Epilepsy & Behavior 31 (2014) 198–208

Contents lists available at ScienceDirect

Epilepsy & Behavior

journal homepage: www.elsevier.com/locate/yebeh

Treatment with melatonin after status epilepticus attenuates seizure activity and neuronal damage but does not prevent the disturbance in diurnal rhythms and behavioral alterations in spontaneously hypertensive rats in kainate model of temporal lobe epilepsy

Zlatina Petkova [1], Jana Tchekalarova *[,1], Daniela Pechlivanova [1], Slavianka Moyanova, Lidia Kortenska, Rumiana Mitreva, Deyan Popov [2], Petya Markova [2], Valentin Lozanov [3], Dimitrina Atanasova, Nikolai Lazarov [4], Alexander Stoynev [5]

Institute of Neurobiology, Acad. G. Bonchev Str., Bl. 23, Bulgarian Academy of Sciences, Sofia 1113, Bulgaria

ARTICLE INFO

Article history:
Received 23 October 2013
Revised 14 November 2013
Accepted 15 December 2013
Available online xxxx

Keywords:
Spontaneous hypertensive rats
Kainate model of temporal lobe epilepsy
Melatonin
Diurnal rhythms

ABSTRACT

Melatonin is involved in the control of circadian and seasonal rhythmicity, possesses potent antioxidant activity, and exerts a neuroprotective and anticonvulsant effect. Spontaneously hypertensive rats (SHRs) are widely accepted as an experimental model of essential hypertension with hyperactivity, deficient sustained attention, and alterations in circadian autonomic profiles. The purpose of the present study was to determine whether melatonin treatment during epileptogenesis can prevent the deleterious consequences of status epilepticus (SE) in SHRs in the kainate (KA) model of temporal lobe of epilepsy (TLE). Spontaneous recurrent seizures (SRSs) were EEG- and video-recorded during and after the treatment protocol. Melatonin (10 mg/kg diluted in drinking water, 8 weeks) increased the seizure-latent period, decreased the frequency of SRSs, and attenuated the circadian rhythm of seizure activity in SHRs. However, melatonin was unable to affect the disturbed diurnal rhythms and behavioral changes associated with epilepsy, including the decreased anxiety level, depression, and impaired spatial memory. Melatonin reduced neuronal damage specifically in the CA1 area of the hippocampus and piriform cortex and decreased hippocampal serotonin (5-HT) levels both in control and epileptic SHRs. Although long-term melatonin treatment after SE shows a potential to attenuate seizure activity and neuronal loss, it is unable to restore epilepsy-associated behavioral abnormalities in SHRs.

1. Introduction

Depression is a common comorbid disorder in epilepsy, but it often remains unrecognized and untreated. Recently, we studied the development of behavioral and neurochemical indices of depressive state in the kainate (KA) model of TLE in two rat strains [1]. The emotional disturbances in epileptic Wistar and SHRs were accompanied by decreased levels of hippocampal serotonin (5-HT) and dopamine (DA). These results were in agreement with the reported compromised serotoninergic neurotransmission in the raphe-hippocampal serotonergic pathway in the pilocarpine post-status epilepticus (SE) model [2]. One factor, which may contribute to the development of depressive state in TLE is rhythm disturbances and, in particular, phase changes in melatonin synthesis. It is widely accepted that mood disorders are related to biological rhythm abnormalities, which can include diurnal mood variation, elevated nocturnal body temperature, lower nocturnal thyroid-stimulating hormone, an overall increased cortisol secretion, and sleep architecture abnormalities as well as an increase in cortisol and melatonin secretion [3]. Products active on circadian rhythms are shown to have chronobiotic effects, and exogenous melatonin is considered one of the best-known

Abbreviations: ABP, arterial blood pressure; BL, basolateral nucleus; C, control; DA, dopamine; DNA, deoxyribonucleic acid; DWRME, "double" working and reference memory error; EEG, electroencephalography; EPM, elevated plus maze; FST, forced swimming test; 5-HT, serotonin; Hip, hippocampus; HPLC, high-performance liquid chromatography; i.p., intraperitoneally; KA, kainic acid; Mel, melatonin; OF, open field; Pir, piriform cortex; PT, pars tuberalis; RAM, radial arm maze; RME, reference memory error; s.c., subcutaneously; SCN, suprachiasmatic nucleus; SCT, sucrose consumption test; SE, status epilepticus; SHRs, spontaneously hypertensive rats; SRSs, spontaneous recurrent seizures; SWDs, spike–wave discharges; TLE, temporal lobe epilepsy; Veh, vehicle; WM, working memory error.

* Corresponding author.
E-mail address: janetchekalarova@gmail.com (J. Tchekalarova).

[1] These authors contributed equally to this project and should be considered co-first authors.

[2] Present address: Department of Physiology, Medical Faculty, Medical University (MU)-Sofia, Bulgaria.
[3] Present address: Department of Medical Chemistry and Biochemistry, Medical Faculty, MU-Sofia, Bulgaria.
[4] Present address: Department of Anatomy, Medical Faculty, MU-Sofia, Bulgaria.
[5] Present address: Department of Pathophysiology, Medical Faculty, MU-Sofia, Bulgaria.

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http://dx.doi.org/10.1016/j.yebeh.2013.12.013

chronobiotic molecules [4]. Besides its primary function as a synchronizer of the biological clock [5], melatonin is a powerful antioxidant that can easily cross cell membranes and the blood–brain barrier. The accumulated clinical and experimental data raise the point about the potential therapeutic role of melatonin in epilepsy. It has been suggested that a lack of sufficient concentrations of melatonin in patients with epilepsy predisposes them to seizure activity, while adaptive increase of melatonin levels may be protective against repetitive seizures [6]. Clinical evidence revealed that melatonin could be used for seizure control in conjunction with antiseizure medications [7].

