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# Lithium/Pilocarpine Status Epilepticus-Induced Neuropathology of Piriform Cortex and Adjoining Structures in Rats is Age-Dependent

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## Summary

Distribution of LiCl/pilocarpine status epilepticus-induced neuronal damage was studied in the piriform cortex and in adjoining structures in 12-day-old, 25-day-old and adult rats. No distinct structural and neuronal alterations were detected in the basal telencephalon in 12-day-old rats surviving status epilepticus (SE) for one week or two months. In 25-day-old rats a decrease in Nissl staining was evident. There was also cell loss and gliosis in the caudal 2/3 of the piriform cortex, in the superficial amygdaloid nuclei, in the dorsal and ventral endopiriform nucleus and in the rostrolateral part of the entorhinal cortical area. In adult animals, the topography of neuropathological changes in the basal telencephalon was comparable to those in 25-day-old rats. The damage in the caudal 2/3 or caudal half of the piriform cortex in adult rats with survival times one week or two months was characterized by a marked loss of neurons and striking glial infiltration. The thickness of the piriform cortex and superficial amygdaloid nuclei was significantly reduced. In 25-day-old and in adult animals the sublayer IIb and layer III of the piriform cortex was more affected, while sublayer IIa was less damaged. Parvalbumin (PV) immunocytochemistry revealed a significant decrease in the number of PV-immunoreactive neurons in the rostral piriform cortex and in the dorsal claustrum in animals surviving for two months.

**Key words**

Epilepsy • Piriform cortex • Endopiriform nucleus • Basal telencephalon • Pilocarpine • Parvalbumin

## Introduction

The piriform cortex localized in the basal telecephalon is a phylogenetically old structure designed as primary olfactory cortex. It is composed of three layers: layer I is characterized by ascending dendrites from deeper layers, layer II is formed by a compact zone of neuronal bodies, and layer III contains neuronal cell bodies at lower density and a large number of dendrites and axons. The piriform cortex receives direct projections from the lateral olfactory tract and is involved in olfactory perception and discrimination (Heimer 1968, Price 1973). Because of its connections with other limbic structures the role of the piriform cortex in memory processing and spread of excitatory impulses was emphasized (Haberly 1990, Datiche *et al.* 2001). The adjacent claustrum and the endopiriform nucleus are anatomically and physiologically related to the piriform



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cortex (Behan and Haberly 1999). Several lines of evidence demonstrated that the piriform cortex possesses a marked epileptogenic potential (Croucher *et al.* 1988, Stevens *et al.* 1988). It was suggested that the piriform cortex might play a crucial role in the generation and propagation of limbic seizure discharges. The piriform cortex and the endopiriform nucleus contain the most sensitive brain regions for chemical or electrical induction of limbic seizures. Recently it was shown that epileptiform events in the endopiriform nucleus (ventral claustrum) precede those in the piriform cortex and a hypothesis was formulated that the great susceptibility of the piriform cortex to epileptogenic agents is due to the ventral claustrum (Hoffman and Haberly 1991, 1996, Löscher and Ebert 1996). It was also demonstrated that the piriform cortex is very prone to develop neuronal injury induced by prolonged epileptic activity (status epilepticus – SE). Rapid development of neuronal damage in the piriform cortex might be due to the fact that epileptic foci from other limbic areas recruit the piriform cortex into epileptic discharge propagation, creating a secondary focus which then serves as a permanent amplifier of seizure activity (Löscher and Ebert 1996). Neuronal destruction in the piriform cortex was shown using various models of status epilepticus (kainate, pilocarpine) in adult animals (Schwob *et al.* 1980, Ben-Ari *et al.* 1980, 1981, Sperk *et al.* 1983, Turski *et al.* 1984, 1986, Clifford *et al.* 1987, Cavalheiro *et al.* 1992).

The piriform cortex is not a homogeneous structure. The functional heterogeneity was described for cortical olfactory information processing (Litaudon and Cattarelli 1995). In addition, the data from several laboratories differ in the localization of the most epileptogenic part of the piriform cortex. Piredda and Gale (1985) functionally localized the crucial epileptogenic site (area tempestas) into a region close to the anterior piriform cortex. Schwabe *et al.* (2000) demonstrated that bilateral lesions of the central but not anterior or posterior parts of the piriform cortex retard epileptogenesis in the kindling model in rats. Löscher *et al.* (1995) using electrical stimulation of different sites in the posterior and anterior piriform cortex (including area tempestas) found the most susceptible area in a posterior part of the piriform cortex. In contrast to functional studies, the distribution of seizure-induced neuronal damage according to detailed morphological parcelation of the piriform cortex has not yet been published.

Different lines of evidence indicate that there is a period of increased seizure susceptibility early in postnatal development. In contrast, the effects of severe epileptic activity on neuronal survival and development still remain controversial. The immature brain is capable of undergoing through status epilepticus without sustaining an adult-like pattern of hippocampal cell loss, mossy fiber sprouting, etc. in several different models (Moshe *et al.* 1983). However, it was recently demonstrated that even in rats younger than two weeks severe epileptic activity leads to irreversible neuronal damage in some brain regions, as in CA1 of the hippocampus (Sankar *et al.* 1998) or in the thalamus (Kubová *et al.* 2001). However, data concerning the development of status epilepticus-induced neuronal damage in other parts of the brain known to be highly susceptible in adults are still sparse. Therefore, our present study was focused on the distribution of neuronal damage induced by LiCl/pilocarpine status epilepticus (SE) in the piriform cortex. SE was elicited at three different stages of postnatal development of the rat (12-day-old, 25-day-old and adults). In addition, three different intervals after SE were used to characterize the development of damage in the acute, silent and chronic period of SE-induced epileptogenesis. Another aim of the present study was to examine age-dependent differences in SE-induced alterations in the immunocytochemical expression of a calcium-binding protein parvalbumin (PV) in the pirifom cortex and the claustrum. Calcium-binding proteins regulate the effects of calcium in cellular reactions by acting as intraneuronal buffering system. During the chronic period of SE-induced epileptogenesis, a decrease of PV- as well as calbindin-immunoreactivity was found in the thalamus and the hippocampus in adult rats (Cavalheiro and Bentivoglio 1991). However, data describing the topography of possible changes of PV-immunoreactivity in the piriform cortex and in the functionally related claustrum are not available.

