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Effects of pentylenetetrazol-induced kindling of seizures on rat emotional behavior and brain monoaminergic systems

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Abstract

The influence of pentylenetetrazol (PTZ)-induced kindling of seizures on the rat emotional behavior, the brain monoamine turnover rate measured in vitro, and correlation between behavioral and biochemical parameters, were examined in rats. The repeated administration of PTZ (35 mg/kg, ip) evoked kindled seizures in rats (Stage 4 or 5 of clonic–tonic convulsions—maximum). PTZ kindling caused selective changes in the rat emotional behavior, present in some models of anxiety only (a decreased freezing time in the conditioned freezing test and a decreased spontaneous and aversively conditioned ultrasonic vocalization). Simultaneously, PTZ kindling decreased the concentration of homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA) in the prefrontal cortex, decreased the DA (HVA/DA ratio) turnover rate in the striatum, and inhibited the serotonin (5-HT) metabolism (5-HIAA/5-HT ratio) in the hippocampus and the prefrontal cortex. Correlations between dopamine (DA) or 5-HT regional metabolic rates in brain structures and animal behavior were either abolished or reversed in PTZ-kindled animals. It is concluded that both DA and 5-HT systems contribute to the emotional effects of PTZ-induced kindling of seizures. The hypothesis is put forward that PTZ kindling-induced inhibition of the serotonergic innervation may lead to the compensatory increase in 5-HT_{1A} receptors in the dentate gyrus of the hippocampus, thus evoking the anxiolytic-like changes in animal behavior.

Keywords: Pentylenetetrazol; Kindling; Seizures; Anxiety; Monoamines; Rats

1. Introduction

A chemically or electrically induced kindling is a model of human epilepsy. The neural mechanisms involved in post-ictal behavioral and biochemical changes in the central nervous system activity remain to be elucidated. Pentylenetetrazol (PTZ) is often used experimentally to induce seizures in animals. This noncompetitive antagonist blocks GABA-mediated Cl^- influx through an allosteric interaction in the Cl^- channel, thus leading to neuronal membrane depolarization, propagation and maintenance of a seizure activity. PTZ is also a known anxiogenic drug, very often used in animal models of anxiety. Convulsions have both short- and long-term effects on animal behavior and brain neurotransmitter system activity. Chemically and electrically induced seizures release neurotransmitters in the brain, leading to the transient post-ictal depletion in the indoleamine and catecholamines stores (Shouse et al., 2001; Yokoi et al., 1986). Simultaneously, kindling is followed by some behavioral effects like antinociception, decreased learning and memory, and anxious-like behavior (Becker et al., 1994; Coimbra et al., 2001; Genkova-Papazova et al., 2000; Kalynchuk, 2000; Rossler et al., 2000). On the other hand, PTZ-induced kindling was reported to be associated with anxiolytic-like effects (File et al., 1996; Stephens et al., 2001). However, no simple correlation between the changes in the hippocampal release of noradrenaline (NA) and

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behavioral effects of PTZ in the elevated plus maze was found (File et al., 1987). After repeated administration of electroconvulsive shocks (ECS), a different pattern of behavioral and biochemical effects was observed. Twenty-four hours after the last ECS treatment, K^+-evoked release of serotonin (5-HT) and NA from cortical slices of the rat brain was significantly inhibited (Green et al., 1987). In human studies, the levels of 5-HT, dopamine (DA), NA, 5-hydroxyindoleacetic acid (5-HIAA), and homovanillic acid (HVA) were higher in the focus (spiking) in comparison to the nonfocus (nonspiking) regions of the temporal neocortex of 20 patients with intractable complex pattern seizures (Pintor et al., 1990). Similarly, 2 h after complex partial seizures or generalized tonic–clonic seizures, concentrations of NA and HVA in the cerebrospinal fluid (CSF) were significantly higher than interictal concentrations or control subjects' levels (Devinsky et al., 1992). These data indicate that the observed behavioral changes may be due to the modification in the synthesis and release of monoamines, caused by altered synaptic regulatory processes, which can occur as a result of neuronal loss, gliosis, or neuronal sprouting.

The aim of the present study was to perform a detailed analysis of some behavioral and biochemical effects of PTZ-induced kindling of seizures in rats. The behavior of fully kindled animals was analyzed in several models of anxiety-like reactions, including the open-field test, the Vogel test, the ultrasonic vocalization test, and the contextual fear conditioning. The specificity of PTZ-induced emotional effects was checked in the step-down passive avoidance test, and the flinch–jump test of the pain sensitivity. Since kindling is a continuous process, and the animals differ in the extent of seizure development, and there are no animals expressing exactly the same degree of kindling, it seemed that the only logical solution to this problem was to apply a strict criterion of seizure development (the fourth or fifth stage of seizures, demonstrated as full clonic–tonic convulsions). Only such homogenously kindled animals can be studied and compared with another homogenous group, i.e., the control, not kindled animals. Moreover, the effects of kindling on monoamine levels and turnover rates were examined in some brain areas. Finally, a correlative analysis between behavioral and biochemical changes was performed. It is considered that such comparison could provide us with new important information. This may also help to characterize better the mechanisms of central effects of kindling, and lead to better understanding of emotional disturbances observed in epileptic patients.

2. Materials and methods

2.1. Animals

Adult male Wistar rats ($n=40$), 12 weeks old, and 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. The weight of kindled and control animals did not differ at the end of the experiment (Fig. 1C). All experiments were approved by the Committee for Animal Care and Use at the Medical University in Warsaw.

2.2. Drugs and treatments

The animals received repeated injections of PTZ—kindled rats ($n=24$) or saline—control rats ($n=16$). The drug was given in a volume of 1 ml/kg of saline. PTZ was injected intraperitoneally at a subconvulsive dose of 35 mg/kg three times a week (Monday, Wednesday, Friday). After each injection, the rats were placed singly in isolated transparent Plexiglass cages and were observed for 30 min. The intensity of convulsions was registered according to a six-point scale: 0 (*no response*), 1 (*ear and facial twitching*), 2 (*myoclonic jerks without rearing*), 3 (*myoclonic jerks, rearing*), 4 (*turn over into side position, clonic–tonic seizures*), 5 (*turn over into back position, generalized clonic–tonic seizures*) (Becker et al., 2000). Control rats received injection of saline and were kept isolated in the same cages as PTZ-kindled rats for 30 min. Animals considered kindled exhibited Stage 4 or (and) Stage 5 seizures on two consecutive trials. The development of kindling is shown in Fig. 1B. After obtaining the convulsive criterion for the remaining period of time, the rats were injected with PTZ once a week (Friday). Behavioral tests were performed 3–5 days after the preceding PTZ administration (Fig. 1A).