Intact SHRs represent a useful model to explore mechanisms underlying disturbed circadian synchronization [8,9]. The disturbances of physiological circadian rhythms underline also a number of impaired processes, some of them resulting in cardiovascular diseases. Therefore, the melatonin system is a good candidate strategy for the treatment of circadian timing system disturbance. Cerebrovascular changes, brain atrophy, loss of nerve cells in cerebrocortical areas, and glial reaction were documented in this strain (reviewed in [10]). In chronic models of epilepsy, SHRs are reported to kindle more rapidly than Wistar Kyoto rats in amygdala and piriform kindling [11]. It is documented that the enhanced seizure susceptibility observed in the pilocarpine model of TLE correlates with neuropathological alterations in the hippocampal formation of SHRs [12]. Clinical data showed disturbances of melatonin biosynthesis in patients with hypertension [13], while experimental studies reported an antihypertensive effect of melatonin in SHRs [14,15]. Taken together, these data are in agreement with the idea that melatonin may have potential for preventing neuronal damage after brain insults such as brain trauma and cerebral ischemia-induced SE, including hypertension-evoked brain damage. Acute administration of melatonin in rats before and during SE induced by either KA or pilocarpine displays neuroprotective effects by reducing neuronal death, supragranular mossy fiber sprouting, lipid peroxidation, and microglial activation [16–18].

Recently, we reported that unlike in the epileptic Wistar rats, which were characterized with depressive behavior only during the light phase, depressive-like patterns were without diurnal variations in epileptic SHRs [1]. Furthermore, the long-term administration of melatonin alleviated the seizure activity during the period of treatment, decreased the neuronal loss in the hippocampus and the piriform cortex, and exerted changes in the behavioral responses specifically during the inactive period in the chronic epileptic phase of normotensive Wistar rats [19]. The important function of melatonin in the circadian timing system and epileptic phenomena, on the one hand, and the significance of synchronization of circadian rhythms both in epilepsy and depression, on the other hand, prompted us to further explore whether long-term melatonin treatment after SE could alleviate the deleterious consequences of SE and the diurnal behavioral disturbances during the chronic epileptic state in SHRs.

2. Material and methods

All experiments were executed in compliance with the European Communities Council Directive of 24th November 1986 (86/609/EEC), and the experimental design was approved by the Institutional Ethics Committees of Sofia Medical University and the Institute of Neurobiology for the National Science Fund grant DTK 02/56 2009-1012.

2.1. Subjects

The experiments were performed on eight-week-old male spontaneously hypertensive rats (SHRs) obtained from the local breeding house (Medical University, Sofia). Following their arrival in the laboratory, the animals were habituated for one week (12/12-h light/dark cycle, lights on at 08:00 h), individually housed under standardized conditions (20 ± 3 °C, 40–60% relative humidity), and handled daily. Food and water were available ad libitum throughout the study except during the tests. All experiments were carried out in the autumn–winter season.

2.2. Experimental design and drug treatment

Animals were randomly divided into four main groups: C-veh (control group treated with vehicle, n = 15); C-mel (control group treated with melatonin, n = 15); KA-veh (rats treated with KA and vehicle, n = 16); and KA-mel (rats treated with KA and melatonin, n = 17). Treatment with melatonin (Sigma-Aldrich, Bulgaria) started 3 h after the beginning of SE at a dose of 10 mg/kg, previously shown to have neuroprotective and antioxidant activities [20]. During the first three days, when the animals were unable to drink via bottles, melatonin was injected subcutaneously (s.c.), dissolved in a lactated Ringer's solution (2 ml/100 g of body weight/day, s.c.). Matched animals not treated with KA were also injected with melatonin or vehicle during the first three days. Later, melatonin was diluted in the drinking water for a period of 8 weeks. Control rats were given tap water. Daily preparation of drinking water containing melatonin was adjusted dependent on the individual consumed volume of a liquid. Behavioral tests started about 14 weeks after SE, when the rats developed a stable chronic epileptic state. The time interval between each test was at least 2 days. The order of the behavioral tests was as follows: open-field (OF) test, elevated plus maze (EPM) test, and forced swimming (FS) test. Ten days after the last testing, rats were tested with the radial arm maze (RAM) test to evaluate whether the chronic melatonin treatment was able to improve learning disability of epileptic rats in a hippocampal-dependent spatial task.

2.3. Surgery

Four rats from the KA-veh group and the KA-mel group were monitored for electrographic seizures affecting the frontal and parietal cortices and the dorsal hippocampus using either epidural or depth chronic recordings. Electrodes for electroencephalography (EEG) recording were implanted on the rats under a mix of ketamine (40 mg/kg) and xylazine (20 mg/kg, intraperitoneally (i.p.)) anesthesia. The animals were placed on a rectal temperature feedback-controlled pad (Digitherm, Yukon-PC, Sofia, Bulgaria), which maintained body temperature at 37 °C. Following local anesthesia with procaine 0.5% and fixation in a stereotaxic device (Narishige Sci. Inst. Labs, Japan), a midline incision over the skull was made, and the skin and the periosteum were removed with aseptic precautions. For monopolar EEG recordings, pieces of teflon-coated (275 μm) stainless steel wire (Medwire Corp, N.Y.) were inserted into premade small holes in the calvaria bilaterally of both hemispheres above the parietal cortical areas (A = −4.2, L = ±3.0). For the hippocampal electrodes, we used two sets of twisted wires, from which the tips were separated vertically by 1 mm and inserted into the left and the right hemispheres with the coordinates for the dorsal hippocampus as follows: A = −4.0, L = ±2.5, and H = −3.3. The other ends of the wires were soldered to the pins of a female miniature socket. Two miniature stainless steel screws, one fixed on the skull above the frontal bone and the other posterior to the lambda, were used for ground and common references, respectively. All wires and screws were covered and fixed to the skull with dental acrylic cement. The two screws served also for anchoring the cement mound to the skull. The rats recovered after the surgery for about one week, during which they were handled once daily and then placed in a recording cage for habituation. Recording of control EEG (before the KA treatment) started 7 days after the surgery and was performed on entirely conscious rats. The female connector fixed on the animal's head was matched with a male connector, to which a flexible shielded cable was soldered. The opposite end of the cable was connected to a swivel commutator mounted on the box's ceiling allowing EEG to be recorded continuously in awake, unrestrained animals. The output of the connector was fed to a Nihon Kohden electroencephalograph (Japan), and EEG was recorded by means of the

MP150 System (BIOPAC Systems, Inc., USA) connected to the amplifiers of the EEG machine.

