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Diurnal variations in depression-like behavior of Wistar and spontaneously hypertensive rats in the kainate model of temporal lobe epilepsy

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ABSTRACT

The purpose of this study was to explore whether the kainate (KA) model of temporal lobe epilepsy (TLE) can be used as a model of comorbid epilepsy and depression to study diurnal behavioral variations in rats. Development of chronic epilepsy was confirmed by the detection of spontaneous motor seizures (SMS) with video monitoring (24 hours/3–5 months after status epilepticus [SE]). KA-treated spontaneously hypertensive rats (SHRs) exhibited higher seizure frequency than Wistar rats during the light phase in the fourth and fifth months after SE. Although epileptic Wistar rats showed depression-like behavior and reduced anxiety mostly during the light phase, there were no diurnal variations in depression-like patterns in SHRs. Anxiety levels of control and epileptic SHRs were similar. Decreases in serotonin, tryptophan, and dopamine concentrations in the hippocampus were detected in epileptic Wistar rats compared with naive controls. However, monoamine levels of epileptic SHRs were close to those of their controls. Wistar rats and SHRs develop stable depression-like behavior during the chronic epileptic phase with strain-dependent diurnal differences.

1. Introduction

Clinical and experimental data have revealed that depression represents one of the most common affective disorders associated with temporal lobe epilepsy (TLE) [1,2]. Epilepsy occurs with approximately fivefold greater frequency among individuals with a history of depression than among the general population, indicating that the bidirectional relationship is more than a psychosocial phenomenon and that the two disorders likely share common pathogenic mechanisms [3,4]. Mood disturbances such as feelings of despair and depressive mood are among the psychiatric features common to both patients with TLE and patients with major depression, but they may have a more abrupt start in persons with epilepsy than in those without epilepsy [1].

In humans almost all physiological and behavioral functions occur on a rhythmic basis. Spontaneously hypertensive rats (SHRs) are widely accepted as an experimental model of essential hypertension, and the chronobiological aspects of cardiac parameters have been explored in this model [5–8]. Compared with the patterns in Wistar–Kyoto (WKY) rats, diurnal heart rate, locomotion, and respiration patterns of SHRs are inverted [5]. However, circadian rhythms of blood pressure in SHRs have been reported to vary from normal [6], to nonexistent [5], to enhanced [7], to inverted [8]. Hypothalamic nuclei have been considered a crucial area in the regulation of circadian rhythms, which are usually abnormal in patients with depression [9]. The antidepressant efficacy of both pharmacological and nonpharmacological strategies affecting endogenous circadian rhythms, such as new antidepressant medications, light therapy, and sleep deprivation, is consistent with the idea that circadian alterations may represent a core component of depression, at least in a subgroup of depressed patients. Moreover, desynchronizations of circadian rhythms may play a role in the disturbed behavior associated with depressive conditions in epilepsy.

Neuroimaging observations suggest that lesions or functional abnormalities in specific brain areas are associated with more severe symptoms of depression, including those in patients with epilepsy [1]. The hippocampus, known to be a stress-vulnerable and plastic brain region, has been considered to play a pivotal role in the pathophysiology of depression [10]. Several lines of studies focused on hippocampal atrophy in patients with recurrent depression have converged to support the idea of a correlation between mesial temporal sclerosis and depressive state in epilepsy [11–14]. On the other hand, it has been demonstrated that there is a correlation between deleterious

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effects of hypertension and abnormalities of the hippocampal structure, neurochemistry, and behavior in SHRs [15–17].

Despite a clear epidemiological link between the two diseases, there is scarce experimental evidence and few validated animal models to support a shared pathology and the potential underlying mechanism in both phenomena. Although some studies have revealed no changes in anhedonia (i.e., inability to experience pleasure) of kindled rats [18,19], Mazarati et al. showed a loss of taste preference for sweet solutions in rats post-status epilepticus (SE) [20]. With respect to another major symptom of depression, despair-like behavior, evaluated in a test commonly used for screening antidepressants, the forced swimming test, the results varied from no changes in amygdala kindling [21], to increased immobility in kainic acid (KA)- and pilocarpine-treated rats [20,22], to improved performance in pilocarpine-treated epileptic mice [23]. The discrepant results could be due to variability in the model, the strain, and/or the experimental design. Dysregulation of the hypothalamic–pituitary–adrenocortical (HPA) axis correlates with behavioral depressive symptoms (anhedonia and despair) in post-SE rats [2].

Deficiency of the serotoninergic system has been suggested to play a crucial role in the mechanism of depression [24]. On the other hand, several reports indicate that serotonin may participate in seizure development and control in the pilocarpine model of TLE in Wistar (WIS) rats [20,25]. A compromised serotoninergic system was suggested to underlie a depressive predisposition in epilepsy [26].

The relationships between depression and epilepsy are still obscure and adequate experimental models are needed to understand the mechanisms underlying depression in epilepsy. Recently, we demonstrated that normotensive WIS rats and SHRs could be explored as a useful model for studying the diurnal rhythms of different behavioral patterns in the KA model of TLE [27]. In addition to being considered as a model of essential hypertension, SHRs have been suggested to be a model of attention-deficit/hyperactivity disorder (AD/HD) [28]. The usefulness of this strain as a model of AD/HD is related to the resemblance of behavioral symptoms (hyperactivity, an attention deficit, and impulsivity) [28] and to biochemical differences from normotensive controls, for example, decreased serotoninergic (5-HT) functioning [29] and dopamine (DA) activity in the frontal cortex of SHRs [30].

The commonly used WIS rat served as an appropriate control to overcome the previously described difficulties associated with comparative behavioral analysis using WKY rats as controls. Whether WKY rats constitute a true normotensive genetic analog of SHRs [31,32] raises the point of the validity of the WKY rat as a valid control animal for SHRs. Comparing WKY rats and SHRs with other rat strains, it seems that SHRs are not as hyperactive, but WKY rats are significantly hypoactive and are very susceptible to learned helplessness [33].

We designed this study to elucidate and compare behavioral patterns characteristic of the depressive condition in WIS rats and SHRs in the KA model of TLE, focusing particularly on their diurnal rhythms. In addition, to explore the development of depression-like symptoms during the stable period of the chronic phase of epilepsy, we characterized neurochemical correlates of comorbid epilepsy and depression.

2. Methods

2.1. Subjects

Sixty-day-old male normotensive Wistar rats and SHRs were habituated for 10 days (12/12-hour light/dark cycle with lights on at 08:00 hours) and individually housed under standardized conditions (20 ± 1 °C, 50–60% humidity). Food and water were available ad libitum throughout the study except during the tests. All experiments were carried out during the autumn–winter season. The experimental design was approved by governmental authorities and was in full accordance with the European Communities Council Directives of 24 November 1986 (86/609/EEC). The study design is depicted in Fig. 1.

