain excitability was assessed in paired-pulse stimulation protocol by transcranial recording, parallel in 16 points of the frontal cortex including the insulted and surrounding areas. The cortical lesion caused a reduction of inhibition in the extended frontal cortex with some minutes delay. Unilateral facial nerve transection however accelerated the widespread disinhibition. Though, the mechanism is not known, both the peripheral and central injury-induced disinhibition may have a significant impact on recovery of function.

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THE EFFECT OF SALSOLINOL, A PUTATIVE PROLACTIN RELEASING FACTOR, ON DOPAMINERGIC SIGNAL TRANSDUCTION

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Evidences have been described supporting the physiological role of salsolinol as a prolactin releasing factor[a]. Salsolinol has a characteristic binding site with the highest affinity in the median eminence[b]. The maximal binding capacity was depressed by suckling. L-DOPA, carbidopa, benserazide and α-methyl-DOPA were potent competitors of the ^{3}H-salsolinol binding. This displacement profile suggested an intimate relationship between the binding site and aromatic amino acid decarboxylase (AADC). Indeed pretreatment of rats with inhibitors of AADC inhibitors augmented and prolonged the prolactin releasing activity of salsolinol. The role of the known specific dopamine receptors in salsolinol's action may probably be excluded. However, the inhibition of the vesicular monoamine transport by reserpine or tetrabenazine counteracted the induced prolactin release. It is concluded that salsolinol may act as a modifier of the monoamine distribution or transport, consequently it may act also by decreasing the activity of the AADC enzyme.

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ORIGIN OF THE COCAINE- AND AMPHETAMINE-REGULATED TRANSCRIPT (CART)-IMMUNOREACTIVE INNERVATION OF THE HYPOTHALAMIC PARAVENTRICULAR NUCLEUS (PVN)

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Neurons synthesizing CART, densely innervate the PVN. This peptide may exert a number of important local effects on the PVN including regulation of eating behavior, thyroid and adrenal functions. Recent data from our laboratory have demonstrated that CART-IR neurons of arcuate nucleus origin are involved in the innervation of the PVN. Furthermore, a large number of CART-immunoreactive (IR) terminals in the PVN seem to originate from neurons residing outside the arcuate nucleus. To identify the location of CART-IR neurons innervating the PVN, cholera toxin B subunit (CTB) was delivered by iontophoresis into the PVN of adult rats. After 5-7 d survival, the animals were perfused. The CART- and CTB-containing neurons were visualized in serial, coronal sections using double-labeling immunofluorescence. The most prominent groups of double-labeled cells were found in the retrochiasmatic area, arcuate nucleus, lateral hypothalamus, zona incerta, ventrolateral medulla and nucleus tractus solitarius (NTS). In the diencephalon, the majority of the double-labeled neurons were localized ipsilateral to the injection site, however, in the ventrolateral medulla and NTS, CART/CTB-containing neurons were found in both sides. In addition, scattered retrogradely labeled CART-IR neurons were found in the parabrachial nucleus. Since CART-IR neurons in the lateral hypothalamus and zona inzerta colocalize with melanin concentrating hormone (MCH) and adrenergic neurons of the ventrolateral medulla project to the PVN, we performed triple labeling immunocytochemistry using antisera against CART, CTB and MCH or the PNMT, the keyenzyme of adrenaline synthesis. In the zona inzerta and lateral hypothalamus, all neurons co-containing CTB and CART also contained MCH. In the ventrolateral medulla, all CART-IR neurons projecting to the PVN were found to be PNMT-IR. This study indicates that innervation of the PVN by axons containing CART-IR derives from several different neuron populations, which collectively may contribute to the complex regulation of homeostatic functions of the PVN.

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FUNCTIONAL ROLE OF VERY SLOW (0-0.5HZ) BRAIN POTENTIAL OSCILLATIONS IN PRIMARY VISUAL CORTEX OF FREELY MOVING RATS DURING DARKNESS, LIGHT, AND AFTER LATERAL GENICULATE NUCLEUS ELECTRICAL STIMULATION

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BACKGROUND AND OBJECTIVE. Some recent reports demonstrated the presence of very slow oscillatory phenomena (frequencies less than 1 Hz) in the lateral geniculate nucleus (LGN) neurons [1]. Additionally, it was shown that illumination suppresses these very slow oscillations [1]. This study was performed to test the predictions following from these findings that extracellular very slow (0-0.5 Hz) brain potentials in the primary visual cortex (PVC) accompany illumination changes and that electrical stimulation of LGN modulates the very slow potential patterns in the PVC.

METHODOLOGY. Chronic implanted stereotaxic electrodes in 10 rats were used for intracerebral recordings and stimulations. Data acquisition and analysis were performed on the computerized system (multichannel bioamplifier, multichannel analog-to-digital converter, and software). Very slow brain potentials (VSBP) were studied under darkness (0 lux), during continuous light (2000-2500 lux), and after local electrical LGN stimulation (rectangle stimuli, with amplitude of 90 μA, stimulus duration time of 3 ms, and stimulation time of 5 s).

RESULTS. In darkness, the results revealed the presence of oscillations in this area of cortex with periods in the range of seconds (main ca. 1 Hz oscillations were within 0.1-0.21 Hz frequency domain), multiseconds (main multisecond oscillations were within 0.0167-0.03 Hz frequency domain) and occasionally minutes (mainly within 0.001-0.002 Hz frequency domain) plus negative (relatively to the rostral electrode) gradients of relatively steady potential in the millivolt range (19.3±4.5 mV). Continuous light exposure induced statistically significant ($P<0.001$) dramatic changes only in the frequency domain of seconds, manifested by a remarkable decrease in the power of 0.1-0.21 Hz oscillations according to the power spectral analysis. There were no statistically significant differences in the other VSBP ranges in primary visual cortex in compare to darkness. After LGN local electrical stimulation, there were observed strong alterations in all PVC oscillation frequency domains manifested by 3 prominent peaks on the frequencies of 0.001 Hz, 0.018 Hz, and 0.1 Hz. These VSBP changes were significantly different ($P<0.001$) from those observed in the darkness or light exposure.

CONCLUSIONS. These observations confirm the aforementioned predictions from thalamic recordings and permit two conclusion: (1) that in primary visual cortex VSBP reproducible oscillations in the range of seconds, plus significant changes in them occur in response to light and darkness; (2) LGN significantly modulates very slow potential oscillation patterns of the PVC mainly in the frequency domain of seconds and minutes. Basing on these findings and on the recent publications [2] on slow oscillation in cortical neurons, it could be proposed that VSBP can contribute to the processes of visual environment neuroprocessing in the PVC, obviously, trough the extracellular Ca^{2+} signaling mechanisms modulating synaptic transmission in the cortical neuronal networks.

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MEMBRANE POTENTIAL-DEPENDENT INHIBITION OF VOLTAGE-GATED SODIUM CHANNELS BY TOLPERISONE

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Tolperisone hydrochloride (Mydocalm) is a centrally acting muscle relaxant relatively free from side-effects. Clinical studies have shown that it relieves painful spasticities associated with neurological or rheumatic diseases. Tolperisone suppresses spinal segmental reflex transmission and potently decreases C-fiber mediated afferent nerve conduction *in vivo* as well as *in vitro*. The drug has a weaker blocking effect on conduction of A-fibers compared to lidocaine, a local anesthetic agent. In the present study the effects of tolperisone and lidocaine were investigated on the voltage-gated sodium channels of acutely dissociated dorsal root ganglion (DRG) neurons from 6-day-old rat pups using standard whole-cell patch-clamp recording.

Both tolperisone and lidocaine blocked tetrodotoxin (TTX)-sensitive, and TTX-resistant currents in reversible and concentration-dependent way (IC50 values respectively: 198 and 264 μM for tolperisone and 260 and 225 μM for lidocaine). The inhibition of tolperisone was strongly holding potential dependent, indicated by a considerable leftward shift of the steady state inactivation curves of the currents. Maximum currents recorded at high negative membrane potentials were also blocked by tolperisone.

These results show that tolperisone has a membrane potential dependent inhibitory effect on both types of voltage-activated sodium channel studied. Since under some pathological conditions neurons are in a sustained depolarized state, their sodium channels are more sensitive to the inhibitory action of the drug, offering an opportunity of ameliorating muscle spasticities and pain with a relatively favorable side-effect profile.

MOLECULAR REGULATION OF THYROXINE ACTIVATION IN THE CNS: **FUNCTIONAL ROLE OF UNTRANSLATED REGIONS OF THE TYPE 2 DEIODINASE (D2) mRNA**

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Thyroxine (T4) has to be converted to 3,5,3'-triiodothyronine (T3) via deiodination to bind effectively to the thyroid hormone receptor. 5' deiodination generates the majority of the available T3 in the CNS. The type 2 deiodinase (D2) catalyzes the conversion of T4 to T3, playing a crucial role in the regulation of intracellular T3 content in the pituitary and brain and affecting the hypothalamic/pituitary feedback. D2 is a low abundance selenoenzyme, containing the rare amino acid selenocysteine (Sec) in its active center. Sec is encoded by a UGA codon that serves as a stop codon in non-selenoprotein mRNAs. D2 mRNAs contain a stem loop structure close to its 3' end called Sec Insertion Sequence (SECIS) that inserts Sec at the in frame UGA codons.

The goal of this study was to address the role of the unusually long 5' and 3' D2 untranslated regions (UTR) in the generation of the enzyme. The D2 mRNA in higher vertebrates is at least 6 kb long, although the length of the open reading frame coding for the enzyme is ~800 nt. The D2 5'UTRs are >~600 bp and contain 3 to 5 short open reading frames (sORF). To evaluate their functional significance for D2 translation, 5'UTR fragments were cloned 5' to a chicken D2 (cD2) coding region followed by a SECIS element and tested in a transient expression system in HEK-293 cells. The full-length human and chicken 5'UTRs decreased D2 activity ~5-fold. A truncation study of the hD2 5'UTR located the suppression primarily to the region containing the first sORF encoding a tripeptide - MKG. A TTG for ATG replacement in the start codon of this sORF abolished the inhibitory effect. Enhanced D2 translation also occurred if the efficiency of the translational initiation sequence was reduced by replacing the -3 "A" for C but retaining the wild-type start codon. The inhibition of D2 translation by the sORF works only in *cis*, since cotransfection of sORF-containing vectors with the cD2-SECIS reporter did not decrease D2 activity. The wild-type D2 sORF also decreased rat D1 translation indicating that the inhibition is not D2 specific. In the extremely long (~ 5 kb) 3'UTRs of the D2 mRNAs contain numerous AUUUA putative RNA instability motifs. The wild-type cD2 mRNA was truncated to remove ~3.7 kb between the coding region and the SECIS element. As a consequence, D2 activity increased ~3.8-fold. To establish that this was due to a reduction in D2 mRNA degradation, the decay of D2 mRNA was studied using a tetracycline repressible promoter and Northern blots. The message of the truncated cD2 mRNA was ~15-fold higher than that of wild-type.

Our data indicate a novel level of D2 regulation. This is the first demonstration that the translation efficiency of D2 mRNA is significantly down-regulated by sORFs in its 5'UTR. This process can reduce the D2 activity expected from the mRNA level. The mRNA is unstable as well ensuring limited expression of this key regulator of thyroid hormone activation.

GENDER-DIFFERENCES OF GFAP-IR OUTSIDE THE "ENDOCRINE BRAIN"

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The cellular units of the central nervous system are the nerve cells (neurons) and the glia cells (neuroglia) – called summarizingly neural cells. Unlike nerve cells, glia undergo shape alterations, regression or hypertrophy, migration, etc. also in the mature central nervous system. Therefore, it is conceivable that glial cytoskeleton has to adapt rapidly to structural changes of the cell.

In the present work we concentrated on changes in the cytoskeleton of astrocytes. In contrast to oligodendrocytes, astrocytes contain a characteristic system of intermediate filaments in both the perinuclear cytoplasm and processes. A major structural protein synthesized by astrocytes is the glial fibrillary acidic protein (GFAP).

To study the effect of gonadal steroids on astroglial cytoskeleton we were interested to find out if there is a region outside the "endocrine brain" where astroglial cytoskeleton shows a sexual dimorphism and if different hormonal states can alter astroglial cytoskeleton and its reaction as revealed by GFAP-immunoreactivity (-IR). For this set of experiments the interpeduncular nucleus (IPN) was chosen displaying a high GFAP-IR but not been described having steroid receptors. Comparing male and female rat brains, a sexual dimorphism for GFAP could be pointed out in the interpeduncular nucleus – a brain region that is not involved in hormonal regulatory mechanisms.

It has been observed that astrocytes react to brain injuries with hypertrophy in the surrounding of the lesion and with the accumulation of cytoplasmic fibrillary material in order to fill the space of the tissue discontinuity or replace spatially the lost neurons. Thus, an increase in GFAP-immunoreactivity might be indicative for "reactive gliosis". Beside the local reaction to a neural damage, a hypothesis was advanced concerning the effects of astroglial reaction also in the projection areas of the injured neurons. These areas might be at considerable distance from the site of the lesion therefore, the term "remote astroglial response (RAR)" was introduced. The phenomenon of remote astroglial response, as revealed in the geniculo-cortical system used as a model, highlights the involvement of the projection area of lesioned neurons in the impairment caused to the brain by a focal lesion, thus it should be regarded as a holistic phenomenon. Accordingly, where secondary synaptic degeneration occurs, astrocytes react with a cytoskeletal hypertrophy. This hypertrophy could be influenced by gonadal hormones.

The fact that the cytoskeleton of astrocytes in areas lacking steroid hormone receptors responded to alterations in the hormonal state of the animal, argues for a more widespread effect of gonadal steroids in the brain than earlier believed.

The main perspective of the present findings is the possible manipulation of gonadal steroid levels to reduce the detrimental effects of the astrocyte reaction, primarily in cerebral edema, focal epilepsy and neurodegenerative disorders.

HIPPOCAMPO-SEPTAL GABAERGIC PROJECTION, AS A POTENTIAL SOURCE OF SYNCHRONY BETWEEN HIPPOCAMPAL AND SEPTAL INHIBITORY NEURONS

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The hippocampus and the medial septum are reciprocally connected. The GABAergic component of the septo-hippocampal projection terminates on all known classes of hippocampal interneurons and is thought to be important in the timing of hippocampal theta activity. A reverse projection originates from cells, whose somata are located in str. oriens and posses dendrites that run horizontally. They massively innervate the GABAergic, and to a smaller extent, the cholinergic neurons of the medial septum. Horizontally oriented neurons in the CA1 area str. oriens form a heterogeneous group of non-principal cells. Neurons containing the markers CB, somatostatin (SOM) and the type 2 muscarinic receptors show similar morphologies. One of their characteristic member is the SOM containing OLM cells, whose axons terminate in str. lacunosum-moleculare within a relatively restricted column.

The relationship among horizontal neurons with different features is not clear however. One important question is, whether does the projecting neurons, at the same time have local axonal arbors as well, that would make them suitable to synchronize the activity in the septo-hippocampal system? More specifically, does the OLM cells with local axonal arbor also project to the medial septum, or the two cell populations are distinct?

The morphology and pharmacology of non-principal cells giving rise to the hippocampo-septal projection were studied by injecting fluorescent microbeads into the medial septal area of young rats to retrogradely label these projecting cells. Two days later *in vitro* hippocampal slices were prepared. The retrogradely labeled HS cells were identified in the CA1 area str. oriens with a fluorescent microscope. Cell-attached and subsequent whole-cell recordings were performed to investigate the response properties of HS cells to different modulatory transmitter agonists. The effect of ACh, ACPD, NA and 5HT has been tested on the cells using a quick application system in the presence of ionotropic glutamate and GABA receptor blockers. The pharmacological modulation of the cells' firing and agonist-induced currents were measured. The cells were filled with biocytin, visualized and reconstructed using camera lucida. In the vast majority of cells all agonists increased the firing of the cells and induced an inward current. The HS cells had local axon collaterals in strata oriens and radiatum. A portion of the cells also projected into the CA3 area, crossing the hippocampal fissure or the CA2 area. Correlated electron microscopical analysis of identified axon segments revealed that the cells selectively target inhibitory neurons both locally and in the CA3 area.

Double retrograde tracing with fluorescent microbeads from the medial septum and the ventral hippocampus revealed, that non-principal cells in the dorsal hippocampus project to both areas simultaneously.

Thus, the identified cells are distinct from the well-characterized OLM cells and selectively innervate interneurons locally, in remote areas of the hippocampus, and at the same time project to the medial septum. The highly target selective dual projection might play an important role in the effective synchronization of hippocampal and septal neuronal activity.

THE ROLE OF GAP JUNCTIONS IN GENERATION OF SEIZURE DISCHARGES AND IN THE TRANSITION FROM ICTAL TO INTERICTAL STATE

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Recently published studies have shown that nonsynaptic mechanisms like gap junction communication can synchronize neurons in the absence of chemical synaptic transmission. In this study, the assumption that gap junctional communication may contribute to the induction and manifestation of ictal discharges and also the transition from brief interictal to ictal activity was tested on the 4-aminopyridine epilepsy model in *in vivo* experiments on rats by combining electrophysiological, pharmacological manipulation and molecular biological techniques.

The effect of a gap junction blocker (carbenoxolon) or a gap junction opener (trimethylamine, TMA) was investigated and compared on the induction, manifestation and propagation of cortical epileptiform activity in young and in adult animals during maturation of the epileptic focus. When electrical synaptic transmission was depressed relative to the initial baseline prior to the induction of epileptic focus, only a mild influence on the induction of seizure discharges occurred in adults, in contrast to youngest, where a significant reduction in the amplitudes of seizure discharges was detected both in the primary and mirror focus. The small amplitudes can reflect the synchronous activation of a restricted, small group of neurones. In another series of experiments, a significant decrease in the intensity of seizure activity of the already active epileptic foci was detected when electrical synaptic transmission was blocked by carbenoxolone both in adults and young animals.

Local application of TMA at the intact cortex of adults induced interictal like discharges, and had a weak influence on the induction of seizure activity. Thirty minutes pretreatment with TMA had a delicate influence on the induction of epileptiform discharges, indicated by gradually longer ictal periods. When TMA was applied at an already active epileptic focus, the duration of ictal periods was significantly increased, and in some cases the well-separated ictal and interictal periods were transformed into a state called status epilepticus. These observations could be indication of the different size of gap junction pool available in the adult or immature brains, and a possible increase in the number of gap junction channel-forming proteins in adults by hyperexcitability during the initial seizure activity.

This theory and the role of the gap junctional communication in the epileptiform activity was further investigated by following the expression pattern of two connexin genes. Both, connexin32 and connexin43 mRNA levels were significantly elevated at the primary focus as well as at the mirror focus, after 60-mins of repeated ictal discharges.

These findings suggest that, the activation of gap junctions appears to be involved in the maintenance of rhythmic ictal discharges. Their extended open stage can increase the duration of ictal periods, induce interictal activity and facilitate a transition from the interictal to the ictal state. Their fewer number or closed stage on the other hand can reduce seizure activity or contribute to termination of an ictal period.

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PROPERTIES OF HIPPOCAMPAL INTERNEURONS EXPRESSING CALRETININ-EGFP IN TRANSGENIC MICE

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In the hippocampus, calretinin (CR), a Ca^{2+} binding protein, is found in a unique interneuron type innervating only other GABAegic cells (Gulyás et al., J. Neurosci., 1996), but their physiological properties are unknown.

To promote the study of these neurons, transgenic mice were generated that express enhanced green fluorescent protein (EGFP) in CR-containing cells. The immunocytochemical analysis revealed that in one of the transgenic lines 1/3 of CR-positive interneurons expressed EGFP, but all EGFP expressing cells contained CR. This line has been chosen for further electrophysiological studies using whole-cell patch-clamp technique. EGFP-CR interneurons responded to a constant current pulse at a membrane potential of –65mV either with burst firing (n=103) or with fast spiking (n=51). The burst firing changed to fast firing pattern at a membrane potential of –45mV and could be blocked by 400 μM Ni^{2+}, which suggests the presence of low threshold Ca^{2+} currents in these interneurons. Using paired recordings, we identified electrical coupling between EGFP-CR interneurons (5 out of 6 cases), but no synaptic contact could be found among them (n=40). On the other hand, 17 synaptically coupled pairs were recorded between EGFP-CR and other unlabelled interneurons (n=85). IPSPs between these cells were bicuculline-sensitive (20 μM, n=6), thus $GABA_A$ receptors mediated the synaptic transmission. Trains of action potentials at 10, 20 or 100 Hz in the EGFP-CR cells evoked facilitating IPSPs in other interneurons. This is in line with the prediction that the presence of a fast Ca^{2+} buffer in the axon terminals, as for instance calretinin, should reduce the probability of the transmitter release and thus give rise to facilitating PSPs.

CANNABINOIDS DO NOT INHIBIT GLUTAMATERGIC NEUROTRANSMISSION VIA CB1 CANNABINOID RECEPTORS

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Resent electrophysiological results have suggested that both the hippocampal glutamatergic and GABAergic synaptic transmission could be reduced by synthetic cannabinoids, thought to be specific for CB1 cannabinoid receptors in the CNS. In contrast, anatomical observations have found CB1 receptors only in the axon terminals forming symmetrical (presumably

inhibitory) synapses. No labeling for CB1 receptors could be seen at asymmetrical (presumably excitatory) synapses. To elucidate the role of CB1 receptors in glutamatergic synaptic transmission, we investigated the effect of the synthetic cannabinoid WIN55,212-2 on the amplitude of postsynaptic currents in both wild type and CB1 receptor knockout mice. Excitatory postsynaptic currents evoked by stimulation of Schaffer collaterals were recorded in CA1 pyramidal cells. Bath application of 1 μM WIN reduced the amplitude of evoked EPSCs by 50% in both wild type and CB1 receptor lacking mice. In both cases, this reduction could be reversed by 1 μM SR141716A (widely used as a CB1 receptor specific antagonist). In contrast, 1 μM WIN caused a 40% decrement in the amplitude of inhibitory postsynaptic currents only in wild type, but not in knockout animals. IPSCs were evoked by stimulation of fibers in str. pyramidale. These results are consistent with a CB1 cannabinoid receptor-dependent modulation of GABAergic neurotransmission, but a novel cannabinoid-sensitive receptor must be responsible for the inhibition of glutamatergic transmission.

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OREXINERGIC FIBERS INNERVATE CHOLINE ACETYLTRANSFERASE-SYNTHESIZING NEURONS IN THE MEDIAL SEPTUM- DIAGONAL BAND (MSDB) COMPLEX

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The neuropeptide orexin (hypocretin) has been implicated in various physiological functions such as the regulation of sleep-wakefulness, neuroendocrine control and feeding behavior. Orexin is synthesized in neurons of the perifornical region and lateral hypothalamic area and orexin fibers innervate widespread regions of the brain including the medial septum-diagonal band complex (MSDB). Cholinergic and GABAergic neurons in the MSDB project to the hippocampus. Infusion of orexin into the medial septum has been reported to increase behavioral and electroencephalographic arousal (Espana et al., 2001). To find morphological evidence that the neurons containing orexin may exert their effect via the MSDB cholinergic cells, we performed a correlated light and electron microscopic double immunolabeling study using antibodies against orexin and choline acetyltransferase (ChAT), the enzyme synthesizing acetylcholine. ChAT-immunopositive neuronal structures were embedded in a rich network of orexinergic axons throughout the MSDB complex. Orexin-containing boutons were found in close proximity to cholinergic cell bodies and dendrites. The ultrastructural analysis revealed several axodendritic asymmetric type synapses between orexin-immunopositive terminals and cholinergic dendrites. Our results suggest that orexin inputs to cholinergic neurons in the MSDB may be an important link in the regulation of sleep-wakefulness, the control of hippocampal excitability and thus learning and memory functions.

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OPIOIDERGIC NERVE ELEMENTS IN THE LATERAL SEPTUM OF THE RAT

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The septal complex, maintaining reciprocal connections with several brain areas, plays a decisive role in the formation of several behavioral patters like food intake, maternal behavior, anxiety, etc. The synchronization of cortical neuronal activity at various frequencies was also found to originate from this brain area. In order to understand the neuronal background of the above functions it is necessary to reveal the connectivity of the septal neuronal network. Using correlated light and electron microscopic pre-embedding immunocytochemical methods, in this study we present data on the distribution and synaptology of the leucine-enkephalin(leu-enk)-immunopositive elements in the septal area in the rat brain.