2.4. Induction of status epilepticus with kainic acid

Details of KA-induced SE methodology were recently published [1,23]. In brief, SE was induced by repetitive KA (Ascent Scientific, UK) injections (5 mg/kg/h, i.p.) according to the protocol of Hellier et al. [21]. Matched controls were treated with an equivalent volume and number of injections of saline. Kainate was diluted in sterile saline (0.9% NaCl) at 5 mg/ml. Seizure intensity was evaluated by a modified Racine's scale [22]. Class I and class II (immobility, facial automatism, head nodding, and wet-dog shakes) were grouped as partial seizures and were not scored. Class III (forelimb clonus with lordotic posture), class IV (rearing and continued forelimb clonus), and class V (forelimb clonus and loss of posture) were grouped as secondary generalized seizures. Kainate treatment continued until sustained seizures of class III, IV, or V (i.e., >9 motor seizures/h) were observed in rats. The number of motor seizures, i.e., classes III, IV, and V, was registered and used as a criterion for an additional KA injection. The severity and duration of SE during the KA treatment protocol were evaluated by means of EEG and video-monitoring (video recorder DVR-4 with AVTECH camera, Taiwan, no. AVC307R). Only the rats that developed SE (recurrent seizures with bilateral forelimb clonus for at least 3 h) and survived thereafter were included in the subsequent analyses. Electroencephalography recording was performed for at least 1 h three times a week. The KA-treated rats received lactated Ringer's solution (2 ml/100 g of body weight/day, s.c.) to prevent dehydration, as well as apple slices and moistened rat chow until they recovered from SE.

2.5. Video and EEG monitoring of spontaneous recurrent seizures

The KA-treated rats were placed in individual transparent labeled kennels for optimal video observation. Video monitoring (24 h/day for up to 20 weeks starting 48 h after SE) was accomplished using an infrared-sensitive colored camera (S-2016, AVTECH, Taiwan, no. AVC307R) connected to a computer. The recordings were visually analyzed by independent observers to detect SRSs. The SRSs during the experimental manipulations when the animals were outside of their boxes were also noted. The evaluated parameters were as follows: seizure latent period for onset of the first SRSs, variation in seizure occurrence related to circadian rhythm, and frequency and progression of SRS of classes IV and V. During the period of video observation, the EEG of KA-treated rats implanted previously with electrodes was recorded with concomitant video monitoring for at least 1 h three times a week. Visual detection of ictal events was performed through inspections of the EEG files recorded by means of the AcqKnowledge software ACK100W (BIOPAC Inc., USA). The criteria for epileptic seizures were abrupt onset of epileptiform activity that lasted at least 5 s and amplitude that was two times higher compared with the baseline EEG.

2.6. Body weight and arterial blood pressure measurements

Because of a slight variability in weight among individual rats before the start of experiments, the effect of melatonin in control and epileptic SHRs on body weight gain in % was analyzed every week up to the 10th week following SE.

Systolic arterial blood pressure (ABP) was measured noninvasively in conscious unrestrained rats by a tail-cuff method (Ugo Basile Blood Pressure Recorder 5800): two times in naive rats, before the start of treatments (KA and melatonin) and on treated rats, immediately after interruption of the drug treatment. The ABP value for each rat was calculated as the mean of three measurements.

2.7. Sucrose consumption test

Sucrose preference behavior was evaluated using a sucrose preference test at the end of the third month post-status. On the first day (habituation), each cage was supplied with two identical graduated bottles filled with water at a volume of 100 ml. On the 2nd (pretest) and 3rd (test) days, water in one of the bottles was replaced with 1% sucrose. The test started at 08:00 a.m. Both bottles were weighed after 12 h and replaced by a second pair of preweighed bottles. Sucrose preference was expressed as the percentage of the volume of sucrose solution of the total volume of fluid (sucrose plus regular water) consumed during a 12-h period (light phase — 8:00–20:00 h and dark phase — 20:00–8:00 h, respectively).

2.8. Behavioral tests

All behavioral tests were carried out under artificial diffused light during the light phase and in red dim light during the dark phase. Open-field test, elevated plus-maze test, and forced swimming test were performed at two time points 6 h after lights on/off (i.e., at 15:00 p.m. and 03:00 a.m., respectively). The rats of each experimental group were divided in two subgroups for OF, EPM, and FST conducted during the light and during the dark period, respectively. The behavioral experiments were conducted in a soundproof room, where the animals were moved into at least 30 min before each test. The rats that exhibited SRSs 1 h before starting the tests were excluded from the experimental procedure. The behavior of the rats in the OF, EPM, and RAM tests was recorded using an infrared sensitive CCD camera and a video tracking system (SMART PanLab software) from Harvard Apparatus, USA.

2.8.1. Open-field test

The open-field apparatus consisted of a gray polystyrene plexiglas box (100 × 100 cm × 60 cm) divided into two zones: outer square (periphery) and inner square (center). The calculated standard measures were the following: 1) total distance traveled (cm), 2) ratio of distance (cm) center/total distance traveled in %, and 3) ratio of time spent (s) center/total time in %. The number of rearings was recorded manually by the experimenter. The rat was placed in the center of the box and was allowed to explore it for 5 min.

2.8.2. Elevated plus maze test

The apparatus of black wood had two open arms (50 × 10 cm), two enclosed arms (50 × 10 × 50 cm), and a central platform (10 × 10 cm). The apparatus was elevated 50 cm above the floor level. The calculated standard measures were as follows: 1) ratio of distance (cm) of the open arms/total distance traveled in % and 2) ratio of time spent (s) in open arms/total time in %. At the beginning of the test, the rat was placed on the central platform facing an open arm. The test lasted 5 min.

2.8.3. Forced swimming test

The test was carried out in a clear and transparent cylinder (50 cm tall–25 cm in diameter) filled to a level of 30 cm from the bottom with 24 °C tap water. Two swimming sessions were conducted: 15 min on the 1st day and 5 min on the 2nd day. After each test, the rat was dried and kept warm by a heating device for 10 min. Behavior during the 2nd day (test) was recorded by two skilled experimenters unaware of the treatment conditions. The parameter measured was immobility in seconds, which occurred when the rat remained motionless or made only movements necessary to keep its head above the water.