2.2. Measurement of arterial blood pressure

Systolic arterial blood pressure was measured noninvasively in conscious unrestrained rats using the tail cuff method (Ugo Basile Blood Pressure Recorder 5800). The arterial blood pressure value for each rat was the mean of three measurements.

2.3. Procedure for induction of status epilepticus with kainic acid

Fifty-two male Wistar rats and SHRs were randomly divided into the following four subgroups: control WIS rats, $n = 10$; control SHRs, $n = 11$; KA-treated WIS rats, $n = 15$; KA-treated SHRs, $n = 16$. Seizures were induced by repeated KA (Sigma–Aldrich, Bulgaria) injections (5 mg/kg/h, ip) according to the protocol of Hellier et al. [34]. KA was diluted in sterile 0.9% saline at 2.5 mg/mL. Rats were continuously monitored for convulsive motor seizures scored from III to V using a modification of Racine's scale [35]. Seizure intensity was defined as follows: class III, forelimb clonus with lordotic posture; class IV, rearing and continued forelimb clonus; and class V, forelimb clonus and loss of posture. Hourly KA treatment continued in rats exhibiting convulsive seizures until class III, IV, or V seizures were evoked for at least 3 hours (i.e., >10 motor seizures per hour). KA treatment was interrupted if a total dose of 35 mg/kg was reached. Matched controls were treated with an equivalent volume and number of injections of sterile saline. The KA-treated rats received lactated Ringer's (2–3 mL/100 g/day, sc), apple slices, and moistened rat chow for up to 6–7 days.

2.4. Long-term video monitoring of spontaneous motor seizures

Forty-eight hours after KA treatment, experimental rats were placed in labeled kennels and video monitored (24 hours/day) for a period of 5 months. The following parameters were evaluated: latency to onset of the first spontaneous seizure, weekly seizure frequency between the third and the fifth months, and distribution of seizures relative to circadian rhythm. In addition to the video monitoring, all spontaneous motor seizures (SMS) detected during the routine and experimental manipulations of the animals were recorded. Video

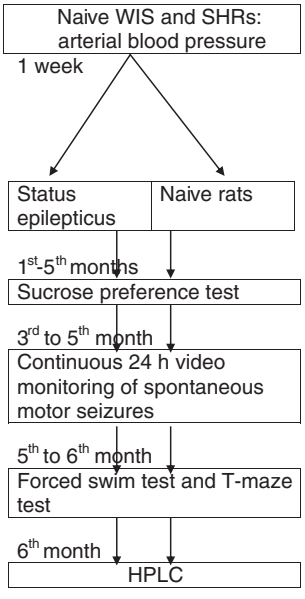

Fig. 1. Scheme of the experiments.

monitoring was performed with a light-sensitive black and white camera (S-2016, AVTECH, Taiwan, No AVC307R), and video recordings were visually analyzed. Motor seizures were scored on the same scale used during KA treatment (i.e., class III/IV/V seizures).

2.5. Behavioral evaluation

During the light phase, all behavioral tests were carried out under artificial light, and during the dark phase, under infrared light. The Porsolt test and elevated T-maze were performed at two time points, that is, 6 hours after lights on/off (15:00 and 03:00 hours, respectively). At least 30 minutes before each test, the rat was transferred to the adjacent soundproof room where the behavioral experiments were conducted. Rats who exhibited SMS 1 hour before, during or after starting the test were excluded from the experimental procedure.

2.5.1. Sucrose consumption test

Impairment of the "hedonic" state of an animal, or the ability to experience pleasure, is considered an index of clinical depression (American Psychiatric Association, 2000). Taste preference behavior was evaluated using the sucrose consumption test at the end of every month from the first to fifth months after SE. On the first day (habituation), each cage was supplied with two identical 100-mL graduated water bottles. On the second (pretest) and third (test) days, regular water in one of the bottles was replaced with 1% sucrose. Tests started at 8:00 AM and ran for 24 hours. During the test, both bottles were removed after 12 hours for weighing, and replaced with a second pair of preweighed bottles. Taste preference was expressed as the percentage of the volume of sucrose solution to total volume of fluid (sucrose plus regular water) consumed over 12 hours (light phase, 8:00–20:00 hours; dark phase, 20:00–8:00 hours).

2.5.2. Forced swimming test

Despair-like behavior was evaluated 5 months after KA-induced SE, that is, during the chronic epileptic state, with the classic forced swimming test (FST) [36], which has been shown to be relevant both for examining depression-like behavior and for screening antidepressant agents. In brief, the FST was conducted in a 2-day procedure; rats had to swim under conditions in which escape was not possible. On the first day, animals were placed in clear, 50-cm –tall, 25-cm-diameter cylinders filled to 30 cm with 23 °C water. The normal rats initially struggle to escape the water, but eventually adopt a posture of immobility in which they make only the movements necessary to keep their heads above water. The first training session lasted 15 minutes. Behavior on the next day (test) of the FST was scored for 5 minutes by two skilled experimenters unaware of the treatment conditions. A rat was judged to be immobile if it was making only movements necessary to keep its head above water, if it was climbing, and if it was making forceful thrashing movements with its forelimbs directed against the walls of the cylinder.

2.5.3. Elevated T-maze

The elevated T-maze was made of wood and had three arms of equal dimensions (50×12 cm). One arm, enclosed by walls 40 cm high, was perpendicular to two opposed open arms. To prevent falls, the open arms were surrounded by a 1-cm-high plexiglas rim. The entire apparatus was elevated 50 cm above the floor. Luminosity at the level of the maze arms was 50 lux during the light phase. The test, executed according to the protocol of Graeff et al., was initiated by inhibitory avoidance measurement [37]. Each animal was placed at the distal end of the enclosed arm of the elevated T-maze facing the intersection of the arms. The time taken by the rat to leave this arm with the four paws (baseline latency) was recorded. The same measurement was repeated in two subsequent trials (avoidance 1 and 2) at 30-second intervals. Following avoidance measurement (30 seconds), each animal was placed at the end of one of the open arms, and the time taken to leave this arm with the four paws was recorded in two consecutive trials (escape 1 and 2), again with 30-second intertrial intervals. A cutoff time of 300 seconds was established for the avoidance and escape latencies.

2.6. High-performance liquid chromatography

The rats were decapitated during the light period; brains were quickly dissected on ice and hippocampi were bilaterally removed. Tissue samples were frozen in liquid nitrogen, lyophilized, and stored at −70 °C before analysis. Dry tissues were accurately weighed and homogenized in precooled 0.5 M formic acid using a MICRA D-8 (ART, Germany) homogenizer. The samples were centrifuged (13,000 rpm at 4 °C) for 20 minutes and a 10-μL aliquot of the supernatant was used for analyses.