2.3. Contextual fear-conditioning test

The test was performed in two boxes [$30\times30\times60$ (height) cm] made of Plexiglass, with three opaque walls, with a grid floor made of stainless steel bars connected to a shock generator. The boxes were cleaned after each trial with 95% ethanol. The experiment was performed during three consecutive days in the same testing boxes 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 after the animals were placed in the box, they were observed and videotaped for 5 min, via a short-circuit television, for spontaneously occurring freezing behavior (baseline freezing). Immediately afterwards, the animals received three footshocks (stimulus: 0.7 mA, 150/300 ms, repeated every 60 s). The animals were removed from the testing boxes 3 min after the last shock. On the following day, the freezing behavior of rats was examined for two consecutive, 5-min-long periods, in the same box. The conditioned response was recorded with the help of a video camera for a later

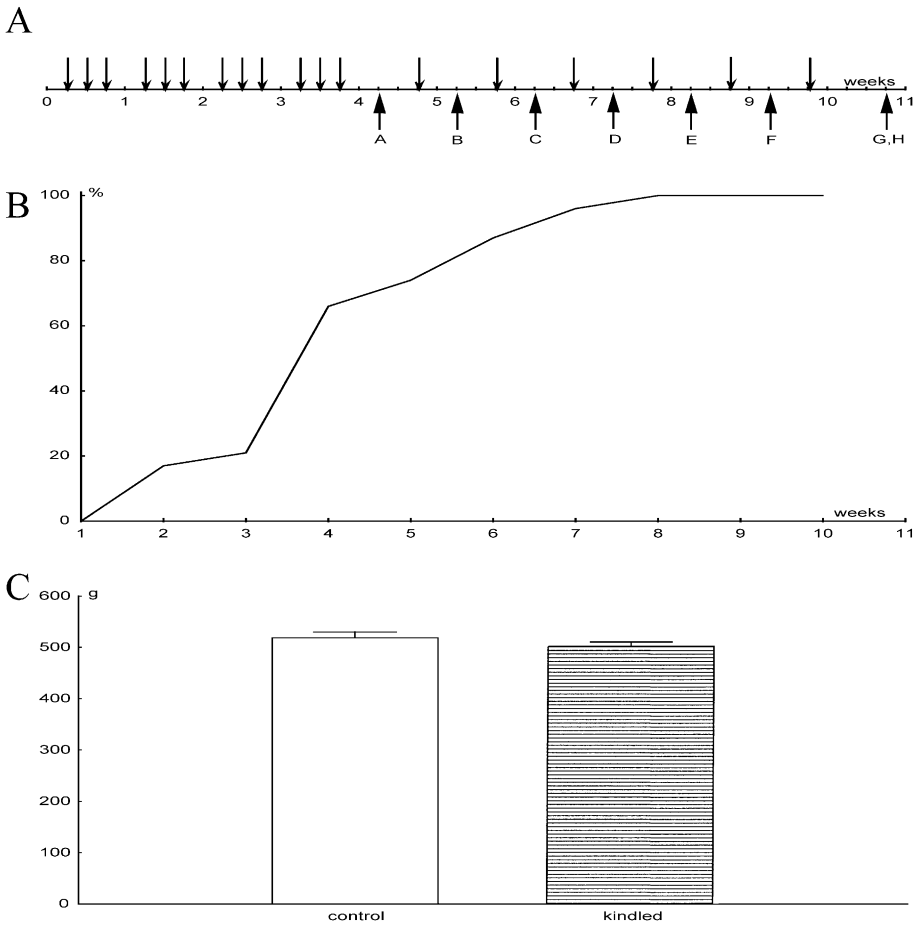

Fig. 1. (A) Schedule of the experiment. Upper arrows mark the injections of PTZ, lower arrows indicate the time of successive experiments. A—contextual fear test; B—open-field test; C—Vogel's test; D—step-down passive-avoidance test; E—flinch–jump test; F—ultrasonic vocalization test; G—measurement of body weight and biochemical analysis. (B) Kindling development. The figure shows the percentage of rats with the fourth or fifth stage of convulsions, across 11 weeks of PTZ administration. (C) The weight of animals at the end of the experiment. The data are shown as means ± S.E.M. Open bars—control rats; striped bars—kindled rats.

analysis of the freezing reaction. Freezing behavior was defined as the absence of any visible body movements except for those required for respiration, and it could be clearly separated from resting behavior characterized by small movements of the whiskers, closed eyes, and relaxed posture of the body (Fanselow, 1980). An experimenter unaware of group membership performed the behavioral observation.

2.4. Open-field test

The test was performed in a soundproof chamber under dim light and continuous white noise (65dB). The open-field apparatus consisted of two separate, round arenas (80 cm diameter) with 30-cm high walls. Open-field behavior of pairs of rats (examined separately): locomotor activity, the number of central entries, and the time spent in the central sector of the open field (50 cm diameter), was recorded on video tape, and then the image was analyzed by PC-based Videomot System, tracking the position of an animal (software—TSE, Bad Homburg, Germany).

2.5. Vogel's conflict test

The test was made in four boxes [30 × 30 × 60 (height) cm], with a grid floor made of stainless steel bars. A water-drinking tube was mounted on the wall of cages. An electric shock generator was connected with the grid floor and the metal end piece of the drinking tube. Through a hole in the wall the rats had access to a stainless steel drinking tube connected with a shock device. The grid floor comprised the other pole to complete the shock circuit through the rat body. The rats were prehabituated for 4 days. During the first 2 days, animals were deprived of water 23 h daily in the home cages. During the following 2 days, the subjects were placed separately in the experimental cages for 15 min without delivery of electric shocks, and the amount of drunk water was recorded (pretest session). Subsequently, the rats were allowed to drink water in their home cages for 45 min. After training, the drinking of water for all animals usually stabilized. The animals that did not reach the criterion of spontaneous drinking (1 ml/15 min) were

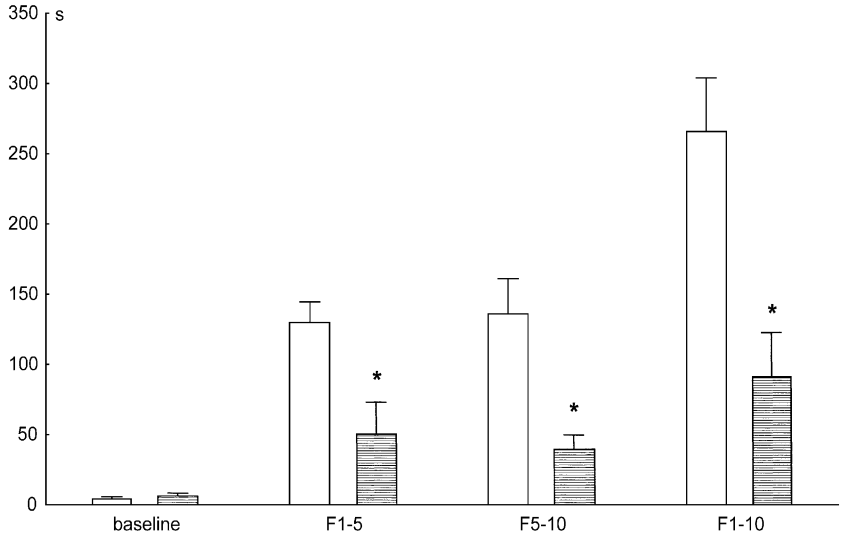

Fig. 2. The effect of PTZ kindling on rat freezing behavior in the contextual fear test. The data are shown as means ± S.E.M. Ordinate: duration of freezing behavior measured during preconditioning session (baseline); postconditioning session (the first 5 min of the test, F1–5; the second 5 min of the test, F5–10; and the whole test, F1–10). Open bars—control rats ($n = 10$); striped bars—kindled rats ($n = 5$). * Differs from control, * $P < .05$; Mann–Whitney U test.