2.8.4. Radial arm maze (RAM) test

Visuospatial learning and memory was assessed using an 8-arm radial maze (RAM) (Harvard Biosci. Comp., USA). The stainless steel RAM consisted of a central octagonal platform (30 cm in diameter) from which eight identical closed, transparent arms (42 × 12 × 12)

radiated from the platform. The maze was elevated 50 cm above the floor level. A variety of environmental cues (wall pictures, table, cupboards, door, and window) were available for facilitation of spatial navigation. Seven days before the start of training and during the test, rats were put on a diet for 15% reduction in body weight. Before the memory testing, all animals were habituated (shaping) before the memory testing to the maze and the experimental set-up for up to 3 days (adapted from [51]). Rats were placed onto the central platform and allowed to explore the maze for 15 min per day. Reinforcements were initially scattered at various distances along all the arms. On the last day of habituation, the amount of reinforcements was reduced to one piece at the end of every arm, and the session was ended when all arms had been visited or after 15 min. Following habituation, the animals were trained in the standard RAM task with one session per day for 18 trials with two days off over the weekend. Four arms, marked with symbols, were baited with one piece of sweet food pellet placed at the end of each arm. The session ended when all baits were found or after 10 min had elapsed. Memory acquisition was assessed through the decrease in the number of errors between each trial. Reentry into a baited arm from which the food pellet had already been retrieved was scored as working memory errors. The total number of arms visited per session was also recorded.

After every test, OF, EPM, and RAM apparatuses were thoroughly cleaned with 0.1% acetic acid solution.

2.9. Histology

After about 20 weeks post-status, under deep anesthesia with Nembutal (50 mg/kg, i.p., Abbott) in a first step, the rats ($n = 5$ per group) were transcardially perfused initially with 0.05-M phosphate buffered saline at pH 7.3 followed by 4% paraformaldehyde in 0.1 M phosphate buffer at pH 7.3. The brains were dissected out and postfixed overnight at 4 °C in the same fixative solution. After postfixation, the brains were sliced in the coronal plane and cryoprotected in 20% phosphate-buffered sucrose solution. After an overnight incubation at 4 °C, the brains were cut on Reichert Jung freezing microtome, and 30-μm thick serial sections were taken in subsets of 4. Every first section of the subset was mounted on chrome–gelatin-coated slides, and Nissl staining with cresyl violet was performed. After staining, the sections were dehydrated in graduated series of ethanol, cleared in xylene, and embedded in Entellan. The sections were investigated on a Nikon Eclipse 80i light microscope (Japan) and photographed with a digital camera (Nikon DMX 1200). Sections were analyzed for major cell loss with special attention to the dorsal and ventral hippocampi and the piriform cortex and basolateral nucleus (BL) of the amygdala as previously described [23]. The staining intensity and density of nerve cell bodies in the hippocampus, piriform cortex, and basolateral nucleus of the amygdala (BL) were estimated by the Nikon's NIS Elements Digital Imaging software. The relative neuronal densities of the selected brain areas were quantified by determining the percentage of the measurement grid occupied by stained cells. The resulting values provide a relative index of the number of stained neurons in the selected brain areas.

2.10. High performance liquid chromatography

An additional subgroup of rats ($n = 8$–10 per every group) was used for HPLC data. After decapitation, brains were quickly dissected on ice, and the frontal cortex and hippocampi were bilaterally removed. The 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 min, and an aliquot of 10 μl of the supernatant was used for analyses. Each pooled sample (from both hemispheres) was analyzed for 5-HT content as measured by LC/MS/MS. The measurements were performed through electrospray ionization in positive mode on a LTQ Orbitrap Discovery® (Thermo Fisher, Germany) connected to the Surveyor®HPLC system (Thermo Fisher, Germany). The analyzed compounds were separated on a ZIC®-HILIC (Merck, Germany) analytical column in isocratic elution mode with mobile phase of 70% acetonitrile, containing 15-mM formic acid at a flow rate of 200 μl/min. The quantitative analyses were performed using "selected ion monitoring" mode with external calibration. The data acquisition and processing were performed by means of Xcalibur® software.

2.11. Statistical analysis

Parametric (for normally distributed data) or nonparametric tests (for data not normally distributed) were used for the statistical analysis (SigmaStat® 11.0). To effectively display the individual differences of seizure frequency data, box plots were generated. The central line in each box plot represents the median, whereas the lower and upper halves of the box are the 25th and 75th percentiles, respectively. The whiskers on the box are the minimum and maximum scores for the group. The significance of differences in behavioral, histological, and biochemical data among groups was calculated by analysis of variance (ANOVA), followed by post hoc testing conducted by Bonferroni or Holm Sidak *t*-test for individual differences. If data were not normally distributed, ANOVA for nonparametric data (Kruskal–Wallis on ranks) followed by the Mann–Whitney *U* test was used. For the RAM test, with the exception of the first session day, data from blocks of three sessions were combined for each animal to obtain more stable results as compared with a single session day. In addition, the data from the first session day (for working memory error) were presented separately, as this day reflects the first exposure of the rat to the maze task. A $p < 0.05$ value was accepted as indicating statistically significant differences.

3. Results

3.1. KA-induced SE

During the baseline electrographic recordings, no seizures were detected in SHRs of both groups. In accordance with previous reports, the development of SE in SHRs was reached after 3–4 repetitive intraperitoneal injections of KA, which was accompanied with increased seizure activity (motor seizures, class IV/V, i.e., >9 motor seizures/h for at least of 3 h). The dose of KA (mg/kg) required to induce SE was as follows: median ± S.D.: 22.5 ± 5.3 (range: 12.5–32.5). In the waking EEG, low-voltage fast waves alternated with generalized theta wave bursts (Figs. 1Aa, Ba) of about 7 Hz (see the inset in Fig. 1Ba where a representative power spectrum is shown). These waves were expressed more in the hippocampal leads. During the treatment with KA, an onset of continuous extensive EEG spiking activity occurred in all SHRs with implanted electrodes, then wet-dog shakes and repetitive electrographic seizures developed in the cortical and hippocampal leads, which later culminated in a typical SE (Figs. 1Ab, Bb) ranked behaviorally as classes IV–V of Racine's scale. After the last KA injection, seizures gradually subsided, and during the next few days, only some paroxysmal EEG signs remained.