Thereafter, each pooled sample (from both hemispheres) was analyzed for content of the monoamines (MA) serotonin and dopamine, and their precursors tryptophan and tyrosine were measured by LC/MS/MS. Measurements were performed by electrospray ionization in positive mode on an LTQ Orbitrap Discovery (ThermoFisher, Germany) connected to a Surveyor HPLC system (ThermoFisher, Germany). The analyzed compounds were separated on a ZIC-HILIC (Merck, Germany) analytical column in isocratic elution mode with a mobile phase of 70% acetonitrile containing 15 mM formic acid at flow rate of 200 μL/minute. Quantitative analyses were performed using the "selected ion monitoring" mode with external calibration. Data acquisition and processing were performed with Xcalibur software.

2.7. Statistical analysis

For statistical evaluation of FST data, a three-way analysis of variance (ANOVA), was used with strain (WIS rats vs SHRs), treatment (saline vs KA), and phase (light vs dark) as independent factors. For frequency of SMS and biochemical data, a two-way ANOVA was used with strain (WIS rats vs SHRs) and treatment (saline vs KA) as factors. For such measures as sucrose preference, avoidance, and escape, a repeated-measures factor (month or trial) was used for the light and dark periods of the cycle, respectively. For each behavior, post hoc Bonferroni *t* tests, if appropriate, were used to examine individual group differences between controls and KA-treated WIS rats and SHRs, as well as differences between WIS rats and SHRs receiving the same treatment (saline treated vs KA treated). For samples that did not have a normal distribution, the Mann–Whitney or Wilcoxon test was employed. Fisher's exact test was used to calculate the incidence of mortality during SE and the incidence of rats with SMS. Spearman correlation was employed to evaluate potential associations between different measures. $P<0.05$ was accepted as an index of statistically significant differences.

3. Results

3.1. Arterial blood pressure

Control SHRs had significantly higher arterial blood pressure (175.1 ± 1.4 mm Hg, $P<0.005$) compared with normotensive WIS controls (121.36 ± 1.66 mm Hg).

3.2. Seizure activity and circadian rhythms in Wistar and spontaneously hypertensive rats treated with kainic acid

Behavioral motor seizures during SE in KA-treated WIS rats and SHRs were similar and did not differ from those reported earlier [27]. Four of 15 WIS rats and 3 of 16 SHRs died in the course of KA-induced SE. There was no significant difference in the average dose of KA needed to induce SE between WIS rats (median ± SD: 18.75 ± 6.8, range: 10–30) and SHRs (median ± SD: 22.5 ± 5.36, range: 12.5–32.5). Among the surviving animals, spontaneous seizures occurred in all

WIS rats and SHRs, respectively; however, two WIS rats and two SHRs died in the course of video monitoring. During the following days, the behavior of KA-treated animals returned progressively to normal, although some aggressive response on handling was observed in both groups during the latent period.

A negative correlation was detected between latency to the first SMS and seizure frequency for SHRs (Spearman correlation coefficient, $\rho = -0.669$, $P<0.029$). In total, 20 rats were continuously monitored for a period of 3 months starting 2 months after SE. Spontaneous motor seizures were detected in 9 WIS rats (total number of SMS = 1201) and 11 SHRs (total number of SMS = 3077), after latent periods of 4–83 days (median = 19 days) for WIS rats and 7–14 days (median = 10.5 days) for SHRs. Two WIS rats (25%) had a high seizure frequency (third month: 105–110 SMS, fourth month: 80–132 SMS; fifth month: 179–226 SMS) after KA-induced SE. Four SHRs (40%) were characterized by frequent seizures (75–301, 136–371, and 172–389 SMS) during the same periods. Six WIS rats (75%) had low seizure frequency (2–17, 6–62, and 13–21 SMS in the third, fourth, and fifth months after SE) whereas six SHRs (60%) had occasional seizures (1–53, 16–48, and 26–63 SMS, respectively).

The distribution of SMS during the 24-hour light–dark cycle is illustrated in Fig. 2. From this figure it is evident that there is a circadian rhythm in the occurrence of SMS, with a prevalence of seizures during the light phase (08:00–20:00 hours). In the third month after SE, both WIS rats and SHRs exhibited higher seizure frequency during the light phase (81%). The same tendency was in force in the fourth (66%) and fifth (70%) months for WIS rats and SHRs (78 and 72%), respectively. Although more SMS occurred during the light than the dark phase, the difference was significant only in the third month (WIS rats: $P<0.047$, SHRs: $P<0.013$) and the fourth month (SHRs: $P<0.001$), respectively. The total number of SMS statistically differed between WIS rats and SHRs in the third month (11:00 PM), the fourth month (08:00, 10:00–16:00 PM), and the fifth month (12:00 and 14:00 PM) ($P<0.05$) (Fig. 1). In 63% of WIS rats and 100% of SHRs, seizures occurred in clusters (i.e., >3 seizures per day) [38]. In 100% of WIS rats and 63% of SHRs, the occurrence of clusters was followed by a seizure-free period of 2–5 days.

Two-way ANOVA revealed a significant main effect of strain on seizure frequency during the light phase (third month: $F[1,63] = 4.854$, $P<0.0321$; fourth month: $F[1,87] = 15.304$, $P<0.001$; fifth month: $F[1,87] = 3.765$, $P<0.056$) and during the dark phase (fourth month: $F[1,87] = 3.765$, $P<0.056$; fifth month: $F[1,78] = 6.375$, $P<0.014$). Post hoc analysis showed statistically that SHRs experienced more seizures than WIS rats during the third and fourth weeks (fourth month) and second and third weeks (fifth month), respectively ($P<0.05$), during the dark phase (Fig. 3). However, comparison of mean seizure frequency during the dark phase week by week revealed only a tendency toward more seizures in SHRs than in WIS rats.