rejected from the experiment. On the fifth day, the control rats and kindled rats were placed separately in the cages, and the electric impulses were delivered in 4-s long trains, with the intervals lasting an average of 5 s (4, 2, 5, 8, and 6 s). Shock current was set at 0.4 mA. The amount of consumed water during the 15-min test session was considered a measure of the conflict behavior (test session).

2.6. Step-down passive avoidance

The test was performed in one box [30 × 30 × 60 (height) cm] made of Plexiglass, with a grid floor made of stainless steel bars connected to a shock generator. The box was cleaned after each trial with 95% ethanol. The experiment was performed during two consecutive days in the same testing box and experimental chamber. After adaptation, the

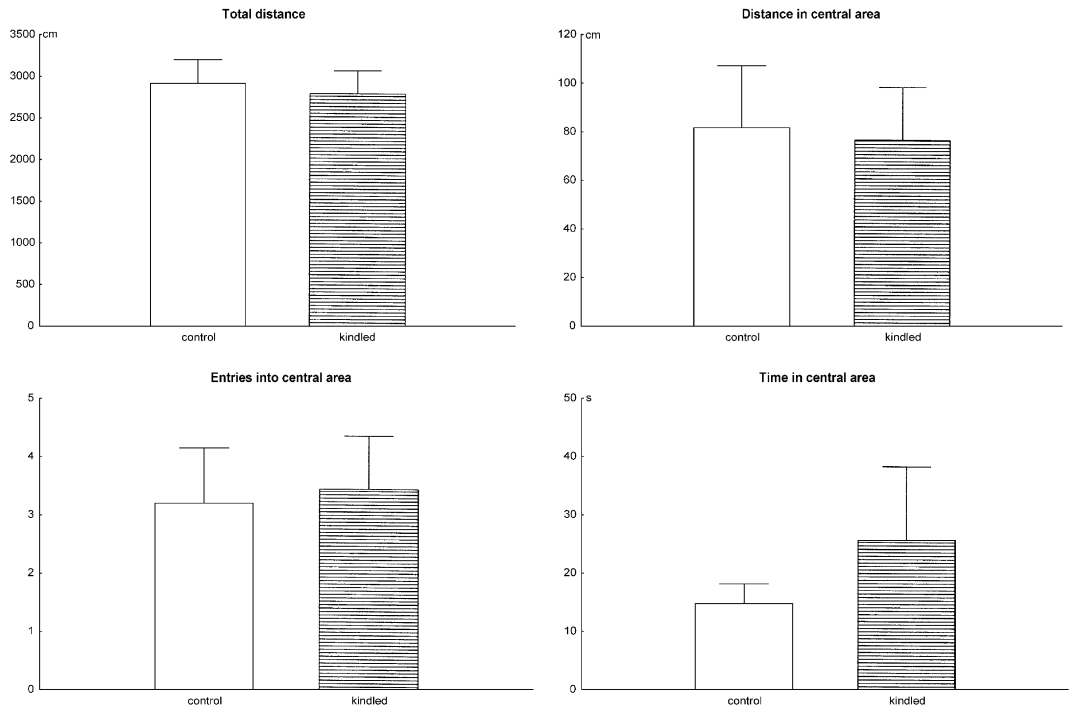

Fig. 3. The behavior of PTZ-kindled rats in the open-field test. The data are shown as means ± S.E.M. Open bars—saline-injected rats ($n = 16$); striped bars—kindled rats ($n = 16$); Mann–Whitney U test.

animals were placed in the experimental box on a wooden platform ($5 \times 10 \times 15$ cm) and time of spontaneous step down was measured in seconds (latency 1). Immediately afterwards, rats received electric shock (0.8 mA, 2 s). The animals that did not reach the criterion of a spontaneous step-down latency of 15 s were rejected from the experiment. After 15 min, the procedure was repeated and time of step down was measured with a cutoff of 180 s (latency 2). On the following day, time of spontaneous step down from the platform was measured for the third time (latency 3).

2.7. Flinch–jump test

The test was performed in the footshock boxes used in the part of the experiment on contextual fear conditioning. The rats were placed individually into the box. Shocks were delivered to the grid floor of the test box through a shock generator. After a 3-min period of habituation to the test box, shock titrations were continued upwards and downwards in a stepwise manner (0.05 mA, 0.05–0.85 mA range) depending upon responsiveness of the rat. 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, the cutoff of 1.0 mA was established. In this way, the flinch and jump thresholds in milliamperes were defined for each rat. The time gap between shocks was 10 s, and each animal was tested only once.

