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Behavioral, biochemical and histological studies in a model of pilocarpine-induced spontaneous recurrent seizures

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Abstract

Although the presence of profound cognitive disturbances in lithium–pilocarpine-induced spontaneous recurrent seizures (SRS) has been well documented, much less is known about changes in emotional behavior, in this model of temporal lobe epilepsy. To that end, a lithium–pilocarpine model of SRS was used to evaluate behavior of experimental animals (SRS, non-SRS and saline-treated rats) in different tests of anxiety (open field test, fear conditioning freezing and footshock-induced ultrasonic vocalization). Flinch-jump test, allowing determination of pain threshold, was employed to confirm specificity of data from anxiety tests. Moreover, neurotransmitters' (dopamine, serotonin and their metabolites) concentration was measured in selected brain structures involved in emotional and motor processing (hippocampus, frontal cortex and striatum). Finally, different brain structures were examined histologically in order to determine structures likely to be involved in behavioral changes. It was found that SRS rats, tested in a seizure free period, revealed an increase in motor activity, and a decrease in fear-related reactions (a freezing response to the aversively conditioned context, and a spontaneous, emotion-related ultrasonic vocalization). No changes in the pain threshold were present. The activity of dopamine and serotonin systems in examined brain structures remained unchanged. The neuropathological effects were widespread and involved a loss of neurons, proliferation of astroglial cells and the presence of activated ramified and ameboid microglial cells in the hippocampus proper, piriform cortex, amygdala and lateral posterior thalamic nuclei. The obtained results suggest a prevalence of disinhibitory effects on behavior in SRS rats, as shown by the results of contextual fear and aversive vocalization tests (i.e. a release of rat behavior controlled by fear). It is conceivable that the lesions to the limbic structures involved in the origin of emotions; the hippocampus, amygdala, and piriform cortex, may underlie changes in anxiety reactions in SRS rats. These results indicate that lithium–pilocarpine-induced SRS are also accompanied by profound alteration of animal emotional behavior.

Keywords: Pilocarpine; Seizures; Monoamines; Histology; Behavior; Anxiety

1. Introduction

The lithium–pilocarpine model of chronic seizures replicates several of the features of human temporal lobe epilepsy (TLE), i.e. spontaneous recurrent seizures (SRS), hippocampal cell loss, supra- and intra-granular mossy fiber sprouting, dentate gyrus cell dispersion, and can be used as an animal tool to understand the basic mechanisms of epileptogenesis (Honchar et al., 1983; Liu et al., 1994; Mathern et al., 1995; Turski et al., 1983). A review of literature data indicates numerous publications on the lithium–pilocarpine-induced SRS, most of them concentrated on the morphological and electrophysiological aspects of this phenomenon. Surprisingly, much less work has been done on the delayed behavioral and biochemical conse-

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quences. For example, Hort et al. (1999) found that cognitive functions, tested in the Morris water maze, deteriorated during convulsion development, and preceded the appearance of spontaneous recurrent seizures. Similarly, Wu et al. (2001) reported deficits in the Morris maze and radial arm maze test in lithium–pilocarpine treated rats, accompanied by cell loss in hippocampal CA-1 area. However, there are very scarce literature data on changes in animal emotional behavior, an important aspect of TLE in humans (Marsh and Rao, 2002; Vazquez and Devinsky, 2003).

Local concentration of monoamines and amino acids has been evaluated most frequently in the acute period of lithium–pilocarpine action (Alam and Starr, 1996; Freitas et al., 2003; Smolders et al., 1997), showing multidirectional changes probably related, among others, to the acute stress-, hypoxia- and ischemia-induced release of transmitters and modulators. The only study on the chronic effects of lithium–pilocarpine treatment on hippocampal monoamines and amino acids in acute, silent and recurrent seizure periods, was published by Cavalheiro et al. (1994). It was found that 5-HT was increased in the acute period only, whereas the increased NE utilization and decreased DA metabolism were present during all phases. On the other hand, in the SRS period, there appeared increases in concentrations of all amino acids measured (GABA, glutamate and aspartate).

Given the scarcity of appropriate data, the aim of the present study was to evaluate behavioral changes in the lithium–pilocarpine-induced SRS model, in SRS, non-SRS and saline-treated control rats in different tests of anxiety (open field test, fear-conditioned freezing and footshock-induced ultrasonic vocalization) (Borsini et al., 2002; Millan, 2003; Sanchez, 2003). Flinch-jump test, allowing determination of pain threshold, was employed to confirm specificity of data from anxiety tests. The working hypothesis was that SRS are accompanied also by changes in anxiety-related behavior. Furthermore, the alterations in 5-HT and dopamine systems activity in the brain structures likely to be involved in emotional and motor processes, i.e. frontal cortex, hippocampus proper and striatum, as well as histopathological abnormalities in different brain structures were examined in parallel, to learn more about the neurobiological background of the behavioral effects.