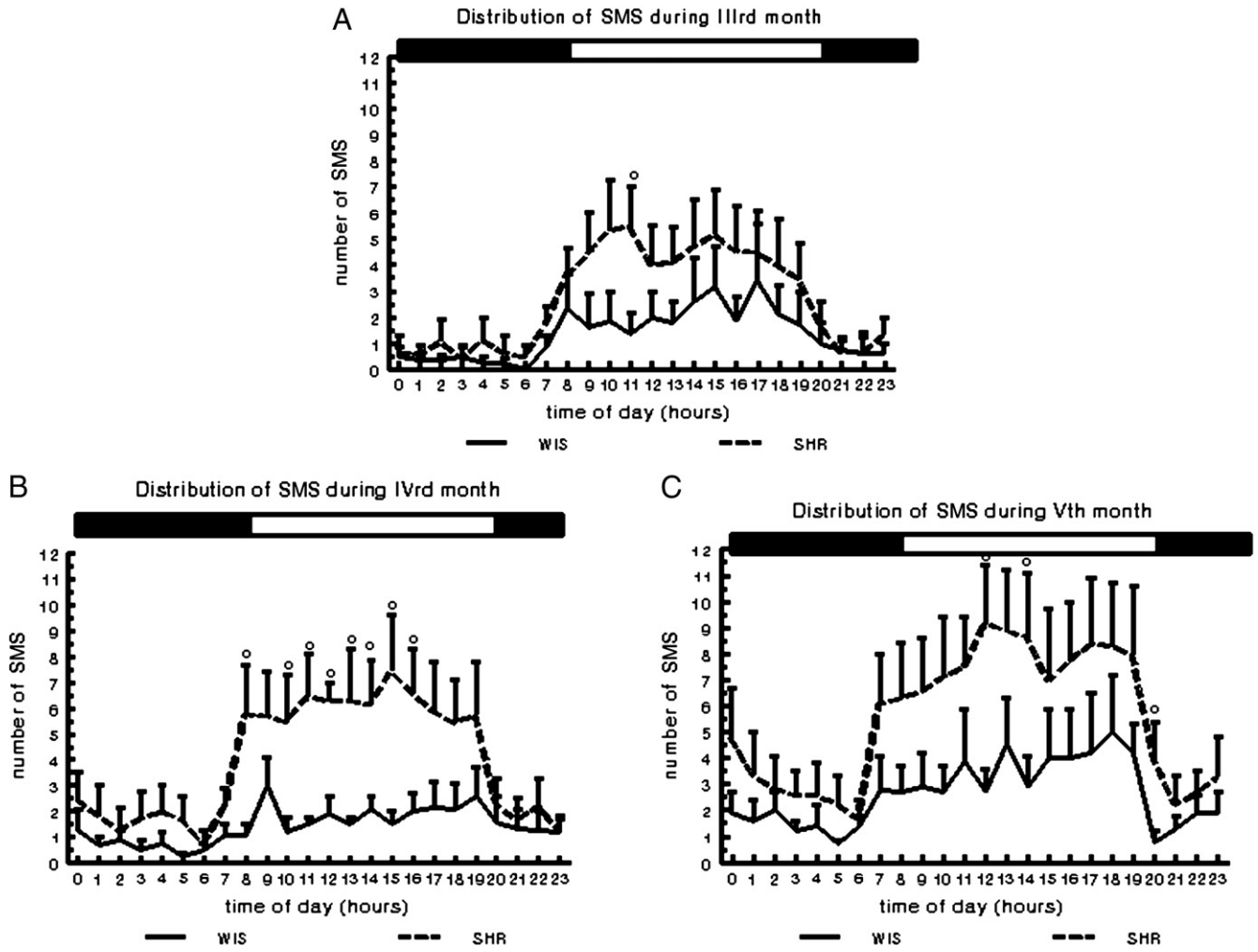

Fig. 2. Circadian rhythm of spontaneous motor seizures (SMS) recorded for Wistar (WIS) rats ($n = 10$) and spontaneously hypertensive rats (SHRs) ($n = 11$) during the third (A), fourth (B), and fifth (C) months after kainic acid (KA)-induced status epilepticus (SE). Each time point is the mean ± SEM; 81% of SMS were found to occur during the light phase in the third and fourth months after SE in both strains; 70 and 72% during the light phase in the fifth month after SE in WIS rats and SHRs, respectively. $^{o}P<0.05$ versus WIS rats during the same hour.

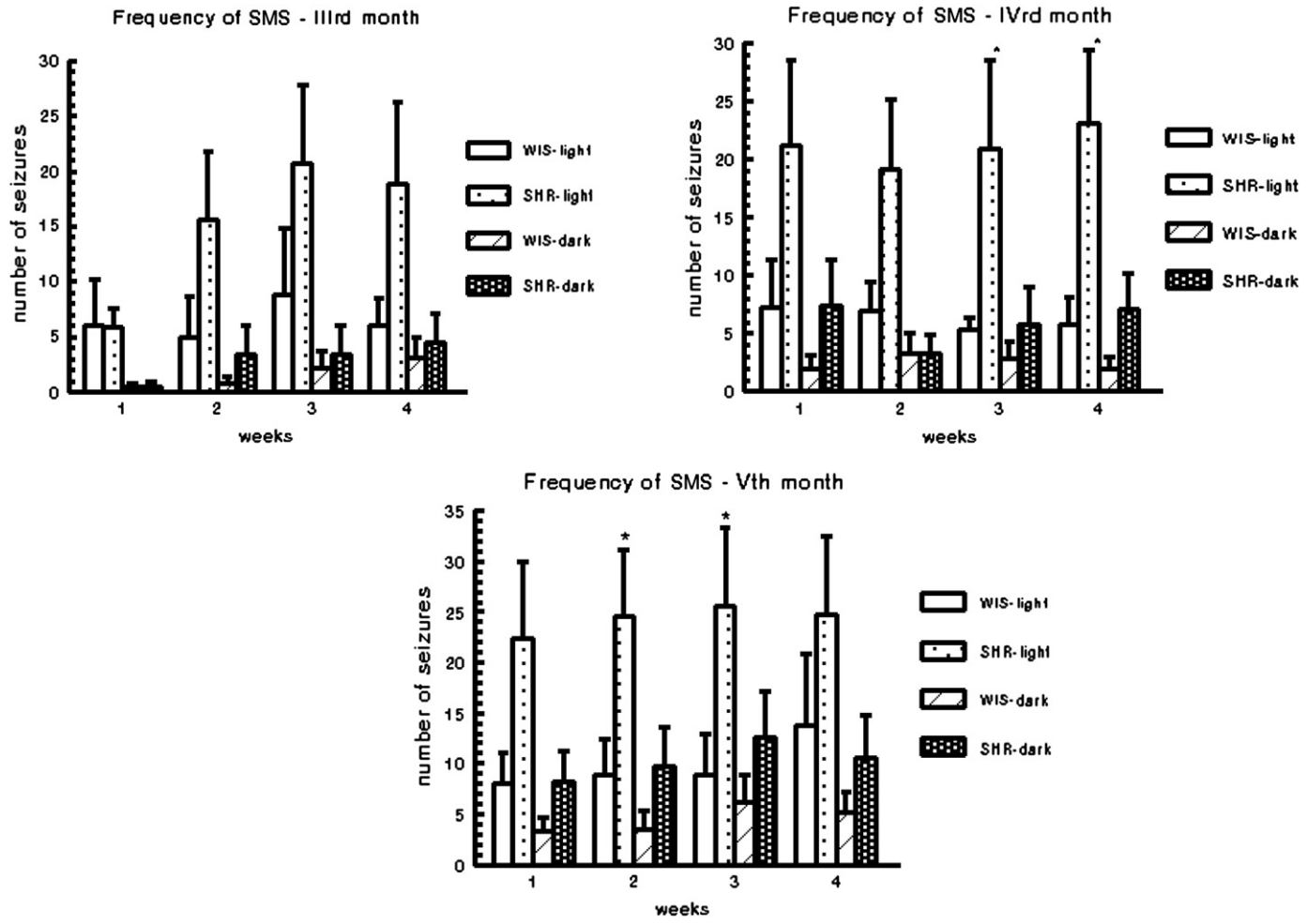

Fig. 3. Dynamics of spontaneous motor seizures (SMS) during the chronic phase of epilepsy counted for 12 weeks (starting 3 months after KA-induced SE) in WIS rats and SHRs. Data are the mean ± SEM weekly seizure frequency during the light (WISd, SHRd) and dark (WISn, SHRn) phases of the 24-hour period of observation. *$P<0.05$, light phase versus dark phase; °$P<0.05$ versus WIS rats given the same treatment.