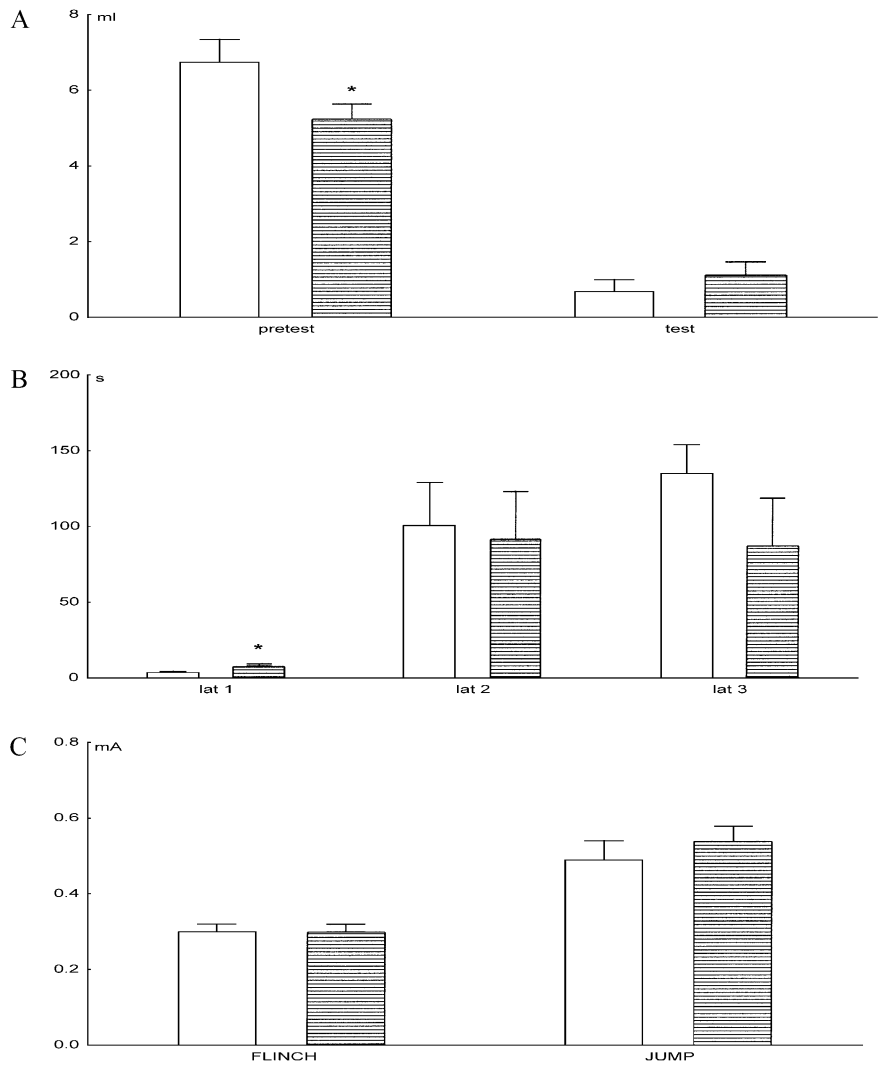

Fig. 4. The effects of PTZ kindling on rat punished water consumption in the Vogel test (A), step-down latency in the step-down passive-avoidance test (B), pain perception in the flinch–jump test (C). The data are shown as means ± S.E.M. Ordinate: the water intake in milliliters (ml), the step-down latency in seconds (s), and the shock threshold in milliamperes (mA). Pretest—spontaneous drinking during a 15-min long session; test—punished drinking during a 15-min long session, LAT1—latency 1; LAT2—latency 2; LAT3—latency 3. Open bars—control rats; striped bars—kindled rats. The number of rats in each group varied from 7 to 17. * Differs from control, * $P < .05$; Mann–Whitney U test.

2.8. Ultrasonic vocalization test

Polycarbonate cages [17 × 17 × 40 (height) 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 ($W = 45$ cm, $L = 45$ cm, $H = 50$ 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 the test cage for a 2-min period. On the three following days, the rats received one stimulation session daily. A stimulus session consisted of an adaptation period of 5 min in the test cage, followed by five electric footshocks (500 ms, 1.0 mA) delivered via the grid floor and scrambled during a 1-min time period, followed by a 5-min shock-free period. The experiment on the fourth day (testing phase), consisted of a 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 testing phase was repeated on the eighth day. In both 5-min periods, the frequency and total duration of ultrasonic vocalizations were automatically recorded.

2.9. Biochemical analysis

Seven days after the last PTZ administration, the rats were killed 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. 5-HT, NA, DA, 5-HIAA, HVA, 3,4-dihydroxyphenylacetic acid (DOPAC) concentrations in the appropriate structures were assayed by using a fully automated high-pressure liquid chromatography system (Shimadzu, Japan) with electrochemical detection, using the standard method described in detail previously (Stefański et al., 1993). The turnover rates of monoamines were calculated as the proportion of HVA to DA (DA turnover rate) and 5-HIAA to 5-HT (5-HT turnover rate).

2.10. Statistical analysis

The data are shown as means ± S.E.M. The distribution of the results was checked with the help of the Kolmogorov–Smirnov test, and because in some cases nonnormal distribution was found, the data were analyzed by the nonparametric Mann–Whitney U test. The data involving multiple comparisons (ultrasonic vocalization test) were checked using two-way ANOVA with repetitions, followed by post hoc LSD test. The biochemical data were analyzed by Student's t test. The correlation between behavioral and biochemical effects of kindling, obtained from the same animal, was performed using the Pearson's r correlation test. The confidence limit of $P < .05$ was considered statistically significant.

3. Results

During the whole experiment, three animals died: two from the control and one from the PTZ group. The remaining animals did not differ with respect to body weight and

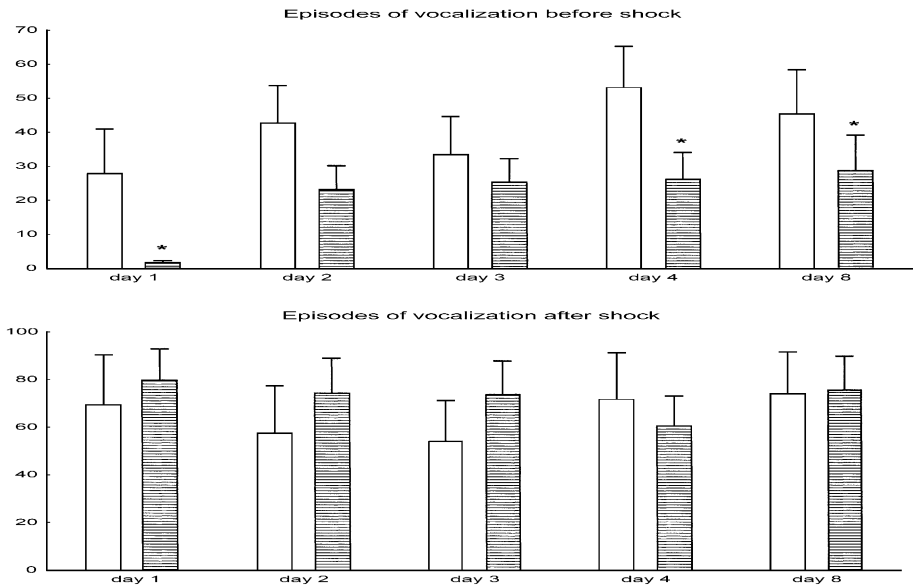

Fig. 5. The effects of PTZ kindling on rat vocalization in the conditioned ultrasonic vocalization test. The data are shown as means ± S.E.M. Ordinate: the number of episodes of vocalization measured before first shock (Day 1—baseline vocalization); episodes of vocalization measured before shock on Days 2, 3, 4, and 8 (conditioned vocalization); episodes of vocalization measured after shock on Days 1, 2, 3, 4, and 8 (a direct reaction to the stressor). Open bars—control rats ($n = 13$); striped bars—kindled rats ($n = 24$). * Differs from control, * $P < .05$; two-way ANOVA.