A very dense leu-enk-immunoreactivity was revealed associated to the ventral subdivision of the lateral septal area. Leu-enk was present in a network of varicose nerve fibres very often forming pericellular baskets around fusiform cell bodies. Some perikarya and proximal dendrites were also weakly labelled. In the electron microscope multiple enkephalinergic synapses were found on immunonegative neuronal somata and dendrites. The leu-enk-positive perikarya and proximal dendrites received numerous synapses from immunonegative axonal profiles.

This arrangement suggests a very efficient opioidergic control of the lateral septal activity. Further studies are needed to elucidate the neurochemical nature of the postsynaptic targets and the functional role of the opioids in the lateral septum.

MEMBRANE PROPERTIES OF TYPE I SPIRAL GANGLION NEURONES OF THE GUINEA PIG

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The spiral ganglion has an important role in the audition as it is responsible for connecting the receptor cells of the organ of Corti to the central nervous system. Two cell types have been identified hitherto in the spiral ganglion: while type I neurones faithfully relay the activity of the inner hair cells to the cochlear nucleus, type II neurones ensure the afferent innervation of the outer hair cells. In this study we studied the potassium conductances determining the membrane characteristics of type I cells and we were particularly interested in the pharmacological separation of the various K^+ current components.

Guinea pig spiral ganglion neurons were isolated by enzymatic treatment of separated modiolus for 10 min. The enzyme solution contained 0.3 mg/l collagenase and 0.12 mg/l pronase (T = 31 °C). The enzyme exposure was terminated by the application of trypsin inhibitor. The neurons were allowed to sediment to a poly-D-lysine coated cover-slip and were examined by whole-cell patch-clamp technique.

The resting membrane potential of the neurones was around -63 ± 9 mV (n = 19), and they fired a single action potential at the beginning of a sustained depolarization. The cells exhibited a slowly activating outward current component on depolarization, which could be blocked partially by application of 1 mM TEA^+. From a holding potential of -60 mV 1 mM TEA^+ inhibited 67 ± 8% of the peak current and 75 ± 13% of the current measured 100 ms after the onset of the stimulus (mean ± S.D.; n = 6). On the other hand, 30 μM 4AP inhibited 27 ± 4% of the peak current, and 26 ± 7% of the current measured at 100 ms (n = 6). To make further separation of the various current components possible, dendrotoxin-I (DTX-I; inhibitor of some Shaker-gene related K^+ channels) was also employed. The spiral ganglion neurones clearly presented a DTX-I-sensitive current component as approximately 30% of the peak and steady-state current was inhibited in the presence of this drug. Possible changes in the action potential firing was also investigated in the presence of 4-AP and DTX-I, but quite remarkably neither compound transformed the typical, rapidly adapting action potential configuration of the ganglion cells.

Summing up, there are some similarities between the membrane properties of the spiral ganglion neurones and those of certain other cells in the central auditory pathway. However, unlike bushy cells in the cochlear nucleus and principal cells in the trapezoid body, spiral ganglion neurons do not exhibit 4-AP sensitive alteration of the firing pattern.

HISTAMINERGIC NEURONS IN THE PERIPHERAL NERVOUS SYSTEM OF GASTROPODS (*HELIX PORM ATIA, LYMNA EA STAGNALIS*)

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The chemical-neuroanatomy, distribution and biochemical properties of histaminergic neurons in different peripheral organs, such as the lip, foot, tentacles, mantle edge, heart and salivary gland, of the pulmonate snails, *Helix pomatia* and *Lymnaea stagnalis,* were studied, applying immunocytochemistry and HPLC assay. HA-immunoreactive (IR) sensory and gland cells could be demonstrated in the lip, foot, tentacles and mantle edge, but not in the heart and salivary gland. The majority the HA-IR sensory and glandular elements was found in the lip and tentacles where numerous groups and individual bipolar sensory cells were located within the epithelial layer. Afferent axons of these cells formed bundles projecting towards and through the deeper regions of the peripheral organs. In addition to the HA-IR sensory cells, a network of fine varicose fibers also occurred beneath the epithelium and in the deeper, muscular regions of the peripheral tissues. In the foot and mantle edge fine single varicose

fibers were also found running parallel with the surface close beneath the epithelial layer. Applying HPLC low but detectable HA concentrations could be assayed in the different peripheral organs of both species. In *Helix,* these HA concentrations were the following: foot 1.39 pmol/mg tissue, lips 0.45 pmol/mg tissue, tentacles 0.41 pmol/mg tissue, whereas in *Lymnaea,* foot 0.27 pmol/mg tissue and lips 0.56 pmol/mg tissue. (For comparison, the CNS [incl. buccal ganglia] contained 14.14 pmol/mg tissue HA in Helix, and 5.83 pmol/mg tissue HA in *Lymnaea* CNS.) Based on our finding a multifunctional role including sensory efferent/afferent regulatory and endocrine functions is suggested for HA in the peripheral nervous system of gastropods.

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ANALGESIC AND ANTI-INFLAMMATORY EFFECTS OF TT-232, A HEPTAPEPTIDE SOMATOSTATIN ANALOG, IN THE RAT

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We have demonstrated earlier that somatostatin released from the activated peripheral endings of the capsaicin-sensitive sensory nerves exerts systemic anti-inflammatory and anti-nociceptive actions. In the present series of experiments the anti-inflammatory and analgesic effects of TT-232 (D-Phe-Cys-Tyr-D-Thr-Lys-Cys-Thr-NH_2), a synthetic and selective heptapeptide somatostatin analog with a cyclopenta ring structure, was studied in the rat.

TT-232 (2.5–20 μg/kg i.v.) dose-dependently inhibited carrageenin-evoked paw oedema examined by plethysmometry and significantly decreased bradykinin-induced Evans blue accumulation in the ankle joint. It was a potent analgesic agent in the neuropathic pain model of Seltzer using Randall-Selitto test (Ugo Basile analgesimeter). Treating the rats with TT-232 (2.5–20 μg/kg i.p.) dose-dependently inhibited mechano-nociceptive hyperalgesia of the hindpaws induced by partial ligation of the sciatic nerve. Diclofenac, a prominent representative of non-steroidal anti-inflammatory agents, exerted significant inhibitory action on articular plasma protein extravasation evoked by bradykinin, but it had no effect on carrageenan-induced oedema and neuropathic pain. Baclofen, a GABA-B receptor agonist, decreased hyperalgesia after Seltzer operation.

As TT-232 is free of endocrine activity mediated through SST 2, 3 and 5 receptor subtypes, SST 1 and/ or 4 subtypes located on sensory nerve terminals and vascular walls are likely to be responsible for the above-described actions. Since there is no effective compound for the treatment of neuropathic pain and neurogenic inflammatory component of different diseases, TT-232 opens new horizons for the development of a new type, broad spectrum anti-inflammatory and analgesic drug.

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SCL, GATA2 AND LMO2 TRANSCRIPTION FACTOR EXPRESSION IN THE DEVELOPING CHICKEN BRAIN

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The SCL/tal-1(SCL) gene, encoding a bHLH transcription factor, was originally identified in T-cell acute lymphoblastic leukemia. SCL expression was also detected in sites involved in early embryonic and foetal hematopoiesis and in the vascular endothelium. In the adult, SCL expression is maintained in endothelia and in some hematopoietic cell types. During the last decade SCL expression has been shown in specific regions of the developing central nervous system (CNS) of mouse, zebrafish and chicken. While SCL is known as a key regulator of early hematopoiesis and seems to play an important role in the development of the CNS, its exact function remains unknown.

In the present study the expression pattern of SCL was compared between regions displaying high proliferation activity and those containing postmitotic matured neurones in developing chicken brains during the main neurogenetic period (E3-E9). The distribution of SCL transcript was compared to the expression of other transcription factors, GATA-2 and Lmo2. Our recent comparative study showed that in the CNS, SCL is coexpressed with GATA-2 in developing neurones, while Lmo2 is found only in endothelial and erythroid cells. On the other hand, we showed that SCL is co-expressed with GATA-2 and Lmo2 during yolk sac erythropoiesis in chicken. Additionally previous *in vitro* studies (undertaken by other laboratories) have suggested that SCL, Lmo2 and GATA-factors (GATA-1 or GATA-2) are members of the same complex in erythroid cells. These results suggest that SCL participate in different transcriptional complexes within the developing CNS in comparison with the hematopoietic system.

NINE-HUNDRED MHz PULSED ELECTROMAGNETIC FIELD ALTERS FUNCTIONING OF THE RODENT PREFRONTAL CORTEX

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It has been broadly discussed so far that electromagnetic fields may be hazardous to health. It is also proposed, that the proximity of cellular mobile phones to the user s head leads to the absorption radiofrequency (RF) irradiation. We created a simplified animal model to

investigate the effects of RF waves on functioning of the prefrontal cortex (PFC). In our present examinations were aimed to assess 1) spontaneous flung activity of PFC neurons in the rat; 2) motor reaction times (RTs) and learning capabilities of freely moving animals in a visual discrimination/reaction time learning task. According to the results of our animal model experiments, reversible decrease/increase of spontaneous firing rates of single/multiple units were observed in the PFC of the rat. In RT studies, increases of response latencies with decreases of task accuracy were observed after lower output power (0.25 W/s, 2 W i.p.p.) which was not observed after higher output power (2 W/s, 16 W i.p.p.). Our present results indicate that RF irradiation caused by GSM output device elicits short depression followed by rebound in spontaneous neural activity, coupled with changed behavior of subjects. In addition, the dynamics of basal neural firing rate changes caused by non-thermal, pulsed RF fields may be a good indicator of time course of transient intracellular biochemical alterations. The above observations may provide clues to further understanding why PFC functions are altered when subjects are exposed to pulsed RF fields.

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THE ANTINOCICEPTIVE POTENCIES AND INTERACTIONS OF ENDOGENOUS LIGANDS AT SPINAL LEVEL

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Increased understanding of endogenous pain modulatory systems that alter nociceptive transmission in the spinal cord of animals and humans has led to the development of specific agents in humans. Opioids are the most widely used drugs to manage different pain states, but bothersome and potentially dangerous side-effects limit their use. One approach to improve analgesia and reduce side effects at the same time is to combine small doses of different drugs. Another possible way of antinociception is the local application of endogenous ligands. The purpose of the present study was to investigate the antinociceptive effect of different endogenous ligands (the μ-opioid agonist endomorphin-1, the NMDA-antagonist kynurenic acid, and a nocistatin derivative bPNP-3-8P acting unknown receptor) and their interactions at spinal level in awake rats.

Methods: After institutional ethical approval had been obtained from our animal care committee, intrathecal catheters were implanted into male Wistar rats. After 4 days of recovery, for the inflammatory heat pain test (paw withdrawal test) the rats were placed on a glass surface in a plastic chamber and allowed to acclimatize to their environment for 20 min before testing. Then the baseline hindpaw withdrawal latencies were obtained (pre-carrageenan values at -180 min). Unilateral inflammation was induced by intraplantar injection of 1.5 mg/0.1 ml carrageenan into the right hindpaw. The paw withdrawal latencies were obtained

again 3 h after carrageenan (post-carrageenan baseline values at 0 min), and then every 10 min for 130 min. After the determination of the post-carrageenan baseline value, the drugs were administered continuously in different doses via the intrathecal catheter for 60 min using a microinfusion pump. Drugs were injected at a rate of 1 μl/min. Groups were compared by ANOVA with $P<0.05$ considered significant.

Results: Continuous administration of endomorphin-1 (0.1, 0.3, 1 or 2 μg/min) dose-dependently decreased the thermal hyperalgesia. After the cessation of the infusion the hyperalgesia reappeared. None of the doses of endomorphin-1 influenced the paw withdrawal latencies of the non-inflamed paws. No side-effects could be observed. bPNP-3-8P (0.003-30 μg, administered cumulatively) significantly decreased the thermal hyperalgesia at higher doses (3 and 30 μg). Continuous infusion of bPNP-3-8P (0.03, 0.1 and 1 μg/min) did not potentiate the antinociceptive effect of endomorphin-1, even if shortened the duration of its effect. Kynurenic acid at higher doses (1-4 μg/min) significantly decreased the thermal hyperalgesia and increased the paw withdrawal latencies at the non-inflamed side. These doses also associated with motor impairments at both sides. The cessation of its administration resulted in a gradual decrease in the antinociceptive effect. Continuous administration of kynurenic acid (0.01-0.1 μg/min) potentiated but did not prolong the antinociceptive effect of endomorphin-1 (0.1-1 μg/min) at the inflamed side. There were no sign of motor impairment during the combined treatment.

Discussion: Our results demonstrate that the μ-opioid agonist endomorphin-1 has the highest potency, while kynurenic acid and bPNP-3-8P have lower efficacy. The co-administration of endomorphin-1 and bPNP-3-8P is no advantageous, although the combination of endomorphin-1 and kynurenic acid shows beneficial antinociceptive interactions.

TRANSCALLOSAL TRACING WITH PSEUDORABIES VIRUS IS INFLUENCED BY A PERIPHERAL NERVE INJURY

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Pseudorabies virus (PRV) is a member of the neurotropic subfamily (alphaherpes viruses) of the herpesvirus family. During the past decade, it turned out that the transsynaptic tracing with an attenuated strain of the pseudorabies virus (Bartha strain) is a suitable method to study the function of neuronal connections within the central nervous system. Entry of alphaherpes viruses into the cells usually requires multiple interactions between the viral envelope proteins and the cell surface proteins. At least two groups (syndecans and nectins) of these cell surface proteins are known to play a role in the development of tissues of neuroepithilial origin. Since the neuronal plasticity in adulthood is thought to recapitulate the developmental phenomena, and the nectins and syndecans are expressed in the adult nervous system, it is supposed that they are possible involved in the injury-induced neuronal plasticity. The present study has demonstrated that a peripheral nerve injury may influence the transcallosal (transneuronal)

spread of PRV from the left side motor cortex to the contralateral motor cortical area of the rat. The main observations were as follows: in controls, PRV injection primarly infected several neurons around the penetration channel, but hardly any secondarily infected neuron was found in the contralateral motor cortex. In contrast, after right side facial nerve injury, PRV was transported from the primarily infected neurons in the left side motor cortex to the contralateral side, and resulted in labelling significantly higher number of neurons due to a secondary infection. On the basis of these results, we suggest that a peripheral nerve injury influences the PRV infection pattern in related cortical areas, wich phenomenon might be related with the changes in the expression of the cell adhesion molecules and/or other cell surface molecules.
This work was supported by a grant from the OTKA (T031893).

MORPHOLOGICAL EVIDENCE FOR DIRECT REGULATION OF LUTEINIZING HORMONE-RELEASING HORMONE NEURONS BY THE BETA ISOFORM OF ESTROGEN RECEPTOR

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Feedback regulation of luteinizing hormone-releasing hormone (LHRH) neurons by estradiol plays important roles in the neuroendocrine control of reproduction. Recently, we found that the majority of LHRH neurons in the rat contain estrogen receptor-β (ER-β) mRNA, whereas, they seemed to lack ER-α mRNA expression. In addition, we observed nuclear uptake of ^{125}I-estrogen by a subset of these cells. These data suggest that ER-β is the chief receptor isoform mediating direct estrogen effects upon LHRH neurons. To verify the translation of ER-β protein within LHRH cells, the present studies applied dual-label immunocytochemistry (ICC) to free-floating sections obtained from the preoptic area of rats. The improved ICC method using the silver-gold intensification of nickel-diaminobenzidine chromogen, enabled the observation of nuclear ER-β-immunoreactivity in the majority of LHRH cells. The incidence of ER-β expression was similarly high in LHRH neurons of ovariectomized female (87.8±2.3%, mean±SEM), estradiol-primed female (74.9±3.2%) and intact male (85.0±4.7%) rats. The presence of ER-β mRNA, ER-β immunoreactivity and ^{125}I-estrogen binding sites in LHRH neurons of the rat provide strong support for the notion that these cells are directly regulated by estradiol, through ER-β. The gene targets and molecular mechanisms of this regulation remain unknown.

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INVESTIGATION OF THE VIP-CONTAINING AREAS IN THE CENTRAL NERVOUS SYSTEM OF VARIOUS SPECIES BY A NEW RADIOIMMUNOASSAY

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Vasoactive intestinal polypeptide (VIP) is a basic straight-chain polypeptide consisting of 28 amino acids and occurs in high concentrations throughout the gut. Distribution of VIP in the central nervous system in a number of species has also been described mainly by immunohistochemistry. The aim of the present study was to determine the VIP content in areas of the central nervous system of various vertebrate and invertebrate species using a newly developed radioimmunoassay (RIA). Different areas of the brains and spinal cords were removed from fish, frog, turtle, chicken and rat. Tissue samples were homogenised, centrifuged and the VIP contents of supernatants was measured. We also determined the VIP concentrations in the cerebral ganglia and the ventral ganglionic chain of earthworm.

Our results show that VIP antiserum raised in rabbit against a conjugate of porcine VIP-bovine thyroglobulin turned to be C-terminal specific based on cross-reaction studies. Average ID_{50} value of the calibration curves was 6.21±0.49 fmol/ml determined in 10 consecutive assays. Detection limit of the assay proved to be 0.2 fmol/ml. The highest VIP level was measured in the hypothalamus of turtle, followed by other brain areas of turtle and rat. Less concentrations of VIP were determined in the brain areas of chicken, frog and fish.

GLUTATHIONE RECEPTORS IN THE MAMMALIAN CENTRAL NERVOUS SYSTEM

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Gamma-glutamylcysteinylglycine (GSH and GSSG, reduced and oxidized glutathione) is present both intra- and extracellularly in the mammalian central nervous system (CNS). Intracellular GSH is an antioxidant which protects cells but the role of extracellular GSH is less well known. Already almost 50 years ago GSH was suggested to play a role in signal transduction in *Hydra* and recently extracellular GSH has been shown to modulate the functions of ionotropic N-methyl-D-aspartate (NMDA) and (S)-2-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) and metabotropic group III glutamate receptors. We have

found in pig cerebral cortical synaptic membranes two (high and low affinity) binding sites for GSH not displaceable by glutamate, GABA and glycine receptor ligands. However, many analogs of cysteine and glutathione (e.g., cysteine, cysteamine, cystamine, cysteinylglycine and glutamylcysteine) effectively inhibit the binding of labeled GSH. The binding is strongly allosterically activated by thiokynurenate. These specific binding sites for GSH thus differ from any known excitatory or inhibitory amino acid receptor. In the rat cortical wedge preparation, GSH evokes a Ca^{2+} -independent depolarizing potential probably by enhancing Na^{+} influx. The effect is not blocked by the antagonists of AMPA and NMDA receptors 6,7-dinitroquinoxaline-2,3-dione (DNQX) and L(+)-2-amino-5-phosphonovalerate (L-AP5), respectively, but again greatly potentiated by thiokynurenate. The cysteine moiety is mandatory in eliciting excitation since dipeptides or tripeptides containing neutral amino acids instead of cysteine give no depolarizing responses. While GSH modulates glutamate receptors via its ©-glutamyl moiety, GSH binds to and activates these sites of its own via the cysteinyl moiety. Our results show the presence of specific ionotropic excitatory GSH receptors involved in synaptic transmission in the mammalian CNS.

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NITRIC OXIDE DISPLAYS A STRESSOR-DEPENDENT EFFECT ON THE ACTIVATION OF THE HYPOTHALAMO-PITUITARY-ADRENAL AXIS

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Nitric oxide (NO) appears to play a rather elusive role in the regulation of the hypothalamo-pituitary-adrenal (HPA) system. Its function differs clearly depending on the site of action, and conflicting data have also been presented regarding the action of this gaseous neurotransmitter on the central organization of the stress response. It appears that such contradictions might be resolved, at least partly, assuming the diverse actions of nitric oxide on the separate neural routes utilized by different stress paradigms. In a view to assessing the relative importance of nitric oxide in the transmission of different stress signals in the present experiments four stressors were applied: electric footshock, ether-stress, restraint and hypertonic saline. Thirty min before the application of the stressors the NO precursor L-arginine (L-Arg) or its competitive antagonist N^{G}-nitro-L-arginine (L-NNArg) were administered intracerebroventricularly to male rats. Neither of these amino acids altered the basal secretion in the dose (20 μg) applied and had a significant impact on the HPA activation evoked by hypertonic saline and restraint. On the other hand L-Arg dose-dependently attenuated the increase in plasma corticosterone brought about by ether or electric footshock, while L-NNArg did not augment significantly the HPA response. These findings clearly demonstrate the inhibitory and stressor selective action of NO on the HPA activation and suggest that profoundly different neural mechanisms might be involved in the processing of distinct nocuous stimuli.

STUDIES ON THE GLUTAMATE TRANSPORT IN THE COURSE OF *IN VITRO* NEURON FORMATION

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Small "classical" neurotransmitters, among them glutamate, were shown to influence the differentiation of neural progenitors, well before the formation of synapses. However, mechanisms underlying the early regulatory functions of glutamate are not well understood.

In the present study, *in vitro* induced neuron formation by NE-4C neuroectodermal progenitor cells (Schlett 1997) was used to study glutamate transport in various phases of early neural development. Continuously dividing NE-4C cells cloned from primary forebrain cultures of p53-deficient mouse embryos (E9) give rise to neurons and astrocytes if exposed to *all trans* retinoic acid (RA). The extracellular glutamate-level, the expression of various glutamate transporters, the uptake of glutamate together with the glutamate responsiveness of the cells were investigated in various stages of neuronal commitment, and were compared to the data obtained on primary neural cells in cultures of embryonic or perinatal rat forebrains.

The data showed that the cells strictly regulated the extracellular glutamate-concentration and established a developmental stage-dependent glutamate-level in their fluid environment. The shifts in the set-points of [Glu]e were accompanied by changes in expression patterns of the glutamate-transporters and in the cell-responsiveness to glutamate. In some stages of neural development, retinoic acid could directly increase the glu-uptake, indicating some stage-specific characteristics of the glutamate-transport.

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DIFFERENTIAL PRESENCE OF IMMUNOREACTIVITY FOR ESTROGEN RECEPTOR-BETA IN CALCIUM-BINDING PROTEIN-CONTAINING CORTICAL NEURONS OF THE RAT

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The rat cerebral cortex has been reported to contain the mRNA for the beta subtype of the estrogen receptorβ (ER) (Shugrue et al., 1997). Given the accumulating evidence that estrogen can affect higher functions, the present study was designed to characterise cortical neurons that

express ERβ-immunoreactivity (IR). Using double-immunohistochemistry we have examined cells immunoreactive for parvalbumin (PV), calbindin (CB) or calretinin (CR), the three main calcium-binding proteins found in cortical interneurons, for the presence of ERβ-IR. Most of the cortical neurons with nuclei displaying ERβ-IR were found in layers 5 and 6; smaller numbers were also seen in layers 2, 3 and 4. Non-pyramidal PV- and CB-positive neurons containing ERβ-IR were widely observed. ERβ-IR was particularly intense in CB-positive neurons in the pirifom and insular cortices and less intense in the cingulate, frontal and parietal cortices. Cortical neurons with CR-IR were rarely found to contain ERβ-IR. Earlier work (DeFelipe, 1997) indicated that the subpopulations of interneurons immunoreactive for these calcium-binding proteins make contributions to cortical circuitry that differ according to the cortical area. The variation in ERβ-IR in these three classes of interneurons at different sites suggests multiple area-specific functions for estrogen that remain to be investigated.

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NEW TECHNICAL SOLUTION FOR ELIMINATION OF ELECTROMAGNETIC ARTIFACTS EMITTED BY GSM MOBILE PHONES IN ELECTROPHYSIOLOGICAL RECORDINGS

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At least 40% of the total output power of a commercial GSM cellular mobile phone is absorbed in the head of the user. There is already ample data available concerning neurobiological effects of electromagnetic fields (EMF), we still have little knowledge about how EMF modifies *in vivo* electric properties of neurons. The absorbed pulsed radio frequency (RF) energy might be rectified in the electrically non-linear brain tissue, and DC impulses are created. The level of DC impulses is often grater then the usually recorded electrical brain activity itself and produce huge artifacts. Artifacts usually overload the main AC amplifier, and may also block it for several seconds. To eliminate these artifacts and to enable electrophysiological recording under RF exposure *in situ,* we aimed to construct an electric gating system. For continuous pulse generation, we used a modified, standard GSM mobile phone at 915 MHz, 217 Hz, 1/8 duty cycle (pulse ratio). Ketamine anaesthetized Long Evans rats were exposed in the near-field RF in an animal holder. The maximum SAR values in the brain (measured in liquid phantom, where e'=42.5, rho=0.85) were 1.35 W/kg brain tissue. Under continuous RF exposure extracellular firing rates of neurons were measured in various cortical areas by single/multi unit recording technique by means of glass microelectrodes. RF pulses produced high energy DC impulses (artifacts) which were short and their rise time was very fast. The recorded DC impulses and the cellular activity have a time shift (delay time) via passing through the high impedance fluid filled microelectrode and the RF filtered preamplifier. The preamplifier, in its frequency range, acts as a high-pass filter, and gives a differentiated impulse curve. This impulse-curve is not suitable for driving a gating electronic system for our purposes.