A positive correlation was detected between time and mean seizure frequency per week for both WIS rats ($\rho = 0.944$, $P<0.001$) and SHRs ($\rho = 0.91$, $P<001$) (Fig. 3). The rate of increase in SMS during the third, fourth, and fifth months after SE is summarized in Table 1. A higher rate of increase in seizure frequency was detected during the fourth month (71%) for WIS rats and at the end of the third month (110%) for SHRs (Table 1).

3.3. Sucrose consumption test

During the light phase, repeated ANOVA revealed a main effect of strain, treatment, and phase, respectively, on preference for sucrose solutions over tap water as well as interactions among them (Fig. 4A, statistical data are provided in the respective figure legends). Control SHRs showed a lower preference for sucrose solutions during the light phase compared with control WIS rats (Bonferroni *t* test: °$P<0.05$) (Fig. 4A). Post hoc analyses demonstrated that 2, 4, and 5 months after KA-induced SE, WIS rats consumed statistically smaller amounts of sucrose solution compared with their naive controls (*$P<0.05$) (Fig. 4A). However, in KA-treated SHRs, after the first month there was a tendency for a decline in sucrose intake which reached significance only during the fourth month after SE (Bonferroni *t* test: *$P<0.05$) (Fig. 4A). During the light period, the sucrose preference demonstrated no correlation with frequency of SMS for both epileptic WIS rats and SHRs (Spearman correlation: $P>0.05$).

During the dark phase, ANOVA showed a main effect of strain and phase, as well as a strain × treatment interaction (statistical data in legend to Fig. 4B). In contrast to the light phase, epileptic WIS rats did not exhibit a lack of preference for sucrose solution compared with their controls, whereas KA-treated SHRs were characterized by anhedonia toward sweet solutions during the second, fourth, and fifth months after SE (*$P<.05$) (Fig. 3B). A negative correlation was demonstrated between affinity for sucrose and total number of SMS during the dark phase in the fifth month after SE for SHRs ($\rho = -0.745$, *$P<0.0108$).

Table 1
Seizure frequency at 4, 8, and 12 weeks after the third month of KA-induced status epilepticus in Wistar and spontaneously hypertensive rats.

Group	4th week (3 rd month)	8th week (4th month)	12th week (5th month)
Wistar	8.9 ± 3.6 (37%↑)	15.2 ± 3.7 (71%↑)	21.3 ± 4 (40%↑)
SHRs	16.4 ± 5 (110%↑)	20.4 ± 4.3 (24%↑)	21.3 ± 4 (20%↑)

Note. Means ± SE and rate of increase (4th vs 1st, 8th vs 4th, and 12th vs 8th week) in percent.

3.4. Forced swimming test

Overall analysis of immobility time demonstrated a main effect of strain, treatment, and phase without interaction among factors (statistical data in legend to Fig. 5). Subsequent pairwise comparison showed that the two epileptic groups exhibited despair-like behavior under the conditions of the FST during the light phase (*$P<0.05$)

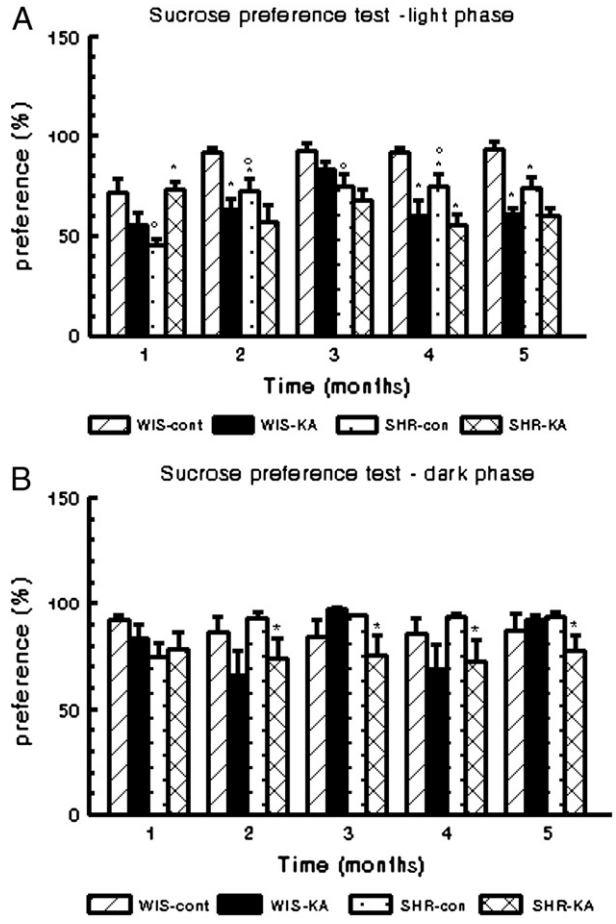

Fig. 4. Sucrose preference during the light phase (A) and dark phase (B) of the day–night cycle. Taste preference is expressed as percentage of volume of sucrose solution to total fluid (water + sucrose) consumed over 12 hours. Data are presented as means ± SEM. Repeated ANOVA + post hoc Bonferroni test: light phase (strain, $F[1,96] = 5.735$, $P<0.018$; treatment, $F[1,95] = 10.824$, $P<0.001$; month, $F[4,195] = 5.624$, $P<0.001$; strain × treatment × month interaction: $F[4,195] = 3.33$, $P<0.012$); dark phase (strain × treatment interaction: $F[1,192] = 4.019$, $P<0.047$). $^{*}P<0.05$ versus controls; $^{o}P<0.05$ versus WIS rats given the same treatment.

(Fig. 5). The duration of immobility exhibited a negative correlation with total number of SMS for KA-treated WIS rats ($\rho = -0.638$, $P<0.0474$).