## Material and Methods

*Animals*

Experiments were performed on albino rats of postnatal (P) ages of 12 and 25 days (P0 defined as the day of birth) and in adult animals (P90). Animals were housed under controlled temperature (23±1 $^{o}$C) and humidity with a 12/12 light/dark cycle. Animals had free access to food and water. The experiments were performed in agreement with the Animal Protection Law of the Czech Republic and were approved by Animal Care Committee of the Institute of Physiology of the Academy of Sciences of the Czech Republic to be in

agreement with the guidelines of the European Community Council directives 86/609/EEC.

*Status epilepticus*

Status epilepticus (SE) was induced using the lithium-pilocarpine model. An aqueous solution of lithium chloride (3 mmol/kg) was injected i.p. 24 h prior to injection of pilocarpine (dissolved in saline) in a dose of 40 mg/kg i.p. After pilocarpine injection, animals were placed into plastic cages and observed in isolation for at least 3 h. All motor as well as behavioral phenomena were recorded. The incidence and latency of the first epileptic seizures, motor SE and generalized tonic-clonic seizures were also registered. After two hours of continuous motor SE, paraldehyde was administered in a dose of 0.3 ml/kg in 12-day-old animals and/or 0.6 ml/kg in 25-day-old or adult rats and the animals were checked every 20 min. Control animals received an equal volume of saline instead of the pilocarpine solution; other conditions including medication, handling and time of isolation from mothers in the case of rat pups were the same as in experimental groups. Body temperature of young animals was maintained at 34 $^{o}$C (i.e. temperature of the nest). After approximately 5 h animals received an injection of saline (3 % of the body weight) and 12-day-old pups were returned to their mothers. Their body weight and behavior were monitored till the end of the experiment. Animals surviving SE induced at the age of 25 days or in adulthood were fed for a few days with a paste made of grounded animal food and glucose.

*Morphological techniques*

Twelve-day-old animals were processed for histological analysis seven days and/or two months after pilocarpine administration, 25-day-old and adult rats were processed 2 days, 7 days or 2 months after SE. Parvalbumin immunohistochemistry was performed in animals sacrificed two months after SE. Only rats with motor SE lasting for at least two hours and without generalized tonic-clonic seizures were included in the study. Experimental and control groups were always processed together and they consisted of at least six animals.

Under deep urethane anesthesia (2 g/kg i.p.), animals were perfused with 0.01 M phosphate buffered saline (PBS), pH 7.4, followed by a fixative solution (4 % paraformaldehyde in PBS). The brains were removed and postfixed in a fixative solution for 3 h at 4 $^{0}$C, soaked in 20 % glycerol in 0.02 M KPBS for 24 h, and frozen in dry ice. Coronal sections (50 μm) were cut through the whole brain in a cryostat, collected in 0.05 M PBS. Every fifth section was used for Nissl staining with cresyl violet. For immunohistochemistry, sections were sequentially incubated in 0.15 % hydrogen peroxide in PBS (0.01 M; pH 7.4) for 10 min, rinsed with PBS four times and then incubated with a blocking solution containing 2 % normal horse serum (Vector Laboratories, Burlinghame, CA, USA) and 0.1 % Triton-X100 in PBS at room temperature. The sections were then incubated with the primary antibody (anti-PVA monoclonal antibody; Sigma, dilution 1:5000 in PBS containing 1.5 % normal horse serum and 0.1 % Triton-X100) for 48 h at 4 $^{o}$C and then rinsed four times in PBS. Thereafter, the sections were incubated for one hour at room temperature with secondary antibody, biotinylated anti-mouse antibody made in horse (Vector) dilution 1:50 in PBS containing 1.5 % normal horse serum and 0.1 % Triton-X100**.** After this step, the sections were rinsed with PBS four times and covered with the ABC reagent (Vectastain Kit, Vector) for one hour at room temperature. After rinsing, sections were incubated for 5-7 min with a mixture of 0.02 % diaminobenzidine and 0.05 % hydrogen peroxide in PBS. Thereafter, sections were mounted onto gelatin-coated slides, dehydrated, coverslipped and examined using light microscopy and the results were compared with data from age-matched controls. Nissl stained sections were analyzed from AP 12.2 mm to AP 2.2 mm.

*Quantitative analysis*

The immunostained sections from 12, 25 and 90 days old animals surviving 8-10 weeks were used for quantitative analysis.

The sections were analyzed with an Olympus AX 70 microscope with bright field optics. To calculate the number of parvalbumin-immunoreactive (PV-ir) neurons in the piriform cortex and in the dorsal claustrum, the boundaries of the piriform cortex and the dorsal and ventral claustrum were drawn from the immunohistochemical sections and from the adjacent Nissl-stained sections using a microscope equipped with a drawing-tube. The sections corresponding to stereotaxic planes AP 10.2, 9.7, 9.2, 8.7, 8.2, 7.7, 7.2, 6.7 and 6.2 for the piriform cortex and sections corresponding to stereotactic planes 10.2 – 7.2 for the dorsal claustrum (Paxinos and Watson 1986) were analyzed. In three groups of experimental animals, the numbers of PV-ir neurons were counted in the piriform cortex and dorsal claustrum (27-30 sections per animal). The numbers of PV-ir neurons in the piriform cortex and PV-ir neurons in

the dorsal claustrum of pilocarpine-treated animals were compared with those of control animals and were expressed as percentage of the controls. Statistical evaluation was performed using the t- test.

*Nomenclature*

The parcelation of the analyzed allocortical fields is based on cytoarchitectonic studies of the piriform cortex according to Filimonov (1949), Price (1973) and Haberly and Price (1978). According to these authors the piriform cortex is divided into an anterior and a posterior part. The anterior part consists of two fields (Filimonov 1949) and the lateral field (Pp 1) is formed by three layers (plexiform or zonal, pyramidal and multiform). The medial field Pp 2 is characterized by a wide zonal (first) lamina with lateral olfactory tract on its surface. The posterior part of the piriform cortex located laterally to periamygdaloid fields is called field Pper (Filimonov 1949) and is characterized by the disappearance of the lateral olfactory tract.