2. Materials and methods

2.1. Animals

Forty adult male Wistar rats (weighing 200±20 g at the beginning of the experiment) were used in the study. The animals were housed two per cage in standard laboratory conditions under 12 h cycle (lights on at 6:00 a.m.) in a controlled temperature (20±2 °C) and 70% humidity. The rats were given free access to food and water. All experiments were performed between 9:00 a.m. and 3:00 p.m. Weights of the animals in all experimental groups did not differ in the end of the study. The Committee for Animal Care and Use at the Medical University in Warsaw approved all experiments. The experiments were performed in accordance with the European Communities Council Directive of 24 November 1986 (86/609 EEC).

2.2. Drugs

Lithium chloride and methylscopolamine were purchased from Sigma-Aldrich (Poland). Pilocarpine hydrochloride was obtained from FARM-IMPEX (Poland), and diazepam was obtained from POLFA Warszawa (Poland). The compounds were dissolved in saline (1 ml/kg) and administered intraperitoneally (IP).

2.3. Treatment

Pilocarpine was administered according to Glien et al. (2001). 24-h before pilocarpine treatment, lithium chloride was given at the dose of 127 mg/kg (IP). Directly before administration of pilocarpine (30 min), the rats were pretreated with methylscopolamine bromide (1 mg/kg, IP). Metylscopolamine bromide was administered as a standard procedure to counteract the peripheral cholinomimetic effects of pilocarpine, i.e. to diminish the mortality rate due to respiratory insufficiency (bronchospasm and exaggerated excretion in the bronchial tree due to cholinergic hyperactivity). Next, the rats received repeated injections of pilocarpine (10 mg/kg, IP) every 30 min until they developed convulsive seizures, at stage 4 or 5 according to Becker et al. (2000), modified after Racine (1972). Stage 4 of seizures was characterized by turn over into side position, and clonic–tonic seizures. In stage 5, rats turned into back position, and showed generalized clonic–tonic seizures. Thus, in both stages, there appeared clonic–tonic convulsions (Becker et al., 2000).

The animals received up to four injections of pilocarpine (maximum), or until they developed SE. SE was blocked after 60 min with IP administered diazepam (10 mg/kg, IP). All experimental animals received injections of 5 ml 0.9% NaCl (IP), directly after SE and twice on the following day after SE to prevent from dehydration. Subsequently, the behavior of animals was videotaped and analyzed 6 h a day (5 days a week, Monday–Friday, for 8 weeks, beginning 2 weeks after SE), for the occurrence of spontaneous recurrent seizures (SRS). After this time, a pilocarpine treated animal was considered epileptic if it presented at least one episode of SRS during the whole observation period (Glien et al., 2001; usually, an animal considered epileptic showed at least three SRS). Only generalized tonic–clonic seizures (stage 4–5 according to Becker et al., 2000) were taken into consideration. Three groups of animals were selected for further research: control-saline treated animals (animals which received lithium, methylscopolamine and saline

instead of pilocarpine, $n=9$), Pilo-rats pretreated with lithium, methylscopolamine and pilocarpine, which did not develop recurrent seizures ($n=17$), SRS-rats pretreated with lithium, methylscopolamine and pilocarpine which developed spontaneous recurrent seizures ($n=8$). Next, after selection of the 3 groups, 11 weeks after SE, the animals were subjected to several behavioral tests in the following order: open field test, freezing test, ultrasonic vocalization test, pain threshold test and finally they were sacrificed for the biochemical and histological parts of the study. The behavioral tests were separated by 1 week long intervals.

2.4. Open field test

The test was performed in a soundproof chamber under dim light and continuous white noise (65 dB). The open field apparatus consisted of two round arenas (80 cm diameter) with 30 cm high walls. Locomotor activity of rats (distance in cm), the number of central entries and the time spent in the central sector of the open field (50 cm diameter) were recorded for 10 min and analyzed with the PC-based Videomot System (TSE, Bad Homburg, Germany), as described previously (Szyndler et al., 2002a).

2.5. Contextual fear conditioning test

The fear-conditioning experiment was performed using a computerized fear-conditioning system (TSE, Bad Homburg, Germany) as described previously (Maciejak et al., 2003). Fear conditioning was performed in the experimental cage ($36\times21\times20$ cm) under constant white noise condition (65 dB). The experiment was performed during three consecutive days in the same testing box and experimental chamber. On the first day, the animals were placed separately for 2 min in a training box, for adaptation to the experimental conditions. The following day, the animal received three footshocks (stimulus: 0.7 mA, 1 s, repeated every 60 s). On the 3rd day, the freezing behavior of rats was examined for two 5 min-long time periods, in the same box. The conditioned response (freezing reaction) was recorded and analyzed by the fear-conditioning system. The freezing behavior was defined as the absence of any movements except for those necessary for respiration. The recording period was divided into two 5 min long periods, to study the time-course of a freezing response, and in this way the strength of a contextual fear.