Therefore, in our device, we pick up the RF signal directly form the radiated electric field by a passive RF receiver. The gating system is located between the preamplifier and the main amplifier. The received gating signal is formed and extended by multivibrator to switch a sample-and-hold circuit. The gating equipment switches off the artifact (DC potentials generated by the RF field), and enables any kind of impulse separator (on any number of recording channels) to detect neuronal activity. Though, our new technical solution causes 25–30% loss of unit discharges, we are able to record extracellular firing activity in a high intensity EMF. The device is also equipped with a free-running, 217 Hz pulse generator, to ensure the same conditions even without RF irradiation. This ensures that analyzed data is fully comparable in all experimental situations. We also provide preliminary electrophysiological recordings indicating reversible firing rate changes of cortical neurons under continuous RF exposure, which depends of the power output of the pulse generator.

These experiments were supported by ETT 049/2000, EU COST 244bis. Electrophysiological set-up was partly funded by Hungarian Res. Fund (OTKA) grant No 029818.

AMINERGIC AND PEPTIDERGIC INNERVATION OF THE SALIVARY GLAND IN GASTROPODS (*HELIX POMATIA, LYMNAEA STAGNALIS*)

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The salivary gland, an important organ of the feeding system of gastropods, is symmetrically located on both sides of the pharynx. Recently, as a first step, we described some immunocytochemical, biochemical and physiological characteristics of the serotonin(5HT)ergic and dopamine(DA)ergic innervation of the salivary gland in the pulmonate snails, *Helix pomatia* and *Lymnaea stagnalis*. In the present study, we have compared the aminergic (5-HT, DA) and molluscan type peptidergic (FMRFamide [Fa]), Mytilus-inhibitory peptides [MW], catch-relaxing peptide [CARP], APWGamide) innervation of the salivary gland, with emphasis onto the primary salivary duct, applying immunocytochemistry, HPLC, uptake-release and bioassay experiments. A rich network of 5-HT- and peptide-immunoreactive fibers, respectively, of CNS origin could be visualized at the level of both the salivary duct and the gland. This innervation is also characterized by a partial colocalization of 5-HT and different peptide immunoreactivities in both the axon processes and varicosities. In the salivary gland of both species, 0.8-1.57 pmol/mg 5HT and 0.2-0.4 pmol/mg tissue DA concentrations, whereas in the isolated salivary duct 14.9-15.5 tissue for 5-HT and 0.38-0.58 pmol/mg tissue for DA were determined by HPLC assay. A single component uptake system and a IC-evoked release of ^{3}H-5HT and ^{14}C-DA were also demonstrated in the isolated salivary duct. Using an *in vitro* isometric technique on the isolated salivary duct of *Helix,* 5-HT was shown to evoke contractions through a 5-HT_3 receptor type and relaxation through a 5-HT_2 receptor type, whereas DA exerted a dose-dependent sustained contraction effect through a D_1 receptor type. Molluscan neuropeptides, Fa, MW, CARP and APWGa, proved to be capable of significantly modulating the effects 5-HT and DA, respectively, on the muscle

contraction activity. Responses elicited by DA or nerve stimulation could be decreased by these peptides, whereas 5HT responses were modified in different ways, depending on the peptide applied. It is suggested that the transport of secretion, that is the salivary duct activity, is regulated by a complex way of aminergic and peptidergic interaction.

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GLUTAMATERGIC AFFERENTATION TO HYPOTHALAMIC MAGNOCELLULAR NUCLEI STUDIED BY [^{3}H]D-ASPARTATE RETROGRADE TRACER METHOD

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Oxytocin and vasopressin neurosecretory neurons of the supraoptic nucleus receive a dense glutamatergic innervation. The localization of the glutamatergic neurons projecting to this prominent hypothalamic cell group is unknown. The aim of the present investigations was to clarify this question. The transmitter-selective [^{3}H]D-aspartate retrograde transport method was used injecting the tracer into the supraoptic nucleus. The radioactive tracer was visualized by autoradiography. The selectivity of [^{3}H]D-aspartate as a retrograde tracer of glutamatergic/aspartatergic neurons has been demonstrated in many brain pathways.

After injection of [^{3}H]D-aspartate into the supraoptic nucleus retrogradely radiolabeled neurons were detected in distinct diencephalic and telencephalic areas and nuclei including supraoptic nucleus itself, suprachiasmatic, hypothalamic paraventricular, ventromedial, dorsomedial, ventral premammillary, supramammillary nuclei, thalamic paraventricular nucleus, lateral septum, median preoptic nucleus, medial preoptic area, bed nucleus of the stria terminalis, medial and basomedial amygdaloid nuclei. No retrogradely radiolabeled cells were found in the brain stem. It should be added that as a whole the number of regions projecting to the nucleus is much larger compared to the structures which project to the cell group and contain glutamatergic/aspartatergic fibers.

Present results are the first neuromorphological data on the origin of glutamatergic/aspartatergic terminals detected in the supraoptic nucleus and representing about 20% of terminals in the cell group. The findings indicate that there are several structures in the diencephalon as well as in the telencephalon which contain glutamatergic/aspartatergic nerve cells projecting to the cell group.

It appears that the localization of retrogradely labeled neurons after injection of [^{3}H]D-aspartate into the supraoptic nucleus is fairly similar to the localization of neurons labeled from the hypothalamic paraventricular nucleus (Csáki et al., Neuroscience 2000; 101: 637-655). This is not surprising, because the magnocellular neurons of both the supraoptic and the paraventricular nucleus (at least the majority of them) project to the posterior pituitary and secrete oxytocin and vasopressin. Neither the supraoptic nor the paraventricular nucleus were

found to receive glutamatergic afferents from the brain stem. Interestingly, in contrast to these two nuclei, the supramammillary nucleus, which projects directly to the hippocampus or indirectly via the septum and participates in the regulation of hippocampal theta activity, does receive glutamatergic afferents from the brain.

The structures containing glutamatergic fibers projecting to the supraoptic and paraventricular nuclei are fairly widespread. This suggest that the glutamatergic innervation of these two cell groups is presumably very complex. There are no well-defined glutamatergic pathways arising from distinct regions of the brain.

A number of experimental data suggest that the glutamatergic innervation of the supraoptic and paraventricular nucleus is of physiological significance and is involved in the control of oxytocin and vasopressin release.

EFFECT OF SURGICAL ABLATION OF THE ENTORHINAL CORTEX ON SEIZURE-INDUCED C-FOS-PROTEIN IMMUNOREACTIVITY IN THE RAT HIPPOCAMPUS AND TEMPORAL ASSOCIATION CORTEX

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The expression of the c-*fos* protooncogene in different seizure models is well documented (Herdegen and Leah, 1998). Previous work from our laboratory showed that in seizures induced with 5 mg/kg 4-aminopyridine (4-AP) in rats, hippocampal c-*fos* expression was detectable first in dentate granule cells (Mihály et al., 2001). We supposed, that the seizure was initiated in cortico-thalamo-cortical circuits then spread to the dentate gyrus through the entorhinal cortex and the perforant path. The aim of the present study was to test this hypothesis by means of the surgical excision of the lateral entorhinal area (lea) in adult wistar rats.

Experimental animal groups

Control group I: 3 rats, were not operated, treated with 4-AP and perfused after 3 h of seizure.

Control group II: 3-3 sham-operated animals, receiving 4-AP injection, and perfused 30 min, 1 h and 3 h later.

Control group III: 3 operated animals with LEA ablation, injected with physiological saline and perfused 1 h later.

LEA ablation and seizure: 3-3 operated animals, injected with 4-AP and perfused 30 min, 1 h and 3 h later.

The animals survived 30-40 days following the surgery. The brains were sectioned in coronal and horizontal planes, and frozen sections were subjected to the immunohistochemical detection of c-*fos* protein. The immunoreactive cell nuclei were counted and analyzed with the Image Pro Plus morphometry program.

Results

1. In "Control group I" the c-*fos* activation patterns of the two hemispheres are nearly identical; there are no significant differences between the two sides as to the counts of immunoreactive cells (data from 3 animals).

2. In operated animals, there are differences between the two hemispheres as to the number of Fos-immunostained cells; the side of the ablation regularly contains more c-*fos*-stained cell nuclei than the contralateral side; most of these differences are not significant.
3. The absolute number of the c-*fos*-stained cell nuclei is significantly lower in operated (LEA ablation) animals (data from 3 animals).
4. In the CA1 region, the number of c-*fos* expressing cells is significantly higher on the operated side following 3 h seizure (data from 3 animals).
5. In the hilus of the dentate gyrus, the number of the c-*fos* expressing cells is significantly higher on the operated side following 30 min of seizure (data from 3 animals).
6. There are no significant hemisphere differences in the sham-operated animals (data from 3 animals).
7. In one animal with LEA lesion, the Fos-immunoreactivity of the granule cell layer is very much stronger than that of the sham-operated animal.
8. In the temporal association cortex (above the perirhinal cortex) the number of Fos-immunoreactive cells was significantly higher on the side of LEA ablation after 3 h of seizure (data from 6 animals).

Conclusions

1. The LEA lesion decreased the seizure induced c-*fos* activation in every region of the hippocampus; this is in accord with the results of Clusmann et al., 1994.
2. In six experiments we found that on the side of the lesion the 4-AP seizure elicited significantly stronger c-*fos* activation; which could be the consequence of the loss of feed-forward inhibition mediated by the perforant path (Freund and Buzsáki, 1996).
3. The increased *c-fos* expression of the temporal association cortex on the side of the lesion raised the possibility that the targets of entorhinal – isocortical projections (Swanson and Köhler, 1986) are inhibitory local circuit neurons.

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PLASTIC CHANGES ON THE CORTEX OF ADULT MICE

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Activity-dependent adaptive changes in the nervous system involve structural and functional changes in the cortical circuitry. In this work the cortical function was studied by repeatedly recording of the somatosensory and motor potentials evoked by whisker deflections after

altered sensory-motor experience in adult mice. The latencies of motor and somatosensory evoked potentials were found to shorten, while their amplitudes decreased, after a behavioural challenge involving the vibrissal apparatus. Sensory deprivation achieved by whisker trimming resulted in a partial reversal of the changes observed after increased activity. The derived parameters imply that cortical information processing speeds up as a result of experience, while decreased activity has the opposite effect. The methods used throughout the experiment were minimally invasive, and thus proved to be sufficient for the long-term follow-up of cortical functions.

NORADRENERGIC RECEPTORS MEDIATE THE ACTION OF Li^+ ON PREFRONTAL NEURONS: AN IONTOPHORETICAL STUDY

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Various lithium (Li^+) compounds are proven as effective in the therapy of manic disorders, but their mechanism of action is not clearly defined. It has been shown that iontophoretical application of Li^+ on hippocampal or cerebellar (Purkinje) cells antagonize norepinephrine (NE) elicited inhibition of neural activity. However, there is no pharmacological explanation provided for this effect, yet. It is also widely excepted that Li^+ blocks the inositol-triphosphate (IP_3) turnover pathway wich is related to the alpha-1 adrenergic receptors. Despite the above observations, alpha-1 receptors are also described elsewhere to exert excitatory effect on neural activity, while alpha-2 receptors cause inhibition of the maintain activity. Since only inhibitory effect can be antagonized by Li^+-salts, we suppose that Li^+ acts on alpha-2 receptors, too. In our present study, we tested the action of Li^+ on NE-elicited activity and/or against adrenergics on neurons located in the prelimbic subregion (PrL) of the prefrontal cortex (PFC).

For extracellular single cell recording and microiontophoresis, six-barreled micropipettes were used (Carbostar-7, Kation Scientific, USA). Drugs: 0.15 M NaCl solution (continuous balancing current), LiCl 0.15 M, Norepinephrine-HCl 50 mM, Phenylephrine 0.1M, Clonidine 0.1 M, Yohimbine 10 mM (dissolved in 30% DMSO), Prazosin 2 mM (dissolved in 50% DMSO), Kainic acid 50 mM, respectively. Each compound, except kainic acid, was ejected as cations.

Our data recorded from single unit activity of 81 PrL neurons demonstrate that lithium can *in vivo* antagonize the inhibition produced by NE. Furthemore we hypothesize that: 1. Alpha-2 agonist Clonidine binds stronger on the alpha-2 receptors than antagonist Yohimbine or lithium. Lithium primarily acts directly on alpha-2 receptors, because it is not able to block firing inhibition caused by Clonidine (acting on second messenger systems). 2. Li^+ and Yohimbine competitively bind on a (putative) subtype of alpha-2 receptors which is present in the PFC but may be absent in the thalamus or hippocampus. This unique mechanism may explain – at least partially – the beneficial therapeutical effects of Li-treatment

POST MORTEM NUCLEOSIDE METABOLISM OF RAT BRAIN

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Experiments under hypoxia and ischemia it is known how converting enzymes can change the concentration of nucleosides. These enzymes convert nucleosides into nucleoside degradation end-products like hypoxanthine and xanthine as in the case of adenosine and inosine. The direction of chemical reactions responsible for degradation has been established but the velocity and the final extent of degradation in *post mortem* conditions is still unknown. It is well known that high rate of degradation process exists in the first 5-10 minutes of hypoxia and hypoxia can induce extensive changes in tissue concentrations of nucleosides. The activity of nucleoside metabolising enzymes is detectable in the brain after death for hours, so changes in extracellular and intracellular nucleoside concentrations were observed after death. As we described earlier, the *post mortem* nucleoside concentrations reflect regional distribution of nucleosides and thus it reflects the regional distribution of converting enzymes, too. However it is unknown whether the nucleoside-converting enzymes work simultaneously at several *post mortem* point of time and these enzymes convert nucleosides to end-products in proportion as normal physiological conditions.

Determination of nucleoside concentrations in the rat brain at various *post mortem* point of time is an available method for demonstration of working of the nucleoside metabolising enzymes. Thus the aim of the present study was to develop a useful sampling method on rat brain and to make a study on *post mortem* nucleoside degradation process.

Our results indicate a continuous degradation of nucleosides between 0 and 120 minutes and simultaneous working of xanthine oxidase, guanine deaminase, adenosine deaminase, 5'-nucleotidase, purine-nucleoside phosphorylase and uridine phosphorylase in the rat brain parietal cortex under *post mortem* conditions.

ENUMERATION AND CLASSIFICATION STRUCTURAL AND DYNAMICAL GRAPHS OF NEURAL NETS. EXAMPLES

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Structure of neural networks and their dynamics are representable with graphical objects like graphs, directed graphs with or without labels attributed to nodes or lines. A way of classification is presented here which permits complete enumeration of network structures satisfying to certain criteria. Independently of structures, dynamical graphs can also be defined.

Dynamics (state transitions, initial, transient and recurring states, stability, etc.) may be predicted in a part on the basis of well-defined structures and motion equations attributed to nodes of graphs. One aim of structural description is to be able to present reliable predictions from feature of architecture to functions or behaviour or dynamics. Answer e.g. that a network wiring permits or prohibits recurrent or even periodic activity. Or carry out selection of theoretical structures which appear impossible or improbable because of acceptable neurobiological arguments or doctrines.

When defining neural net structure, remark that the meaning of a unit may be either a whole real neuron or one of its part, a piece of axon or dendrite, or a synaptic bouton. Also, graph nodes may correspond even to amorphous assembly or to organized net of neurones. Finally, a motion equation, like versions of HH axon-equation which describes membrane patch or axon dynamics may be dependence nets of suitable variables. So concepts of units and nets are regarded in as broad sense as possible. A network structural graphical object is defined in several steps: graph $\rightarrow$ 2-multigraph $\rightarrow$ directed graph $\rightarrow$ [2-node or 2p-labelled digraph or 2-line or 2q-labelled digraph] $\rightarrow$ 2p2q-labelled digraph.

The two node-labels take into account that both autoactive and autosilent units exist. These concepts reflects resting (un) stability of units. Similarly, the two labels applied to interconnections incorporate fact that a unit may exert excitatory and inhibitory influence to other ones. 2p2q-digraphs are already rich enough to provide information about dynamics; e.g. kinds of autoactivity can be predicted supposed that suitable motion equations and coupling was applied to characterize unit-dynamics.

Classification and graphical enumeration is usually tedious mathematical procedure, not easily computerized, often needs individual manipulations. It is also remarkable the combinatorial explosion, which means often hyper-exponential increase in the number of cases. Example: Let enumerate possible 4-neuronal nets of which either units or lines may have one of 2 possible labels. The number of non-isomorphic unlabeled graphs of p=4 nodes is 11; number of similar unlabeled digraphs is 218. If only nodes may have 2 labels then about 3000, when only lines are labelled then 30,000 or so cases can be distinguished. Finally, if 2 kinds of neurons and 2 kinds of interconnections are permitted, then the number of cases with only four neurons is about 360,000. In such a computations asymmetric and symmetric nets are distinguished because the so-called automorphism group of graphs has to be studied to get exhaustive list of cases without erroneous repetitions. Enumeration of asymmetric networks represent "easy cases", while various symmetry types need usually laborious combinatorial considerations. Having a complete list of nets, efficient selection may result interesting or even surprizing cases not easily available with non-formal random search.

The second important graphical object arising in the context of neural nets is the state-transition graph or graphs. A typical classification and enumeration problem is here as follows. In autonomous cases, the states are classifiable as initial (I), transient (T), cyclic (C) or fixed (F) states. Given the number of all states, n, and it is partitioned into numbers of states falling into the four classes, i.e. $n = i + t + c + f$. Enumerate state-transition graphs or dynamics with a given partition. The combinatorial task is as follows: IUT is the transient forest, CUF is the attractor set of states. Allocate possible sets of trees onto the attractor set which consists of circles of recurrent states. Partitions demands advanced combinatorial procedures. Remark also that such and similar transient-steady-state configurations are suitable as well to describe formally architecture of stored items and their recall mechanisms.

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CART mRNA EXPRESSION REGULATED BY THE CREB TRANSCRIPTION FACTOR

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Previous studies have shown that CART mRNA (i.e. cocaine and amphetamine regulated transcript) expression is upregulated in the rat striatum after acute administration of cocaine and amphetamine suggesting its important role in the mechanism of action of psychomotor stimulants. Also, *in situ* hybridization studies have demonstrated that CART mRNA is localized in different neuronal groups involved in feeding, endocrine, neuronal and autonomic functions. Because the CART mRNA and proteins are highly regulated in the different parts of the brain, this study was carried out to characterize the promoter of the CART gene. The mouse CART promoter sequence was analyzed for transcription factor binding sites using the Transcription Factor Database (TRANSFAC) and MatInspectorV2.2. Our data revealed a cluster of transcription factor binding site including cAMP response elements (CRE, TGACGTCA).

To examine the activity of the CRE site in the CART promoter, GH3 rat pituitary cells were treated with 20 mM forskolin (adenylate cyclase activator) and 30 mM H89 (PKA inhibitor) for 0, 1, 3, 6, 12 and 24 h, and Northern-blot analysis was conducted. The result showed a significant (4.5-fold) increase in CART mRNA level after 6 h of forskolin treatment. Moreover, co-incubation of forskolin and H89 reduced this activation up to 50%. To test the hypothesis that the CART promoter is being activated by the CREB transcription factor family, Electrophoretic Mobility Shift analysis (EMSA) was employed. Isolated nuclear proteins from forskolin-treated GH3 cells were incubated wit CRE site oligonucleotide from the CART mouse (GCTGCCGTTTGACGTCAATGCCCGCCG); it revealed a complex binding. This complex was competitively destroyed by coincubation with cold CRE site oligonucleotide and were supershifted by a CREB-1 antibody.

Thus, CART expression is regulated in a cAMP-dependent manner which is likely mediated by CREB binding to the CRE site on the CART promoter.

MODULATION OF CORTICAL ACETYLCHOLINE RELEASE BY VASOACTIVE INTESTINAL PEPTIDE AND NITRIC OXIDE: AN *IN VIVO* MICRODIALYSIS STUDY IN RAT

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Background: Both vasoactive intestinal peptide (VIP) and nitric oxide (NO) are able to modulate the release of various neurotransmitters including ACh. Recently it has been found that VIP increases the cholinergic activity in hippocampus (Masuo et al., 1993). The

involvement of NO in synaptic plasticity in the hippocampus has also been reported (Bohme et al., 1991). Furthermore, it has been recently demonstrated that VIP and NO are colocalized in cortex (Bayraktar et al., 1997).

Aim of the work: The aim of the present research was to elucidate the modulation of cortical ACh release by VIP and NO.

Methods: Male Wistar rats (200-240 g) were anaesthetized with chloral hydrate (400 mg/kg, i.p.) and placed in a stereotaxic frame (Narishige S.I.L.). A transversal microdialysis membrane was positioned into the frontoparietal cortex: AP = +1.0 mm and H = -2.0 mm reffered to bregma, with bregma and lambda on a horizontal plane (Paxinos and Watson, 1982) under chloral hydrate anesthesia. Twenty-four hours after surgery the ACh release was measured in freely moving rats by HPLC with electrochemical detection. The data obtained were expressed as mean ± S.E.M. and analysed using one-way ANOVA followed by the Newman-Keuls *post hoc* comparison test.

Results: After 60 min recovery period the extracellular ACh level found in the microdialysis perfusates collected from the cortex was 9.24 ± 0.81 pmol/40 min (mean ± SEM, n = 30 rats). In control animals the basal outflow of the neurotransmitter was relatively constant throughout the experiment (up to 5 hours, range of variations ± 8%, not significant). VIP (0.1 to 1 μM) administered locally through the microdialysis membrane to the cortex concentration-dependently decreased the basal extracellular ACh levels. On the other hand, sodium nitroprusside (NO donor, concentration of 100 μM) increased the cortical ACh release by 229.01 ± 42.14%. Local perfusion of NO synthase inhibitor N^G-nitro-L-arginine methyl ester (L-NAME, 1 mM) decreased cortical ACh release by 29.49 ± 5.64%. The biochemical effect of L-NAME on cortical ACh release was reversed by local perfusion of semiessential amino acid L-arginine (10 mM).

Discussion: These experiments demonstrated that VIP and NO had opposite effects on cortical ACh release in freely moving rats. On the other hand it is known that the cholinergic system plays an essential role in learning and memory (Bartus et al., 1982). Our recent results indicated that NO synthesis inhibition impairs memory formation (Kalfin et al., 2001), hence an amnestic effect of VIP could be also suggested.

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RECEPTOR BINDING PROPERTIES OF ENDOMORPHINS AND THEIR ANALOGUES

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Endomorphins (Es) are putative mu opiate receptor (MOR) selective endogenous ligands (1, 2, 3). There are indications that the effects of Es are not limited to MOR. For example, E1 induced antinociception was not reversed by MOR selective antagonists naloxonazine and beta-funaltrexamine in normal and diabetic mice (4, 5) and experiments on E1 stimulated viral replication in microglial cell culture also suggested that E1 acts through an "atypical" binding site (6). Based on these observations it was feasible to speculate that Es may bind to more than one site. Therefore, we determined the binding characteristics and biological activities of Es in rat brain homogenates and intact tissues.

Using tritiated Es (7) we found that Es bind to MOR with high affinity (Kd~0.4 nM) but they also label a naloxone insensitive, non-opioid binding site (NOBS) (Kd~8 nM). Apart from Es, several endogenous peptide ligands of different receptor types bound to NOBS, but no synthetic peptide ligands or alkaloids could displace E binding. Using hippocampal slices we observed that the binding to NOBS alone did not potentiate synaptic transmission or induced intracellular calcium changes. However, in cerebellum, that lacks MORs, the binding of E1 to NOBS elevated cAMP level independently from adenylyl cyclase or G-proteins.