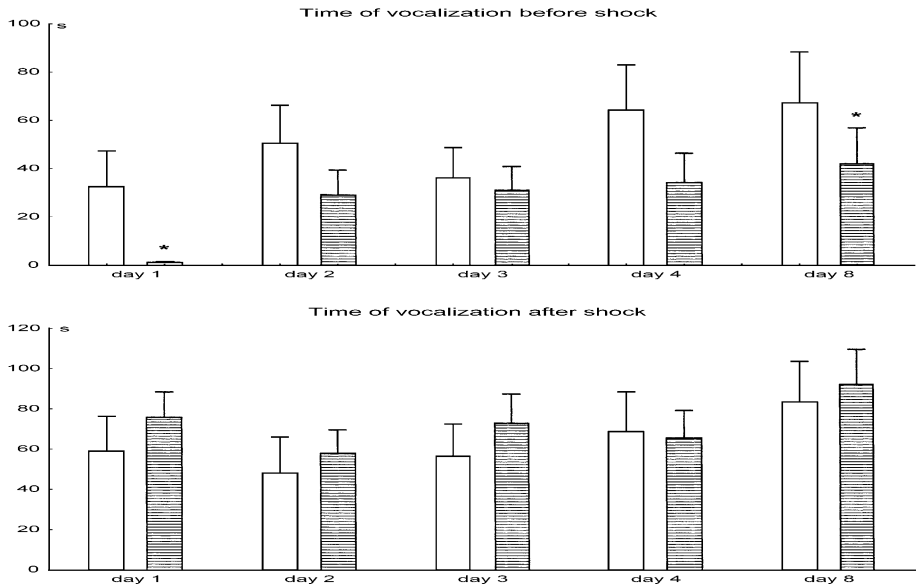

Fig. 6. The effects of PTZ kindling on rat vocalization in the conditioned ultrasonic vocalization test. The data are shown as means ± S.E.M. Ordinate: the time of vocalization measured before first shock (Day 1—baseline vocalization); time of vocalization measured before shock on Days 2, 3, 4, and 8 (conditioned vocalization); time of vocalization measured after shock on Days 1, 2, 3, 4, and 8 (a direct reaction to the stressor). Open bars—control rats ($n = 13$); striped bars—kindled rats ($n = 24$). * Differs from control, * $P < .05$; two-way ANOVA.

general physical appearance (Fig. 1C). All kindled animals developed seizures, but at different time points (Fig. 1B).

Rats which received chronic injections of PTZ did not show any statistically significant difference in time of spontaneously occurring freezing reaction, evaluated for 5 min before fear conditioning ($U = 12.0$, $P > .05$) (Fig. 2). However, PTZ rats showed significantly less freezing behavior examined 24 h after fear conditioning during first interval (5 min) ($U = 6.0$, $P < .05$); during second interval (5 min) ($U = 7.0$, $P < .05$) as well as during the whole 10 min of experiment ($U = 5.0$, $P < .05$).

In the open-field test, the behavior of kindled rats did not differ from the control group (Fig. 3). Total distance crossed and distance in the central area were the same in both examined groups ($U = 127.0$, $P > .05$ and $U = 123.0$, $P > .05$, respectively). Also the number of entries to the central area ($U = 117.5$, $P > .05$) and the total time spent in the central area ($U = 127.0$, $P > .05$) were similar in both group of animals.

In the Vogel conflict test, kindled rats exhibited similar inhibition of punished water consumption in comparison to control rats ($U = 54.5$, $P > .05$), but they differed with respect to baseline water intake ($U = 31.5$, $P < .05$) (Fig. 4).

PTZ-kindled rats did not reveal any significant differences in a short-term (step-down latency 15 min after shock) ($U = 79.5$, $P > .05$) and long-term memory (step-down latency 24 after shock) ($U = 68.0$, $P > .05$), in the passive-avoidance test, but there occurred an increase in a spontaneous, pretest, step-down latency ($U = 53.0$, $P < .05$) (Fig. 4).

Mann–Whitney U test did not reveal any significant differences in the rat's flinch ($U = 151.5$, $P > .05$) and jump reactions ($U = 130.5$, $P > .05$) to painful stimulus between both experimental groups (Fig. 4).

Table 1
The effect of kindling on the concentration of monoamines and their metabolites (ng/g of tissue) in the brain structures

	DA	HVA	DOPAC	NA	5-HT	5-HIAA
Prefrontal cortex						
Control ($n = 12$)	1099.58 ± 107.4	155.25 ± 13.7	262.83 ± 16.5	644.08 ± 55.4	663.25 ± 41.1	481.66 ± 23.7
Kindled ($n = 18$)	981.50 ± 41.8	114.88 ± 7.2 **	312.00 ± 35.2	687.00 ± 42.2	623.38 ± 16.8	391.83 ± 15.2 **
Hippocampus						
Control ($n = 12$)	24.5 ± 10.3	2.58 ± 1.7	204.08 ± 46.2	498.58 ± 39.7	240.75 ± 34.5	303.41 ± 24.6
Kindled ($n = 21$)	12.42 ± 2.4	2.76 ± 1.6	336.66 ± 68.4	528.85 ± 40.1	262.38 ± 27.0	274.09 ± 20.3
Striatum						
Control ($n = 12$)	7731.83 ± 977.9	617.08 ± 87.37	216.08 ± 19.7	267.75 ± 27.2	388.66 ± 49.1	482.91 ± 49.6
Kindled ($n = 18$)	7488.77 ± 708.0	500.67 ± 59.62	274.66 ± 34.8	293.83 ± 21.9	355.66 ± 36.9	383.83 ± 34.6

The data are shown as the mean ± S.E.M., n = number of rats.
** $P < .01$.

Kindled rats differed from controls in the test of ultrasonic vocalization. Preshock number of episodes of vocalization was decreased on the fourth and eighth day of the experiment [episodes of vocalization, $F(4,140)=2.454$, $P<.05$]. Similarly, preshock time of vocalization differed on the eighth day of the experiment [time of vocalization, $F(4,140)=4.795$, $P<.05$]. Post hoc test indicated that rats in the kindled group showed significantly less spontaneous vocalization (episodes of vocalization, $P<.05$; time of vocalization, $P<.05$). Vocalization on the first day of the experiment (preconditioned) was also inhibited in kindled rats (episodes of vocalization, $P<.05$; time of vocalization, $P<.05$). Both groups of animals did not show any significant differences in vocalization after shock, only a weak tendency in kindled rats to have more episodes of vocalization and to increase the time of vocalization occurred (Figs. 5 and 6).

Repeated injection of PTZ caused a significant decrease in concentrations of 5-HIAA ($t=3.35$, $df=28$, $P<.01$), and HVA ($t=2.83$, $df=28$, $P<.01$) in the prefrontal cortex. No significant differences were observed in the remaining structures (Table 1).