The treatment of rats with melatonin (KA-mel group) or vehicle (KA-veh group) began 3 h after the onset of SE, and the recorded EEG was different between the abovementioned two groups during the time course and the manifestation of the epileptogenesis. Three out of 33 SHRs died during the SE or in the post-status period. The final size of KA-treated groups was as follows: KA control (KA-veh, $n = 13$) group and KA melatonin (KA-mel, $n = 17$) group, respectively. During the seizure-latent phase, solitary spike(s) and sharp waves, rhythmic sharp wave bursts, and spike–wave complexes occurred in the EEG recordings (Fig. 1Ac). In the KA-mel group, EEG waves during the

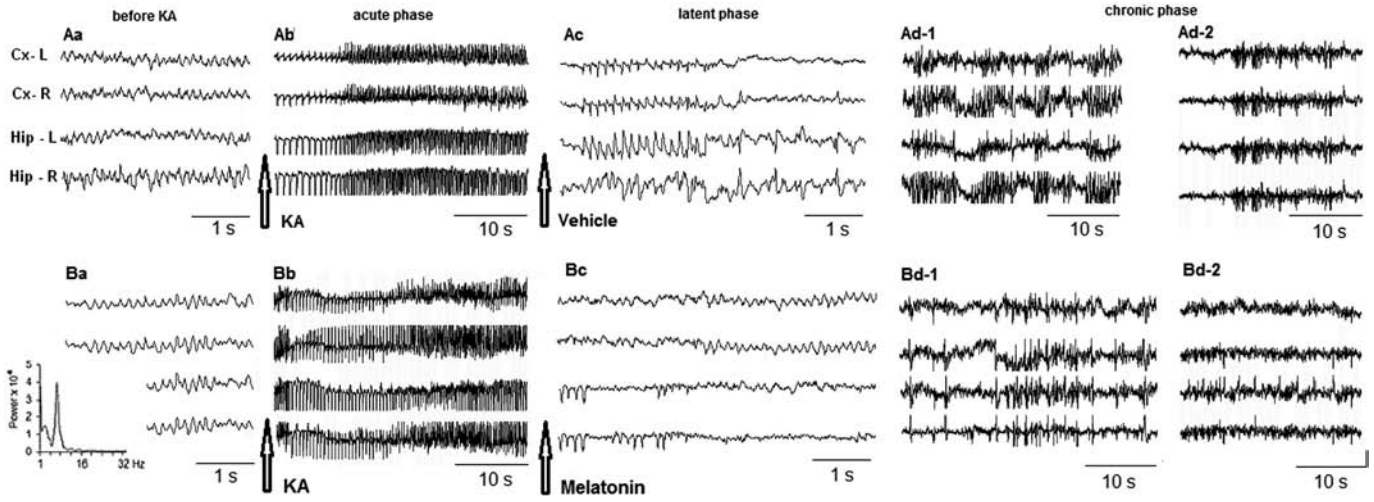

Fig. 1. Electrographic activity from the left and right parietal cortices (Cx-L and Cx-R) and the left and right dorsal hippocampi (Hip-L and Hip-R) in two representative rats, a rat from group KA-veh (Aa, Ab, Ac, and Ad); a rat from the kainate (KA)-mel group (Ba, Bb, Bc, and Bd). Panels a, b, c, and d are EEG examples from baseline recordings before KA (Aa and Ba), during the acute phase of status epilepticus (SE) several hours after KA (Ab and Bb), during the seizure-latent phase of the epileptogenesis (Ac and Bc) before the appearance of the first spontaneous recurrent seizure (SRS), and during the chronic phase after the first SRS (Ad-1 and Ad-2, Bd-1 and Bd-2). No seizures were seen in the spontaneously hypertensive rats (SHRs) prior to KA treatment: the cortical waking EEG typically was low amplitude and fast, but often, theta EEG activity was seen in both cortical and hippocampal leads (Aa and Ba with well-expressed peak at 7 Hz in representative power spectrum shown in an inset). Panels Ab and Bb show high-frequency, large-amplitude spike activity and generalized EEG seizures during the KA treatment. Later, the SE subsided, but irregular spikes, spike–waves, and groups of spike–wave discharges (SWDs) continued during the seizure-latent phase in the KA-veh group (Ac, 10 days after KA). Expended time scale on Ac is used to show the nonconvulsive SWDs followed by isolated spikes in the hippocampal leads. In the KA-mel rat, spikes were seen only in the hippocampal leads during the seizure-latent phase when continuous generalized slow theta cortical activity was also seen (Bc, 21 days after KA). First spontaneous electrographic seizures in the KA-mel rat appeared later (Bd, 29 days after KA) than in the KA-veh rat (Ad-1, 12 days after KA), the seizures appeared more rarely in the KA-mel rat (Bd-1) than in the KA-veh rat (Ad-1); and in the KA-mel rat, they subsided 2–3 months (Bd-2, 98 days) after the first SRS, when isolated spikes remained only in the hippocampal EEG. In the KA-veh rat, EEG seizures (Ad-2, 102 days) were recorded until the end of the EEG experiments (115 days after KA treatment). Calibration: 1 s and 10s, 100 μV.

seizure-latent phase were generally low voltage and of different frequencies with prevailing theta frequencies in the cortical leads and rarely appearing spikes in the hippocampal leads (Fig. 1Bc). The spontaneous recurrent EEG seizures in the KA-veh rats were detected during the whole chronic phase of epilepsy (from 4 to 12 days after KA injection until the end of the EEG experiments, i.e., 115 days after the KA treatment protocol) (Figs. 1Ad-1 and Ad-2). In the KA-mel rats, the spontaneous EEG seizures were characterized by smaller frequency of spikes and recurred more rarely than in rats of the KA-veh group, and after the EEG seizures, spikes remained only in the hippocampal leads (Figs. 1Bd-1 and Bd-2).