Claustral complex is divided into dorsal (or insular) claustrum situated above the level of the rhinal fissure and ventral (or prepiriform) claustrum adjoining the piriform cortex (Narkiewicz 1964, Druga 1966, Druga *et al.* 1993). The term endopiriform nucleus (Krettek and Price 1977) or dorsal endopiriform nucleus (DEn, Paxinos and Watson 1986) is now used for the ventral claustrum. The area located between the ventral claustrum (dorsal endopiriform nucleus, DEn) and the piriform cortex is described as the ventral endopiriform nucleus (VEn, Paxinos and Watson 1986) or the ventral endopiriform region (Behan and Haberly 1999). The anteroposterior extent of the damaged regions was determined according to the atlas of Paxinos and Watson (1986).

*Abbreviations*

| | |
|---|---|
| AB | accessory basal nucleus |
| AE | amygdalo-entorhinal transitional field |
| AI | insular agranular area |
| AHAL | amygdalohippocampal area, lateral division |
| AHAM | amygdalohippocampal area, medial division |
| Apir | amygdalopiriform area |
| B | basal nucleus |
| Bi | basal nucleus, intermediate division |
| Bpc | basal nucleus, parvicellular division |
| CE | central nucleus |
| CL | claustrum, dorsal part |
| Clv | claustrum, ventral part (dorsal endopiriform nucleus) |
| COa | anterior cortical nucleus |
| COp | posterior cortical nucleus |
| DEn | dorsal endopiriform nucleus (claustrum, ventral part) |
| DI | disgranular insular area |
| DIE | dorsal intermediate entorhinal field |
| DLE | dorsal lateral entorhinal field |
| L | lateral amygdaloid nucleus |
| Md | medial nucleus, dorsal division |
| PAC | periamygdaloid cortex |
| PACm | periamygdaloid cortex, medial division |
| PACs | periamygdaloid cortex, sulcal division |
| Pir | posterior part of the piriform cortex |
| Pp l | anterior part of the piriform cortex, lateral field |
| Pp 2 | anterior part of the piriform cortex, medial field |
| PRh | perirhinal area |

## Results

Motor SE was induced in all animals included in this study. It started after a latent period of 530±27, 844±44, and 1790±159 s (mean ± S.E.M.) in 12-day-old, 25-day-old and adult rats, respectively, and it consisted of continuous clonic seizures of the head musculature and forelimb muscles. Rearing as a part of seizure activity was regularly observed in the two older groups only. Mortality was similar in all three age groups and varied in the range from 17 to 20 % of animals with seizures.

In 25-day-old and in adult animals neuronal damage was also observed in other brain regions such as the hippocampal formation, amygdala and thalamus. Our study was focused on the piriform cortex and adjoining structures only.

*12-day-old animals surviving one week after SE*

No obvious changes were found in this interval in any evaluated brain region.

*12-day-old animals surviving two months after SE*

Only sporadic dark and shrunken neurons were found in the 3rd layer of the piriform cortex, whereas the 2nd layer remained intact. Isolated dark neurons were also discernible in the ventral endopiriform nucleus (VEn).

*25-day-old animals surviving for two days*

No severe neuronal damage was observed in 3 out of 6 animals at this time period. However, a slight decrease in Nissl staining and local edemas were apparent in neurons located in the third layer of the piriform cortex

and in the transitional area between piriform cortex and the ventral claustrum (VEn) in these animals. At the same time, nuclei of the glial cells and blood vessels become more prominent in the affected area.

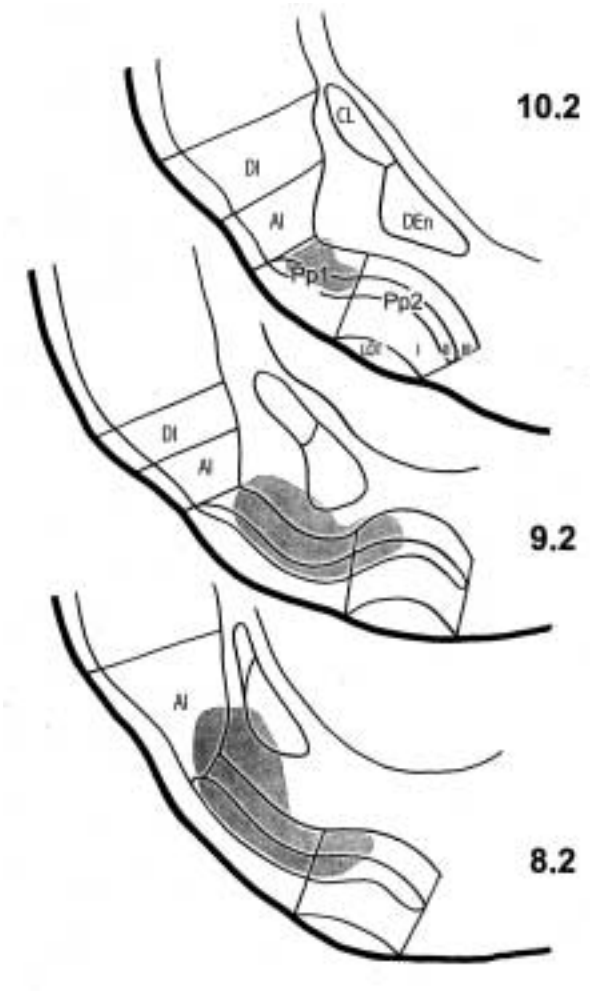



**Fig. 1.** *Schematic drawings of coronal sections of rostral piriform cortex. Numbers at right identify anteroposterior planes according to Paxinos and Watson (1986), shaded areas indicate location and extent of damage (decrease in Nissl staining, neuronal loss, gliosis) in 25-day-old and adult rats one week after status epilepticus. For explanation of letters see the list of abbreviations, numerals I, II and III indicate layers of the piriform cortex.*