In order to better understand the binding of Es to both MOR and NOBS we synthesized E1 and E2 derivatives by side chain modifications or truncation. Most changes resulted in peptides with decreased binding affinity to MOR and decreased level of stimulation of [^{35}S]GTPγS binding. Some of the modified peptides partially, while YPF-phenyl-ethyl-N-allyl amide fully inhibited the E2 or DAMGO stimulated [^{35}S]GTPγS binding. Based on the above features, the sodium indices and the effects on mouse *vas deferens*, the observed changes are related to alterations in the agonist properties of E derivatives. YPF-phenylalanilol showed increased binding affinity to MOR and higher potency in stimulating [^{35}S]GTPγS binding, but its maximal stimulation was lower than that induced by E2 alone. Based on its low Hill coefficient (n_H~0.6) and the large receptor pool reserve this derivative might be a useful tool in understanding the function of endomorphin binding to both MOR and NOBS.

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UNUSUAL DISTRIBUTION OF CONE-SUBTYPES IN THE PERIPHERAL RETINA OF MAMMALS

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The extreme peripheral part of the mammalian retina has never attracted too much attention. Our aim was to fill this gap and present data on the "edge" of the retina.

Two diurnal species, an African rodent (*Otomys irroratus*), and the domestic pig (*Sus scrofa domestica*) were selected for thorough immunocytochemical examination of their photoreceptor content, using PNA lectin and antibodies directed against blue- and red- to green-sensitive photopigments. In both species dichromatic vision was reported with retinas that contained only blue- and green-sensitive cones. Both in the Otomys and the domestic pig, fairly high cone densities were found in all retinal positions examined. The density of green sensitive cones in Otomys peaked at the central retina (55.000/mm^2), and gradually decreased towards the periphery (15.000/mm^2). Similar trend was found in the domestic pig (25,000/mm^2 vs. 5000/mm^2), with slightly lower actual values. The number of the blue-sensitive cones was much lower in both species, and their distribution was not quite as regular. Besides reaching high values at the center, surprisingly high densities were also found at the edge of the dorsal peripheral retina. Using morphological and immunocytochemical criteria, this region strongly resembles the developing retina of other mammals.

The first immunopositive cones present in developing retina are the blue ones. Their number is much higher, and they comprise a higher percentage of the population than in adults. The green-sensitive cones can only be identified a few days later. Their density sharply increases, together with the decrease in the density of blue cones. At this time interval, special elements, containing both visual pigments can also be seen. A probable explanation for this phenomenon is that the green cones develop from the blue ones via transdifferentiation. The double-labeled elements are the ones already producing the green sensitive pigment, but still containing the blue. To see if such elements are indeed part of the photoreceptor mosaic in the Otomys and

porcine retina, we carefully checked the dorsal periphery in these two species, and managed to identify them in Otomys, were they comprise 1.5-1.7 percentage of all cones. No such elements were found in the domestic pig.

Experiments on retinal detachment in cat show that the dorsal periphery shows a high degree of regeneration. Considering this fact and our results presented here, the presence of a few undifferentiated mitotic cells in this region, as candidates for this regeneration cannot be excluded.

STUDIES ON THE ROLE OF GABA IN THE COURSE OF *IN VITRO* INDUCED NEUROGENESIS BY NE-4C PROGENITOR CELLS

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Several *in vitro* studies suggest that GABA, like other classical neurotransmitters, is involved in the early phases of neuronal development. GABA has been shown to influence the proliferation, migration and differentiation of neural progenitors well before the synaptic structures are established. Moreover GABA undergoes a unique switch from being excitatory to inhibitory in its action during neuronal maturation. For studies on the role of GABA in the neuronal cell fate decision, cloned neural progenitor cell lines provide appropriate models.

In the presented study, *in vitro* induced neuron formation by NE-4C neuroectodermal progenitor cells (Schlett, 1997) were used to study the significance of GABA in the course of neuronal cell fate determination. The NE-4C one-cell derived clone was isolated from primary forebrain cultures of p53-deficient mouse embryos (E9). These continuously dividing cells give rise to neurons and astrocytes when exposed to all-trans retinoic acid (RA). The neuron formation proceeds through well reproducible stages characterised by distinct morphological (Schlett et al., 1997), cell physiological (Herberth et al., 2002 in press) and molecular biological (Jelitai et al., 2002) features.

Immunocytochemical and *in situ* ELISA assays indicate that GABA is present in NE-4C cells during all stages of *in vitro* neuron formation. The non-induced cells contained GABA in a consistently low but detectable level. Intracellular accumulation of GABA was detected in a proportion of cells with neuronal morphology, while the GABA-content decreased in substrate-attached, non-neuronal cells in RA-induced cultures. Studies on GABA-uptake were carried out to determine the sources of intracellular GABA.

The effect of GABA on non-induced and neuronally committed cells in various stages of differentiation was evaluated by measuring the intracellular Ca^{2+} levels in Fura 2 loaded cells. GABA did not elicit Ca responses in non-induced cells or in substrate-attached cells in RA-induced cultures. Neuronally committed cells, however, responded with an increase of $[Ca^{2+}]_I$ following the 4^{th} day of induction and the proportion of responding cells increased with the advance of neuronal differentiation. The increase of $[Ca^{2+}]_I$,evoked by KCl, was not reduced by GABA indicating that GABA did not hyperpolarise the *in vitro* formed neurons up to a differentiation-level reached during a 2-week induction period.

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ANALYSIS OF THE FROG HYPOGLOSSAL MOTONEURONS WITH MULTIVARIANT STATISTICAL METHODS

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The movements of the tongue is the part of prey-catching behaviour controlled by three different groups of muscles. The protractor muscles protrude the tongue and this is followed by its withdrawal through the contraction of the retractors. The inner muscles are active both during the protraction and retraction. We have demonstrated that motoneurons innervating the muscles with different functions show a musculotopic organization. The aim of this study was to examine whether motoneurons innervating functionally different muscles show quantitative differences in their dendritic structure and geometry. The nerves supplying the individual muscles were labeled with cobaltic lysine. The dendritic trees of labeled neurons were reconstructed from serial sections with the aid of a camera lucida and x and y coordinates of the dendrites were fed into a computer through a graphic tablet. Z-coordinates were calculated according to a modified akima interpolation. Each reconstructed motoneuron was described by 15 morphological parameters refer to the size of the soma and dendritic trees and 15 orientation variables characterize the arborization pattern and orientation of the dendritic field. The data were subjected to multivariant discriminant analysis to find correspondence between the morphology, orientation and function of the motoneurons. The most important morphological variables in the discrimination of groups were the following: the diameter of the perikaryon, mean diameter of stem dendrites and mean length of dendritic segments. Our results also show significant differences in dendritic arborization pattern of neurons innervating functionally different muscle groups of tongue.

This work was supported by FKFP 0425/99 and OTKA T 034376.

LIGTH- AND ELECTRON MICROSCOPICAL RECONSTRUCTION OF EXCITATORY AND INHIBITORY SYNAPTIC INPUTS ON CCK-CONTAINING BASKET CELLS IN THE RAT HIPPOCAMPUS

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The number and distribution of the excitatory and inhibitory synapses influence the integrative properties of neurons. Therefore, it is important to reveal these parameters. The basket cells are one of the well-characterized interneuron populations concerning their targets, but we know little about the number and arrangement of excitatory and inhibitory inputs converging onto them. In this study we reconstructed at the light microscopical level the entire dendritic tree of the CCK-containing basket cell subpopulation in the CA1 area of the rat dorsal hippocampus, in order to describe the geometry, total length and laminar distribution of their dendrites. This was followed by an electron microscopical analysis to reconstruct the distribution of synapses on serial ultrathin sections and calculate the density of symmetrical and asymmetrical synapses on their somata, axon initial segment and different subclasses of dendrites. In this poster we show the preliminary data collected in strata pyramidale and radiatum.

We found that the CCK-immunoreactive basket cells have the most extensive dendritic tree (~6000 μm) among the distinct interneuron populations examined so far (PV, CB and CR). The light microscopical feature of CCK cells are similar to PV-containing interneurons located in stratum pyramidale, but CCK cells send dendrites only into the lower third of the stratum lacunosum-moleculare. At the electron microscopical level, CCK basket cells resemble the CB and CR-containing interneurons concerning the number and ratio of excitatory and inhibitory synapses terminating on the different type of dendrites. The density of inputs terminating on the somata – as perisomatic inhibition – and axon initial segment of CCK cells are lower than on any of the interneurons examined so far. The ratio of inhibitory synapses on CCK cells is increasing towards the soma, similar to other studied neuron populations.

Our anatomical data indicate that although the CCK-containing basket cells have similar function – perisomatic inhibition – in the hippocampal network to the PV-immunoreactive basket cells, they resemble the CB and CR-containing interneurons according to the number and distribution of their inputs.

SEIZURE-DEPENDENT EXPRESSION OF C-FOS IN NEURONS OF THE RAT HIPPOCAMPUS

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Introduction and methods

Fos-protein (the product of the c-*fos* gene) belongs to the AP-1 regulatory nuclear proteins, which bind to the AP-1 DNA sequence. The c-*fos* gene expression is mediated by the CREB response element, through cAMP and Ca^{++} mediated phosphorylation cascades. C-*fos* is a protooncogene, which is expressed transiently upon the stimulation of nerve cells and therefore widely used as a marker of neuronal excitation. The aim of the present studies was to induce forebrain seizures with the intraperitoneal injection of 4-aminopyridine (4-APY) and detect the localization and dynamic changes of the Fos-protein by means of immunohistochemistry. The changes of the number of immunostained cell nuclei were used as the indicator of neuronal activity. The number of cell nuclei has been counted with the Image Pro Plus morphometry program. The activated inhibitory neuronal populations were investigated with parvalbumin-c-*fos* double labeling in the hippocampus of rats. The relationship between c-*fos* expression and glutamate transmission was evaluated in pharmacological experiments, using the non-competitive open channel antagonists of the NMDA receptor (ketamine and MK 801). The distribution of 4-APY in the brain was investigated with autoradiography of [^{3}H]-4-APY on coronal brain sections. The expression of c-*fos* mRNA was detected by polymerase chain reaction, with nucleic acids prepared from deep-frozen neocortical samples.

Results

The distribution of [^{3}H]-4-APY was homogeneous in the grey matter the silver grain density showed a time dependent decrease from 30 to 60 min following the injection of the compound. The region of the lateral ventricles displayed higher density, indicating that the compound diffused into the CSF. A very strong c-*fos* mRNA signal was detected in brain tissue extracts 1 h following the injection of 4-APY, indicating the continuous expression of the c-*fos* gene. The Fos-protein immunoreactivity was maximal at 1 h in the granule cells of the dentate gyrus, and at 3 h in the pyramidal cell layer of the Ammon's horn. It was still detectable at 5 h following 4-APY administration. Parvalbumin-containing GABA neurons were maximally activated at 3 h following 4-APY injection in every area of the hippocampus. The number of c-*fos*-containing parvalbumin cells decreased thereafter. The number of Fos-like immunoreactive cell nuclei decreased significantly upon the effect of 3 mg/kg ketamine and 1 mg/kg MK 801 in every part of the hippocampal formation.

Our results indicated that 4-APY seizures were initiated probably in the entorhinal cortex and spread to the dentate gyrus through the perforant path. However, the NMDA antagonists did not abolish completely the expression of c-*fos* in the dentate granule cells, therefore the significance of other pathways and AMPA receptors has to be considered.

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NONLINEAR AND LINEAR COMPLEXITY OF FAST AND SLOW POTENTIAL CHANGES

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Introduction
Numerous techniques have been developed which are capable of quantifying brain complexity as revealed by electrophysiological methods. In this paper both nonlinear (point-correlation dimension, PD2) and linear (Omega complexity, OC) methods are used to investigate how the brain deals with increased demands of processing information in circumstances when the time available for this processing changes.
Methods
The nonlinear complexity measure (point-correlation dimension, PD2) allows the estimation of the time-dependent changes of dimensional complexity. OC is as a function of eigenvalues of the covariance matrix of the signal and thus is a global measure of spatial synchrony. An increase of Omega and/or that of PD2 can be explained by the increasing number of independent processes.

All subjects were payed participants. The Neuroscan system (bandpass: 0.15-200 Hz) was used for EEG was recording at 19 electrode sites. Data recorded at Cz are shown in this study only. In the CNV-paradigm pairs of auditory stimuli (S1 and S2, with 2.5 s in between) were delivered binaurally through headphones. In the control condition no instruction was given. In the task condition the subjects had to press a button if they recognized that S2 (1000 Hz or 1100 Hz) was different (i.e. higher) than S1 (1000 Hz). In the P3b-paradigm standard auditory stimuli (1000 Hz) were randomly (probability: 10%) interrupted by 1100 Hz target stimuli which latter had to be signalled by button press. Repetition rate was either 1/s ("slow condition") or 2/s ("fast condition").
Results
In the CNV condition for the whole digitized epoch (3050 ms), the value of Omega in the control situation was not significantly (t-test, Wilcoxon test) different from that found in the task situation. When the analysis was restricted to the period characterized by the CNV itself, significant Omega decrease was found in the task situation with respect to that observed in the control situation. Significant PD2 changes were observed only in the last 750 ms of the CNV.

In the P3b paradigm the amplitude of the P3 event-related potential (ERP) component decreased as repetition rate was increased, as expected. The epoch including the whole ERP in the "fast condition was found to be higher dimensional than that observed in the "slow condition.

Discussion and conclusions

The number of independent neural processes as indicated by changes of OC and PD2 decreased during the CNV, which can be interpreted as a sign of increased cooperativity during this event. With respect to different periods of the analyzed epoch the sensitivity of OC and PD2 was different but yielded complementary information. Increasing the repetition rate of stimulation draws on the available capacity reserve(s) of the system resulting in less allocable capacity for P3 production. This was accompanied by increased dimensional complexity, probably related to the higher attentional demand to be able to execute the task correctly. Additional analysis will be needed to clarify if the described phenomena characterize recordings from other scalp regions as well, or which is more likely regional differences will also be found in this respect.

EFFECTS OF ß-AMYLOID PEPTIDES AND THEIR FUNCTIONAL ANTAGONISTS ON G-PROTEIN ACTIVATION

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Alzheimer's disease (AD) is a neurodegenerative disorder that affects the cognitive function of the brain. Pathological changes in AD are characterized by the formation of amyloid plaques and neurofibrillary tangles as well as extensive neuronal loss. There are diffusible oligomers, low molecular weight intermediates, protofibrils and highly aggregated fibrils that are resistant against proteases. The toxicity of the aggregates seems to depend on the degree of aggregation. The molecular events underlying the toxic effects are largely unknown, but aggregated ß-amyloid (Aß(1-42)), and its shorter fragments, have been shown to stimulate GTPase activity in neurons. Although the connection between G-protein activation and toxicity is yet to be clarified, it was suggested that increasing levels of aggregation of Aß(1-42) may not only alter toxicity, but also the activation of G-proteins. In this study we determined the effects of different aggregated forms of synthetic Aß(1-42) and Aß(25-35) peptides on G-protein activation in crude rat brain membranes, and, using synthetic peptide "antagonists", we were trying to inhibit this activation.

Diffusible aggregates were prepared from 10^{-3} M stock solutions in trifluoro-ethanol (TFE) by dilution in H_2O with or without lyophylisation (final concentration 5×10^{-5} M) followed by one hour vigorous stirring. To obtain highly aggregated fibrils the 10^{-4} M solution was left undisturbed for one week than diluted to 5×10^{-5} M final concentration. We found that diffusible aggregates of Aß(1-42) produced a concentration dependent, saturable activation of G-proteins with an ED_{50} of ~10^{-6} M. Highly aggregated Aß(1-42) fibrils only affected G-protein activity at concentrations above 10^{-5} M, but this stimulation was not saturable. The

Aß(25-35) stimulated G-protein activity was similar to that of Aß(1-42) in the presence of 1% TFE. However, without 1% TFE present no GTPase activity could be detected till 10^{-5} M of Aß(25-35) and the stimulation observed at higher concentrations was not saturable. Inhibition of the actions of Aß(1-42) might have therapeutical relevance. BSB-D(42-38), a putative beta sheet breaker (BSB), did not inhibit G-protein activation by Aß(1-42), but rather acted as a weak stimulator on its own right. However, the functional agonist propionyl-Arg-Ile-Ile-Gly-Leu-NH_2 appeared to be a good inhibitor of Aß's toxicity. In our studies this peptide fully inhibited the Aß(1-42) stimulated GTP binding. In addition it had a profound inhibitory effect on basal G-protein activity (ED_{50}~10^{-5} M). Based on these observations this compound was designated functional-antagonist/inverse-agonist (FAIA). Replacement of propionyl with hexanoyl moiety further potentiated the inhibitory effect of the pentapeptide. Given that these compounds inhibit toxicity as well as G-protein activation, FAIAs might be useful in designing therapeutic agents.

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PLASTIC CHANGES IN THE DISTRIBUTION OF NK1, GLUR1 AND GLUR2/3 IMMUNOREACTIVITIES IN THE RAT SPINAL DORSAL HORN EVOKED BY FREUND'S ADJUVANT INDUCED INFLAMMATION OF THE HINDPAW

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Spinal neurons that express glutamate and neuropeptide receptors are known to play crucial roles in spinal transmission of nerve signals evoked by noxious stimuli. It has also been suggested that chronic inflammation of various areas of the body may evoke changes in the expression of these receptors in the spinal dorsal horn. Progressing along this line, in the present experiment we studied the expression of substance P receptor (NK1R) and two subunits of AMPA-type glutamate receptors (GluR1 and GluR2/3) in chronic Freund's adjuvant evoked inflammation of the hindpaw in rats. Using pre-embedding immunocytochemical methods, we have measured the densities of immunostaining and counted the numbers of immunoreactive perikarya in the dorsal horn both ipsi- and contralateral to the inflamed hindpaw.

Four days after Freund's adjuvant injection into the hindpaw when the evoked inflammation peaks, the densities of the immunoreaction for both GluR1 and NK1R were increased. In addition, the density of GluR2/3 like immunostaining declined in the medial part of the ipsilateral dorsal horn, although the numbers of immunoreactive perikarya did not show any significant change.

The result shows that although chronic inflammation induces plastic changes in the expression of nk1r, glur1 and glur2 subunits of glutamate receptors, the chronic inflammatory condition alter the expression of the three receptors differently in the spinal dorsal horn of rats.

This may also indicate that the functional properties of spinal neurons and information processing mechanisms in the superficial spinal dorsal horn may show distinct features in normal condition and chronic inflammatory state.

DIFFERENTIAL ALTERATIONS IN THE EXPRESSION OF NMDA RECEPTOR SUBUNITS FOLLOWING CHRONIC ETHANOL TREATMENT IN PRIMARY CULTURES OF RAT CORTICAL AND HIPPOCAMPAL NEURONES

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Cellular mechanisms underlying neuronal adaptation to ethanol are only partially understood. Previously, development of alcohol-dependence was observed in primary cultures of cortical neurones. Severe cellular damages and neuronal cell loss were observed following 24 hours of alcohol withdrawal in cultures pretreated with ethanol (50 – 200 mM) repeatedly for 3 days. Increased NMDA induced cytosolic calcium responses and excitotoxicity were also demonstrated in these cultures. Thus, enhancement in functions of NMDA receptors was supposed to be involved in the adaptive changes leading to the neurotoxic effect of alcohol-withdrawal.

In this study we investigated the effect of the 3-day repeated ethanol (100 mM) treatment on the function and subunit composition of the NMDA receptors in primary cultures of cortical as well as hippocampal neurones. Here we demonstrate that the maximal inhibitory effect of ethanol was significantly increased after ethanol pretreatment. Similarly, the inhibitory activity of the NR2B subunit selective antagonists threo-ifenprodil, CP-101,606 and CI-1041 was also enhanced. On the contrary, the efficiency of the channel blocker agent MK-801 and the glycine-site selective antagonist 5,7-dichlorokynurenic acid was the same as in control cultures. These observations suggested a shift in subunit expression in favour for the NR2B subunits. Indeed, according to the results of flow cytometry based immunocytochemical experiments, we observed increased expression of the NR2B subunit proteins in ethanol pretreated cultures. In addition, the expression of the NR1 splice variant forms containing the C1 and/or the C2' cassette was also increased in both ethanol pretreated cortical and hippocampal cultures.

These changes in subunit compositions and the subsequently increased functions of the NMDA receptors may explain the enhanced sensitivity of the ethanol pretreated cultures to excitotoxic insults and the increased neuronal cell loss observed after ethanol-withdrawal. Such alterations may play a central role in the neuronal adaptation to ethanol and in the development of alcohol-dependence, and might form the background of neuronal cell loss in certain areas of the brain during alcohol withdrawal in alcoholics.

LONG-TERM EFFECTS OF NEONATAL MK-801 TREATMENT ON SPATIAL LEARNING AND CORTICAL PLASTICITY IN ADULT RATS

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The long-term effects of neonatal treatment with MK-801 on spatial learning and cortical plasticity were investigated in adult rats. Rat pups were injected twice daily with MK-801 (0.1 mg/kg) on postnatal days 7-19, participated in water maze testing between postnatal days 90 and 102, and were then studied electrophysiologically. Treatment with MK-801 in such a low dose resulted in a very slight impairment of performance in the water maze task, but not in the visual cue response. Besides the slight learning impairment, the electrophysiological study revealed a reduction in the capacity for plasticity in the primary motor cortex of the treated animals, which was pronounced in the controls. The study demonstrates that even a slight impairment in learning and memory function may be accompanied by a cortical plasticity deficiency that is detectable electrophysiologically.

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^{125}I-LABELLING AND PURIFICATION OF NEUROPEPTIDES FOR RADIOIMMUNOASSAY (RIA) MEASUREMENTS

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The iodination and separation of various diagnostically and/or experimentally important peptides including (Tyr[1])-somatostatin-14, rat Tyr-α-calcitonin gene-related peptide (23-37) (CGRP) and vasoactive intestinal peptide (VIP), furthermore bovine serum albumin (BSA) are described. All species were iodinated by iodogen method. The ^{125}I-labelled peptide products were separated by reversed-phase high performance liquid chromatography (HPLC).

Under optimal labelling conditions the proportion of the mono-iodinated forms, as RIA tracers, can be as high as 75-80%. Specific activities of the mono-iodinated forms are near identical with the theoretical value (78.63 TBq/mmol) indicating high efficacy of the reversed-phase HPLC separation technique. The ^{125}I-labelled BSA, used *in vivo* to quantify the extent of plasma extravasation, was separated by Sephadex G-100 gel filtration.

NEUROTOXICITY OF LINDANE AND PICROTOXIN: NEUROCHEMICAL AND

ELECTROPHYSIOLOGICAL CORRELATES IN THE RAT HIPPOCAMPUS *IN VIVO*

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In the present study, we compared *in vivo* changes of extracellular amino acid levels and nucleotide derivatives to a single *ip* dose of lindane (10-60 mg/kg) and picrotoxin (5 mg/kg) in the hippocampus of halothane anaesthetized rat by microdialysis-coupled HPLC analysis. Brain activity was monitored by EEG. The effects of lindane and picrotoxin on EEG pattern of rats as well as on hippocampal amino acid and nucleotide status were studied in 0-50 min, 50-100 min and 100-150 min periods post-dosing. Significant decreases in Glu and Asp were found after picrotoxin treatment. After 50-100 min post-dosing, hippocampal hypoxanthine and inosine levels increased to both lindane (10 mg/kg) and picrotoxin whereas xanthine and uridine levels increased to picrotoxin, only. Lindane elicited a dose-dependent occurrence of negative spikes accompanied with rhythmic activity at 4-5 Hz. The picrotoxin-induced 4-5 Hz activity did not display negative sharp waves and was accompanied by 10 Hz oscillations.

CHOLINERGIC MODULATORY MECHANISMS IN THE DORSAL COCHLEAR NUCLEUS OF THE RAT

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The pyramidal (also known as fusiform or principal) cells of the dorsal cochlear nucleus (DCN) form one of the most important efferent pathways of the DCN projecting to the contralateral inferior colliculus. Pyramidal cells receive excitatory inputs from the acoustic nerve and from the parallel fibre system of the granule cells, but they are also contacted by inhibitory interneurones. Owing to the complexity of their innervation, pyramidal cells may produce significantly different activity patterns depending on the prior activity occurring within the nucleus. Moreover, acetyl-choline has also been postulated as a possible agent modifying the activity pattern of the pyramidal cells, but no specific information is available concerning the exact nature of the cholinergic action. In this work we studied the effects of cholinergic stimulation on both depolarization- and hyperpolarization -activated currents of the pyramidal cells.