3.2. Development of spontaneous recurrent seizures and their circadian distribution in different treatment groups

In total, thirty rats were continuously video-monitored for a period of five months starting two days after SE. Spontaneous recurrent seizures were detected in all KA-treated rats: KA-veh group (total number of SRSs: 2021 and a seizure-latent period: mean ± S.D.: 13 ± 16.7 from 4 to 32 days, median 13 days, n = 13) and in the KA-mel group: (total number of SRSs: 691 a seizure-latent period: mean ± S.D.: 31.3 ± 18.3 from 6 to 60 days, median 39 days, n = 17), respectively. In the KA-veh rats, SRSs appeared in clusters (i.e. >3 seizures per day) in 9 out of 13 (69%) rats, while in the KA-mel rats, 3 out of 17 (18%) were clustered (Fisher exact test: $p < 0.051$). The distribution of seizure activity as a function of day/night period was assessed between the 9th and 20th weeks after SE when the rats displayed a relatively high seizure frequency (Fig. 2). In accordance with our previous studies, the KA-veh group exhibited a circadian distribution of the seizure appearance with greater prevelance when the lights were on (Holm-Sidak method: $p < 0.01$) [1,23]. The proportion of motor seizures during the lights "on" period was 74% in the KA-veh group compared with 54% in the KA-mel group (Fig. 2). Compared with the KA-veh group, the melatonin-treated group experienced flattened diurnal rhythm of spontaneous seizure activity (Holm-Sidak method: $p = 0.145$).

A high variability in the frequency of motor seizures (expressed as an average number of seizures per day) was found among rats treated with either vehicle or melatonin (Fig. 3; Table 1). The median daily seizure frequency was 0.6 (range: 0.13–1.3) for the KA-veh group and 0.2 (range: 0–2.7) for the KA-mel group, respectively, during the first monitoring period, which coincided with the period of the melatonin treatment (1–8 weeks) (Table 1). Twelve out of 17 rats treated with melatonin had less than 0.1 seizures/day, while all vehicle-treated rats had higher than 0.1 seizures/day (Table 1). The median daily seizure

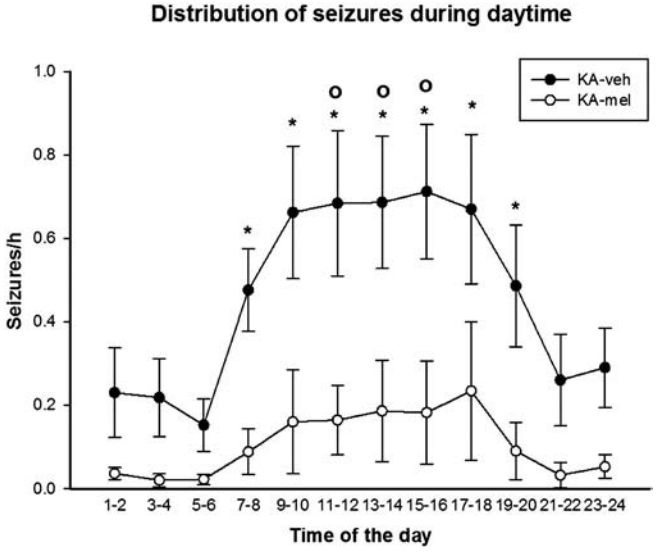

Fig. 2. Distribution of SRSs in rats during 9–20 weeks following KA-induced SE. Based on four selected rats in the KA-veh and KA-mel groups, respectively, displaying the highest seizure frequency (seizure/h), it was determined for each two-hour segment of the day. Abbreviations in legends: KA-veh (group with epilepsy treated with vehicle); KA-mel (group with epilepsy treated with melatonin). $^{*}p < 0.05$, $^{o}p < 0.05$, $^{+}p < 0.05$, $^{\#}p < 0.05$ within group (11–16 h vs. 1–2 h; 09–16 h vs. 3–4 h; 08–18 vs. 5–6 h; 15–16 h vs. 23–24 h, respectively).

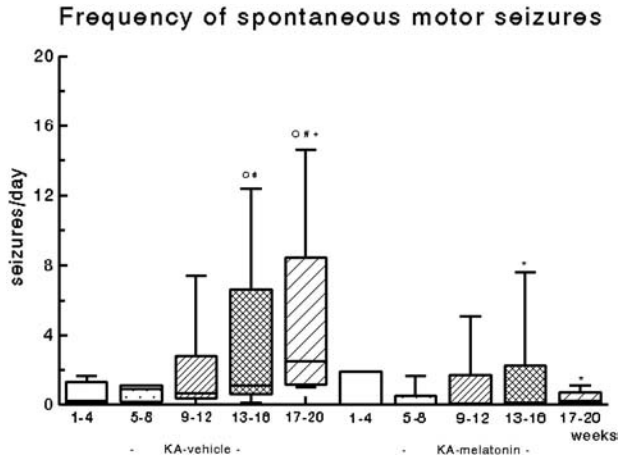

Fig. 3. Seizure frequency in rats during 1–4, 5–8, 9–12, 13–16, and 17–20 weeks after KA-induced SE. The data present seizure frequency (number of seizures per day) as function of time of the KA-veh group (n = 13) and the KA-mel group (n = 17). Vehicle or melatonin was administered for 8 weeks after SE. $^{*}p < 0.05$ between groups (vs. the KA-veh group); $^{\#}p < 0.05$, $^{o}p < 0.05$, $^{+}p < 0.05$ within a group (vs. 1–4, 5–8, and 9–12 weeks post-status, respectively) (Kruskal–Wallis test).