Degenerative changes and structural destruction occurred consistently in the caudal half (or two thirds) of the piriform cortex in the remaining three animals. In addition, neuronal destruction was found in adjacent areas (the amygdaloid nuclei, the part of insular and perirhinal cortex and the entorhinal area) (Figs. 1 and 2). Neurons localized in the deep layer II and in the layer III of the piriform cortex were markedly affected while the superficial part of layer II was less damaged (Fig. 3B). In addition to a paleocortical damage, the ventral part of the claustrum (claustrum ventrale, DEn) and the ventral endopiriform nucleus (VEn) were also affected. At this period, the anterior olfactory nucleus was unaffected. The paleocortical destruction started rostrally at stereotactic planes AP 9.7 – 9.2 (Paxinos and Watson 1986). Destruction affected piriform cortex in the whole mediolateral region of the field Pp 1 as well the lateral half or lateral third of the field Pp 2. Medial part of the Pp 2 and adjoining olfactory tubercle remained unaffected. In addition, tissue damage also occurred in the area VEn, in the ventral claustrum (DEn), in the agranular insular area (AI) and in the perirhinal area (Prh) both below the niveau of the rhinal fissure. Caudally to the level of stereotactic plane AP 5.7, the destruction affected the piriform cortex, the dorsal endopiriform nucleus and the ventral endopiriform nucleus. The allocortical destruction ended at the AP level of 2.9 – 2.7.

*25-day-old animals surviving for one week*

The damage of the piriform and periamygdaloid cortex was detected in five out of six animals of this group. A decrease in Nissl staining, cell loss and moderate gliosis were evident in the affected area. In most cases histopathological changes of the piriform cortex were at first discernible at the AP level 10.2 in the field Pp 1 and lateral half of the field Pp 2. Caudally to AP 9.2 the damaged area involved whole mediolateral region of the piriform cortex, ventral part of the claustrum (DEn) and VEn. Within the piriform cortex sublayer IIb and layer III were damaged more markedly than the other layers. Cortical damage was seen up to the AP level 4.2 – AP 3.7 in four animals. Damage of the entorhinal cortex was inconstantly observed up to the AP level 2.2 and was limited to the rostrolateral parts of this area.

*25-day-old animals surviving for two months*

Five out of six animals of this group exhibited a consistent pattern of paleocortical damage, which differed from that described in the former group. The destruction of the piriform cortex was apparent at the AP level 9.2 – 8.7 and more caudally and affected field Pp 1 and the lateral half of field Pp 2. The destruction of the piriform cortex differed markedly from animals examined 7 days after SE by a marked reduction of its thickness, apparent neuronal loss and massive glial infiltration of layers Ib, II and III. Sublayer IIa was less affected. The destruction extends from the piriform cortex to the area VEn and to

the basal part of the ventral claustrum (DEn). The most caudal part of the piriform cortex was damaged only in its medial half (AP 5.2 – 4.4).

In two out of 6 animals of this group the destruction was restricted only to the layers II and III of the fields Pp 1 and Pp 2 of the piriform cortex without spreading to the VEn and to the ventral claustrum (DEn).