The experiments were conducted on 8-12-day-old rats by employing thin (150-200 μM) slices prepared from the DCN. When the current-clamp configuration of the whole-cell patch-clamp technique was applied, spontaneous activity was noted and recorded in 22% of the cells

(n = 83). The frequency of spontaneous firing was reduced to 12 ± 6% (mean ± SEM; n = 8) of the control in the presence of carbachol (50-100 μM), an effect which could be completely reverted. To reveal the ionic conductances which might be responsible for such changes in the activity, both depolarization- and hyperpolarization-activated currents of the pyramidal cells were investigated. Application of hyperpolarizing stimuli activated an inward current. The half-activating voltage of this current was -99 ± 2 mV with a slope factor of 11 ± 1 mV (n = 12). This current was highly sensitive to 1 mM CsCl (blocking effect: 85 ± 9% at -140 mV; n = 12) but was much less prominently affected by 1 mM $BaCl_2$ (blocking effect: 23 ± 9%; n = 5) hence this current was recognised as h-current. In our experiments carbachol increased both the instantaneous and the steady-state components of the h-current. Moreover, carbachol also affected the depolarization-activated current component as the peak current was reduced by 24 ± 7% (n = 6; +30 mV) in the presence of cholinergic stimulation.

To conclude, although carbachol altered both hyperpolarization- and depolarization-activated currents of the pyramidal cells, these effects may not explain the dramatic changes exerted on spontaneous firing. Further work ought to be carried out, therefore, to reveal some additional targets of the cholinergic activation on the pyramidal cells.

LASTING EFFECTS OF SUBCHRONIC ORGANOPHOSPHATE TREATMENT ON THE CORTICAL ELECTRICAL ACTIVITY OF RATS

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Organophosphates (OPs) are neurotoxic substances widely used as insecticide agents in agriculture and vector control. Accidental human exposure cases and animal experiments (including our previous ones) have shown that OPs in single or repeated dosing induce a number of alterations in the spontaneous and stimulus-evoked electrical activity of the cortex. Decreased activity of acetylcholinesterase was also seen but in some cases the extent and/or time course of enzyme inhibition and the changes in nervous activity were not in parallel. This indicated that certain actions of OPs in the central nervous system may be dependent on some other effects, not connected to the inhibition of acetylcholinesterase. It was supposed that by determining the duration of the changes after cessation of OP administration, valuable information can be obtained for the future identification of the underlying alternative biochemical mechanisms.

Ten young adult male Wistar rats per group were treated with 1/25 and 1/100 LD_{50} (7.0 and 28.0 mg/kg b.w.) of dimethoate (an OP) from their 12^{th} to 20^{th} or 24^{th} week of life. Control groups received distilled water. Following the 8 or 12 weeks treatment, one group from each dose was recorded and dissected immediately and another was kept for further 4 weeks. For electrophysiology, the head of the rats was fixed (in urethane, 1000 mg/kg, anesthesia) in a stereotaxic frame and the left hemisphere exposed. Electrocorticogram was recorded from the primary somatosensory, visual and auditory centres for 5 min and its frequency distribution was determined. Then, one series of 50 evoked potentials was recorded from each centre with

adequate sensory stimulation, latency and duration being calculated off-line.

In the spontaneous activity, a shift to higher frequencies was observed which was slight after 8 but significant after 12 weeks and was dose-dependent. The frequency distribution of the electrocorticogram in the groups recorded after 8+4 and 12+4 weeks was between that of the controls and the corresponding treated groups (8 and 12 weeks). The latency and duration of the sensory evoked potentials was increased by dimethoate, and the comparison of the groups treated for 12 and 12+4 weeks showed the same relationship as with the spontaneous activity. The outcome of groups with 8 and 8+4 weeks treatment was inconclusive.

The results showed that there was only a partial recovery of the effects of Op treatment in 4 weeks. As the restitution of the cholinesterase activity was described to be complete in this length of time, it is possible that another biomolecule, also affected by OPs but having very slow turnover, is responsible for the effects.

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TASTE-RESPONSIVE NEURONS IN THE NUCLEUS ACCUMBENS OF THE RAT

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The nucleus accumbens septi (NAcc) is a well-known element of the forebrain limbic circuitry. It has already been implicated in various regulatory processes, among others, in the central feeding control as well. To date, however, feeding associated functional characteristics of NAcc neurons, with particular emphasis on their exogenous chemosensitivity, are yet to be defined. In the present, experiments, therefore, extracellular single neuron activity of the NAcc of male Wistar rats was recorded by means of tungsten wire multibarreled glass microelectrodes during 1) gustatory stimulations (two concentrations of sucrose, NaCl, HCl, quinine HCl, MSG as umami substance, and orange juice as complex taste), and 2) microelectrophoretic administration of chemicals. Taste responsiveness was examined altogether in case of 30 accumbens cells also tested for their endogenous chemosensitivity. More than the half (17) of these neurons were found to be modulated by gustatory stimuli. An overwhelming majority (beyond 80%) of the taste cells proved to possess specific, feeding-related neurochemical attributes, i.e., they were either of the glucose-sensitive (GS) (whose activity is suppressed by microelectrophoretically applied glucose) or glucose-receptor (GR) (whose firing rate is increased to glucose) type of cells. Chemosensory units of the NAcc appear to have a characteristic functional-topographical distribution: GS neurons were found mainly in the shell , whereas GR cells in the core region of the structure. The predominant type of glucose-monitoring (GM) units, in contrast to the minor population (i.e., GR in the shell and GS in the core), was likely to change in firing rate in response to two or more tastants in both divisions. Furthermore, these chemosensory neurons were also influenced by various neurotransmitters and modulators, such as dopamine, acetylcholine, GABA and

streptozotocin as well. The above findings undoubtedly verify the existence of taste responsive cells in the NAcc of the rat. It appears that the very same neurons are elements of the forebrain GM network. To elucidate exact functions of these accumbens chemosensory units in the central feeding control require further studies.

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EFFECTS OF DIETARY POLYUNSATURATED OR SATURATED FATTY ACIDS ON EPILEPTOGENISITY

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Polyunsaturated fatty acids (PUFA) are reported to have a potential effect in the prevention of coronary heart disease and of some cerebral diseases. Dietary fatty acids, after building into membrane phospholipids, influence the availability of substrate for the arachidonate cascade, and thereby influence prostanoid (PG) synthesis. Our previous experiments demonstrated that a linoleic acid rich diet increased PGD_2 synthesis by 47% in brain microvessels. Endogenous PGD_2 has been shown to inhibit initiation and propagation of pentylenetetrazol-induced seizures in rats. PUFA prevent neuronal death and protect animals against kainic acid-induced seizure and hippocampal lesions. The neuroprotective effects of polyunsaturated fatty acids are associated with decreasing neuronal excitability by opening K^+ channels with two pore-forming domains, by blockage of glutamatergic neurotransmission, and by inhibition of voltage-dependent Na^+ and Ca^{2+} channels.

In the present study we have chosen the 4-aminipyridine (4-Ap)-induced seizure as an *in vivo* model of ictal epileptiform activity to investigate the possible modifying effects of dietary lipids on epileptogenisity.

Male Sprague-Dawley CFY rats were fed *(ad libitum)* a diet of standard rat food pellet supplemented with 10% sunflower seed oil (SSO, as a source of linoleic acid) or pork fat (SF, as a source of saturated fatty acids) for 4 weeks. In an other serial of experiments the diet was continued while behavioural stage 1-5 seizure was induced at every other day during 2 weeks by intraperitoneal injection of 4-Ap to study behavioural seizure activity. After the diet in acute electrophysiological experiments the manifestation and propagation of cortical seizure activity induced by local application of 4-Ap to the exposed cortical surface of anaesthetised animals were tested in both serials of the experiments.

We found that the linoleic acid rich diet remarkable reduced seizure susceptibility, in comparison to saturated fatty acids rich diet. This was indicated by the increased threshold for behavioural seizure and lower mortality during attack, the three times longer latency for both behavioural and electrographic seizure, the 43% smaller averaged amplitudes of the seizure discharges, the 40% shorter duration of individual ictal periods as well as by 72% reduced summated ictal activity. The propagation of epileptiform discharges to other cortical areas was not influenced by linoleic acid rich diet. The repeated behavioural seizures during two weeks

period slightly enhanced the seizure suppressing effect of linoleic acid. These findings suggest that the potential therapeutic value of linoleic acid and other related compounds in neurological diseases associated with neural hyperexcitability be worth exploring.

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SIMULTANEOUS CHANGES OF SPONTANEOUS AND STIMULUS-EVOKED CORTICAL ACTIVITY IN RATS ACUTELY TREATED WITH HEAVY METALS

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A number of heavy metals is known to affect the activity of the nervous system of animals and humans, as indicated by the multitude of neurological signs following e.g. occupational exposure to neurotoxic metals. In animal experiments, including those done earlier at our department, different heavy metals caused a variety of alterations in the central and peripheral nervous system activity. However, a comprehensive mechanistic explanation of the alterations seen is missing in a number of cases. The aim of the work presented was to find correlation between the changes of spontaneous cortical activity (electrocorticogram, ECoG) and cortical sensory evoked potentials (EPs) recorded from rats acutely treated with certain heavy metals.

The experiments were done on adult male Wistar rats (ca. 350 g b.w.). In urethane (1000 mg/kg b.w.) anesthesia, the head of the rats was clamped in a stereotaxic frame and the left hemisphere was exposed. Silver recording electrodes were placed on the somatosensory projection area of the whiskers (barrel field) and of the tail if the animal. The corresponding peripheral sites (whiskery skin and base of tail) were stimulated by electric pulses (ca. 4 V, 0.05 ms, 1 Hz). The pattern of recording consisted of a five minutes ECoG taken from both areas simultaneously, then EPs by applying one train of 20 stimuli to each of the peripheral sites. During tail stimulation, compound action potentials of the tail nerve were also recorded. This pattern was repeated every 20 minutes. After at least 4 control records, mercury ($HgCl_2$, 7 mg/kg) or manganese ($MnCl_2$, 50 mg/kg) was administered via a peritoneal cannula and the recording was continued for further ca. 2 hours.

The effect of the two metals on the ECoG was similar: increased activity of the low and decreased activity of the high frequency bands, in both recording areas. Simultaneously with that the amplitude of the EPs increased. This effect was stronger with Hg than with Mn treatment and generally more pronounced on the whiskers projection area. In case of Mn, the delay between administration of the metal and onset of the above effect was longer. Hg also increased the latency of the EPs while Mn had no such effect. On the tail nerve potential, both metals caused an amplitude decrease and a latency increase, the latter corresponding to a slower conduction velocity. In case of Hg, the latency increase of the cortical EP and the decrease of nerve conduction velocity evolved in parallel.

The amplitude increase of EPs and the observed shift in ECoG band activity both indicate diminished spontaneous activity of the cortex. This is likely due to some specific, and not to a general toxic, effect of Hg or Mn (where both spontaneous and stimulus-dependent activity of

the neurons would be reduced). The EP latency change seen with Hg seems to be correlated with the decreased nerve conduction velocity. It is possible that different mechanisms and/or sites of action of neurotoxic heavy metals can be identified this way.

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LONG-TERM EFFECTS OF CAPSAICIN AND RESINIFERATOXIN ON THE NOXIOUS HEAT THRESHOLD OF THE RAT

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Introduction

In the classical hot plate and tail flick test, commonly used for investigation of thermonociception, reflex latency of nocifensive behavioural reactions evoked by heat stimuli of suprathreshold intensity is measured. We have recently developed and validated an increasing temperature hot plate equipment which allows determination of the noxious heat threshold i.e. the lowest temperature capable of evoking nocifensive reactions. Activation of the capsaicin VR1 receptor located on nociceptive primary afferent neurons causes an initial excitation followed by lasting functional impairment (desensitization) in response to various stimuli including noxious heat. However, the classical hot plate and the tail flick test have provided ambiguous results concerning the thermal antinociceptive effect of capsaicin. The aim of the present study was to investigate the long-term effect of the VR1 receptor agonist capsaicin and resiniferatoxin (RTX) on the noxious heat threshold determined with the increasing temperature hot plate.

Methods and materials

Female Wistar rats (150-220 g) were placed on the metallic plate of the equipment the temperature of which was linearly (6 °C/min) raised by a computer-driven heating system from room temperature until licking of either hindpaw occurred. The temperature evoking this response was regarded as the noxious heat threshold. Chemonociception was also studied by painting the dorsum of one of the hindpaws with increasing concentrations of xylene dissolved in ethanol. The xylene concentration eliciting a nocifensive response (elevating, shaking or licking of the hindpaw) within 3 minutes was considered as the chemonociceptive threshold. The effect of subcutaneously applied RTX (3-30 μg/kg) or capsaicin (10-30 mg/kg) on the noxious heat and chemonociceptive threshold was determined by daily measurements.

Results

The control noxious heat threshold was 44.9±0.27 °C, while the chemonociceptive threshold ranged from 15 to 25%. Both RTX and capsaicin caused a dose-dependent elevation (up to 2.3 °C) of the noxious heat threshold. RTX was found to be over 1000 times more potent than capsaicin. The heat threshold returned to the control level after 2-4 days except for the highest RTX dose (30 μg/kg) which had an effect lasting for 12 days. Both RTX (10 and 30 μg/kg) and capsaicin (30 mg/kg) caused an elevation of the chemonociceptive threshold as well which lasted for not more than 4 days and was shorter than the effect on the heat threshold. The

solvent of RTX and capsaicin failed to alter the noxious heat and the chemonociceptive threshold.

Conclusions

The newly developed increasing temperature hot plate test is suitable for revealing the thermal antinociceptive effect of VR1 agonists and for investigation of its dose dependence and kinetics. A dose evoking a significant but reversible elevation of the noxious heat and the chemonociceptive threshold was found for both RTX (10 μg/kg) and capsaicin (30 mg/kg). The reversible effects of these doses could serve as an appropriate *in vivo* model for the investigation of the biochemical mechanisms underlying the functional sensory neuron blocking action of VR1 agonists.

CHOLECYSTOKININ IN SIGNALING POSTPRANDIAL HYPERTHERMIA

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Food intake is followed by rises in metabolic rate and body temperature. In rats the fasting hypometabolism and hypothermia were converted to such postprandial rises even if the animals consumed calorie-free chow (saccharine-sweetened $CaCO_3$), and also when they were given calorie-rich or calorie-free mixtures (FWG body building formula or $BaSO_4$, respectively) into the stomach through a preimplanted tube (1). However, the course of temperature rise depended on the substance used: it started later in case the calorie-free mixture was applied. From the effects of the calorie-free mixture, a role for gastrointestinal stretch signals has been suggested.

Similar stretch signals have been shown to interact with locally produced cholecystokinin (CCK) in inducing satiety upon feeding, but centrally applied CCK also suppressed food intake (2). The peripheral signals are thought to be transmitted by the vagus nerve.

Central administration of CCK has been shown to induce fever-like elevation of body temperature (3). Although peripherally applied CCK had the opposite (direct) effect, a CCK-induced excitement of abdominal nerves might be assumed to cause additional activation of central CCK-erg mechanisms. Indeed, various phases of the febrile temperature rise have been shown attenuated either by centrally applied CCK type-B antagonist (4) or by intraperitoneally applied CCK type-A antagonist.

In the present experiments it was analyzed whether or not gastrointestinal or central CCK is involved in the development of postprandial hypermetabolism and hyperthermia. In cold-adapted female Wistar rats, peripheral or central application of CCK receptor-antagonists (type-A or type-B, respectively) preceded the gastric injection of nutrient-containing FWG or calorie-free $BaSO_4$ (through a pre-implanted gastric tube), in order to modify the postprandial hyperthermia courses. Type-A antagonist devazepide (ML Laboratories, London) was given intraperitoneally, while type-B antagonist L-365,260 (MSD Research Laboratories, USA) was given into the lateral cerebral ventricle (ICV) through a preimplanted cannula.

While intragastric injection of FWG induced a rapid rise in metabolic rate and body temperature, lasting for over 3-h, similar injection of $BaSO_4$ induced these rises with a latency

of near 1-h. Intraperitoneal injection of CCK type-A antagonist prevented or attenuated the hypermetabolism and hyperthermia to $BaSO_4$ but not to FWG injections. ICV injection of CCK type-B receptor acted similarly.

In conclusion, abdominal stretch activates CCK type-A receptors, which results in enhancement of metabolic rate and rise in body temperature. In transmission of such abdominal signals central CCK type-B receptors are used. Nutrients have other actions besides stretch: they can induce postprandial hypermetabolism and hyperthermia without the contribution of CCK-erg mechanisms.

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PROPRIOSPINAL LUMBO-CERVICAL PATHWAY IN THE RAT SPINAL CORD

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In one of our recent studies we have revealed an extensive propriospinal afferent and efferent system in the lumbar spinal dorsal horn of rats (Petkó and Antal, J. Comp. Neurol., 422:312-325, 2000). In addition to these lumbar inter- and intrasegmental projections, we also found some indications that long projection fibers that arise from the lumbar spinal gray matter and terminate in low cervical segments (C7-C8) may also exist in the spinal cord of rats. In the experiments presented here, applying the anterograde neural tracer *Phaseolus vulgaris* leucoagglutinin (PHA-L) to the dorsal horn of the lumbar spinal cord of rats, we have studied the course, termination, synaptic relations and neurochemical properties of this lumbo-cervical propriospinal projection. We found that the ascending fibers arose mostly, if not exclusively, from the lateral aspect of the dorsal horn at the level of both the upper and lower segments of the lumbar spinal cord. Labelled fibers ascended in the ventral aspect of the ipsilateral lateral funiculus and terminated in C7-C8 segments of the cervical spinal cord where PHA-L labelled axon terminals were confined to the ventral and ventro-lateral areas of the ventral horn (lamina IX). They established both asymmetric (58.6%) and symmetric (41.4%) synaptic contacts with initial dendritic segments or soma (65.7%), dendritic shafts (30.0%), and dendritic spines (4.3%) that likely belong to motoneurons. Most of the labelled terminals as well as their postsynaptic profiles were negative for both GABA and glycine, only a few terminals (3.9%) showed poor immunostaining for glycine. Some of the labelled terminals (2.8%) received synaptic contacts from presynaptic vesicle containing profiles that showed poor immunoreactivity for glycine.

The lumbo-cervical projection may play a role in the sensory driven coordination of motor activities of the forelimbs and hindlimbs.

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MORPHOLOGICAL AND ELECTROPHYSIOLOGICAL CHARACTERISTICS OF MEDIAL PREFRONTAL NEURONS IN THE RAT

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The medial prefrontal cortex (mPFC) receives projections from a number of limbic structures including the basolateral amygdala (BLA). By means of extracellular single unit recordings in the mPFC mainly inhibitory and sometimes excitatory responses were recorded after electrical stimulation of the BLA. The neurons displayed no topographic organization in response to stimulation of the BLA.

The aim of our study was to identify and characterize morphologically mPFC neurons responding to electrical stimulation of the BLA in urethane anesthetized rats. Single unit activity was recorded extracellularly with glass microelectrodes (tip diameter 1 μm, impedance 10-50 MΩ) containing neurobiotin (2% dissolved in 0.5 M NaCl solution). The BLA was electrically stimulated (single monophasic square wave pulses, 0.2 ms duration, 200-400 μA intensity) by means of concentric bipolar electrodes (tip-ring separation 0.5 mm). After electrophysiological characterization, neurons were labeled with juxtacellular application of neurobiotin through the recording electrode. We labeled successfully 19 neurons in the dorsal mPFC. Corresponding to the results of earlier Golgi studies the labeled neurons were classified as 1) regular pyramidal cells with pyramid shaped cell body and extended dendritic tree, 2) so-called "primitive" pyramidal cells with oval shaped cell body and poorly branched dendritic tree, 3) transitional forms between these types, and 4) interneurons. All of the 15 cells belonging to the "primitive" or transitional forms exhibited inhibitory responses with a latency of 20-25 ms. One of the regular pyramidal cells responded with 20 ms latency inhibition, one with long latency inhibition (latency over 60 ms), and one with excitatory responses. The interneuron recorded in the dorsal mPFC exhibited remarkable excitatory responses, followed by inhibition.

Present results suggest that: (1) "primitive" pyramidal cells or transitional forms between "primitive" and regular pyramidal cells that are the most common output neurons of the dorsal mPFC respond characteristically with 20-25 ms latency inhibition to BLA stimulation; (2) as far as their inputs from the BLA are concerned regular pyramidal cells may represent a heterogeneous cell population; (3) at least some interneurons exhibit excitatory response to BLA stimulation.

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ANTI-INFLAMMATORY EFFECT OF A HEPTAPEPTIDE SOMATOSTATIN ANALOGUE TT-232 IN THE RAT, MOUSE AND RABBIT SKIN

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Our previous findings revealed that sufficient amount of somatostatin can be released from the neural pool by activation of primary afferent neurones and exerts systemic anti-inflammatory effects (Helyes et al., 1996; Szolcsányi et al., 1998). The therapeutic use of native somatostatin is limited by its broad range of effects and very short plasma half life-time. Recently a series of new potent, stable, analogues has been synthesised in our laboratories. An analogue with a cyclopentaring structure: D-Phe-Cys-Tyr-D-Thr-Lys-Cys-Thr-NH_2, called TT-232, was found to be unique because it had no endocrine activity. The aim of the present study was to investigate the inhibitory effect of TT-232 on vascular and cellular phases of inflammation in different *in vivo* models of three species (rat, mouse and rabbit).

Methods. Capsaicin-induced ear inflammation in mice. Under ketamin anaesthesia 10-10 μl 2.5% capsaicin solution in 96% ethanol was dropped on the outer and inner surfaces of one ear of BALB/c mice. The other ear was treated with ethanol. Thirty min later the animals were killed by cervical dislocation, ears were cut down and weighed with 0.1 mg accuracy. TT-232 was given s.c. 30 min before capsaicin and ethanol application. Diclofenac was given s.c. 30 min before capsaicin-etnanol application was used for comparison.

Histamine-, bradykinin- and PAF-induced plasma extravasation in the rabbit skin. White rabbits were anaesthetised with diazepam (iv.) ketamine (im.) The dorsal skin was shaved and [^{125}I] bovine serum albumine was iv. injected. The inflammatory mediators and solvents were applied i.d. After 30 min accumulation period animals were killed by exsanguination, the skin around the injection sites were punched out and their radioactivity was counted together with the plasma samples in a ©-counter. The extravasated plasma was expressed in μl/skin site.

Carrageenin- and interleukin 1β-induced neutrophil cell accumulation in the dorsal skin of the rat hindpaw. Wistar rats were anaesthetised with thiopentone sodium i.p. The inflammatory polysaccharide, 1% carrageenin or cytokine, IL-1® (rat recombinant), 3 pmol were injected in 100 μl volume id. into the dorsal skin of the right hindpaw. Tyrode solution was applied into the left, control hindpaw (Pintér et al., 1999; Gao et al., 2000). TT-232, its solvent, or diclofenac were injected iv. three times during the 3 h accumulation period. Animals were killed and dorsal skin of the paws were removed. Neutrophil accumulation was assessed by comparing myeloperoxidase levels in extracts from rat neutrophils and skin sites using a microplate reader. Data were analysed for statistical significance by Mann-Whitney non-parametric test.

Results. Capsaicin caused 24.7% increase of weight compared to the ethanol-treated ears. TT-232 inhibited the capsaicin-induced increase of weight of mouse ears. On the other hand the reference drug, diclofenac in the applied dose range (5-20 mg/kg) failed to induce inhibition in neurogenic ear oedema of the mice. Histamine, bradykinin and PAF injected into the dorsal skin of the rabbit elicited plasma extravasation measured by ^{125}I-labelled radioactive

bovine serum albumin,. TT-232 induced significant inhibition of plasma extravasation evoked by histamine and PAF. Non-significant decrease was observed in case of bradykinin. In contrast, dexamethasone in the applied dose (3 mg/kg) had no effect on the histamine-, bradykinin- and PAF-induced oedema formation. Carrageenin caused significant cutaneous neutrophil accumulation over 3 h accumulation period. Only 3·80 μg/kg TT-232 inhibited significantly the carrageenin-induced leukocyte accumulation. Inhibition induced by diclofenac was non-significant. IL-1® induced expressive recruitment of neutrophil cells in the dorsal skin of the hindpaw over 3 h. 3·80 μg/kg iv. injected TT-232 elicited significant, and 3 · 40 μg/kg iv. a non-significant reduction in the IL-1® 13-induced leukocyte accumulation. Recruitment of neutrophils to IL-1® was also inhibited by diclofenac.