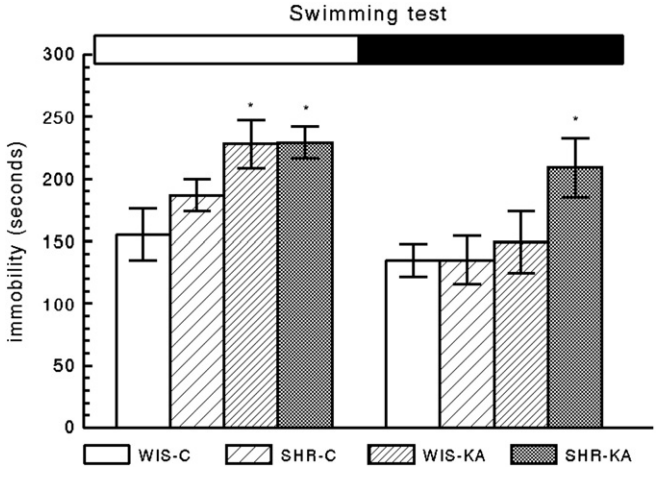

Fig. 5. Duration of immobility (mean ± SEM) in forced swimming test during the light phase (left) and the dark phase (right) of the day–night cycle. Three-way ANOVA + post hoc Bonferroni test: strain, $F[1,75] = 11.93$, $P<0.001$; treatment, $F[1,75] = 4.091$, $P<0.047$; phase, $F[1,75] = 8.208$, $P<0.006$. $^{*}P<0.05$ versus controls.

During the dark phase, despair-like behavior was evident only in epileptic SHRs ($^{*}P<0.05$) (Fig. 5). WIS rats and SHRs did not differ with respect to immobility and passive swimming during the FST.

3.5. Elevated T-maze

3.5.1. Avoidance session

During the light phase, repeated ANOVA showed a main strain and treatment effect on avoidance behavior as well as a strain × treatment interaction (statistical data in legend to Fig. 6A). There were no significant differences among the three avoidance trials (baseline latency, avoidance 1 and 2) ($P>0.005$). Subsequent post hoc analysis demonstrated a statistical difference between control WIS rats and SHRs as well as between KA-treated WIS rats and SHRs, respectively (baseline latency, avoidance 1 and 2) ($^{o}P<0.05$) (Fig. 6A). Although control SHRs did not differ from epileptic SHRs, KA-treated WIS rats exhibited a lower latency to avoidance 1 and 2 compared with their controls ($P<0.05$). Furthermore, latency to avoidance 1 correlated negatively with number of SMS for epileptic WIS rats ($\rho = -0.727$, $P<0.05$).

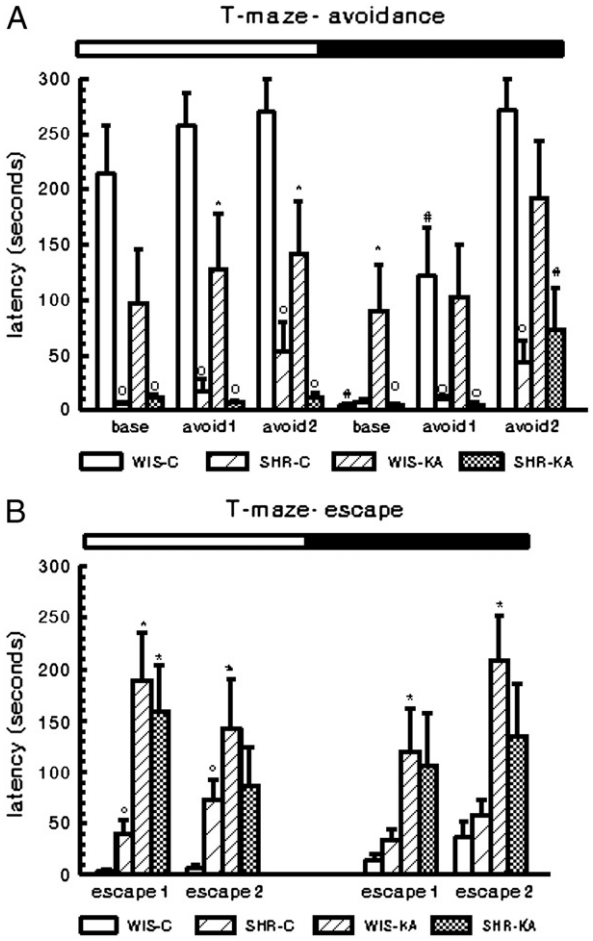

Fig. 6. (A) Latency to inhibitory avoidance in seconds (baseline, avoidance 1 and 2) (mean ± SEM) in T-maze test during the light and dark phases of the day–night cycle. Repeated-measures ANOVA + Bonferroni test: light phase (strain, $F[1,105] = 101.171$, $P<0.001$; treatment, $F[1,105] = 18.139$, $P<0.001$; strain × treatment: $F[1,115] = 10.970$, $P<0.001$); dark phase (strain, $F[1,110] = 32.091$, $P<0.001$; tral, $F[2,110] = 15.365$, $P<0.001$; strain × treatment x trial interaction, $F[2,110] = 4.820$, $P<0.01$). $^{*}P<0.05$ versus controls; $^{o}P<0.05$ versus WIS rats given the same treatment; $^{\#}P<0.05$ versus light phase. (B) Latency to escape behavior in seconds (escape 1 and 2) (mean ± SEM) in T-maze test during the light and dark phases of the day–night cycle. Repeated-measures ANOVA + Bonferroni test: light phase (treatment, $F[1,74] = 26.551$; strain × treatment interaction, $F[1,72] = 6.053$, $P<0.017$; and strain × trial interaction, $F[1,74] = 3.878$, $P<0.053$); dark phase (treatment, $F[1,71] = 24.504$, $P<0.001$). * $P<0.05$ versus controls; $^{o}P<0.05$ versus WIS rats given the same treatment.

During the dark phase, ANOVA demonstrated effects of strain and trials as well as a strain × treatment × trial interaction (statistical data in Fig. 6A). Unlike the control SHRs, which did not demonstrate diurnal anxiety-related fluctuations, WIS rats had lower baseline and secondary avoidance latencies during the dark phase ($^{\#}P<0.05$). As in the light phase, both control and epileptic SHRs demonstrated higher activity compared with WIS rats (controls: avoidance 1 and 2, epileptic rats: baseline latency and avoidance 1, respectively) ($^{o}P<0.05$). Unlike, controls, epileptic WIS rats did not exhibit diurnal changes in the latency on avoidance trials, whereas KA-treated SHRs were characterized by higher latency to avoidance 3 compared with the respective trial during the light phase ($^{\#}P<0.05$).

3.5.2. Escape session

During the light phase, repeated ANOVA revealed a treatment effect as well as strain × treatment and strain × trial interactions for escape 1 and 2 (statistical data in Fig. 6B). Subsequent post hoc analysis demonstrated a significant difference between WIS and SHRs controls ($P<0.05$). However, both epileptic groups, WIS rats and SHRs, exhibited panicolytic activity compared with the respective controls (escape 1), an effect that was maintained in the second trial only for KA-treated WIS rats ($^{*}P<0.05$).