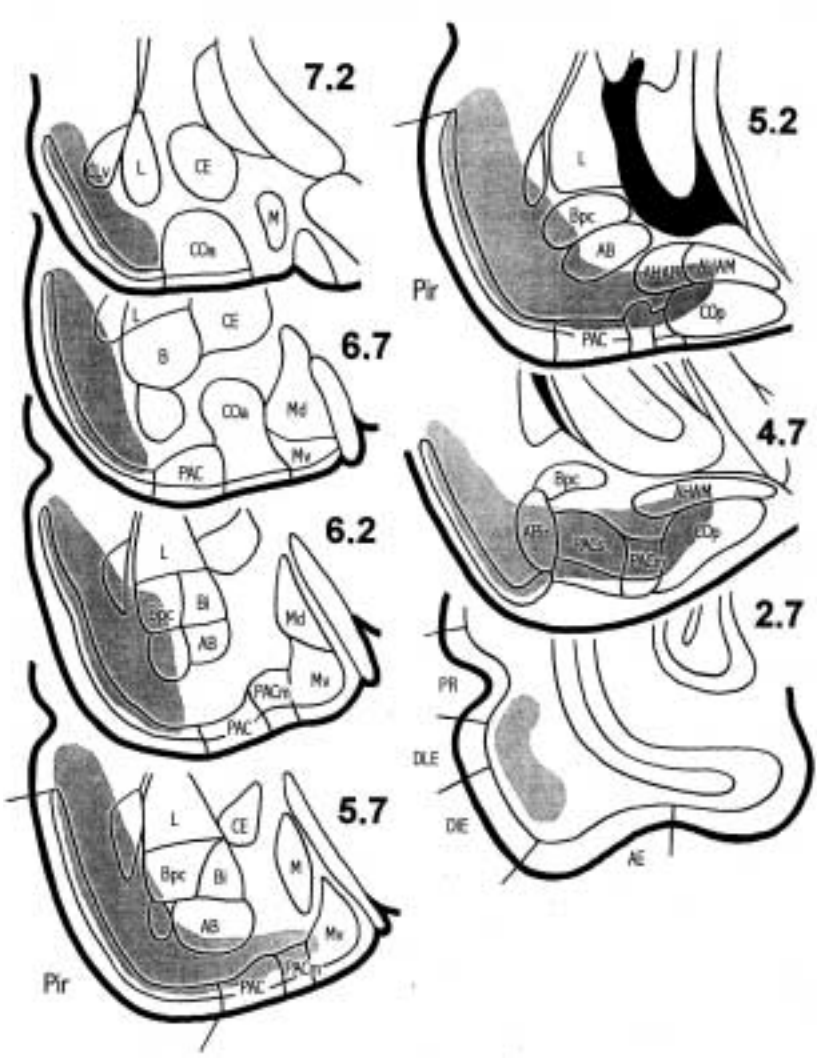


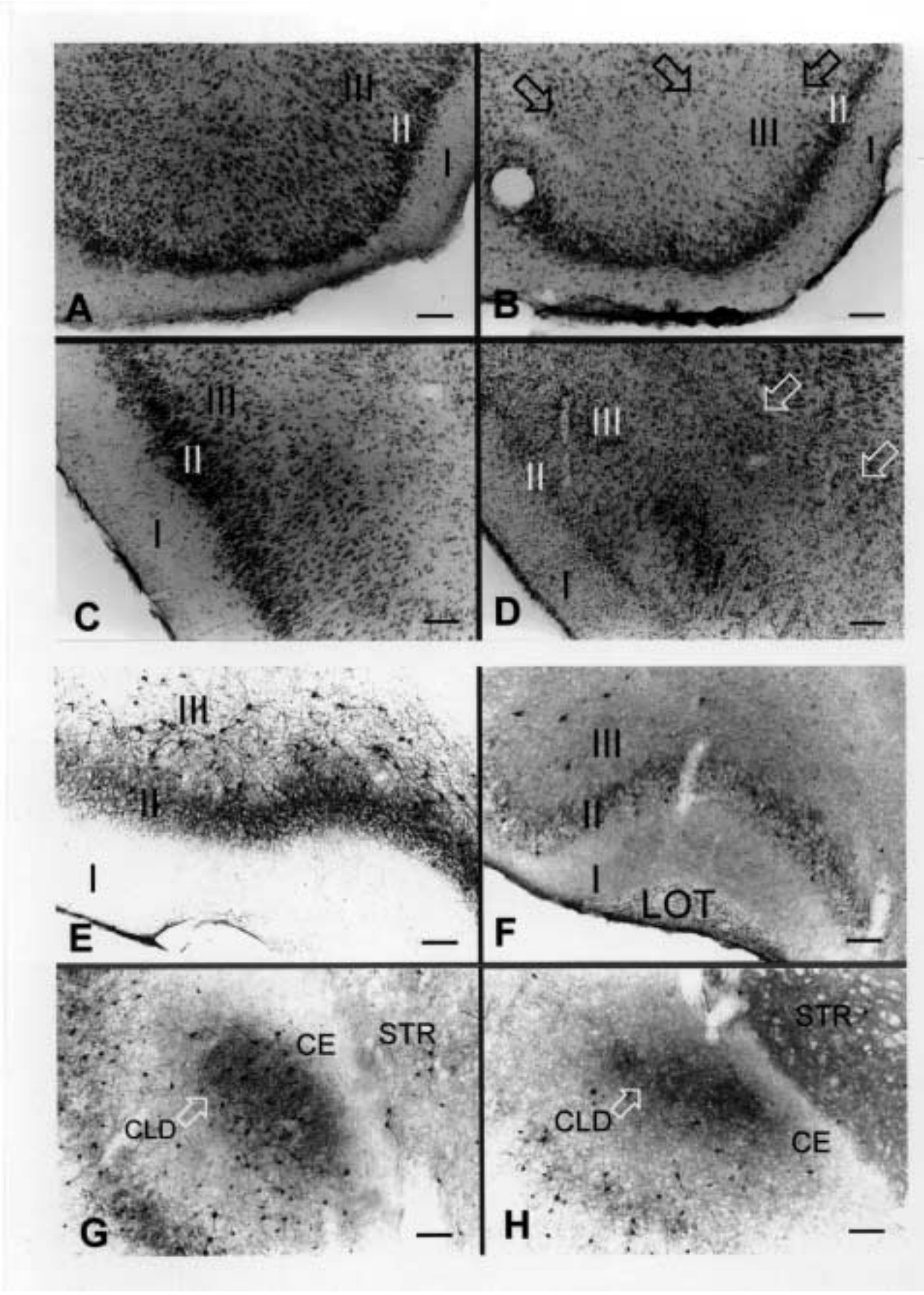

**Fig. 2.** *Schematic drawings of coronal sections of caudal piriform cortex, amygdala, and rostral part of entorhinal cortex of 25-day-old rats two days after status epilepticus. Details as in Fig. 1.*

*Adult animals surviving for two days*

Despite some variation in the anteroposterior region of the lesion, the paleocortical damage was symmetrical and the general characteristics of destruction resembled those of 25-day-old animals surviving for 2 days. The damaged area exhibited local edema, vacuolization and a significant decrease in Nissl staining. Fragmentation of the neuropil with total disruption of the central zone of the affected area was evident in some sections. The damaged area of the piriform cortex was evident caudally from the AP level 5.7.

Exceptionally in one out of 7 animals the damaged area exhibited greater anteroposterior extent from AP 10.7 to AP 2.2. The piriform field Pp 1, lateral half of the Pp 2, nucleus VEn and the basal part of the DEn were affected in these cases.

**Fig. 3.** *Photomicrographs of Nissl-stained coronal sections of the posterior part of piriform cortex (AP 7.2 mm) of 25-day-old rats under control conditions (A) and two days after status epilepticus (B) and of adult rats (control animal – C, rat one week after status – D). Arrows in B and D mark boundaries between the intact and damaged tissue. Photomicrographs of coronal sections of piriform cortex (E, F) and dorsal claustrum (G, H) stained immunohistochemically for parvalbumin. F and H represent sections from an adult animal (AP 9.7 mm) two months after status epilepticus induced at the age of 25 days, E and G demonstrate sections from appropriate controls. Arrows in G and H indicate parvalbumin-positive part of the dorsal claustrum (AP 8.7 mm). Numerals I, II and III indicate layers of the piriform cortex. Abbreviations are explained in their list.*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*Adult animals surviving for one week*

Extensive symmetrical lesions extending from the AP plane 8.7 – 7.7 to 4.2 – 3.7 prevailed in this group (in six out of 7 animals). They were characterized by neuronal loss and a marked glial infiltration sharply delineated against intact regions of the basal telencephalon. In some sections, neurons of layer II of the piriform cortex were completely replaced by strips of glial cells. Lesions affected the posterior half of the piriform cortex, ventral claustrum (DEn), and Ven. The basal telencephalic lesion was more extensive (from AP 10.7 to 2.9) with changing involvement of the piriform cortex (Figs. 3D and 4) in one case from this group.