Conclusions. In conclusion the present findings established a broad spectrum of anti-inflammatory effects of TT-232. Therefore this compound might be effective in diseases where beyond neurogenic inflammation several other mediators produce vascular and cellular inflammatory responses.

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INPUT FROM SUBSTANCE P-CONTAINING PRIMARY AFFERENTS TO LAMINA I PROJECTION NEURONS WHICH EXPRESS THE NK1 RECEPTOR

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Substance P is present in many nociceptive primary afferents, and these terminate mainly in laminae I and II of the spinal cord. They can be distinguished from other types of substance P-containing axon by the presence of calcitonin gene-related peptide, which is only found in

primary afferent axons in the dorsal horn. Lamina I contains many neurons that project to the brain and most of these express the neurokinin 1 (NK1) receptor on which substance P acts. In this study we have examined the input from substance P afferents onto lamina I projection neurons with the NK1 receptor.

Fluorogold was injected into the caudal ventrolateral medulla to label projection cells, and we carried out triple-labelling immunofluorescence staining to identify substance P afferents and retrogradely labelled lamina I neurons with the NK1 receptor. Confocal microscopy revealed that all of the Fluorogold-labelled NK1 receptor-immunoreactive cells received numerous contacts from substance P afferents, although the distribution of these contacts on different parts of the neuron varied. No obvious relation was observed between the density of input and neuronal morphology.

We also carried out combined confocal and electron microscopy and found that asymmetrical synapses were present at many of the contacts between substance P afferents and lamina I projection neurons.

Although substance P is thought to act through volume transmission, the presence of synapses between substance P-containing primary afferents and lamina I projection neurons suggests that glutamate released by these afferents acts as a fast transmitter in this situation.

EFFECT OF SOMATOSTATIN ON DOPAMINERGIC-CHOLINERGIC INTERACTION IN THE STRIATUM: AN *IN VIVO* MICRODIALYSIS STUDY IN FREELY MOVING RATS

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Introduction

Numerous studies of somatostatin (SOM) have suggested that this neuropeptide has a neuromodulatory and transmitter function in the central nervous system. The basal amount of SOM in the striatum of freely moving rats has been found to be 5 25 fmol. It was shown that SOM can stimulate *in vivo* the release of ACh (Rakovska et al., 2002) and dopamine (DA) (Hathway et al., 1999) in the striatum. The ability of dopamine in striatum to inhibit *in vivo* and *in vitro* the release of acetylcholine (ACh) from striatal cholinergic interneurones is well established (DeBoer and Aberchombie, 1996). However, the role of SOM in the regulation of dopaminergic-cholinergic interaction in the striatum is unclear. These data prompted us to study the effect of SOM on the modulatory mechanisms which exist between dopaminergic and cholinergic neurons in the presence of DAergic antagonist sulpiride and after 6-OHDA lesion of Daergic nigrostriatal pathway.

Methods

Male Wistar rats (250-300 g) were used. Under chloral hydrate anesthsia (400 mg/kg) each rat was placed in a stereotaxic apparatus and transversal microdialysis tubing was inserted into striatum (stereotaxic coordinates: AP 0.0 and H +5.0) from bregma. Twenty-four hours after surgery the microdialysis tubing was perfused at a flow rate of 2 μl/min with Ringer solution.

The dialisate was collected at 40 min intervals and ACh content was assayed by HPLC with electrochemical detector (Damsma et al., 1987). Drugs were administered by local application via the microdialysis membrane after collecting 3 basal samples. 6-Hydroxydopamine treatment: rats were injected i.c.v. with 2×250 μg 6-OHDA and used for microdialysis experiments 5th days after the treatment. DA content in the striatum was measured by HPLC with electrochemical detection.

Results and discussion

Basal release of ACh from the striatum was 12.13 ± 2.56 pmol/20 min (mean ± SEM, n = 15). Local application of SOM (1 μM) caused long-lasting increase (about 250%) in the release of ACh from the striatum. Simultaneous perfusion with 1 μM TTX abolished the effect of SOM on ACh release. In the presence of sulpiride, D_2 receptor antagonist (10 μM) SOM-evoked release was potentiated to a much greater extent than it did in the absence of sulpiride. Whereas sulpiride by itself in concentration 1 μM did not enhance release, at a concentration 10 μM the drug significantly enhanced the basal release of ACh. When the nigro-striatal dopaminergic input was destroyed by 6-OHDA treatment, SOM (1 μM) was much more effective in enhancing basal release of ACh. Our finding suggest that SOM is able to release *in vivo* ACh in the striatum, but the ACh release-stimulating effect of SOM is masked unless the tonic inhibitory action of endogenous dopamine is attenuated.

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ASSESSMENT OF NEUROLOGICAL RECOVERY FOLLOWING PERMANENT FOCAL ISCHEMIA IN RATS

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Middle cerebral artery occlusion (MCAO) in rats is the most commonly used animal model for examining mechanisms of focal ischemic brain damage as well as testing the efficacy of candidate neuroprotective drugs. Besides the infarct size, assessment of sensorimotor

performance has become increasingly important in neuroprotective drug research, and it is necessary to carry out parallel functional and histological studies. However, there are a lot of contradiction and discrepancy about appropriate procedures for testing functional outcome following MCAO. In the present study we collected and described a set of functional neurological scoring systems, which investigates sensorimotor performance based on many signs, and yet, does not require any special equipment or pretraining, and can be carried out in less then 5 minutes. Animals underwent permanent occlusion of the middle cerebral artery, and 15 rats were examined for sensorimotor scores for 48 h, and 24 rats were followed for 14 days. Rats were scored for postural signs, gait disturbance, limb placing, beam balance, strength, sensory function and spontaneous activity. Ischemic rats showed significantly higher scores in each test than sham operated animals. It was found that best recovery occurred in climbing, while least recovery in postural signs and lateral resistance. The infarct size best correlated with daily body weight after the 2nd day, and the scores for gait disturbance 4 h after MCAO. The simple scoring system described in the present study could provide a useful screening system for initial functional testing of neuroprotective drugs.

INDIVIDUAL DISTRIBUTION AND COLOCALIZATION OF VIP, NITRIC OXIDE SYNTHASE, GABA, GLUTAMATE AND NMDA RECEPTORS IN THE DEVELOPING HUMAN ENTERIC NERVOUS SYSTEM

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Immunoreactivity for glutamate and NMDA receptors in the enteric nervous system (ENS) of guinea-pig[1] strongly supports the hypothesis that glutamate is an enteric neurotransmitter. Glutamatergic transmission through NMDA receptors is proposed to be important in visceral nociception[2] and control of food intake[3] in rat. The coexistence of mRNA for NMDA and VIP in the enteric neurons of rat[4] suggests that NMDA-mediated glutamate responsivity in the intestine is modulated by VIPergic neurons. Several population of enteric VIPergic neurons have been defined in the guinea-pig small intestine[5] where VIP was costored with GABA and NOS. Information about the distribution of nerve fibres and somata of these neuronal subpopulations and about the presence of glutamate and NMDA receptors in the developing human ENS is scarce. The first aim of this work was therefore to determine the individual distribution of VIP, NOS, GABA, glutamate and NMDA receptors in the ENS of the human fetal intestine. The second aim was to investigate the possible colocalization of these substances in order to find the particular neuronal population expressing NMDA receptors and serving as a target of glutamatergic excitatory input.

Human fetuses at week 18 of gestation were obtained after legally approved abortions in accordance with the declaration of the Medical World Federation proclaimed in Helsinki in 1964. The dissected whole intestines were flushed with phosphate buffer (0.1 M, pH 7.4) and immersed in the fixative (2% parafomaldehyde in 0.1 M phosphate buffer) for 4 h. After fixation wholemounts were prepared form selected intestinal segment. Next to the

preincubation in normal goat serum, wholemounts were incubated overnight in the primary antisera (antibodies were raised against NOS, VIP, glutamate, GABA and NMDA NR1, dilution 1:2000, 1:200, 1:100, 1:250 and 1:200, respectively). Simultaneous incubations with these antibodies were applied in double-labelling experiments. The species-specific secondary antibodies used for visualizing immunopositivity were conjugated to Cy3, TRITC and FITC. Preparations were viewed and photographed with a Zeiss Axioscope 2 MOT fluorescent microscope equipped with a Zeiss AxioCam digital camera.

Enteric neuronal elements displayed immunoreactivity for VIP, NOS, GABA, glutamate and NMDA NR1 in the myenteric plexus of the developing human ENS. NOS-positive neurons were numerous whereas glutamate, GABA and VIP immunoreactive cells were rarely seen. Double-labelling experiments revealed a limited coexistence of NMDA NR1 with VIP and GABA with NOS. In addition, these experiments demonstrated VIP-immunopositive pericellular baskets around a given population of NOS and NMDA NR1-positive myenteric neurons. These results give the first immunocytochemical evidence that NMDA receptor-mediated glutamate responsivity and transmission might be modulated by VIPergic neurons. The question that the VIPergic neurons, which modulate glutamatergic function do or do not coexpress other substances like NOS or GABA is under investigation.

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DOWNREGULATION OF THE NMDA RECEPTOR 2B SUBUNIT DURING POSTNATAL GRANULE CELL MATURATION IS REQUIRED FOR ACCURATE CEREBELLAR DEVELOPMENT

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NMDA receptors (NRs) play a key role in the establishment of functional cerebellar synapses. NRs are heteromers, comprised of NR1 and NR2 (NR2A-2D) subunits, which show characteristic spatial and temporal expression pattern during postnatal cerebellar development. By the 2nd-3rd week of age, NR2B subunits are replaced by NR2C in cerebellar granule cells. To understand the importance of the switch of NMDA receptor subunits during cerebellar development, a gene targeting strategy was used to replace the NR2C by the NR2B subunit

(designated NR2C-2B mice) leading to perpetuated NR2B subunit level in the adult cerebellum, as demonstrated by mRNA- and protein expression and channel function. Thus, the receptor exchange resulted in the prolongation of a NMDA receptor configuration found in early granule cell development.

Despite normal development of homozygous NR2C-2B mice, phenotypical changes in the cerebellum were observed as soon as the expression of the NR2B subunit started from the mutated NR2C gene locus. The thickness of molecular layer was reduced from the early postnatal ages on and synaptic NMDA currents were increased in cerebellar granule cells after three weeks of age. In adult NR2C-2B animals, cerebellar NR2A protein level, the internal granule cell layer and the excitatory innervation of Purkinje cell dendrites were reduced. In addition, adult NR2C-2B mice exhibited motor deficits in contrast to young ones. These findings indicate that the perpetuated expression of the NR2B subunit interferes with accurate synaptic innervation and stabilization between granule cells and Purkinje cells.

cAMP-DEPENDENT MODULATION OF NEUROTOXIC MEDIATORS PRODUCED BY MACROPHAGES

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Cells of the monocyte/macrophage system are normally also present in the CNS but their number is multiplied during brain injury. Upon certain stimuli, these macrophages secrete mediators that might possess neurotoxic effect depending on their location and concentration. As cAMP is regulating a number of cellular responses both in the nervous- and in the immune-system, here we studied the modulatory effect of some commonly used, centrally acting drugs, affecting the cAMP level. RAW 264.7 murine macrophages were preincubated with drugs known to elevate cAMP: the phosphodiesterase-I (PDE-I) inhibitor vinpocetine, or the ß-adrenergic agonist isoproterenol as a control. Changes in the amount of TNF-α, NO and glutamate were followed in their supernatants after LPS induction. We could demonstrate that both vinpocetine and isoproterenol (1) decreased significantly the LPS-evoked production of TNF-α and NO in the 30-100 μM concentration range. The drugs under investigation affected the glutamate transport of RAW 264.7 cells, too. It is interesting to note that while the pretreatment with isoproterenol is resulted in an approx. 20 per cent increase in the glutamate release, the effect of vinpocetine was bimodal, depending on its concentration. Our results on RAW 264.7 cells are in good accordance with those, published in the literature, demonstrating a facilitating effect of isoproterenol on glutamate release in cerebrocortical synaptosomes (2) and an inhibitory effect of vinpocetine in 5-15 μM concentration on the glutamate release in striatal synaptosomes (3).

It can be supposed that the targets of the drugs under investigation are not the same in the regulation of glutamate release and in the modulation of TNF-α and NO production, therefore, they will be the subjects of further investigations. The significant downregulation of the production of the inflammatory mediators both via ß-adrenergic stimulation and by PDE-I

inhibition, reported here, suggests that these drugs might have neuroprotective effect in cases when activated macrophages infiltrates the CNS.

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DISTRIBUTION OF NITRIC OXIDE SYNTHASE MRNA IN THE CNS OF THE DEVELOPING SNAIL, *LYMNAEA STAGNALIS* L.

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Nitric oxide is an important messenger molecule during the development of different invertebrates. The first molluscan nitric oxide synthase (NOS) gene was cloned from the central nervous system (CNS) of the pond snail, *Lymnaea stagnalis,* and a *Lymnaea-NOS (Lym-NOS)* mRNA was demonstrated in the adult nervous system by *in situ* hybridization (Korneev et al., 1998). In this study, the distribution of NOS mRNA was investigated during the embryonic and postembryonic development of *Lymnaea,* using a digoxigenin labeled probe complementer to the repetitive translated sequence of the *Lym-NOS* mRNA.

No hybridization signal could be detected in the CNS during embryonic development. A pair of symmetrically located perikarya in the pedal ganglia were the first *Lym-NOS* mRNA positive elements appearing at PT juvenile (early postembryonic) stage. From PT to P3 stages, a small number (less than 10) of neurons became *Lym-NOS* rnIRNA positive in the pedal, cerebral, and buccal ganglia. From P3 juvenile to the adult stage, the number of perikarya showing the *Lym-NOS* hybridization signal increased significantly in all ganglia of the CNS. Distribution of the labelled cell groups was mainly symmetrical in the cerebral, pedal and buccal ganglia, whereas differences in the location could be observed between the left and right pleural and parietal ganglia. In the cerebral ganglia, the most prominent labeling was found in the anterior lobe at all stages of postembryonic development. Among the most prominent identified giant neurons, the cerebral giant cell (CGC) displayed a Lym-NOS hybridization signal from P3, whereas the B2 motoneuron in the buccal ganglion expressed it from P4 juvenile stage. Other large size or giant neurons, many of them identified physiologically, were found to contain *Lym-NOS* mRNA from the P4 juvenile stage. Transient *Lym-NOS* mRNA signal could not be detected in the CNS during development. In the periphery, the cytoplasm of the mantle epithelial cells exhibited hybridization signal from P3 juvenile stage.

The development of *Lym-NOS* expression correlates well with the appearance of the capability of associating appetitive and aversive stimuli and recalling this association during *Lymnaea* post-embryogenesis (Yamanaka et al., 1999). Hence it is suggested that nitric oxide signalling contributes to the formation of learning and memory in the developing juvenile *Lymnaea*.

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URIDINE RELEASE DURING 3-AMINOPYRIDINE-INDUCED EPILEPSY

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As it has been described in our previous papers, uridine, similarly to adenosine is released under sustained depolarizing conditions and uridine is able to inhibit hippocampal neurons. Since epilepsy is accompanied with sustained depolarization, we supposed that uridine is released during seizures and it may have a neuromodulatory role under depolarized conditions. Thus, we made an *in vivo* microdialysis study in a 3-aminopyridine induced epilepsy model and measured the extracellular changes of nucleoside and amino acid concentrations as well as EEG activity simultaneously. Extracellular uridine, adenosine, inosine, and glutamate levels show a significant increase, while the extracellular concentration of glutamine decreased in rat hippocampus during epileptic state and the release of uridine was stricly correlating with the seizure type activity in the EEG. Following the microdialysis experiments, we made silver impregnation and immunostaining to verify the decrease in calcium binding proteins and the degree of cell loss in the hippocampus to get information about correlation of cell degeneration and uridine release in correlation with seizures. There was a decrease in the number of calretinin-containing interneurons in both dorsal hippocampi. The number of calbindin-containing principal neurons did not show significant changes, and we did not find any loss in the number of interneurons and principal cells with silver impregnation technique. Thus we conclude that uridine is released during epileptic activity which induces mild neuronal degeneration in the hippocampus. Our findings suggest that like adenosine, uridine may contribute to the epilepsy-related neuronal activity.

COMBINATORIAL TRANSMITTER SIGNALLING IN CENTRAL SYNAPSES: A HYPOTHESIS

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Pharmacological and physiological data on heterogeneity in the GABA and glycine receptor-dependent components of miniature inhibitory postsynaptic currents in spinal motoneurons suggest that there may be a corresponding heterogeneity in the transmitter content of afferent terminals (Frerking et al., 1995; Jonas et al., 1998). We examined this possibility by quantifying immunogold labelling for GABA, glycine and the major excitatory transmitter, glutamate, in nerve terminals contacting the dendrites of motoneurons retrogradely labelled from the rat hindlimb muscle, biceps femoris. Virtually all terminals (94%) were immunoreactive for at least one amino acid and 60% contained 2 or 3 amino acids. All possible combinations of GABA, glycine and glutamate were found. Labelling densities for GABA, glycine and glutamate varied over a wide range from terminal to terminal. We argue that the variability in terminal labelling density reflects those in synaptic vesicles and hence in released quanta. Therefore, the amino acid content of released quanta can be a major underlying cause of variability in GABA and glycine receptor mediated components of miniature inhibitory post-synaptic currents in motoneurons. Furthermore, vesicles of highly variable transmitter content can translate axonal electrical signals to chemical messages in the synaptic cleft more efficiently than the chemically homogeneous quanta. Such a quantum content code may also represent a memory which is engraved in the chemical architecture of the synapse.

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CHRONIC ALZHEIMER'S MODEL EVOKED BY MITOCHONDRIAL POISON IN RAT

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Alzheimer's disease (AD) is a form of dementia and accounts for 50-70% of all dementia cases affecting 8-10 million people worldwide. It is reportedly the fourth leading cause of death in the Western world [1]. Although there are drugs available which can delay the progression of cognitive decline, there are no treatments on the market able to protect the affected neurones

and repair the damages. It is due to that the primary causes of disease are presently unknown, however numerous evidences suggest the involvement of mitochondrial damage. Partial inhibition of the mitochondrial respiratory chain produces free radicals, diminishes aerobic energy metabolism, interferes with microglial inflammatory response, and compromises mitochondrial function creating a deleterious spiral that may contribute to neurodegeneration, resembling to AD [2]. Selective reduction in activity of terminal complex of electron transport chain (complex IV or citochrome C oxidase) in *post mortem* AD brain have been reported [3]. In the preclinical animal studies inhibition of this complex could be evoked by chronic sodium-azide (NaN_3) administration (drug causes a selective impairment in complex IV activity inducing chemical hypoxia). According to Bennett and Rose [4] 24 mg/kg daily dose of azide impairs long-term potentiation and spatial memory.

In recent study Sprague-Dawley rats (male, 300-320 g) were treated by various doses of NaN_3 (24-54 mg/kg/day) for 31 days applied via subcutaneously implanted osmotic minipumps. The spatial orientation capability of animals (cognitive function) was tested in Morris water maze test (MWM) between the 12^{th}-14^{th} and the 19^{th} treatment-day. Spontaneous motor activity was measured on the 17^{th} and 21^{st} day. On the 31^{st} day animals were sacrificed and the brains were removed for biochemical and histological analysis.

Animals received doses under 45 mg/kg/day of azide did not show cognitive or movement deficits, but histopathological changes were already present. Doses above 45 mg/kg/day proved to be toxic in 4-week long application causing early mortality. In the group of 45 mg/kg/day animals showed:

- significant cognitive defects in Morris water maze acquisition and retention tests, without diminished swimming ability;
- significant decrease of spontaneous locomotor activity in open field test;
- histopathological changes: dendritic thickening, nerve loss in the frontal cortical area, corkscrew-like dendrites, pycnotic nerve cells

The biochemical results (ChAT and AChE activities in the cortical and striatal samples) were controversial.

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PERINATAL DEVELOPMENT OF THE EQUINE BRAIN MOTOR SYSTEM

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The purpose of the present study was to demonstrate the structural maturation of the horse brain in the critical period of development emergence of coordinated locomotion. Equine brains from 14 days before expected birth to adulthood were fixed in formaldehyde and

embedded in paraffin. After taking the outer parameters of the brains, full series of large-area coronal sections were prepared on a special microtome and stained with Nissl's cresyl violet and Haidenhain's iron-haematoxylin. Microscopic images of sections were digitized and were subjected to computer-aided image analysis.

The gross morphology of the brains and the image analysis of histological preparations suggest that in the perinatal period studied there is no substantial increase in brain size and mass, while the amount of Nissl substance and myelin grows rapidly till postnatal day 45. Then a relative decrease of both is observed till adulthood accompanied by a doubling of brain size and mass.

It is concluded that during the maturation of the equine brain, decisive changes of the motor system such as up-regulation of protein sysnthesis and full myelination of motor tracts takes place during the critical period of onset of coordinated locomotion.

EFFECTS OF AGMATINE ON ON AND OFF CELLS OF THE RVM

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Agmatine (AG) is an endogenous polyamine metabolite formed by decarboxylation of L-arginine. AG is present in the brain and the spinal cord, and meets many criteria for a neurotransmitter or neuromodulator. This compound binds to alpha$_2$-adrenoceptors and imidazoline binding sites and may modulate NMDA receptor channel functions. In behavioral studies, AG exerts an antinociceptive effect and potentiates antinociception evoked by administration of more traditional drugs such as morphine or clonidine. The ON and OFF neurons of the rostral ventromedial medulla (RVM) play a significant role in the descending modulation of spinal nociceptive processing. In anesthetized rats, ON cells are activated by nociceptive stimulation of the peripheral receptive fields or nerves. In contrast, OFF cells, which are continuously active at about 15-20 spikes/s, temporarily cease firing under the same conditions.

In the present experiments, the effects of iontophoretically applied AG were tested on the functions of ON and OFF cells of the RVM. Adult Sprague-Dawley rats (250-300 g) of either sex were used under general anesthesia (4% chloral-hydrate, *i.p.*). Extracellular recordings were made using carbon fiber containing multibarrel micropipette arrays. Spikes were counted, digitized and recorded using a LabVIEW-based, computer-controlled system. Peristimulus time histograms were created on-line and stored for subsequent analyses. Thirty-eight ON cells and sixteen OFF cells were characterized as described using noxious heat or mechanical (pinch) stimulation delivered to the tail or the paw. Cells were also stimulated by iontophoretically applied excitatory amino acids (EAAs), NMDA and kainic acid (KA).

In 70% of the ON cells, iontophoretic ejection of AG resulted in a significantly decreased response to NMDA and kainic acid, both applied by microiontophoresis. However, the responses of these neurons to noxious pinch of the tail did not show a significant alteration in the presence of AG. In OFF cells, the basal firing rate was not changed by AG application. Their excessive firing evoked by NMDA or KA iontophoresis was inconsistently affected by

AG ejection. Cessation of OFF cell firing in response to noxious peripheral stimulation, however, were significantly decreased or completely abolished by AG iontophoresis.

Our results demonstrate that AG may modulate NMDA and AMPA/kainate receptor functions of ON and OFF neurons in the RVM. The inhibitory actions of AG on the EAA-evoked responses are more pronounced and consistent in ON cells than in OFF cells. In case of noxious peripheral stimulation, cessation response of the OFF cells is inhibited by AG as opposed to the unaltered ON cell firing in the presence of AG. Our data show that AG can influence functions of ON and OFF neurons in the RVM and these effects may be due to changes in their EAA receptor functions. Moreover, effects of AG on the peripheral stimulation-evoked ON cell and OFF cell responses suggest the involvement of non-EAA receptors that control the function of these neurons.

NEUROPEPTIDE SIGNALS IMPLIED IN REGULATORY PATTERNS OF ENERGY HOMEOSTASIS

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Neuropeptides are important in signal transduction both in the regulation of body temperature and in the regulation of food intake. These regulations are connected with energy homeostasis: the first might be described as short-term, the second one as long-term regulation of energy balance. Neuropeptides may affect either of the two regulations separately, in an independent manner, or simultaneously, in a coordinated fashion. Only those peptides can be regarded general regulators of energy homeostasis, which affect the two types of regulation according to "coordinated" regulatory patterns. Thus, the anabolic peptides are assumed to cause metabolic and thermal suppression (energy conservation) coupled with increased calorie (food) intake, while the catabolic peptides should induce hypermetabolism and hyperthermia (energy dissipation) coupled with anorexia (satiation, decreased food intake).