2.6. Ultrasonic vocalization test

Polycarbonate cages ($17\times17\times17$ cm) were located on a metal grid floor connected to a shock-generator, and the whole cage was placed in a sound-protected test chamber ($45\times45\times50$ cm). Ultrasonic vocalizations were recorded by a microphone (Mini-3 Bat Detector, Noldus Information Technology) attached to the ceiling of the chamber and processed by an interface (Ultravox, Noldus Information Technology) to select 22 ± 4 kHz signals and to digitize them in an IBM compatible PC.

On the first day, each rat was placed in a test cage for a 2-min time period. On the three following days, the rats received one stimulus session daily. A stimulus session consisted of an adaptation period of 5 min to the test cage, followed by a 5 electric footshocks (500 ms, 1.0 mA) delivered via the grid floor and scrambled during 1 min, followed by a 5 min shock-free period. The experiment on the fourth day (testing phase), consisted of 5-min shock-free period, followed by one electric footshock (500 ms, 1.0 mA) and terminated with another 5-min shock-free period. The

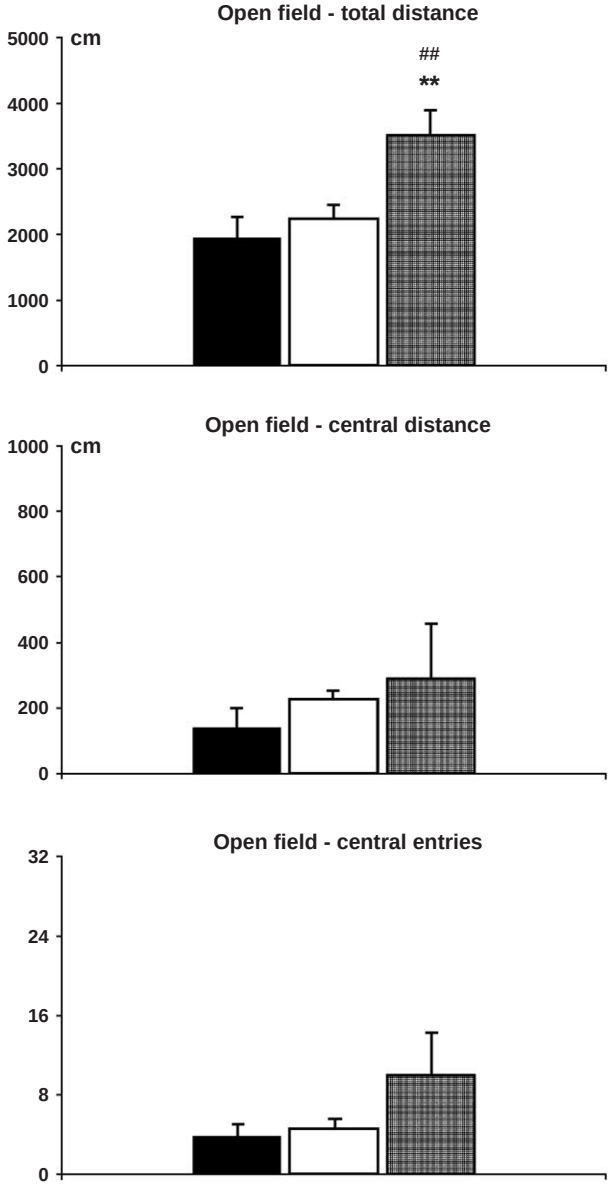

Fig. 1. The behavior of rats in the open field test. The data are shown as means ± S.E.M. Closed bars—Control, saline pretreated animals, $n=9$; open bars—Pilo, lithium-pilocarpine pretreated rats which did not develop recurrent seizures, $n=17$; gray bars—SRS, lithium–pilocarpine pretreated rats which developed recurrent seizures, $n=8$. *—differs from Control; #—differs from Pilo group. **, ##=$p<0.01$.

testing phase was repeated on the 8th day. In the 5-min long pre-shock periods, the frequency and total duration of ultrasonic vocalizations were recorded automatically, as described previously (Szyndler et al., 2002a).