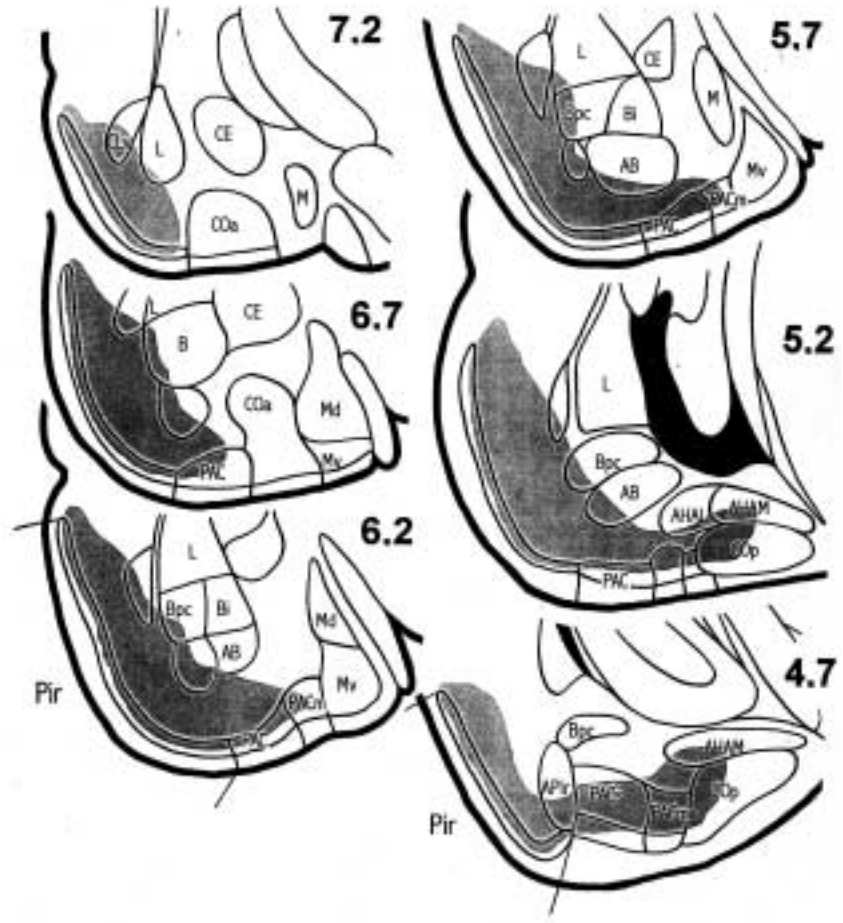


**Fig. 4.** *Schematic drawings of the coronal sections of the caudal piriform cortex and amygdala showing the location and extent of damage in adult rats one week after status epilepticus. Details as in Fig. 1.*

*Adult animals surviving for two months*

Symmetrical lesions extending from AP 8.7 to AP 3.7 – 2.9 were characteristic for this group. The lesions were filled by amorphous weakly stained material surrounded by a thick border of condensed glial cells and thus delineated from the intact tissue. Isolated nuclei of glial cells were also discernible inside this amorphous glial scar. The lesion affected layer II and a superficial part of layer III of the piriform cortex, whereas deeper parts of the layer III remained relatively unaffected. Typically, the whole mediolateral area of the piriform cortex was affected (fields Pp 1, Pp 2) and it protruded to the VEn and to the marginal part of DEn. Exceptionally, in one of 7 rats the lesion originated more rostrally (AP 10.2 – 9.7) affecting the lateral half of the piriform cortex (field Pp 1). An intact piriform cortex was found in one animal.

*Changes of parvalbumin-immunoreactivity in the piriform cortex*

In control animals, PV-immunoreactive neurons were identified only in layers II and III and majority of them were in layer III as described by Kubota and Jones (1993). A decrease in the number of PV-ir neurons and fibers was evident in 12-, 25-day-old as well as adult animals two months after the SE (Figs. 3F,I). A significant difference between the control and experimental animals with seizures at the age of 12 days was found only in the rostral piriform cortex, whereas the differences in caudal piriform cortex did not reach the

level of statistical significance. Significant differences could only be demonstrated in rostral piriform cortex of the two older groups because of a complete destruction of the caudal part of this structure (Fig. 5).

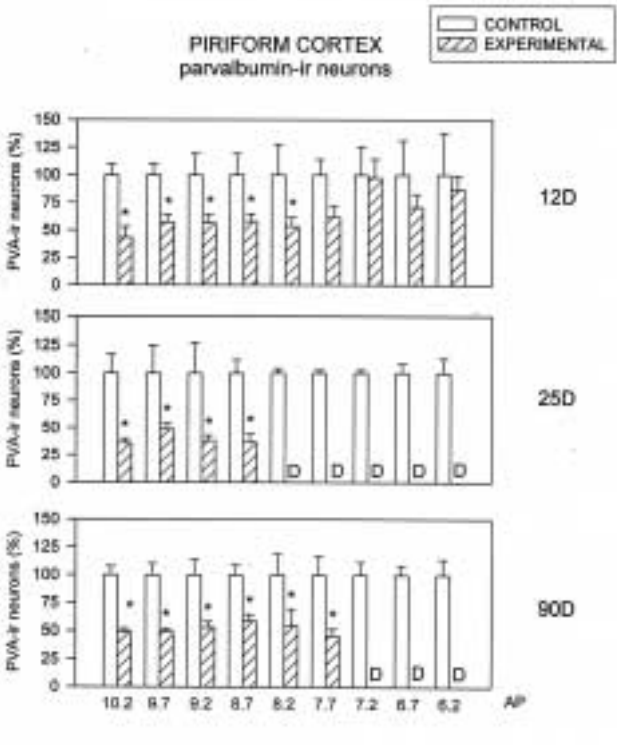


**Fig. 5.** *Changes of the relative number of parvalbumin-immunopositive neurons in the piriform cortex (mean ± S.E.M.). Age when status epilepticus was induced is indicated at right, the animals were sacrificed two months later. Abscissa: AP levels of sections; ordinate: relative number of neurons (number of neurons in control animals is always taken as 100%). Asterisks denote a significant difference between control and experimental group, D marks sections with total destruction (where no neurons were found).*

*Changes of parvalbumin -immunoreactivity in the dorsal claustrum*

Intense PV-immunostaining in neurons and neuropil was characteristic for dorsal claustrum while PV-ir neurons were rare in the ventral claustrum (DEn) in control rats in agreement with previous findings (Druga *et al.* 1993). The density of PV-ir neurons and intensity of neuropil staining within the dorsal claustrum was lower in the experimental animals than in the controls. This difference was significant; the number of PV-ir neurons in experimental animals reached 50 % or less of the control values (Figs. 3G,H and 6).