In Wistar rats chronic cannula was preimplanted into the lateral cerebral ventricle (ICV). Neuropeptides were injected ICV through this cannula, and their effects were analyzed on thermoregulation and on food intake. Thermoregulatory studies were performed on semi-restrained rats at thermoneutral or moderately cool environment: temperatures of the colon (T_c) and tail skin (T_s), along with the metabolic rate, were continuously measured for at least 3-h post-injection. To assess food intake of unrestrained animals, the cumulative 3-h chow consumption was measured following injection and the body weight was also measured every 30-min in this period. Orexigenic substances were given to well-fed animals, while anorexigenic actions were checked during re-feeding of 24-h food deprived rats.

Neuropeptide Y induced hypometabolism and hypothermia and also elicited or enhanced food intake, suggesting a coordinated "anabolic" pattern of energy homeostasis (retaining plus gaining energy). Both the hypothermic and the orexigenic actions were confined to about the first 30-min post-injection period. Similar response was seen to ICV application of orexin-A, but not orexin-B. In contrast, coordinated "catabolic" patterns (hypermetabolism, hyperthermia and suppressed food intake) followed the injections of cholecystokinin, amylin, calcitonin-

gene-related peptide, substance P. Neurotensin or bombesin induce hypometabolism and hypothermia with hypophagia, i.e., they affect energy homeostasis according to an incoordinated pattern – such pattern appears to be similar to that seen in hibernation. Hyperthermia and hyperphagia could be regarded another incoordinated pattern, however, thus far no definite data supports this type of neuropeptide action.

Complex regulation of energy metabolism, rather than separate regulation of either food intake or body temperature, may be primary target for many neuropeptides.

Further analysis of anabolic and catabolic peptides (and their connections) may be useful for studying the overall regulation of energy balance.

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CHOLECYSTOKININ AND PROSTAGLANDIN SIGNALS IN THE BRAIN: CIRCADIAN BODY TEMPERATURE (CBT) AND ACTIVITY MODIFICATIONS COMPATIBLE WITH THEIR FEVER-MEDIATOR ROLE IN THE RAT

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Cholecystokinin octapeptide (CCK8) has been shown to induce a fever-like increase in body core temperature mediated by CCK2 receptors when injected intracerebroventricularly (icv) in slightly restrained rats. As a further evidence for the possible role of this peptide in thermoregulation it was found that endotoxin-induced fever could be attenuated by a CCK2 receptor antagonist. To gain a deeper insight into the possible role of this CNS peptide signal, biotelemetry (Minimitter-VMFH, Series 4000, Sunriver, OR) has been used and – instead of acute injections – chronic infusions (using ALZET minipump connected to an icv cannula) of CCK8 or of the reference central pyrogen mediator prostaglandin E1 (PGE1) was applied. Peripheral bolus injection of *E. coli* endotoxin (LPS) was also carried out to allow comparisons of circadian thermoregulatory and activity modifications in these unrestrained animals held at thermoneutral ambient temperature.

In female Wistar rats with free access to standard laboratory chow and tap water and with a 12/12 hour day-night schedule, an icv infusion of lower doses (2.4 or 4.8 μg a day) of CCK8 (Sigma) induced a significant rise in CBT amplitude together with an increase of acrometron, while the day minima remained unchanged. The rise of acrometron was most expressed on the first 2-3 days of CCK-infusion and again some 7 days later when icv infusion was over. Icv infusion of a higher dose (24.0 μg a day) of the peptide resulted in similar rises of CBT acrometron with a tendency of more stable values throghout the period of infusion. Icv infusion of PGE1 induced even more marked rises in core temperature than those observed after infusion of comparable doses of CCK8. Simultanenous recording of general activity in these rats during control periods showed parallel changes to those of body temperature with rises at night (dark phase) and falls during the day. A remarkable temporary divergence from the virtual parallelism between the two parameters was however obvious at the height of fever response, when induced either by icv infusion of CCK8 or PGE1 with a reduction of general activity at the time of the rise of body temperature. An intraperitoneal (ip) injection of LPS

(100 μg per kg body mass) given at the first hours of the light period led to a short-lasting fever as expected, again with a decrease of general activity at the height of fever.

It is concluded that in unrestrained rats icv infusion of CCK8 or PGE1 induced a fever-response characterized by a rise in body core tempareture, characteristically with an increase of circadian body temperature amplitude. In either example the febrile nature of this rise in core temperature was supported by a simultaneous decrease in general activity, a phenomenon accepted as another sign of the so-called "sickness behaviour". All three ways of inducing a rise in body core temperature (icv infusions of CCK8 or PGE1 as well as ip injection of LPS) can thus be regarded genuine fevers rather than hyperthermias. If so, along with the established fever mediator nature of LPS or the central fever signal role of PGE, CCK8, one of the more abundant CNS neuropeptide, may also be regarded as a central fever signal in the rat.

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LIGHT ADAPTATION PROCESSES ARE DIFFERENT IN BALB/cJ AND CXBI INBREAD MOUSE STRAINS DUE TO THE QUANTITATIVE DIFFERENCES IN TYROSINE-HYDROXYLASE ACTIVITY

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Dopamine (DA) influences retinal processing of visual information, controlling occulomotor movements, photoreceptor disc shedding, electrical coupling of horizontal cells and photoreceptor to second order neuron communication. DA also has effect on bipolar, amacrine and ganglion cells expressing dopaminergic receptors. In general it is believed that DA switches the retina from scotopic to photopic vision.

BALB/cJ and CXBI highly inbread mouse strains are thyrosine hydroxilase (TH) quantitative trait loci mutants and thus the monoaminergic transmission is highly different in the two strains. TH expression has been investigated mostly in the mesencephalic region of the BALB/cJ and CXBI strains and it was reported to be low in CXBI and high in BALB/cJ strain. Brain TH activity in the BALB/cJ strain was 1.4-1.7 fold higher than those found in CXBI strain respecting to the anatomical location and to the sex of mice. Dopaminergic cells, however, do not necessarily differ in other regions of the nervous system but there is a good chance for that. Thus we predicted that the two strains can be used to study the role of dopamine in the visual system since differences might be detectable in the dopamiergic cells of the retina.

In the present study we demonstrate that TH activity difference occurs in the retina of the BALB/cJ and CXBI mouse strains, and thus it influences retinal light adaptation. Electroretinogram (ERG) recordings were performed on freely moving mice and the b-wave amplitude of the ERG was used to create adaptation curves. Adaptation curves show an

increment treshold function for the two strains and a higher treshold sensitivity was observed for CXBI mice. As a result of higher TH activity in BALB/cJ mice, a greater elevation of light induced L-Dopa synthesis was found while the levels of L-DOPA in darkness were identical in the two strains. Total TH content of retinas was also investigated by Western blot analysis. In BALB/cJ mice there is a 1.36 fold higher TH content than those of found in CXBI mice. As a consequence of wholemount immunohistochemistry this difference is not due to the eleveted DAergic cell number in the retina but rather due to the increased TH content of amacrine cells. Thus we suggest the TH quantitative trait loci mutant mouse strains as a promissing subject for studying cellular mechanisms of light adaptation.

ELECTROPHYSIOLOGICAL AND MORPHOLOGICAL CHARACTERISTICS OF NEURONS IN THE INTERMEDIATE GRAY MATTER (LAMINAE V-VII) OF THE NEONATAL RAT LUMBAR SPINAL CORD *IN VITRO*

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Interneurons in laminae V-VII of the spinal gray matter are known to play vital roles in spinal motor functions. Many of these neurons participate in reflex pathways from various types of primary afferents to spinal motoneurons. Some of them have been identified as last-order premotor interneurons. Despite of the extensive studies, our present knowledge is limited about the correlations between the electrophysiological and morphological properties of these neurons in the mammalian spinal cord.

To collect further data in this matter, whole cell patch clamp recordings were performed on 300-400 μm thick horizontal spinal cord slices obtained from neonatal Wistar rats (age P0-P7) with pipettes containing 0.5% biocytin. After registering the discharge patterns of the recorded neurons the slices were fixed and processed for morphological analysis including computer aided 3-D reconstruction of the dendritic and axonal arbors.

Based upon their discharge pattern three groups of neurons were distinguished: neurons with phasic, repetitive and single firing pattern, that has been previously demonstrated by other laboratories. In addition we described a fourth group of neurons with slow spiking. Of the 27 interneurons recorded, 6 showed phasic, 8 repetitive, 10 single and 3 slow discharge patterns. Cells in the phasic and repetitive groups were evenly distributed in laminae V-VII and presented very variable morphology. Neurons with single discharge pattern had the smallest cell bodies among the recorded cells and were mainly located in laminal V and VI. Neurons with slow firing pattern were recovered in lamina VII and had the largest cell bodies with extensive dendritic arbors.

Our findings that neurons with single and slow firing pattern appear to be confined to laminal V-VI and VII, respectively, suggest that they might contribute differently in the formation of spinal neuronal circuits. This hypothesis is under further experimental clarification in our laboratory.

COMPARATIVE NEUROPROTECTIVE EFFECTS OF PREISCHEMIC ICV VIP AND PACAP ADMINISTRATION IN RAT PERMANENT FOCAL ISCHEMIA

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Vasoactive intestinal polypeptide (VIP) and pituitary adenylate cyclase activating polypeptide (PACAP) belong to the same peptide family (secretin/glucagon/VIP family) and show closest structural homology among these related peptides. VIP and PACAP have a wide range of functions, among them both show *in vitro* and *in vivo* neurotrophic and neuroprotective effects. Recent studies have shown the protective effect of PACAP in transient focal cerebral ischemia, when treatment started with 4 hours delay after the ischemic insult.

The aim of the present study was (1) to investigate the effects of PACAP in a different experimental paradigm: preischemic intracerebroventricular (icv) administration of a single bolus of PACAP in permanent middle cerebral artery occlusion (MCAO), (2) to examine whether VIP has similar neuroprotective effects in the same ischemic model, and (3) to examine the effects of both peptides before the final infarct volume is reached e.g.: 12 hours after MCAO.

Male rats were given 450 pmol VIP, pacap38 or vehicle into the lateral ventricle. Immediately after the bolus injection, the animals underwent permanent MCAO, using the intraluminal suture technique. Blood pressure was measured in 6 randomly chosen animals by catheterizing the aorta (not included in final analysis). Blood pressure was monitored for 1 hour following icv administration of pacap or vip. 12 or 24 hours after MCAO, animals were decapitated and 2-mm-thick coronal sections were stained with ttc. Infarct size is expressed as % of total brain volume.

Icv administration of either PACAP or VIP did not alter the systemic blood pressure throughout 1 h following MCAO. Infarct size was 14.85% in control, 15.25% in VIP-treated animals, and 5.85% in PACAP-treated animals 12 h after MCAO. 24 h after MCAO, infarct sizes were 21.53% in control, 20.68% in VIP-treated rats and 10.24% in PACAP-treated animals. Thus, icv PACAP38 reduced the infarct size by more than 50% measured both 12 and 24 hours after MCAO, while the same doses of VIP did not alter the infarct size. The present results provide further proof for the neuroprotective effects of PACAP in ischemic brain damage, and show that PACAP has better protective effects in focal cerebral ischemia than VIP.

IN VITRO EFFECT OF NR2B SELECTIVE NMDA ANTAGONISTS ON THE PLASTICITY OF THE SPINAL CORD

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Repetitive stimulation of the primary afferent fibres results in a cumulative depolarisation and increased responsiveness of spinal neurons. This can be demonstrated at both the sensory and motor levels. The phenomenon is called windup and plays a role in the induction of clinical pain states related spinal hyperalgesia. NMDA antagonists seem to exert a greater inhibition on these plastic changes than on normal, physiological nociception. However, most presently available NMDA antagonists have serious adverse effects limiting their usage as analgesic drugs. NR2B selective NMDA antagonists offer more adventageous side-effect profile than the nonselective ones. The expression of NR2B receptor subunits in the spinal dorsal horn is restricted to the superficial layers, which are thought to be involved in nociceptive transmission. Some NR2B specific NMDA antagonists have been reported to reduce neuropathic pain and also inhibit windup of C-fibre related motor responses in rabbits *in vivo*. In this study the involvement of NR2B receptors on spinal neurotransmission was investigated *in vitro*, under normal and windup conditions. Experiments were performed on hemisected spinal cords isolated from 6-day-old rat pups. Evoked ventral root potentials were recorded from the L_5 ventral root following electrical stimulation of the corresponding dorsal root (exceeding C-fiber threshold) at 0.016 Hz (low frequency) or 1 Hz (windup frequency). Single responses comprised an initial sharp peak, which corresponds to a monosynaptic population action potential (MSR), followed by a motoneuronal EPSP-related long-lasting tonic potential.

Studies with selective antagonists indicated that MSR was predominantly mediated by AMPA-type glutamate receptors, while both AMPA and NMDA receptors were involved in generating the long-lasting EPSP related potential. At low-frequency stimulation the NR2B selective NMDA antagonists CP 101,606, Co 101,244 and CI-1041 (all at 1 μM) partially depressed an early component of the EPSP. The inhibition confined to the 10-300 ms post-stimulus time window with a maximal effect at around 100 ms. In contrast, APV (40 μM) blocked both the early and late (>300 ms) EPSP components. Stimulation with windup frequency resulted in a cumulative depolarisation, which was significantly depressed by APV but not by the NR2B selective blockers.

These findings suggest that NMDA receptors containing the NR2B subunit are not involved in the late part of the potential, and therefore they play no role in the wind-up response under our *in vitro* experimental conditions. This observation contradicts with some *in vivo* observations.

CIRCADIAN EXPRESSION OF BMAL1-CLOCK AND SEROTONIN N-ACETYL TRANSFERASE TRANSCRIPTS IN CHICKEN RETINA CELLS AND PINEALOCYTES

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The abundance of chicken serotonin N-acetyltransferase (arylalkylamine N-acetyltransferase, cAANAT) mRNA in the pineal gland and retina exhibits a circadian rhythm, which is translated into a daily oscillation in melatonin production. cBMAL1 and cCLOCK are putative transcription factors which are involved in circadian rhythm generation in chicken. These clock genes are co-expressed in the chicken retina and pineal gland, and may enhance cAANAT transcription. In this study, the expression patterns of *cBmal1*, *cClock* and cAANAT mRNA were evaluated and compared in retinal and pineal photoreceptor cells in chickens housed for 7 days on a 14-h light/10-h dark cycle (lights on zeitgeber time (ZT) 0-14). We also studied the effect of constant lighting conditions on *cBmal1* and cAANAT expressions. Quantitative analysis of mRNA levels was performed by RT-multiplex PCR. *cBmal1* mRNA is expressed in a rhythmic manner with a slightly different profile in the retina and pineal gland, with peak levels between ZT 8 and ZT 10 preceding the amplitude in cAANAT expression (ZT 16). However, retinal and pineal *cClock* mRNA levels fail to exhibit detectable rhythm. The peak values of *cBmal1* expression were higher in retina cells than in pinealocytes. In contrast, pineal cAANAT mRNA displays a more robust cycling than retinal cAANAT transcript. Temporal profile of *cBmal1* and cAANAT mRNA has not been changed in chickens released into constant darkness (DD) or constant light (LL) for 1 day, however, the peak values of *cBmal1* and cAANAT expressions have been decreased with the exception of retinal *cBmal1* transcripts in LL. In contrast, rhythmic pattern of cAANAT expression has been phase-shifted and/or altered in animals kept in DD or LL for 1 week. Rhythmic *cBmal1* expression with peak values at ZT 9 still persisted in retina and pineal gland in animals maintained for 1 week in LL and DD, respectively. Our data suggest that rhythmic expression of *cBmal1* mRNA in these cells is primarily regulated by a circadian oscillator. However, rhythm in cAANAT mRNA in avian retinal and pineal cells is under dual control: cAANAT expression is driven by an intrinsic clock (clock genes) and this rhythm is entrained by lighting conditions. Their relative importance in cAANAT mRNA rhythm varies in retinal and pineal cells.

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REINNERVATION OF DENERVATED LARYNGEAL MUSCLES IN ANIMAL EXPERIMENTS

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Introduction

The paralysis/immobility of the larynx can be unilateral or bilateral. In most of the cases medialisation can't support suitable voice quality, moreover laterofixation makes the voice quality worse. After the failure of the previously mentioned two surgical methods reinnervation could become the best solution. There are several techniques and outcomes of laryngeal reinnervation. Methods include direct anastomosis, nerve grafting with nerves such as the phrenic nerve or ansa cervicalis and laryngeal nerve-muscle pedicle. So far there is no technique that has wide clinical application. In this study we adopted the nerve-muscle pedicle.

Methods

Five mongrel dogs (2 male, 3 female, 12-23 kg) were used. After midline incision the strap muscles were retracted laterally and the sternohyoid muscle with the ansa cervicalis was identified on the affected side. The left recurrent nerve was dissected and resected, so we paralysed the left vocal fold. We prepared the ansa cervicalis-sternohyoid muscle pedicle and sutured to the left posterior cricoarytenoid muscle (PCA) with three interrupted stiches of 6-0 Prolene, then the wound was closed. One year later in average, the dogs were returned for a second surgery, at which videolaryngoscopy, electromyography (EMG) data were collected. The nerve pedicle and its attached muscular fragments were harvested for histological examination. We used an immunohistochemical reaction for neurofilament with a monoclonal anti-neurofilament 200.

Results

The video records demonstrate the return of motion of the denervated PCA muscle by using the muscle-nerve pedicle. EMG data from the four animals showed evidence of reinnervation of the PCA muscle with polyphasic potencials. With the immunhistocemical reaction we could demonstrate neurofilaments and motor endplates in both sides. The smallest diameters of at least 200 fibers were measured from the operated and the intact side. The average value and the standard deviation were obtained in each group. Significant difference between the operated and the intact muscle couldn't be noticable.

Conclusion

By using an ansa cervicalis-sternohyoid muscle pedicle for selective reinnervation of the PCA muscle, a remobilization obtained of the previosly denervated vocal cord after one year as it was documented by videolaryngoscopy, EMG recording and histological examination.

BINDING AND SIGNALLING PROPERTIES OF A SYNTHETIC ENKEPHALIN ANALOGUE

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Met-enkephalin-Arg-Phe (Tyr-Gly-Gly-Phe-Met-Arg-Phe; MERF) is a proenkephalin-derived opioid heptapeptide. In radioligand binding experiments performed with [^{3}H]MERF in frog and rat brain membranes κ/δ-binding profile of the compound was demonstrated[1,2]. In search for more stable analogues of MERF, which are resistant to enzymatic degradation, new derivatives with D-amino acid substitutions were prepared and tested in binding assays as well as in *in vivo* algesiometric tests. Replacement of Gly^2 for D-Ala^2 results in inhibition of cleavage in the first position (Tyr is essential for opioid activity). Met^5 which may be sensitive to oxidation, was replaced with its isosteric amino acid, norleucin (L- or D-Nle^5). One compound with the structure of Tyr-D-Ala-Gly-Phe-D-Nle-Arg-Phe (DADN) showed high potency in producing antinociception following intrathecal administration[3]. In binding assays (using [^{3}H]MERF and [^{3}H]naloxone) lower affinities were observed when compared to the parent peptide. On the other hand the new ligand showed higher affinity for μ binding sites as measured by [^{3}H]DAMGO. DADN was also prepared in tritiated form having 41 Ci/mmol specific radioactivity. Binding properties of [^{3}H]DADN were measured in crude membrane fractions of rat brain and spinal cord tissues and in homogenates of chinese hamster ovary (CHO) cells expressing either κ- or μ-opioid receptors. Specific binding of [^{3}H]DADN was reversible, saturable, stereo-selective and of high affinity. The results obtained in the *in vitro* agonist-stimulated [^{35}S]GTPγS binding assay confirmed the peptide's opioid agonist effect seen in the *in vivo* pharmacological tests. Despite the original preference of MERF for κ- and δ-opioid receptors, DADN possesses definite selectivity toward μ-opioid binding sites. This is probably due to the presence of the D-Nle side chain in the heptapeptide backbone. Chemical stability, increased μ-receptor selectivity and hydrophobicity of the compound all contribute to the high potency observed in the pharmacological assays.

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IMMUNOFLUORESCENT CHARACTERIZATION OF AFFERENT PROJECTIONS TO PREOPTIC LHRH NEURONS FROM THE HYPOTHALAMIC ARCUATE NUCLEUS OF THE GFP-LHRH TRANSGENIC MICE

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Recent development of molecular technologies enabled the generation of transgenic mice which selectively express the fluorescent jellyfish protein, green fluorescent protein (GFP) under the control of the luteinizing hormone-releasing hormone (LHRH) gene promoter. The present experiments addressed the value of the GFP-LHRH transgenic model in morphological studies of afferent neuronal projections from the hypothalamic arcuate nucleus (AN) to LHRH neurons of the preoptic area. Adult male animals were sacrificed by transcardiac perfusion using 4% paraformaldehyde, and then their brains were removed and sectioned at 20 μm on a cryostat. Histological specimen was processed for dual-label immunofluorescent detection of various neurotransmitter/neuromodulator substances, using Cy3 (red) and AMCA (blue) as fluorochroms. Triple-fluorescent studies revealed the close apposition of fibers expressing neuropeptide Y (NPY), agouti-related peptide (AGRP), alpha-melanocyte stimulating hormone (MSH), and the noradrenergic marker dopamine beta hydroxylase (DBH), to GFP-positive LHRH neurons. The presence of AGRP in a population of NPY fibers indicated that NPY afferents to LHRH neurons partially originate in the AN where all AGRP producing perikarya reside. A second major subset of NPY fibers was devoid of AGRP, but contained DBH, clearly indicating their origin from the catecholamine pool of the brainstem. In addition to the finding of AGRP and NPY in afferents to LHRH neurons, the proopiomelanocortin-derived opioid peptide MSH was also revealed in afferents to LHRH neurons.

These data indicate that the hypothalamic AN innervates LHRH neurons via at least two chemically distinct projection systems.

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ANIMAL MODELS FOR NEURODEGENERATIVE DISEASES

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With the aging population, the burden on healthcare systems and society the treatment of neurodegenerative diseases is predicted to increase substantially, and in the next decades these diseases will be the major healthcare problem. In the developed world, the most numerous

cohort of people, already now belongs to the 60 to 65 years age range. It is well known that the prevalence of Alzheimer's disease (AD), Parkinson's (PD) and other neurodegenerative diseases with age is increasing rapidly.

No disease-modifying drugs are currently on the market. It is clear that even the most successful symptomatic treatments (such as cholinesterase inhibitors and L-dopa) cannot halt or reverse the disease development, only alleviate the cognitive or motor decline. New disease-modifying neuroprotective drugs and diagnostic methods for early recognition are necessary for effective treatment.

In the last decade, important achievements in neurodegenerative disease research have included the discovery of genes for disease-related proteins, such as the amyloid precursor protein, the high-risk factor apolipoprotein E4, the presenilins, and the tau protein. New, valid pharmacological target was described by recognizing the enzymatic cleavage of APP by secretases, and among them, the pharmaceutically most promising, beta-secretase.

Common feature is in the neurodegenerative diseases the damage of the mitochondrial electron transport chain. Damage of different complexes, located in the inner mitochondrial membrane are involved in different diseases. Animal models employing this feature can be developed besides the knock out and transgenic animals, and could give us important information about disease development and about relevant therapeutic targets. Partial blocking of the mitochondrial electron transport by specific poisons leads to generation of reactive oxygen species, declined energy production and activation of apoptotic pathways. Clinically AD and PD are the most important neurodegenerative diseases.

For Alzheimer's disease model we applied the complex IV blocker, sodium-azide (Na_3N). As it is demonstrated on one of our posters Sprague-Dawley rats were treated by various doses of Na_3N (24-54 mg/kg/day) for four weeks applied via subcutaneously implanted osmotic minipumps. The spatial orientation capability of animals (cognitive function in Morris water maze) declined in non treated animals. Spontaneous motor activity, biochemical and histopathological changes are analysed in the model.

As a Parkinson's disease model we applied a chronic partial and selective inhibition of complex I of mitochondrial respiratory chain by low-dose intravenous infusion of rotenone in Lewis rats (for detailed description see our poster). Rotenone is a potent inhibitor of mitochondrial Complex I, and widely used as a common pesticide. During our introductory experiments the animals showed many PD-like features: in behavioral studies decrease of locomotor activity in open field test, decreased motor coordination on rotarod, increased rigidity in rod suspension test and cognitive deficit in Morris water maze acquisition. Histopathological changes, as neuronal loss in substantia nigra pars compacta and biochemical alterations in dopamine concentration were also present.