2.7. Flinch-jump test

Reactivity to the footshock was evaluated in the same apparatus as used for contextual fear conditioning (Evans, 1961). The rats were placed individually into a box. Shocks were delivered to the grid floor of the test box through a shock generator. Each animal was allowed for a 3-min habituation period prior to start of a series of shocks (0.5 s), delivered at 10 s intervals. Shock intensities ranged from 0.05 to 0.85 mA in 0.05 mA increments. The flinch threshold was defined as the lowest shock intensity that elicited any detectable response. The jump threshold was defined as the lowest shock intensity that elicited simultaneous removal of at least three paws (both hindpaws) from the grid. To avoid foot damage, a cut-off threshold of 1.0 mA was established. In this way, the flinch and jump thresholds in mA were defined for each rat. The time gap between shocks was 10 s, and each animal was tested only once, as described previously (Szyndler et al., 2002a).

2.8. Biochemical analysis

14 days after the last behavioral experiment, the rats were sacrificed and biochemical analysis was performed. The brains were rapidly removed and the hippocampus, striatum and prefrontal cortex were dissected and frozen in isopenthane (−30 to −40 °C) cooled with dry ice. Serotonin (5-HT), dopamine (DA), 5-hydroxyindoleacetic acid (5-HIAA), homovanillic acid (HVA), 3,4-dihydroxyphenylacetic acid (DOPAC) concentrations in the appropriate structures were assayed by using a fully automated high performance liquid chromatography (Shimadzu, Japan) with electrochemical detection, using standard method described in detail previously (Stefański et al., 1993).