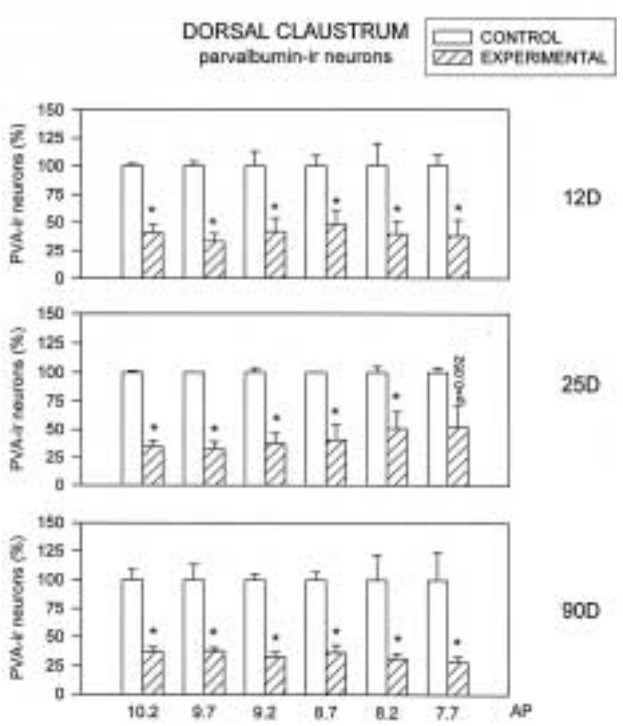


**Fig. 6.** *Changes of the relative number of parvalbumin-immunopositive neurons in the dorsal claustrum. Details as in Fig. 5.*

## Discussion

The present data indicate that histopathological consequences of lithium/pilocarpine-induced status epilepticus (SE) in the basal telencephalic area are age-dependent. The development of histopathological changes in the basal telencephalic area of 25-day-old and adult animals was similar in many aspects. In both age groups the characteristic features of the short survival time (2 days) were edema, vacuolization and fragmentation of the neuropil and a significant decrease in Nissl staining (bleaching). Our results support previous findings obtained in kainate-induced SE in adult animals (Schwob *et al.* 1980, Ben-Ari *et al.* 1981, Sperk *et al.* 1983, Lassman *et al.* 1984, Ben-Ari 1985). In contrast to the kainate model of SE (Schwob *et al.* 1980), dark and shrunken neurons were observed only in long survival intervals, whereas they were rare during the acute period of lithium/pilocarpine-induced SE. Similarly, the hemorrhages common in the kainate model were seen only exceptionally in our model. In longer survival times

(one week and more) the morphological reaction to lithium-pilocarpine in 25-day-old and adult animals is similar. The affected area exhibits a loss of neurons and moderate gliosis. Glial infiltration is more apparent in adult animals especially after longer survival time (2 months) where it is accompanied by a considerable reduction in thickness of the piriform cortex and adjoining superficial amygdaloid nuclei. It was shown that neuronal damage is accompanied by gliosis involving a marked astroglial reaction (Turski *et al.* 1984, 1986, Lassmann *et al.* 1984). However, it has recently been demonstrated that the glial response is a more complex phenomenon with important participation of the microglial cells. Activation of microglia was described in animal studies after neuronal cell death as well as in hippocampal sclerosis associated with human temporal lobe epilepsy (Jorgenssen *et al.* 1993, Beach *et al.* 1995, Acarin *et al.* 1999).

Numerous studies have clearly demonstrated that the development of structural damage is preceded by metabolic activation and changes in cellular activation monitored by heat shock proteins, protooncogenes and their encoded protein products (Cavalheiro *et al.* 1992, Kiessling and Gass 1993, Motte *et al.* 1998, Dubé *et al.* 1998, Suzukawa *et al.* 1999). Increased metabolism was detected in the piriform cortex even in very early stages of SE. In contrast, at a late period of SE (24 h), glucose utilization was depressed as a consequence of early necrosis (Handforth and Treiman 1995). Similarly, Dube and coworkers (2000) reported a significant increase of glucose metabolism followed by extensive neuronal degeneration as a consequence of SE in rats since the age of 3 weeks. In contrast, younger animals also showed a metabolic increase in various brain regions including the piriform cortex, but only a few injured neurons were detected (Fernandes *et al.* 1999). In addition, no neuronal damage was found during the silent period in animals surviving SE during the first two weeks of life (Ben-Ari *et al.* 1984, Cavalheiro *et al.* 1987, Dube *et al.* 2000). These data are also supported by our results, because no obvious neuronal loss was found in Nissl-stained sections in animals surviving SE on postnatal day 12 at any time period studied. Only isolated dark and shrunken neurons occurred in the layer III of the piriform cortex and in the VEn two months after SE.

Despite some variations, the regional distribution of damage exhibited a rather consistent pattern in older rats. In all animals in which degenerative changes were detected in the basal telencephalon the lesion constantly affected the piriform cortex, the amygdaloid and periamygdaloid nuclei and the entorhinal cortex.

In the piriform cortex, the cortical destruction was limited to posterior two thirds in a majority of animals. Rostral part of the piriform cortex (rostrally to AP plane 10.7) including the anterior olfactory nucleus was never damaged. Similarly, we have never observed destruction in the part of the deep rostral piriform cortex called "area tempestas" by Pirreda and Gale (1985). Destruction of the piriform cortex invariably affected only its lateral part (field Pp 1, Filimonov 1949). Medial part of the piriform cortex (field Pp 2) characterized by the presence of the lateral olfactory tract in the first (molecular) layer was partly preserved. In 25-day-old and adult animals the medial half of the field Pp 2 was relatively unaffected while the lateral half of this field adjoining to the field Pp 1 exhibited distinct histopathological changes similar to those observed in the field Pp 1. Sublayer IIa was less affected than sublayer IIb and layer III in 25-day-old and adult animals especially after shorter survival times.