Repeated injections of PTZ caused a significant decrease in 5-HT turnover rate (5-HIAA/5-HT) in the hippocampus ($t=2.321$, $df=31$, $P<.05$) and in the prefrontal cortex ($t=3.449$, $df=28$, $P<.01$). The changes in 5-HT metabolism in the striatum were not significant, but the trend in the same direction ($t=1.450$, $df=28$, $P>.05$) was present (Fig. 7). DA turnover rate (HVA/DA) also changed in kindled animals. A significant decrease in HVA/DA coefficient was recorded in the striatum ($t=2.784$, $df=28$, $P<.01$) (Fig. 7).

Rat's behavioral parameters examined in open field, contextual fear conditioning, conditioned ultrasonic vocal-

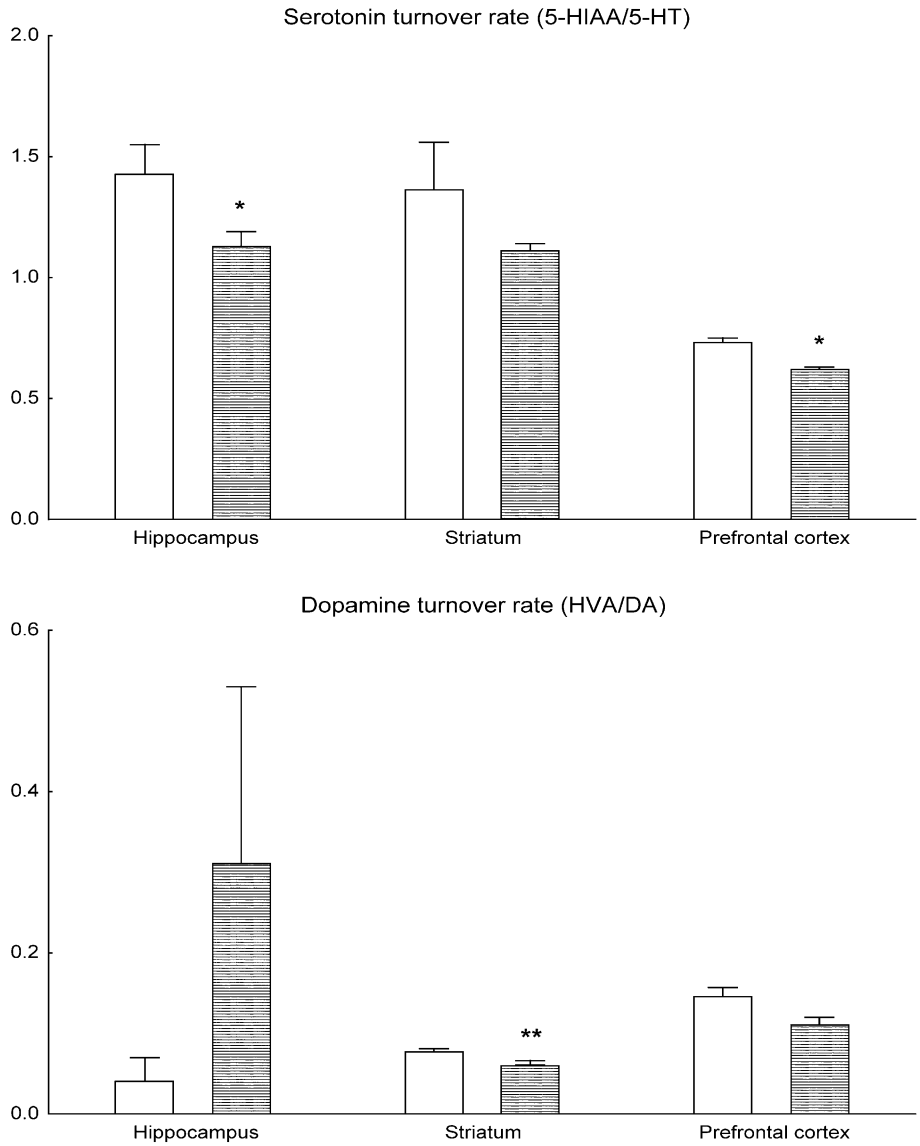

Fig. 7. The effects of PTZ kindling on rat 5-HT and DA turnover rate in brain structures. The data are shown as means ± S.E.M. Open bars—saline-injected rats, striped bars—kindled rats; * Differs from control, * $P<.05$; Student's t test.

Table 2
Correlations between behavioral parameters and the monoamines' metabolism in the prefrontal cortex, hippocampus, and striatum

	5-HIAA/5-HT (control)	5-HIAA/5-HT (kindled)	HVA/DA (control)	HVA/DA (kindled)
Prefrontal cortex				
OFT (Dyst C)	−.70; *P*=.005	−.13; *P*=.64	−.33; *P*=.23	−.34; *P*=.21
OFT (Entries)	−.68; *P*=.006	−.006; *P*=.82	−.34; *P*=.23	−.37; *P*=.16
OFT (Time)	−.72; *P*=.003	−.17; *P*=.54	−.39; *P*=.16	−.25; *P*=.35
USV (TV8-1)	+.18; *P*=.51	+.30; *P*=.15	+.53; *P*=.04	−.007; *P*=.97
Hippocampus				
OFT (Time)	−.35; *P*=.21	−.59; *P*=.02	−.22; *P*=.56	+.52; *P*=.06
USV (EV1-1)	−.04; *P*=.87	+.55; *P*=.006	−.18; *P*=.63	+.02; *P*=.91
USV (TV1-1)	−.08; *P*=.76	+.62; *P*=.001	−.14; *P*=.70	−.06; *P*=.81
Striatum				
OFT (Dyst T)	+.06, *P*=.83	+.12; *P*=.64	−.34; *P*=.22	−.56; *P*=.02
CFT (F1−5)	−.19, *P*=.71	−.13; *P*=.66	−.81; *P*=.04	+.45; *P*=.11
CFT (F5−10)	−.41, *P*=.41	−.17; *P*=.57	−.82; *P*=.04	+.59; *P*=.03
CFT (F1−10)	−.33, *P*=.52	−.15; *P*=.60	−.83; *P*=.04	+.55; *P*=.04
USV (EV1-1)	−.26, *P*=.36	+.23; *P*=.28	+.63; *P*=.01	−.52; *P*=.009
USV (TV1-1)	−.27, *P*=.33	+.24; *P*=.26	+.66; *P*=.01	−.46; *P*=.02
USV (TV2-1)	−.29, *P*=.30	−.008; *P*=.96	+.67; *P*=.008	+.08; *P*=.68

The data are shown as Pearson's coefficients (*r*). OFT = open-field test; Dyst T = total distance; Dyst C = distance in central area; Entries = number of entries to the central area; Time = time spent in the central area; CFT = contextual fear test; F(1−5) = duration of freezing behavior in the first 5 min of postconditioning session; F(5−10) = duration of freezing behavior in the second 5 min of postconditioning session; F(1−10) = total duration of freezing behavior in the postconditioning session; USV = ultrasonic vocalization test; EV1-1 = episodes of vocalization on the first day of experiment, before shock administration; TV1-1 = time of vocalization on the first day of experiment before shock; TV2-1 = time of vocalization on the second day of experiment before shock; TV8-1 = time of vocalization on the eighth day of experiment, before shock.