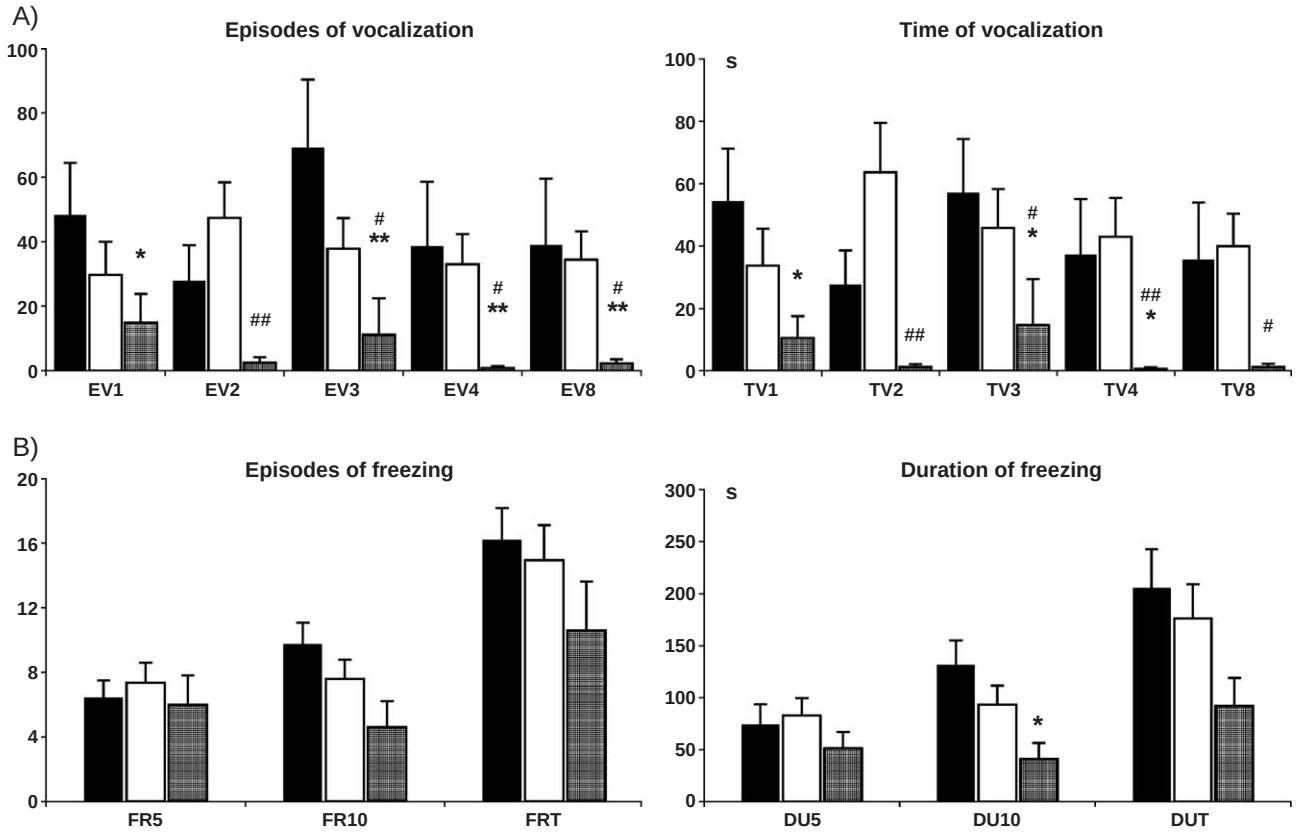

Fig. 2. The behavior of rats in the aversive ultrasonic vocalization test (A) and conditioned freezing test (B). The data are shown as means±S.E.M. Closed bars—Control, saline pretreated animals, $n=9$; open bars—Pilo, lithium–pilocarpine pretreated rats which did not develop recurrent seizures, $n=17$; gray bars—SRS, lithium–pilocarpine pretreated rats which developed recurrent seizures, $n=8$. EV1—number of episodes of ultrasonic vocalization recorded before first aversive stimulation; EV2, EV3, EV4, EV8—number of episodes of conditioned vocalization on day 2, 3, 4, 8, recorded before aversive stimulation; TV1—time of ultrasonic vocalization recorded before first foot-shock; TV2, TV3, TV4, TV8—time of conditioned vocalization on day 2, 3, 4, 8, before aversive stimulation. FR5—number of episodes of freezing behavior measured during post-conditioned session (first 5 min of the test); FR10—freezing episodes during the second 5 min of the test session (5–10 min); FRT—total number of freezing episodes (1–10 min); DU5—time of freezing during first 5 min of the test session; DU10—time of freezing during the second half (5 min) of the test; DUT—total time of freezing (1–10 min). *—differs from Control; #—differs from Pilo group. *, #=$p<0.05$; **, ##=$p<0.01$.

Table 1
Pain threshold in the flinch-jump test

Footshock reactivity threshold		
Groups	Flinch	Jump
Control	0.43±0.03	0.66±0.03
SRS	0.43±0.04	0.66±0.05
Pilo	0.40±0.02	0.59±0.03

Data are shown in mA as means±S.E.M. Control, saline pretreated animals, $n=9$; SRS, lithium–pilocarpine pretreated rats which developed recurrent seizures, $n=8$; Pilo, lithium–pilocarpine pretreated rats which did not develop recurrent seizures, $n=17$.

2.9. Histology

For the purpose of assessment by means of light microscopy, the brains were fixed in 4% buffered paraformaldehyde (pH 7.4), embedded in paraffin and cut serially at 8 μm sections. Routine staining of sections with hematoxylin-eosin (H&E) was performed. In addition, sections were subjected to immunohistochemical and histological reactions with an anti-glial fibrilary acid protein antibody (GFAP, DACO, 1:4000) and ferritin (DACO, 1:3000), and lectin from Lycopersicon esculentum (LE, Sigma, 3 μg/ml). After counterstaining with H&E, dehydration and clarification in xylene, the specimens were embedded in balsam, and examined in light microscope for analysis of neurodegenerative and neuroadaptive changes (Szyndler et al., 2002b). The following brain structures were represented on brain slices: nucleus lateralis thalami, piriform cortex, amygdala, the hippocampal formation, thalamus and hypothalamus.

2.10. Statistical analysis

The data are shown as means±S.E.M. A one-way analysis of variance (ANOVA) with repetitions (groups x days as contributing factors) was used to assess differences in the ultrasonic vocalization experiment. LSD post hoc test was performed to identify the origin of any significant differences. The data from all other experiments were checked with the help of one-way ANOVA followed by a post-hoc test (LSD). The basis of statistical decision was a significance level of 0.05.

3. Results

3.1. General findings

From among 30 rats subjected to the procedure of lithium–pilocarpine kindling of seizures, 5 animals were lost, 8 developed SRS and 17 did not show any seizure-like activity. It is noteworthy that 30% of rats with SRS are much lower than that reported previously by Glien et al. (2001). It is conceivable that the differences in a gender and strain-dependent sensitivity of rats to cholinergic stimulation may determine the percentage of rats developing SRS. The lethality rate was 16.6%, and the physical condition of the remaining animals, including those with SRS, was good, as the body weight at the end of the experiment was not different among the experimental groups (the starting weight, mean±S.E.M., for the whole cohort of animals was 200±20 g; the final weight: saline −486.25±8.49; Pilo −493.23±6.99; SRS −475.00±7.13; n.s.).

3.2. Behavioral tests

3.2.1. Open field test

In the open field test, the analysis of variance showed significant differences among groups in the parameter of total distance crossed [$F(2,31)=6.95$, $p<0.01$]. Post-hoc test revealed that SRS rats showed enhanced locomotor activity in comparison to control animals ($p<0.01$), and to the lithium–pilocarpine pretreated rats, which did not

Table 2
Concentration of monoamines and their metabolites in the brain structures