Application of new animal models of chronic mitochondrial poisoning, bearing the major features of neurodegenerative diseases, may help in recognizing the critical steps of disease development and testing of promising neuroprotective compounds.

SYNAPTIC CONNECTIONS OF CALRETININ-IMMUNOREACTIVE INTERNEURONS IN THE HUMAN HIPPOCAMPUS

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Interneurons in the hippocampus are functionally heterogeneous, with every cell type having characteristic calcium binding protein or neuropeptide contents and unique input-output properties. They most frequently innervate principal cells with a few exceptions, the most notable of which are the rat hippocampal calretinin-containing cells: they were shown to selectively innervate other interneurons. However, no electron microscopic data are available about the synaptic connections of the human hippocampal calretinin-immunoreactive neurons. In the present light and electron microscopic study we aimed to provide these data to establish whether interneuron-selective interneurons indeed present an essential feature of hippocampal circuits across distant species. Six control hippocampi were examined light and electron microscopically. CR-positive cells were present in all layers and subfields; they were more numerous in the CA1 region. Cells had a radial orientation in the stratum radiatum, whereas some cells had horizontal (in str. oriens) or multipolar (str. lacunosum-moleculare) dendritic trees. Input of dendrites were moderate, CR-positive terminals contacting CR-positive dendrites were frequently seen. Two types of CR-immunostained terminals were found in the CA1 region: one of them presumably derived from the thalamic reuniens nucleus, and established asymmetric synapses on dendrites and spines. The other type formed symmetric synapses on both pyramidal- and interneuron dendrites mainly in the strata pyramidale and radiatum. Analysis of postsynaptic targets of these latter synapses revealed that at least 26% of the targets were CR-positive interneuron dendrites, and 27% of the targets proved to be proximal pyramidal dendrites. CR-negative interneuron dendrites were also contacted (12%). Small caliber postsynaptic dendrites were not classified (27%). Somata were rarely contacted (7.5%). Our results suggest that calretinin-positive boutons do show a preference for other interneurons, but innervated pyramidal cells are also abundant. Our data suggest that the human Ammon's horn containes calretinin-positive interneuron-selective inhibitory cells, and boutons that innervate pyramidal cells likely derive from another calretinin-positive cell type, which may not be present in rodents.

THE NORADRENERGIC ALPHA-2 ANTAGONIST YOHIMBINE MODULATES BEHAVIORAL CORRELATES OF ATTENTION AND ALERTING TO NON-TARGET STIMULI IN VISUAL DISCRIMINATION TASK

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The alpha-2 noradrenergic system play an important role in prefrontal cortical cognitive functions (Arnsten et al., 1996). Alpha-2 receptor agonists, such as clonidine improve spatial working memory (Franowicz et al., 1999) and better memory functions in schizophrenia

(Fields et al., 1988). Guanfacine, another alpha-2 agonist, is used in the treatment of Attention Deficit Hyperactivity Disorder. The prefrontal alpha-2 sites are also involved in visual discrimination task that required monkeys to initiate or inhibit a motor response according to visual guidance (Li et al., 1998). In the present study we were interested how the very selective, highly compative alpha-2 antagonist yohimbine effects the performance of animals in visual discrimination task.

Male hooded rats (n=9) divided in two groups were used, each group had to learn a different visual discrimination task. Group One's task was to press the bar when all the LEDs in the cage were on, in order to get water. For Group Two there were two levers at the sides and a liquid-feeder in the middle on one side of the cage, with LEDs above each. The rat had to press the lever above which the LED was on and should not press when the LED in the middle was on. Each session lasted 30 minutes. After learning a cannula was implanted in their lateral cerebral ventricle. Following recovery the rats were tested: yohimbine-DMSO (50 nmol/10 μl) or 35% DMSO-saline were injected ICV in 10 μl volume. Data were recorded and analysed.

Results: Group 1. ICV yohimbine significantly increased the reaction time from light onset to bar press, and the total number of bar-presses decreased, especially in the first 15 minutes compared to controll or not treated rats. The learning rate (target per overall number of bar-presses) also decreased significantly, and remained under the rate of controll animals all throught the 30 minutes. Group 2. In animals that learned the task yohimbine caused an increased number of bar-press for non-target stimuli. But those rats that had a base learning rate ca. 34% in average made less mistakes for ICV yohimbine.

Conclusion: Group 1. The alpha-2 receptor antagonist yohimbine reduced the attention of animals, their performance was worse, they made more mistakes. Group 2. In the case of the "clever animal" significantly more mistakes were made, but the performance of the "dumb animal" improved after yohimbine injection!

PROJECTIONS OF PRIMARY AFFERENT FIBERS TO LAST-ORDER PREMOTOR INTERNEURONS IN THE LUMBAR SPINAL CORD OF RATS

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It is well established that last-order premotor interneurons (LOPIs) have crucial importance in the integration of activities generated by the spinal motor apparatus, sensory information and volleys arising from higher motor centers, indicating that they play a substantial role in spinal motor functions. Despite the extensive studies, there has been no attempt to make an accurate account concerning the morphological substantiation of the synaptic input systems of these neurons. Accordingly in the present experiments, we have made an attempt to visualize possible contacts between primary afferents and LOPIs in the lumbar spinal cord of rats using double label neural tracing methods. Here we demonstrate that terminals of primary afferents do really establish close appositions with LOPIs, although in limited numbers.

HISTOCHEMICAL CHARACTERIZATION OF MAST CELLS IN THE FEMALE RAT BRAIN

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MC are studied widely for being able to secrete in IgE dependent and independent manner, and to enter the CNS under normal physiological conditions. In ring doves after two hours of courtship MC number increases dramatically in the medial habenula. It is more difficult to find these granular cells in the mammalian brain. Their number changes with seasons, circadian periods, in the estrus cycle, or postpartum, therefore it is very difficult to locate them in the thalamus.

In our study six weeks old (120-150 g) CFY female rats (n=3) were used. In their brain MC are numerous. With the aid of acidic toluidine-blue staining MC counts ranged from 3550 to 6900. The cells appeared in the entire thalamus especially in the dorsal part, and few cells were always in the medial habenula. MC were filled with cytoplasmic granules of different density. They were found freely in capillary vessels, partly embedded in the capillary walls, or partly in the neuropil. Their true nature was studied with regular electron microscopy, too.

Immunocytochemistry was performed with anti-5HT, anti-IP3R, and anti-RyR antibodies. Immunoreactivity for 5-HT was found only in some, but not all, MC so 5HT-positive cells were counted in two animals. Only the double-stained cells (5HT and toluidine-blue) were scored. In one animal 8.29%, in the other 10.06% of the counted MC were 5HT-positive. As MC are in clusters, 5-HT-positive cells were visible also in small clusters. Few of the cells were darkly stained, while most of them had reaction product only in some granules, and these were generally situated at one pole of the MC. The darkly stained cells were mostly close to the third ventricle.

IP3R has been shown to exist in MC earlier. In our study we found only a very small population of IP3-receptor and Rhyanodine-receptor immunoreactive brain MCs. The IP3R and RyR staining was seen in very few cells only, while IP3R-positive granules seemed to be always outside of cells, or at the membrane of cells. Cells stained for RyR were very scarce, but many granule like structures seemed to be around and away from cells. Around active MC (light blue staining) most of the capillary endothelial cells were RyR-positive.

To show that immunoreactivity is real and was found indeed in MC 5-HT and IP3R, and 5-HT and RyR double-staining were performed for fluorescent microscopy.

It has been shown that in these cells IP3-receptors have different activational states. It is possible that not secreting MC mask their receptors. Further invstigations are needed especially at the electronmicroscopy level to see the distribution of these receptors in brain MCs.

MEDULLARY ADRENERGIC NEURONS CONTRIBUTE TO THE NEUROPEPTIDE Y-ERGIC INNERVATION OF HYPOPHYSIOTROPIC THYROTROPIN-RELEASING HORMONE SYNTHESIZING NEURONS

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The arcuate nucleus gives rise to approximately 80% of the neuropeptide y-(NPY) immunoreactive (IR) innervation to thyrotropin-releasing hormone (TRH) neurons in the hypothalamic paraventricular nucleus (PVN). However, the source of the remaining 20% is unknown. Since medullary adrenergic neurons synthesize NPY and also innervate the hypophysiotropic TRH neurons, we raised the possibility that adrenergic neurons contribute to the NPY-ergic innervation of TRH neurons in the PVN. Triple-labeling immunofluorescence was performed to study the colocalization of NPY and phenylethanolamine N-methyltransferase (PNMT) – the key enzyme of adrenaline synthesis – in axons in association with hypophysiotropic TRH neurons. NPY-immunoreactivity was observed in 74% of PNMT-ir axon varicosities apposed to proTRH-IR neurons, comprising 26% of all NPY-IR axons in contact with proTRH perikarya and dendrites in the PVN. We conclude that at least two distinct populations of NPY neurons innervate hypophysiotropic TRH neurons, the NPY neurons of the hypothalamic arcuate nucleus and the medullary adrenergic neurons that co-contain NPY.

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POPULATIONAL ACTIVITY OF SPINAL CPG NEURONS IN A COMPUTER MODEL FOR SWIMMING IN THE *XENOPUS* TADPOLE

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Swimming is one of those rhythmic activities that are vital for many animals. The underlying cyclic patterns are produced by neural networks, called Central Pattern Generators (CPGs). We study the spinal CPG for swimming in the young *Xenopus* tadpole at hatching. We used anatomical and physiological data from experimental studies to build a large-scale population

model of the tadpole's CPG using GENESIS software. Part of the 3.5 mm long model spinal cord can be cut off to study its rhythm producing capabilities without its rostral and/or caudal end(s). Activity was initiated by mimicking impulse patterns in the pathway from the skin sensory neurons to CPG neurons. Population activity of hundreds of CPG neurons can be simultaneously visualised vs time.

We found distributions of motoneurons and premotor interneurons that produce swimming-like self-sustaining alternating activities of the neurons on the left and right side of the cord with rostro-caudal delays in our 'cut off spinal cord' model. Details of the model and the neural activities with the underlying synaptic connections will be presented.

This work was supported by the Wellcome Trust and by the Hungarian National Research Fund OTKA.

MORPHOLOGIC ALTERATIONS IN SENSORY NEURONS FOLLOWING NERVE INJURY

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Peripheral nerve injury causes marked changes in expression of variety of peptides, channels, receptors and enzymes in sensory ganglia and in spinal neurons postsynaptic to the injured afferents. These neurochemical alterations together with other morphological and physiological changes are thought to play an important role in development and maintaining of different pain states such as hyperalgesia or allodynia. In this study, we have examined the structural alterations in the sensory neurons following spinal and cranial nerve injury.

The sciatic nerve was transected and the proximal stamp was ligated in adult rats. After survival times of 5-7 weeks the animals were perfused and the L_4-L_5 dorsal root ganglia (DRG) were removed and embedded in plastic. Semithin sections stained with toluidine blue and lead stained ultrathin sections were studied. Large size (34-40 μm in diameter) DRG neurons were found with cytoplasmic vacuolar changes. The nucleus and the nucleolus of the neurons seemed intact even in cases when the vacuole, replacing the cytoplasm, filled almost the entire neuron. The vacuoles were lined by membrane towards the cytoplasm and contained cytoplasmic debris. The initial portion of axons of the neurons with vacuoles showed significant atrophy (1.5-2.5 μm in diameter) with respect to those of the intact large size neurons (3.5-5.5 μm in diameter). Neurons with vacuolar changes were encountered in the largest number five weeks following the surgery. No changes were found when the sciatic nerve was severed but not transected. Repeated transection of the proximal stamp after a five-week survival time resulted in vacuolar changes in large number of neurons two weeks after the second operation. Similar vacuoles were detected within large size neurons in the jugular ganglion following the transection of the vagus nerve at the cervical portion.

In order to study the effectiveness of axonal transport of injured sensory neurons, another series of experiments were carried out. After seven weeks following the transection Cholera Toxin β subunit (CTb) was injected into the proximal stamp of the sciatic nerve. Retrograde

transport of CTb within the sensory neurons in L_4-L_5 dorsal root ganglia was detected with antibody against Cholera Toxin β subunit and visualized with DAB chromogen reaction. CTb-filled large and small neurons were seen throughout the ganglia. CTb immunoreactivity was also detected in large size neurons with vacuolar changes.

Loss of the distal portion of axon causes severe changes in a population of large size primary sensory neurons, such as cytoplasmic vacuoles and axon atrophy. Since vacuoles appeared also in neurons of jugular ganglion, it may imply that, after nerve injury, vacuolization is characteristic not only for dorsal root ganglia. Although the severe cytoplasmic alterations seem to indicate that the neurons are slowly deteriorating, the unimpaired transport capacity and the 5-week reaction time may also suggest that the cytoplasmic vacuoles (chromatolysis in the true sense) are signs of regenerative processes in the neurons.

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BOOK REVIEW

CYTOSKELETON METHODS AND PROTOCOLS

(ed. by Gavin, R.H., "Methods in Molecular Biology" series, Vol. 161, Humana Press, 2001)

Our view of the cytoskeleton has been modified recently from the classical concept of a rigid network of individual fibers to the recent view of a highly dynamic structure composed of interconnected fibers, molecular motors and accessory proteins. These achievements were to a great extent due to the emergence of sophisticated experimental procedures and tools. In this book the reader can find excellent, detailed, reproducible protocols that use biochemical, immunological, genetical or microscopical techniques. The protocols are always preceded by a short introduction outlining the scientific and practical backgrund of the topic. The spectrum of the subjects covered and the impact of the contributors is impressive.

The identification of cytoskeletal proteins is addressed in Part I. Appropriately designed PCR is clearly the method of choice for this purpose. Inverse PCR enables the investigators to clone contiguous DNA fragments (Chapter 1), while degenerate PCR is put forward as a key step in the strategy for the characterization of gene families, as described in Chapter 3 for the dynein heavy chain gene family in Tetrahymena.

Microscopy, the irreplaceable tool to visualize and characterize movements of whole cells as well as the dynamics of cytoskeletal filaments and the movements of organelles on them is covered in Part II. A description of the techniques of the video-enhanced differential interference microscopy (Chapter 4) and the computer-assisted systems (Chapter 5) gives a useful guideline for the interested reader and serves as a good introduction for the following chapters which treat the different aspects of the quantification of the observed phenomena. Mathematical models and calculations are presented for the characterization of the motile behavior of cells (Chapter 6) and for the evaluation of cell morphology (Chapter 7).

An upsetting display of reagents is given, mainly in Part III, for studying cytoskeletal function. These tools involve the production of site-specific antibodies (Chapter 10), the Tat-mediated delivery of antibodies into cultured cells by (Chapter 12), and the preparation of colloidal gold-conjugated primary antibodies for using to electron microscopic identification of the position of subunits within protein complexes (Chapter 11).

The possibilities of studying cytoskeletal dynamics are delt with in Part IV. Green Fluorescent Protein (GFP) tagging of proteins is used extensively for this purpose. An account of advantages and disadvantages of this technique is given in Chapter 13, along with a compilation of its use to study cytoskeletal filaments and motor proteins. A detailed description of the construction of GFP tagged motor proteins is given in Chapter 14. The third chapter of this part two approaches are discussed to study IF dynamics: the formation of cell hybrids and transient gene transfection.

Part V is a survey of several cellular systems used for investigating the structure and function of the cytoskeleton. In Chapters 16-19 chromatophores, Spisula solidissima oocyte lysates, Xenopus egg extracts and detergent-extracted cilia and flagella are discussed as models of organelle transport, centriole, centrosome and spindle dynamics, cytoskeletal filament interactions and cilial and flagellar motility, respectively.

Finally, genetic approaches are described in Part VI. From the multitude of studies in the line, the editors have chosen to present three successful projects to illustrate the potential of this approach. A generally applicable technique is described for probing cytoskeletal protein interactions by the yeast two-hybrid system (Chapter 20), followed by two chapters dealing with more specific themes, i.e. the dynein genes in Tetrahymena (Chapter 21) and the cytoskeletal components of the developing visual system of Drosophila (Chapter 22).

This book is an outstanding tool for those who want to be well-informed about up-to-date techniques for studying structure and function of the cytoskeleton.

STEM CELLS AND CNS DEVELOPMENT

(ed. by Mahendra S. Rao, Humana Press, 2001)

Stem cell biology has already produced results which raised a lot of enthusiasm and debate within and outside of the scientific community. Numerous scientific concepts were modified, new hopes and fears were raised. Serious ethical and religious aspects are to be treated. We are currently wittnessing a fundamental breakthrough in understanding the function, evolution and development of stem cells. Recent reports suggest that tissue-specific adult stem cells have the potential to contribute to replenishment of multiple adult tissues. We have thus to consider, that stem cells may arise late in development, acting principally in tissue renewal. Even within the CNS, where Cajal's dogma of "no-new-neurons" is still largely correct, i.e. at birth the neurogenesis is downregulated, in several anatomically restricted areas (subventricular zone, hippocampal granule cell layer and the olfactory bulb) new neurons are added continuously during adulthood.

Stem cell biology is still speeding: high time for us to be well-informed about recent developments.

"Stem Cells and CNS Development" gives an account of the wealth of knowledge accumulated about the nervous system's stem cells. Ten chapters are devoted to the description of the various kinds of stem cells, progenitor cells and precursor cells, followed by two chapters dealing with therapeutic perspectives. In the well-organized appendices the interested reader will find informations of practical importance, e.g. neural stem cell companies, patents and US Federal Guidelines.

The first chapter, written by S. Temple, the questions of stem cell potency are discussed in the context of the regional distribution of the different cell types within the developing nervous system. This interesting chapter is followed by two likewise informative discussions on the multipotent stem cells in the embryonic and in the adult nervous system. Further readings involve the description of the stem cells of the adult subventricular zone, as well as the characterization of the neuronal restricted and the glial restricted precursors. Resident neural progenitor cells of the adult human brain are described by S.A. Goldman in a separate chapter. The goal of their research is to characterize and "utilize these cells for neuronal and oligodendrocytic replacement in the damaged CNS". McDonald surveys recent achievements of the *in vitro* ES cell system, including the methods for isolating such cells. The possibilities and ways of mobilizing endogenous stem cells are described in Chapter 10.

Cultured stem cells are considered as potential powerfool tools and "reagents" for medical innovation. The main fields of interest are transplant therapy, gene therapy and the use of stem cells in drug discovery. These subjects are treated in the last two chapters.

The results summarized in "Stem Cells and CNS Development" are revealing and encouraging, the discussions are clear-cut. This book is worth reading for those interested in new achilvements and perspectives in biology.

TRANSGENICS IN ENDOCRINOLOGY IN CONTEMPORARY ENDOCRINOLOGY SERIES OF HUMANA PRESS IN 2001

(Ed. by Matzuk, MM, Brown CW, and Kumar TR)

The technology to produce genetically engineered animals has advanced considerably since 1980, when the first transgenic mice were produced. The possibility to manipulate selected genes, available nowadays in an increasing number of laboratories, offers us a powerful tool to explore their function within the living animal. "Transgenics in Endocrinology", published as a part of the Contemporary Endocrinology Series of Humana Press in 2001 (Ed. by MM Matzuk, CW Brown and TR Kumar) provides a compilation of the contribution of this technique to endocrinology.

In the first chapter a concise up-to-date review is given on the technologies used in the introduction, manipulation and replication of the mammalian genome, focusing on the most widely used mouse model.

Most of the subjects treated in this book are related to the molecular mechanisms of controlling mammalian sex determination and sexual differentiation.

Intelligent reviews are included e.g. about the role of The Mullerian-Inhibiting Substance (MIS) on selection between Müllerian and Wolffian ducts - a decisive step in the developing the male/female reproductive system, or about the remarkable achievements on pre- and postnatal ovary development, furthermore about recent advances in understanding the ways of hormonal control of spermatogenesis by the hypothalamo-pituitary axis.

A molecular dissection of apoptosis is provided as well, dealing mainly with the functional analysis of the Bcl2 gene family and the role of these proteins during the development of the testis, prostate, ovary, uterus and mammary gland.

Promising results on the development, differentiation, and homeostasis of the mammalian germline are described in the following chapter, with apecial emphasis on the c-kit/KL axis.

Several chapters are devoted to the use of a multitude of knockout mouse strains to study the different aspects of the genetic regulation of hormonal homeostasis, including that one of steroid hormons, glycoprotein hormones, prolactin, oxitocin, vasopressin, FSH etc.

State-of-the-art account is given on the deciphering of the factors governing mammary gland morphogenesis, using a combination of classical biological techniques with modern methods of mouse genetics.

Besides the above outlined aspects of reproductive endocrinology several chapters describe recent endeavours to understand other hormone-regulated physiological and pathological processes, as growth, food intake, obesity or skeleton patterning.

"Transgenics in Endocrinology" convincingly shows the impact of our ability to manipuate the mammalian genome in the different branches of endocrine physiology and pathology. The range of the subjects treated is remarkably broad, the individual chapters are informatives and

well-written, making the book an interesting and stimulating reading for both endocrinologists and non-endocrinologists.

MOLECULAR MECHANISMS OF NEURODEGENERATIVE DISEASES

(ed. by Chesselet, M.-F., "Contemporary Clinical Neuroscience" series, Humana Press, 2001)
and

PATHOGENESIS OF NEURODEGENERATIVE DISORDERS

(ed. by Mattson, M.P. Humana Press, 2001)

More reliable screening methods and better understanding of the molecular and cellular mechanisms leading to neurodegeneration have no doubt contributed enormously to recent developments in the field of neurodegenerative diseases. These subjects are summarised in two books published by Humana Press in 2001: "Molecular Mechanisms of Neurodegenerative Diseases" ("Contemporary Clinical Neuroscience" series, ed. by Chesselet, M.-F.) and "Pathogenesis of Neurodegenerative disorders" (ed. by Mark P. Mattson). Despite of the necessarily overlapping subjects the reader won't find him/herself bored by overlapping informations as the viewpoints are clearly different in these two books.

"Molecular Mechanisms of Neurodegenerative Diseases" focuses on Alzheimer's, Parkinson's and CAG triplet repeat diseases.

As far as Alzheimer's disease is concerned, the controversial issue of A toxicity, the role of glial cells, amyloids, inflammation, proteolysis and aging are reviewed by prominent authors. These discussions provide the basis of the chapter which gives an account on recent treatment approaches to Alzheimer's disease.

Three chapters discuss common mechanisms that contribute to the pathogenesis of several distinct neurogenetic diseases. Such mechanisms include proteolysis, filamentous tau and synuclein accumulation and dopamine toxicity.

Among the subjects dealing with the still poorly understood pathophysiology of Parkinson's disease, the reader will find an interesting review on the central role of mitochondria, while another chapter illustrates the utility of different types of PET-based measurements in this disease.

Since the discovery of a novel mutational mechanism, the expansion of an unstable trinucleotide repeat, in 1991, similar mechanism has been found to be associated with 16 neurodegenerative diseases. The largest category of these disorders, the CAG repeat diseases include spinobulbar muscular atrophy (SPMA), Huntington disease and the spinocerebellar ataxias (SCA1, SCA2, SCA3, SCA6, SCA7 and Machado-Joseph Disease). Distinct and common aspects of these diseases are presented and discussed in the last four chapters.

"Pathogenesis of Neurodegenerative Disorders" deals mainly with fundamental mechanisms involved in particular neurodegenerative disorders, summarising results obtained by *post mortem* brain studies together with animal and cell-culture investigations. The individual chapters chapters describe the clinical and histopathological features, as well as the cellular

mechanisms involved in the pathogenesis and the animal models that have allowed investigation of the pathophysiology of the given disorder.

As cell death is a central feature in several neurodegenerative diseases, the two main cell death forms: neural apoptosis and excitotoxicity is discussed in the first two chapters.

Special features and implications of the basic neuronal cell death mechanisms in age-related neurodegenerative disorders as Alzheimer's, Parkinson's and Huntington's diseases, amyotrophic lateral sclerosis (ALS) and ischemic stroke are treated in well-written, informative chapters. Other disorders discussed involve Down syndrome, spinal cord injury and Duchenne muscular dystrophy (DMD). In this latter case the authors discuss the role of the dystrophin protein and its binding partners in the functioning of the neuromuscular junction. Dystrophin mutations, their phenotypic correlations and the mutations in the genes encoding associated proteins are also reviewed.

The books reviewed here describe distinct aspects of the large progress in the understanding of the fundamental features of the neurodegenerative disorders. It seems that basic science starts to get closer to therapeutic applications, leading to an improvement of life expectancy and quality of life of the patients.

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