The destruction of the piriform cortex was frequently associated with a destruction of the ventral endopiriform nucleus and a partial or total destruction of the ventral claustrum (dorsal endopiriform nucleus). Anteroposterior extent of destructions in the VEn and DEn was similar to destruction of the piriform cortex. This means that the rostral part of the ventral claustrum (DEn) and VEn (from AP 11.2 to AP 10.2) remains unaffected.

The extent and topography of destruction in the piriform cortex and in the ventral claustrum (DEn, VEn) corresponds to a decrease of NADPH-diaphorase positivity in the neuropil and neurons described in 25-day-old rats after lithium-pilocarpine SE (Kubová *et al.* 1999).

The extent of the histopathological changes after systemic administration of the kainic acid is more extensive than in our material; the anterior olfactory nucleus, whole anteroposterior and mediolateral region of the piriform cortex, superficial and deep amygdalar nuclei, the ventral and dorsal claustrum and the whole extent of the entorhinal cortex and temporal and occipital cortices are affected (Schwob *et al.* 1980, Ben-Ari *et al.* 1981, Ben-Ari 1985).

Several studies indicate that the piriform cortex and adjoining structures play a more important role in epileptogenesis than previously suspected. It was shown in slice preparations that epileptiform EPSP is driven by deep cells located in the ventral claustrum (endopiriform

nucleus) and probably also by cells in the deep part of layer III of the piriform cortex (Tseng and Haberly 1989a,b, Hoffman and Haberly 1991). Epileptiform events in the endopiriform nucleus preceded those in the piriform cortex and they could be triggered by local application of glutamate into the claustrum and/or endopiriform nucleus but not into the piriform cortex (Hoffman and Haberly 1996). From the endopiriform nucleus, epileptic activity may be projected by means of direct monosynaptic pathways to neighboring cortical and subcortical structures including the amygdala, piriform cortex, entorhinal cortex, perirhinal and insular cortex (Krettek and Price 1977). The epileptiform activities may further spread to many limbic and neocortical areas *via* successive bi- and multisynaptic projections. These connections are mostly excitatory using glutamate as a neurotransmitter (Demir *et al.* 2001), hence the hyperacitivity may increase glutamate release with a subsequent cascade of neurotoxic events resulting in the destruction of large basal telencephalic areas.

Recently published data (Behan and Haberly 1999) indicate that the connectivity of the ventral claustrum (endopiriform nucleus) could explain the topography of at least some basal telencephalic lesions in our experiments. Efferent projections from the middle part of the endopiriform nucleus terminate approximately in the caudal two thirds of the piriform cortex while projection to the rostral third is weak. Efferent fibres from the caudal part of the endopiriform nucleus are oriented to the caudal third of the piriform cortex, while they are lacking in the rostral third of the piriform cortex. Intermediate and caudal parts of the endopiriform nucleus project heavily to the periamygdaloid cortex and to the posterior cortical nucleus of the amygdala. In contrast to this, the projections to the nucleus of the lateral olfactory tract, to the anterior cortical and the medial nucleus of the amygdala are significantly weaker, similarly as projections to the deep amygdaloid nuclei. The middle part of the endopiriform nucleus projects massively to the agranular insular area and to the perirhinal area. In the perirhinal area, the majority of fibers terminate below the rhinal fissure. On the other hand, projections from the anterior and the posterior part of the endopiriform nucleus to the agranular insular and to the perirhinal area are scarce. The central and caudal part of the endopiriform nucleus also project massively to the rostrolateral part of the entorhinal cortex. The distribution of these projections (Behan and Haberly 1999) is in agreement with areas showing destructions in our experiments.

The present results allow a number of explanations concerning the extent of destructions and severe gliosis within the basal telencephalic area of pilocarpine-treated animals. Nevertheless, there is a considerable overlap between the extent and topography of destructions affecting piriform and entorhinal cortex and amygdalar nuclei on one side and the projection field of the middle and caudal part of the endopiriform nucleus and deep amygdalar nuclei on the other. On the basis of such an overlap, it might be hypothesized that hyperactivity (i.e. propagation of seizure discharges) in projections of the middle and caudal part of the ventral claustrum (endopiriform nucleus) and of the deep amygdaloid nuclei could be considered as a posible mechanism leading to the destruction of several basal telencephalic structures (Okazaki and Nadler 1988, Meldrum and Garthwaite 1990).

The fact that the rostral part (approximately one third) of the piriform cortex remains undamaged may be explained by the fact that projections from the ventral claustrum are directed preferentially to the caudal two thirds of the piriform cortex. Furthermore, the structural and functional heterogeneity of the piriform cortex (Litaudon *et al.* 1997) should be taken into consideration. At present the available data do not allow to explain why the medial half of the piriform field Pp 2 remains unaffected.

PV-ir neurons in the rat piriform cortex represent a subpopulation of GABAergic neurons (Kubota and Jones 1993). A decrease of PV-immunoreactivity of the neuropil and neurons in experimental animals indicates that at least some of GABAergic neurons located in the anterior piriform cortex exhibited a down-regulation of PV that may be caused by neuronal degeneration. The loss of GABAergic neurons in the anterior piriform cortex may lead to a hyperactivity in association fibers directed caudally and terminating in the posterior piriform cortex, superficial amygdaloid nuclei and rostrolateral entorhinal cortex (Haberly and Price 1978), i.e. in the structures with severe neuropathological changes in 25-day-old and adult rats. Thus a decrease in the level of inhibition in the anterior piriform cortex might be an additional factor leading to severe damage of the posterior part of the piriform cortex and adjoining structures. Decreased PV-immunostaining coincident with decreased immunostaining for the GABA-synthesizing enzyme glutamic acid decarboxylase in the neocortex was repeatedly associated with temporal lobe epilepsy (for review see DeFelipe 1999).

Decreased immunostaining for parvalbumin in the dorsal claustrum indicates that neuronal network responsible for epileptic discharge propagation is larger than was expected. Epileptiform discharges originating in the dorsal endopiriform nucleus propagate *via* direct projections to the dorsal (insular) claustrum and may lead to consequent neuronal degeneration affecting PV-IR neurons (Lipowska *et al.* 2000, Demir *et al.* 2001).

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