Neurotransmitter levels					
	5-HT	5-HIAA	DA	HVA	DOPAC
Hippocampus					
Control	687.77±74.71	565.77±45.97	432.66±27.36	146.22±10.20	63.11±45.75
SRS	705.62±57.67	578.12±33.48	392.50±24.63	150.12±16.25	99.75±24.37
Pilo	687.35±57.45	495.52±21.74	393.58±18.08	136.76±9.03	74.35±13.60
Striatum					
Control	1268.22±65.91	879.11±79.31	21151.22±1493.30	1207.66±120.48	3363.33±240.27
SRS	1237.62±77.46	877.75±51.26	18155.25±1860.77	867.50±94.34	2766.00±291.47
Pilo	1384.23±93.84	888.00±56.57	19112.94±1355.35	974.76±74.53	2859.70±154.44
Prefrontal cortex					
Control	1251.00±111.07	655.66±50.52	4534.44±509.86	283.33±41.96	1120.66±139.75
SRS	1609.50±91.3	756.75±50.03	5206.75±622.97	263.62±38.83	944.50±114.63
Pilo	1537.29±236.56	700.82±88.19	5215.94±932.84	301.35±53.32	1174.88±294.91

Data are shown in ng/g of wet tissue as means±S.E.M. Control, saline pretreated animals, $n=9$; SRS, lithium–pilocarpine pretreated rats which developed recurrent seizures, $n=8$; Pilo, lithium–pilocarpine pretreated rats which did not develop recurrent seizures, $n=17$.

develop recurrent seizures ($p<0.01$). Distance crossed in the central area [$F(2,31)=0.40$, $p>0.05$], and the number of entries to the central area [$F(2,31)=2.59$, $p>0.05$], in spite of some tendency to be enhanced in the SRS group, did not reach the criterion of a statistical significance (Fig. 1).

3.2.2. Contextual fear conditioning test

SRS rats showed a tendency to express fewer episodes of freezing, and shorter time of freezing behavior, however, a statistically significant difference was found in the duration of freezing behavior, in the second half of the test only [DU10, $F(2,31)=3.77$, $p<0.03$; SRS group vs. control., $p<0.05$, LSD test]. As far as the total time of freezing was concerned, the differences were not statistically significant (Fig. 2).

3.2.3. Ultrasonic vocalization test

Significant differences appeared in the number of episodes of ultrasonic vocalization [EV 1,2,3,4,8; $F(2,155)=3.42$, $p<0.05$], as well as in time of vocalization among experimental groups [TV 1,2,3,4,8; $F(2,155)=4.21$, $p<0.05$]. Post-hoc tests indicated that SRS rats showed significantly decreased spontaneous vocalization before shock (preconditioning session on the first day; episodes and time of vocalization, $p<0.05$). Vocalization on the 3rd, 4th and 8th day of experiment was also inhibited in SRS rats (episodes of vocalization, 3rd, 4th, 8th day, $p<0.01$; time of vocalization, 3rd and 4th day, $p<0.05$) (Fig. 2). Post-hoc tests indicated also that SRS rats vocalized less on the 2nd, 3rd, 4th and 8th day of experiment in comparison with the lithium–pilocarpine pretreated rats, which did not develop recurrent seizures (Fig. 2).

3.2.4. Flinch-jump test

One-way ANOVA did not reveal any significant differences in the rat flinch [$F(2,31)=0.23$, $p>0.05$], and jump reaction [$F(2,31)=0.94$, $p>0.05$] among experimental groups (Table 1).

3.3. Biochemical analysis

In the biochemical part of experiments, no significant differences in the concentration of DA, 5-HT and their metabolites were observed in the examined structures: hippocampus, striatum and prefrontal cortex (Table 2).

3.4. Histology

Light microscopy demonstrated structural abnormalities in the brains of SRS animals. Neuropathological changes were found in the dentate gyrus of the hippocampus, piriform cortex and amygdala, and the lateral posterior thalamic nuclei. The loss of neurons and proliferation of astroglial cells in hippocampus, lateral posterior thalamic nuclei and piriform cortex is shown in Fig. 3. Some of the remaining neurons appeared shrunken, pycnotic and dark