During the dark phase, ANOVA showed a main effect only for the factor treatment without interactions (statistical data in Fig. 6B). Control WIS rats showed diurnal fluctuations with respect to escape latency, whereas both control and epileptic SHRs, as well as epileptic WIS rats, were characterized by a lack of diurnal variations in this anxiety pattern. Epileptic WIS rats showed lower anxiety levels compared with controls for escape 1 and 2 ($^{*}P<0.05$) (Fig. 6B). A positive correlation between latency to escape 1 and escape 2 and number of seizures was detected for KA-treated SHRs ($\rho = 0.925$, $^{*}P<0.0001$; and $\rho = 0.825$, $^{*}P<0.00526$, respectively).

3.6. Levels of monoamines and their precursors in the hippocampus

Hippocampal changes in levels of monoamines and their precursors in epileptic WIS rats and SHRs are illustrated in Figs. 7 and 8. There was a significant treatment effect as well as interaction strain × treatment interaction effect on tryptophan level in the hippocampus (statistical data in Fig. 7A). KA-treated WIS rats exhibited diminished levels of the respective precursor of 5-HT compared with their controls ($^{*}P<0.004$), whereas control SHRs were characterized by lower tryptophan levels compared with control WIS rats ($^{*}P<0.004$) (Fig. 7A). For 5-HT levels, two-way ANOVA revealed a main strain effect with strain × treatment interaction (statistical data in Fig. 7B). A post hoc test indicated that in the hippocampal tissue of epileptic WIS rats, the concentration of 5-HT was significantly decreased as compared with that of controls ($^{*}P<0.003$), whereas the 5-HT level of control SHRs was lower than that of control WIS rats ($^{*}P<0.006$) (Fig. 7B). Latency to avoidance 2 during the light phase exhibited a negative relationship with 5-HT levels for epileptic WIS rats ($\rho = -0.847$, $^{*}P<0.006$).

Hippocampal tyrosine levels did not undergo significant changes in KA-treated rats (Fig. 8A). There was an overall effect of strain and treatment as well as a strain × treatment interaction on DA level in the hippocampus (statistical data in Fig. 8B). Similarly to 5-HT and its precursor, DA levels were diminished in epileptic WIS rats compared with their controls ($^{*}P<001$), whereas control SHRs were characterized by lower concentrations compared with control WIS rats ($^{*}P<0.001$) (Fig. 8B). The level of hippocampal DA showed a negative correlation with the number of SMS during the light phase in the fifth month after SE for WIS rats ($\rho = -0.74$, $^{*}P<0.05$) and for SHRs ($\rho = -0.733$, $^{*}P<0.02$). In addition, the Spearman test showed a significant correlation between hippocampal DA levels and total number of SMS during the light phase for SHRs ($\rho = -0.661$, $P<0.0428$).

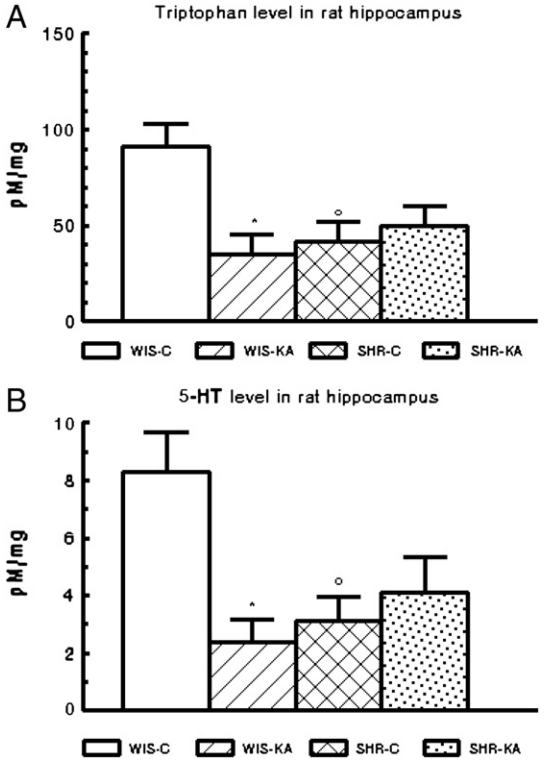

Fig. 7. Hippocampal concentrations of (A) serotonin and (B) tryptophan in picomoles per milligram of wet tissue, measured with the HPLC method. Each bar represents the mean ± SEM. Two-way ANOVA + Bonferroni test: serotonin (strain, $F[1,36] = 4.119$, $^{*}P<0.05$; strain × treatment interaction, $F[1,36] = 7.908$, $^{*}P<0.008$); tryptophan (treatment, $F[1,36] = 5.041$, $^{*}P<0.032$; strain × treatment interaction, $F[1,36] = 8.996$, $^{*}P<0.005$).

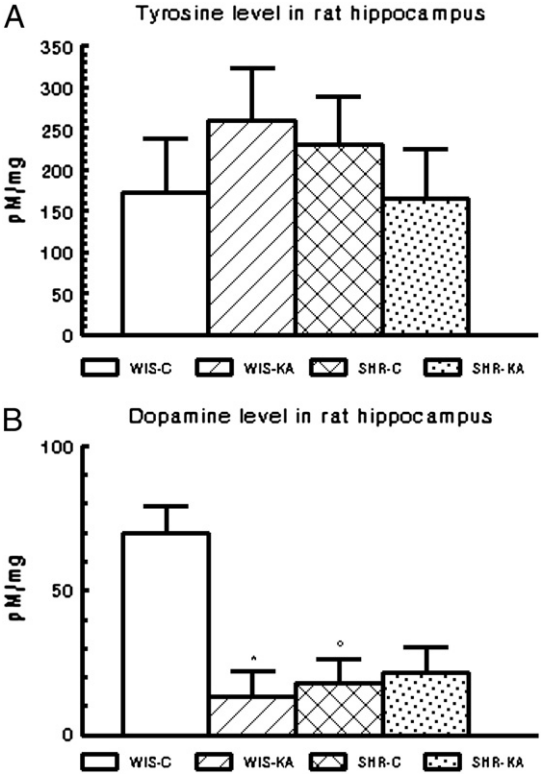

Fig. 8. Hippocampal concentrations of (A) tyrosine and (B) dopamine in picomoles per milligram of wet tissue, measured with the HPLC method. Each bar represents the mean ± SEM. Two-way ANOVA + Bonferroni test: dopamine (strain, $F[1,37] = 5.95$, $^{*}P<0.02$; treatment, $F[1,37] = 8.964$, $P<0.005$; strain × treatment interaction, $F[1,37] = 11.575$, $^{*}P<0.002$]. $^{*}P<0.05$ versus controls; $^{o}P<0.05$ versus WIS rats.