ization, and Vogel's tests were correlated with the monoamines turnover rate in different brain structures (prefrontal cortex, hippocampus, striatum), in both control and kindled animals (Table 2). Strong negative correlations were present between 5-HT turnover rate in the prefrontal cortex, and open-field test parameters (total time spent in central area, $r=-.72$, $P=.003$; entries to the central area, $r=-.68$, $P=.006$; and total distance in the central area, $r=-.70$, $P=.005$) in saline-treated rats. A significant negative correlation was found between the time spent in central area of the open-field test and 5-HT turnover rate in the hippocampus ($r=-.59$, $P=.02$) in kindled rats. There was also a strong positive correlation between the number of episodes and time of vocalization (measured on the first day), and 5-HT turnover rate in the hippocampus ($r=+.55$, $P=.006$; $r=+.62$, $P=.001$, respectively) in the same groups of animals. In the striatum, DA turnover rate was correlated with freezing reaction in a negative way in control rats (in the first 5 min, $r=-.81$, $P=.04$; in the second 5 min, $r=-.82$, $P=.04$; in all tests, $r=-.83$, $P=.04$), but in a positive way in kindled animals (in the second 5 min, $r=+.59$, $P=.03$; in all tests, $r=.55$, $P=.04$). There also was a positive correlation between the preshock episodes of USV and time of vocalization (measured on the first day) and the DA turnover rate in the striatum of the control group ($r=+.63$, $P=.01$; $r=+.66$, $P=.01$, respectively), whereas the same parameters correlated negatively in kindled rats ($r=-.52$, $P=,009$; $r=-.46$, $P=.02$). On the second day of the test, time of preshock vocalization correlated in a positive way with DA turnover in control animals only ($r=+.67$, $P<.008$).

4. Discussion

The main finding of the present study is a strong disinhibition of rat behavior in the contextual fear-conditioning test in a group of PTZ-kindled animals. This finding corresponds with a previously published report on the short-term rebound anxiolytic effect in the social interaction and the elevated plus-maze tests after PTZ kindling in rats (File et al., 1996). The effect of PTZ was selective, independent of changes in rat motor and exploratory activity (open-field test), learning and memory (step-down passive-avoidance test), and pain perception (flinch−jump test). These results indicate that chronic administration of PTZ leads to two opposite effects: sensitization in structures that underlie seizure activity and desensitization in structures that underlie anxiety. PTZ-treated animals also exhibited small, but significant, changes in the spontaneous water intake in the Vogel test and pretraining latency of a step down in the step-down test. However, these effects did not have any consequences on animal behavior in both tests.

It is noteworthy that the lack of another control group, i.e. animals pretreated with PTZ which do not fully developed seizures, does not allow us to exclude the

role of PTZ alone in the observed effects, independently of seizure progression. Such possibility seems, however, less likely given previously published reports on the selective anxiolytic-like effects of PTZ kindling (File et al., 1996; Stephens et al., 2001). In all models of seizures: PTZ-, lithium/pilocarpine-, and kainic acid-induced kindling, the animals gradually develop seizure activity, differing in frequency and intensity. Therefore, it can be said that there are no animals expressing exactly the same degree of kindling, and an arbitrary method of a cutoff for animal qualification is necessary to have a group of homogenously kindled animals. Interestingly, the other method of kindling, i.e. long-term electrical stimulation of amygdala, was repeatedly shown to result in the hyperemotionality, increased defensive and withdrawal responses, the exaggeration of conditioned fear-potentiated startle, and the decreased exploration in the elevated plus maze (cf. Kalynchuk, 2000). The reasons for the opposite changes in emotional processes accompanying chemically or electrically induced kindling are not clear. Most probably, they reflect the involvement of different brain limbic structures in the process of the initiation, propagation, and sensitization of seizure activity. Accordingly, anatomical localization of electric kindling-induced inhibition of rat exploratory activity in the elevated plus maze was found (Adamec and Shallow, 2000).

Interestingly, PTZ-kindling also produced changes in rat behavior in the ultrasonic vocalization test. Pretreatment with PTZ decreased significantly the spontaneous and conditioned vocalization of animals, leaving the unconditioned shock-evoked reactions of animals unchanged. Results of both the conditioned freezing and vocalization tests indicate that the animal fear-controlled behavior was disinhibited by pretreatment of rats with PTZ, secondarily to the seizure sensitization. This effect was limited only to some behavioral models of anxious reactions.

In some morphological studies, it was shown that PTZ kindling caused neuronal loss in many brain structures. Neuronal necrosis was found in hippocampal CA1, CA3, and DG regions (Franke and Kittner, 2001). Therefore, it is conceivable that the shorter freezing time in the contextual fear-conditioning test could be due to an impairment of the acquisition and/or retention of contextual cues, caused by damage to the hippocampus. However, the lack of changes in animal behavior in the step-down passive-avoidance task, involving also contextual cues, indicates a more selective effect of PTZ kindling on rat freezing behavior.

The mechanisms of PTZ-induced emotional effects may involve changes in the activity of brain dopaminergic and serotonergic systems. Both the concentrations of HVA and 5-HIAA in the prefrontal cortex were lowered in the PTZ pretreated animals. Furthermore, the metabolic turnover rates of DA (HVA/DA ratio) and 5-HT (5-HIAA/5-HT ratio) were decreased in the striatum, the hippocampus, and the prefrontal cortex, respectively. The small decrease in dopaminergic activity in the striatum after PTZ kindling seems to be a local phenomenon, with no direct behavioral consequences, as PTZ has been found to significantly increase DA concentration in the nucleus accumbens, even 10 weeks after kindling completion (Becker et al., 2000). In vivo, PTZ kindling was found to enhance the basal activity of DA in different brain structures (Dazzi et al., 1997). Interestingly, there appeared also a negative correlation between DA metabolism rate (HVA/DA) and rat freezing behavior, and a positive correlation between DA metabolism and spontaneous and conditioned vocalization in the striatum of the control group. The meaning of this biochemical versus behavioral correlation remains unclear. Importantly, all these correlations were reversed in PTZ-kindled animals. Thus, these data add more arguments for the involvement of brain DA systems in the control of emotional behavior, and the pathomechanism of emotional disturbances accompanying kindling.