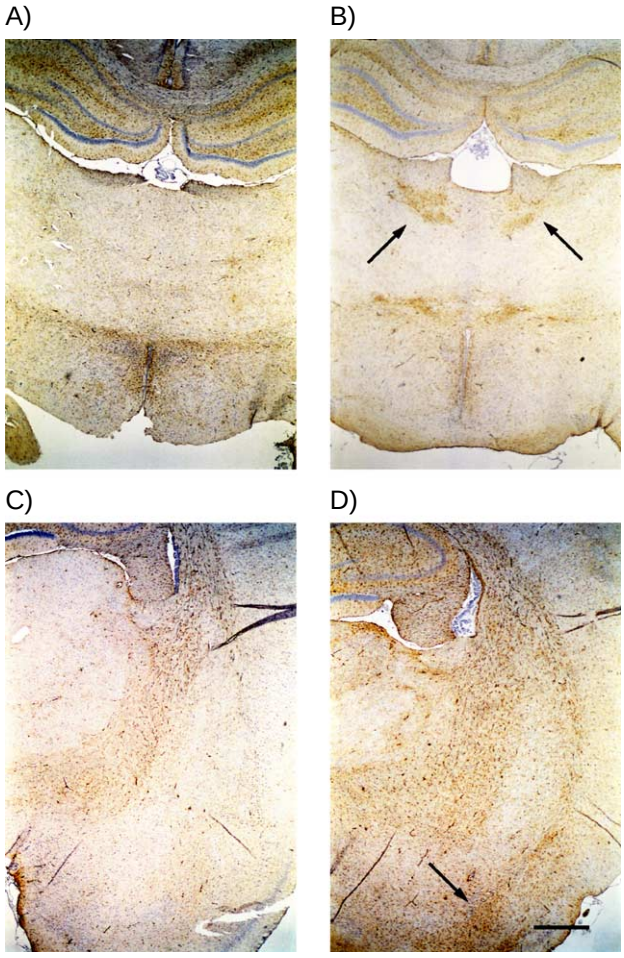

Fig. 3. (A) Control rat, the hippocampal formation, thalamus and hypothalamus (GFAP counterstaining with hematoxylin, ×20); (B) SRS rat, proliferation of astroglia in the nucleus lateralis thalami—arrows (GFAP and H×20); (C) control rat, piriform cortex and amygdala (GFAP counterstaining with hematoxylin, x20); (D) SRS rat, piriform cortex, proliferation of astroglial cells—an arrow (GFAP counterstaining with hematoxylin, ×20). Bar indicates 500 μm.

(i.e. characteristic of ischemia-like changes) (Fig. 3). The astroglial cells were observed as dark cells with long and thin processes, especially in the granular layer of dentate gyrus of hippocampus and piriform cortex (Fig. 4). Activated ramified and ameboid/macrophages-like microglial cells were present in all regions with structural abnormalities (Fig. 4).

4. Discussion

The obtained results indicate a characteristic profile of behavioral changes in SRS rats. In the open field test, the SRS rats were more active (locomotor activity), in comparison with control animals (saline and Pilo groups). In the aversive ultrasonic vocalization test (USV), SRS rats vocalized less frequently and intensively already during the first, pre-conditioning session, and on some of the subse-

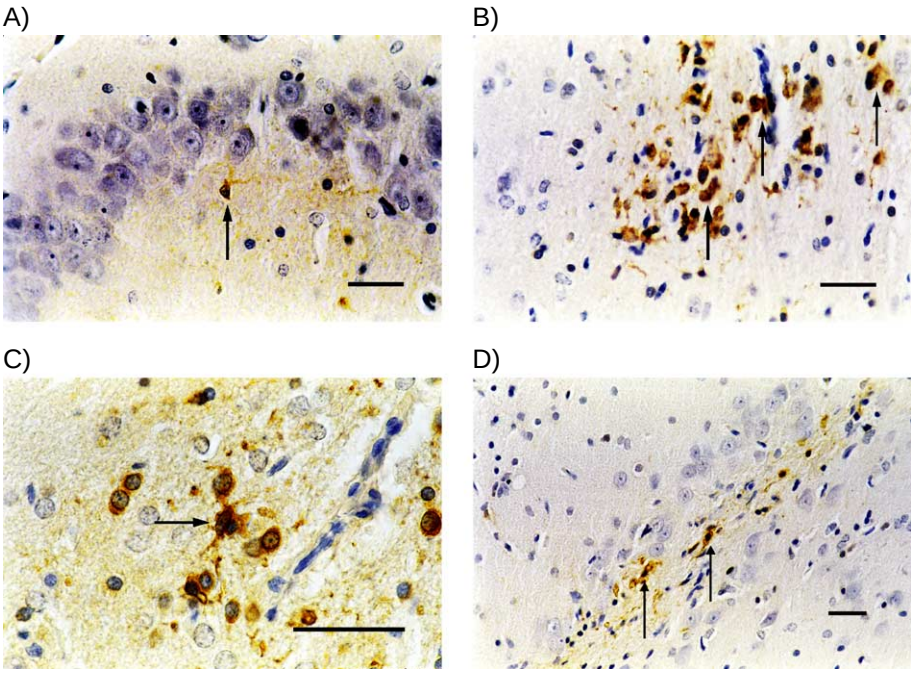

Fig. 4. SRS rats. (A) Dentate gyrus, pycnotic neurons and microglial cells—an arrow (Ferritin and H, x500); (B) nucleus lateralis thalami, activated microglia—arrows (Ferritin and H, ×500); (C) the hippocampal formation, activated microglia—arrow (Ferritin and H, ×900); (D) piriform cortex, activated microglia—arrows (Ferritin and H, ×320). Bar indicates 20 μm.