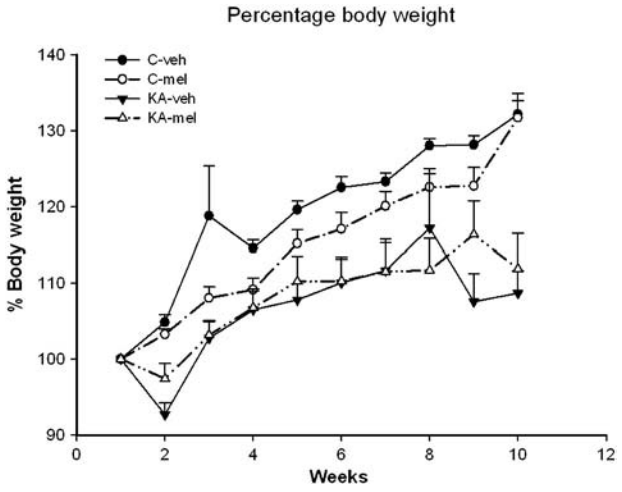

Fig. 4. Percentage of body weight over a 10-week period of observation. The graphs represent the mean ± SEM. Four groups of rats are illustrated: controls (n = 8–10) — C-veh and C-mel rats that received vehicle or melatonin (10 mg/kg), respectively; KA-treated rats — KA-veh and KA-mel, in which SE was induced on week 1 followed by once-daily treatment with vehicle/melatonin for 8 weeks. A repeated measures ANOVA showed a significant main effect of Epilepsy [$F_{1,346} = 105.996$, $p < 0.001$] and a Week [$F_{9,346} = 26.221$, $p < 0.001$] with Epilepsy × Week interaction [$F_{9,346} = 3.293$, $p < 0.001$] and Epilepsy × Drug interaction [$F_{1,346} = 8.589$, $p < 0.004$]. The KA-veh and KA-mel groups weighed significantly less than the C-veh group at all time points (measured from week 2 to 10) ($^{*}p < 0.05$). The C-mel group weighed significantly less than the C-veh group at weeks 3 and 4 ($^{o}p < 0.05$).

frequency was 1.22 (range: 0.8–10.6) for the KA-veh group and 0.2 (range: 0.01–4.3) for the KA-mel group during the second monitoring period (9–20 weeks after SE). Moreover, the frequency of seizures was significantly decreased in the KA-mel group compared with the KA-veh group during the last two months, i.e., 13–16 and 17–20 weeks post-status ($^{*}p < 0.05$) (Fig. 3). In addition, a significant progression in seizure frequency as a function of time was evident only for the KA-veh group ($^{o\#+}p < 0.05$) (Fig. 3).

3.3. Body weight gain and arterial blood pressure

Epilepsy significantly altered the body weight gain during the 1-week period of measurement after SE (Fig. 4) ($p < 0.05$). The exposure to melatonin significantly decreased the body weight during the 3rd and the 4th week of treatment in control conditions ($^{o}p < 0.05$). The epileptic rats (KA-veh and KA-mel groups) showed less weight gain than naive SHRs during the whole period of measurement ($p < 0.05$).

Table 1
Daily seizure frequency (mean and range) for each rat during the first (1–8 weeks) (left) and the second (9–20 weeks) (right) monitoring periods in the KA-veh group (n = 13) and the KA-mel group (n = 17), respectively. The average number of seizures per day and the range of daily seizures were determined by taking the seizure recorded on the first day as the starting point. Abbreviation: "n.d." — not detected.

Group	First monitoring period		Second monitoring period	
	KA-veh	KA-mel	KA-veh	KA-mel
Rat 1	0.68 (0–11)	0.00 (0–0)	4.28 (2–11)	0.17 (0–3)
Rat 2	1.10 (0–9)	0.00 (0–0)	1.26 (0–9)	0.06 (0–2)
Rat 3	1.30 (0–9)	0.00 (0–0)	1.13 (0–13)	0.01 (0–1)
Rat 4	0.45 (0–6)	0.00 (0–0)	1.00 (0–4)	0.04 (0–5)
Rat 5	0.61 (0–7)	0.00 (0–0)	1.10 (0–6)	0.01 (0–4)
Rat 6	1.00 (0–11)	0.00 (0–0)	2.25 (1–14)	0.02 (1–14)
Rat 7	0.54 (0–9)	0.00 (0–0)	0.96 (0–9)	0.03 (0–4)
Rat 8	0.72 (0–5)	0.08 (0–2)	10.58 (6–22)	0.20 (0–5)
Rat 9	0.55 (0–3)	0.00 (0–0)	8.73 (2–23)	0.02(0–2)
Rat 10	0.50 (0–9)	0.05 (0–1)	1.17 (0–6)	0.03 (0–1)
Rat 11	0.28 (0–4)	0.02 (0–1)	1.10 (0–6)	0.20 (0–1)
Rat 12	1.10 (0–11)	0.02 (0–8)	0.84 (0–6)	0.02 (0–2)
Rat 13	0.13 (0–2)	0.15 (0–3)	n.d.	0.03 (0–1)
Rat 14	n.d.	0.17 (0–2)	n.d.	0.22 (0–1)
Rat 15	n.d.	2.66 (1–10)	n.d.	4.26 (0–2)
Rat 16	n.d.	0.17 (0–1)	n.d.	0.30 (0–2)
Rat 17	n.d.	0.44 (0–3)	n.d.	0.75 (0–6)
Median	0.61	0.20	1.22	0.17

The naive SHRs were characterized by a significantly high ABP (180 ± 1 mm Hg, $p < 0.005$). Furthermore, epilepsy exerted an additional increase in the ABP of SHRs (188.8 ± 1.8, $p < 0.05$). The long-term melatonin treatment during epileptogenesis significantly decreased the ABP in epileptic SHRs (167 ± 3.7, $p < 0.001$).

3.4. Sucrose consumption test (SCT)

Diurnal variation in sucrose consumption test was detected during the 3rd month in the C-veh group, KA-veh group, and KA-mel group, respectively (Fig. 5) ($^{\#}p < 0.05$). Three months after SE, a decrease of the sucrose consumption was detected in the KA-veh group during the

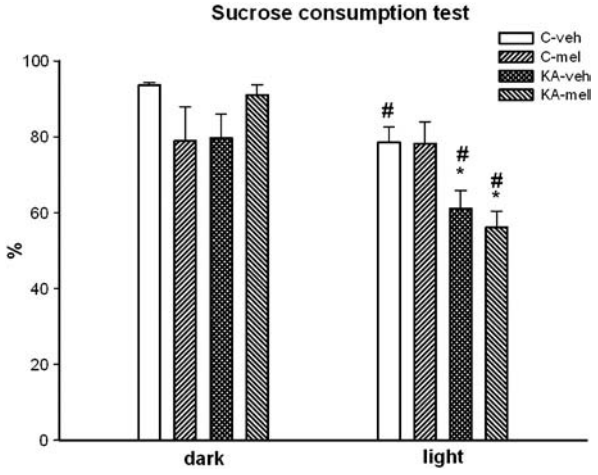

Fig. 5. Diurnal variations in sucrose consumption test during the 3rd month after status epilepticus (SE). Data are means ± SEM (n = 10). Analysis of data by three-way ANOVA indicated an Epilepsy effect [$F_{1,95} = 6.712$, $p = 0.011$], a Phase effect [$F_{1,95} = 18.812$, $p < 0.001$], and an Epilepsy × Phase interaction [$F_{1,95} = 5.629$, $p = 0.020$]. $^{*}p < 0.05$ vs. C-veh group; $^{\#}p < 0.05$ within a group (Light vs. Dark phase).

light phase (*p < 0.05). One month after discontinuation of the drug administration, the epileptic SHRs exhibited depressive-like behavior during the light phase similar to nontreated-with-melatonin epileptic rats (*p < 0.05).