The hypoactivity of serotonergic innervation of the hippocampus and prefrontal cortex can be more directly related to the emotional changes after PTZ pretreatment. It was found that 5-HT decreases in the frontal cortex and the hippocampus after *p*-CPA (a 5-HT synthesis inhibitor), 5,7-DHT (a 5-HT neurotoxin), or benzodiazepines. This finding is significantly and inversely correlated with rat behavior controlled by fear in different models of anxiety (Nazar et al., 1999a,b). Accordingly, in the present study, rat exploratory behavior was found to inversely correlate with serotonergic activity (5-HIAA/5-HT ratio) in the prefrontal cortex. This correlation was abolished in the PTZ-kindled animals. In this group of rats, there appeared a negative correlation between time spent in the central sector of the open field, and a positive correlation between spontaneous and conditioned vocalization, and 5-HT turnover rate in the hippocampus, respectively. The intrinsic mechanism of these phenomena may involve changes in density of the 5-HT_{1A} receptors in the dentate gyrus of the hippocampus. It has been recently found that kindling leads to long-lasting increases in 5-HT_{1A} receptors in the kindled rat dentate gyrus (Cagnotto et al., 1998). This effect may be responsible for the anxiolytic-like changes in animal behavior in the ultrasonic vocalization test, as limbic 5-HT_{1A} receptors are considered to exert a tonic inhibitory influence on emotional processes (Plaznik et al., 1994).

Summing up, the present results indicate a direct involvement of serotonergic and dopaminergic innervation of the brain in the behavioral effects of chemically induced kindling. PTZ kindling caused selective changes in animal emotional behavior limited to some models of anxiety only (a decreased freezing time and an inhibition of spontaneous vocalization). Some of them may be reminiscent of emotional outbursts, i.e. disinhibition of emotional behavior, observed in epileptic patients.

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References

Adamec R, Shallow T. Rodent anxiety and kindling of the central amygdala and nucleus basalis. Physiol Behav 2000;70:177–87.

Becker A, Grecksch G, Brosz M. Naloxone ameliorates the learning deficit induced by pentylenetetrazol kindling in rats. Eur J Neurosci 1994;6(9): 1512–5.

Becker A, Grecksch G, Thiemann W, Hollt V. Pentylenetetrazol-kindling modulates stimulated dopamine release in the nucleus accumbens of rats. Pharmacol, Biochem Behav 2000;2:425–8.

Cagnotto A, Crespi D, Mancini C, Manzoni C, Presti ML, Gariboldi M, Vezzani A, Mennini T. Lasting increase in serotonin 5-HT_{1A} but not 5-HT_4 receptor subtype in the kindled rat dentate gyrus: dissociation from local presynaptic effects. J Neurochem 1998;70:850–7.

Coimbra NC, Castro-Souza C, Segato EN, Nora JE, Herrero CF, Tedeschi-Filho W, Garcia-Cairasco N. Post-ictal analgesia: involvement of opioid, serotonergic and cholinergic mechanisms. Brain Res 2001;888: 314–20.

Dazzi L, Serra M, Porceddu ML, Sanna A, Chessa MF, Biggio G. Enhancement of basal and pentylenetetrazol (PTZ)-stimulated dopamine release in the brain of freely moving rats by PTZ-induced kindling. Synapse 1997;26:351–8.

Devinsky O, Emoto S, Goldstein DS, Stull R, Porter RJ, Theodore WH, Nadi NS. Cerebrospinal fluid and serum levels of DOPA, catechols, and monoamine metabolites in patients with epilepsy. Epilepsy 1992;33: 263–70.

Fanselow MS. Conditional and unconditional components of post-shock freezing. Pavlovian J Biol Sci 1980;15:177–82.

File SE, Curle PF, Baldwin HA, Neal MJ. Anxiety in the rat is associated with decreased 5-HT and glycine from the hippocampus. Neurosci Lett 1987;83:318–22.

File SE, Mabbutt PS, Becker A, Gonzales LE, Fluck E. Short-term rebound anxiolytic effects and long-term changes in platelet benzodiazepine binding after pentylenetetrazol-kindling in two strains of rats. Anxiety 1996;2:109–16.

Franke H, Kittner H. Morphological alterations of neurons and astrocytes and changes in emotional behavior in pentylenetetrazol-kindled rats. Pharmacol, Biochem Behav 2001;70:291–303.

Genkova-Papazova MG, Petkova B, Shishkova N, Lazarova-Bakarova M. The GABA-B antagonist CGP 36742 prevent PTZ-kindling-provoked amnesia in rats. Eur Neuropsychopharmacol 2000;10:273–8.

Green AR, Heal DJ, Vincent ND. The effects of single and repeated electroconvulsive shock administration on the release of 5-hydroxytryptamine and noradrenaline from cortical slices of rat brain. Br J Pharmacol 1987;92:25–30.

Kalynchuk LE. Long-term amygdala kindling in rats as a model for the study of interictal emotionality in temporal lobe epilepsy. Neurosci Biobehav Rev 2000;24:691–704.

Nazar M, Siemiątkowski M, Bidziński A, Członkowska A, Sienkiewicz-Jarosz H, Plaznik A. The influence of serotonin depletion on rat behavior in the Vogel test and brain 3-H zolpidem binding. J Neural Transm 1999a;106:355–68.

Nazar M, Siemiątkowski M, Członkowska A, Sienkiewicz-Jarosz H, Plaznik A. The role of the hippocampus and 5-HT/GABA interaction in the central effects of benzodiazepine receptor ligands. J Neural Transm 1999b;106:369–81.

Pintor M, Mefford IN, Hutter I, Pocotte SL, Wyler AR, Nadi NS. Levels of biogenic amines, their metabolites, and tyrosine hydroxylase activity in the human epileptic temporal cortex. Synapse 1990;5:152–6.

Plaznik A, Kostowski W, Stefański R. Limbic mechanisms of anxiolytics acting on 5-HT receptors. Pol J Pharmacol 1994;46:473–7.

Rossler AS, Schroder H, Dodd RH, Chapouthier G, Grecksch G. Benzodiazepine receptor inverse agonist-induced kindling of rats alters learning and glutamate binding. Pharmacol, Biochem Behav 2000;67:169–75.

Shouse MN, Staba RJ, Ko PY, Saquib SF, Farber PR. Monoamines and seizures: microdialysis findings in locus coeruleus and amygdala before and during amygdala kindling. Brain Res 2001;892:176–92.

Stefański R, Pałejko W, Bidziński A, Kostowski W, Plaznik A. Serotonergic innervation of the hippocampus and nucleus accumbens septi and the anxiolytic-like action of midazolam and 5-HT_{1A} receptor agonists. Neuropharmacology 1993;32:977–85.

Stephens DN, Brown G, Duka T, Ripley TL. Impaired fear conditioning but enhanced seizure sensitivity in rats given repeated experience of withdrawal from alcohol. Eur J Neurosci 2001;14:2023–31.

Yokoi I, Yamamoto Y, Fujikawa N, Shirasu A, Mori A. Determination of neurotransmitter release into the caudate nucleus during convulsions induced by pentylenetetrazol using in vivo differential pulse voltametry. Brain Res 1986;385:212–8.