quent training and retention sessions as well. In the conditioned fear test, the behavior of SRS animals was significantly less inhibited by aversively conditioned context. No difference in the pain threshold among experimental groups was found, thus proving the selectivity of reported changes in rat emotional behavior. Altogether, the obtained results are in some important aspects similar to those previously found in pentylenetetrazol (PTZ)-kindled rats (Szyndler et al., 2002a), and indicate a decrease in fear-related reactions: freezing and ultrasonic vocalization. Since PTZ-induced kindled seizures may also serve as a model of TLE, the similarity of behavioral effects allows for a more general conclusions about the central processes accompanying TLE. Both SRS and PTZ-kindled animals showed less spontaneous vocalization, less freezing behavior examined 24 h after fear conditioning and no differences in the flinch and jump reaction (i.e. in general reactivity to the external stimulation). However, contrary to PTZ-rats, SRS animals were more active in the open field, and therefore one cannot exclude a contribution of locomotor disinhibition to the results of the conditioned freezing test. Neuropathological changes in the hippocampus, the brain structure involved in spatial memory, might also contribute to the results of the contextual fear test. The effects in the USV test can be interpreted more univocally, given the much lesser influence of animal motility in this model of anxiety. SRS rats vocalized less in response to the novel environment in the frequency band (22.0 kHz), recognized as specifically linked to the expression of emotions (Borsini et al., 2002; Knutson et al., 2002; Sanchez, 2003). Thus, both specific and non-specific factors might contribute to the changes in emotional behavior of SRS rats.

In the biochemical part of the experiment, no changes of 5-HT and DA systems activity were observed in SRS animals, in the three brain structures examined. These results do not replicate biochemical effects found in PTZ-kindled rats (Szyndler et al., 2002a). In those animals, there appeared a significant decrease in 5-HT turnover rate in the hippocampus and in the prefrontal cortex, and a significant decrease in HVA/DA metabolic ratio in the striatum, in the period of time between fits of seizures (Szyndler et al., 2002a). It was also previously reported that in SRS period, following pilocarpine pretreatment, noradrenaline level was increased, dopamine content decreased and serotonin concentration unchanged, in the rat hippocampus (Cavalheiro et al., 1994). Utilization rate measurement of monoamines showed increased noradrenaline- and decreased dopamine metabolism, in this stage of seizures development (Cavalheiro et al., 1994). The inconsistent changes in 5-HT and DA systems indicate that their tonic activity, measured in a silent period between fits of seizures, probably does not significantly contribute to the behavioral effects in the studied model of epilepsy, or that their dysfunction is compensated in a non-challenging situation (i.e. resting conditions). Accordingly, we did not recently observe changes in the monoamines and their metabolites in the rat striatum, measured between fits of convulsions, in the PTZ model of kindled seizures (in vivo microdialysis, data not published). Importantly, monoaminergic neurons have been repeatedly shown to be potently activated at the time

proximal to the tonic/clonic convulsions, probably playing a role in the control of seizures spread (see Introduction) (Alam and Starr, 1996; Naffah-Mazzacoratti et al., 1996).

The histological changes reported in the present paper are in agreement with previously published papers (Klitgaard et al., 2002; Mathern et al., 1995; Turski et al., 1983), and are also similar to the neuropathological effects found in PTZ-kindled animals (Franke and Kittner, 2001; Szyndler et al., 2002b). They involved a loss of neurons in the hippocampal formation and other brain structures, including amygdala and piriform cortex, a proliferation of astroglial cells, and a presence of activated ramified and ameboid microglial cells, with some of the remaining neurons shrunken and pycnotic. These data illustrate a widespread pattern of histopathological abnormalities accompanying SRS (Liu et al., 1994; Scharfman, 2002). The neurodegenerative effects of SRS in the parts of the limbic system contributing to the emotional memory processing; hippocampus, amygdala and piriform cortex, may explain also the disinhibition of animal behavior in the anxiety tests. Importantly, the reported histopathological changes are similar to human pathology in the TLE (cf. Kalynchuk, 2000; Scheepers and Kerr, 2003; Tisher et al., 1993).

The intrinsic mechanisms of behavioral effects of SRS may also involve a concomitant activation of inhibitory processes related to emotion regulation as represented, for example, by an increase in hippocampal GABA levels (Cavalheiro et al., 1994). However, the presented data suggest a prevalence of disinhibitory effects on behavior in SRS rats, as shown by the results of the contextual fear and aversive vocalization tests (i.e. a release of rat behavior controlled by fear). The behavioral effects were accompanied by histopathological but not biochemical changes in the brain structures involved in the control of emotional input and memory (hippocampus, amygdala and piriform cortex).

In sum, the obtained results indicate that lithium–pilocarpine-induced SRS are also accompanied by profound changes in animal emotional behavior.

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