4. Discussion

The major findings of this study are the observed interstrain differences in the diurnal rhythms of depression- and anxiety-related behavior in the KA model of TLE. Compared with epileptic WIS rats exhibiting depression-like behavior during the light phase, epileptic SHRs showed depression-like responses without diurnal fluctuations and a lower anxiety level. Our study indicated that control and epileptic SHRs are characterized by hippocampal monoamine and tryptophan levels comparable to those of epileptic WIS rats, which were lower than those of WIS controls. An unexpected finding was that SHRs demonstrated a significantly higher frequency of SMS during the chronic epileptic phase compared with WIS rats. Recently, we reported that SHRs exhibit lower seizure activity accompanied by attenuated responses in hyperexcitability tests during the light phase of the early stage of the chronic phase after KA-induced SE [27]. We suggested that this alleviation in seizure frequency during the first 10 weeks of the chronic epileptic phase might represent a transient remission process shifted in time in SHRs compared with WIS rats [27].

Our data are in accordance with other studies focused on validating an animal model of comorbid epilepsy and depression [2,20,39,40]. Previously, anhedonia-like symptoms tested in the sucrose consumption test were detected in Wag-Rij rats, a model of absence epilepsy [41], in the kindling model [42], in a rat model of genetic generalized epilepsy [39], and in the pilocarpine model of TLE [20]. Our study provides additional evidence on the diurnal characteristics of behavioral disturbances in WIS rats and SHRs in the KA model of TLE. We have reported that epileptic WIS rats and SHRs demonstrate hyperactivity without diurnal fluctuations. In this work we found that although KA-treated WIS rats exhibit depression-like behavior during the light phase, the affective responses of epileptic SHRs are not characterized by diurnal fluctuations. We could assume that SHRs develop heavier depressive symptoms than WIS rats with epilepsy. Moreover, a predisposition to anhedonia in this strain could be proposed because control SHRs demonstrated a reluctance to consume sweet solutions during the light phase compared with WIS controls.

A previous study [40] indicated that depression-like behavior does not correlate with spontaneous seizure activity in the absence model of epilepsy. In this study, the relationship between seizure frequency and depression-like and anxiety-related behavior was phase dependent in WIS rats and SHRs. The negative correlation of seizures with despair- and anxiolytic-like behavior in WIS rats was evident only when depression-like behavior was exhibited, that is, during the light phase. In contrast, epileptic SHRs displayed a seizure frequency-dependent exacerbation in depression- and panicolytic-like behavior during the dark phase. Our data confirmed our previous finding in the KA model of TLE [27] as well as others considering a decreased anxiety level after a 5-month period of epilepsy induced by pilocarpine in rats during the light period [43,44]. The decreased anxiety level displayed by control SHRs in the T-maze test corroborates data from previous studies showing that hypertensive rats show decreased fear/anxiety responses in different behavioral tests [45,46]. However, although we confirmed the data of Conceicao et al. showing that SHR controls exhibit a significant decrease in the latency to exit the enclosed arm of the T-maze compared with WIS rats [47], we detected panicolytic activity in SHR controls versus normotensive WIS rats. It is worth noting that control SHRs appear much like epileptic SHRs with respect to inhibitory avoidance behavior. Furthermore, the higher latency in escape trials of SHR controls versus WIS controls, as well as comparable responses of the epileptic WIS rats and SHRs, revealed that the impulsivity and hyperactivity of SHRs and epileptic WIS rats do not distract from the escape response in the T-maze test.

Our results are in agreement with numerous experimental and clinical data revealing compromised 5-HT transmission in the hippocampus as a consequence of comorbid epilepsy and depression [2,26,48,49]. In addition, we have found that epileptic WIS rats and SHRs developing depression-like behavior have lower hippocampal DA levels. The hippocampus is considered a key limbic structure associated with development of the epileptic state in models of TLE. Electrophysiological data have revealed an inhibitory effect of 5-HT in hippocampal neurons [50], whereas elevated 5-HT levels have been proposed to contribute to the therapeutic actions of numerous anticonvulsants commonly used in clinics such as carbamazepine, valproate, lamotrigine, citalopram, and zonizamide [51]. The role of the 5-HT system in rat strains differing in their emotionality/anxiety was considered earlier [52]. Thus, anxious Lewis rats showed significantly higher basal 5-HT levels compared with SHRs, which are characterized by low anxiety levels. Low levels of 5-HT are associated with the impulsive and aggressive behavior commonly seen in the TLE model of epilepsy [53]. Recently, Kondziella et al. postulated that a deficiency in 5-HT may modulate neuronal hyperexcitability in the limbic system, responsible for reduced impulsive control and aggressive behavior [48]. Levels of 5-HT increased directly after induction of SE with pilocarpine, whereas DA levels decreased during the acute, latent, and chronic periods [54]. We have found that low anxiety levels in control and epileptic SHRs as well as epileptic WIS rats are associated with low levels of 5-HT, DA, and tryptophan compared with normotensive WIS controls. Previous data revealed a compromised dopaminergic system [30] and decreased 5-HT functioning in SHRs [29]. We could speculate that the anhedonia-like responses during the light phase and anxiolytic behavior in the T-maze of control SHRs are associated with diminished 5-HT levels in the hippocampus, whereas other systems would be involved in behavioral deviation in epileptic SHRs. The observed close relationship between the frequency of SMS and hippocampal DA levels in WIS and SHRs suggests a particular role for the DAergic system in epileptogenesis-induced biochemical alterations in epileptic rats.

In addition to 5-HT levels, we found diminished levels of the serotonin precursor tryptophan in the hippocampus of epileptic WIS rats and SHRs. Although almost all the serotonin in the brain results from classic projections out of the raphe cells, numerous studies also demonstrate the presence of tryptophan protein levels or enzyme activity in several other brain areas in animal studies. Tryptophan activity in the amygdala and hippocampus was detected in male rats after pinealectomy [55]. Moreover, the activities of a rate-limiting enzyme in the biosynthesis of serotonin, tryptophan hydroxylase (TPH), and a second isoform, TPH2 mRNA, could be measured in the frontal cortex, hypothalamus, thalamus, and hippocampus [56,57]. These findings may suggest alteration of axonal transport mechanisms or the existence of other serotoninergic neurons in the aforementioned brain regions.

In conclusion, behavioral disturbances during the chronic phase in the KA model of TLE involve depression-like symptoms, anxiolytic activity, and panicolytic-like activity associated with increased seizure activity in WIS and SHRs. However, although a significantly higher frequency of spontaneous seizures in SHRs were accompanied by exacerbations in depression- and panicolytic-like behavior with abolished diurnal rhythms, the negative correlation of SMS with despair and anxiolytic behavior was evident only during the light phase in WIS rats. In addition, reduced hippocampal levels of 5-HT and DA in control SHRs as well as epileptic SHRs and WIS rats may provoke disturbances in emotional responses and higher seizure activity during the chronic epileptic phase.

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