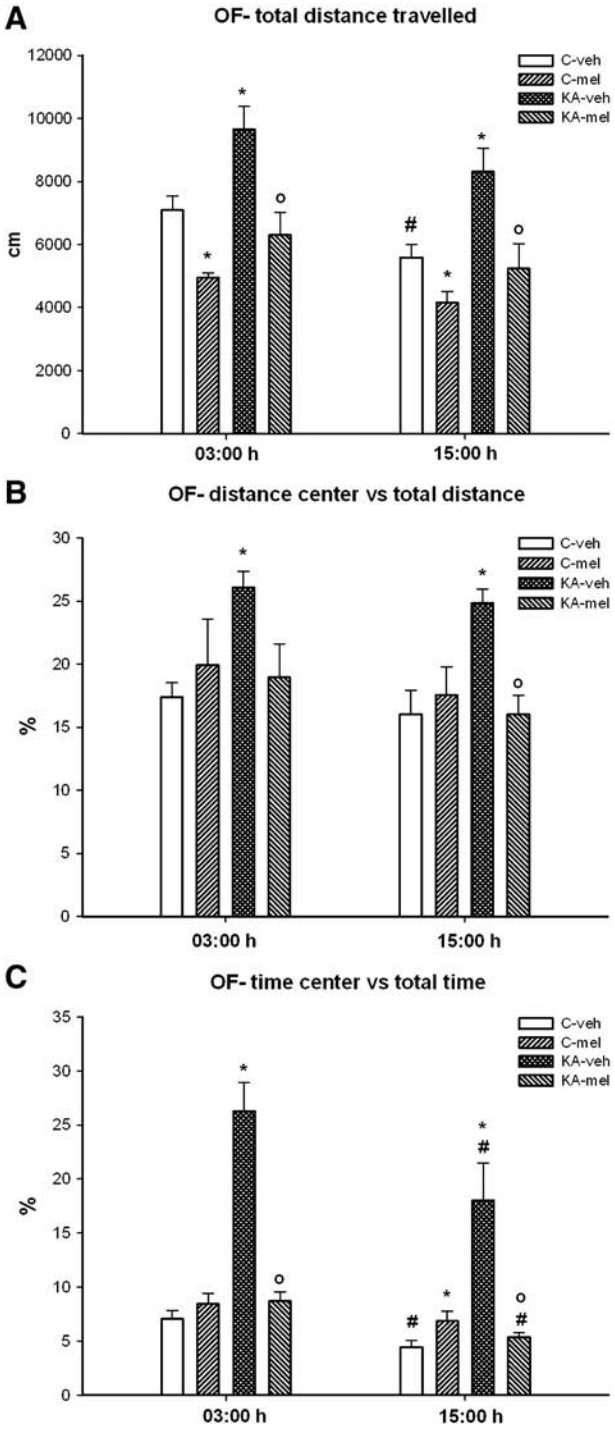

Fig. 6. Diurnal variations of activity in the open-field test: total distance traveled (A), ratio of distance center traveled vs. total distance (B), and ratio of time spent in center vs. total time (C). Data are means ± SEM (n = 10); analysis of data by three-way ANOVA indicated a main Epilepsy effect [$F_{1,96} = 13.208$, $p < 0.001$], a Drug effect [$F_{1,96} = 22.229$, $p < 0.001$], and a Phase effect [$F_{1,96} = 4.936$, $p = 0.029$] for data illustrated in (A); a main Epilepsy effect [$F_{1,142} = 6.811$, $p = 0.010$], an Epilepsy × Drug interaction [$F_{1,142} = 11.992$, $p < 0.001$] for the data illustrated in (B); a main Epilepsy effect [$F_{1,100} = 22.118$, $p < 0.001$], a main Drug effect [$F_{1,100} = 15.587$, $p < 0.001$], a main Phase effect [$F_{1,100} = 5.585$, $p = 0.020$], and Epilepsy × Drug interaction [$F_{1,100} = 25.777$, $p < 0.001$] for the data illustrated in (C). *p < 0.05 vs. C-veh group, °p < 0.05 vs. KA-veh group, #p < 0.05 within a group (15:00 h vs. 03:00 h).

3.5. Behavioral tests

3.5.1. Open-field test

The control rats were characterized by diurnal variations of locomotor activity and exploratory activity measured by total distance traveled (Fig. 6A) (#p < 0.05). The melatonin treatment abolished this diurnal activity rhythm (#p > 0.05). The total distance traveled was decreased in the C-mel group during both the light and the dark phase (*p < 0.05). The KA-veh group was hyperactive with abolished diurnal fluctuations. The increased locomotor activity was attenuated to control level in the KA-mel group (°p < 0.05). The KA-veh group was less anxious than the C-veh group without diurnal variations, which was indicated by longer distance traveled and increased time spent in the aversive central zone of the field (*p < 0.05) (Figs. 6B, C). The KA-mel group showed lower activity in the aversive central zone compared with the KA-veh group (°p < 0.05).

3.5.2. EPM test

The KA-mel group had a lower anxiety level with increased distance traveled and time spent in the open arms during the light phase compared with controls (*°p < 0.05) (Figs. 7A, B).

3.5.3. FS test

The control rats treated with melatonin (C-mel) exhibited diurnal fluctuations with less immobility time during the dark phase (#p < 0.05)

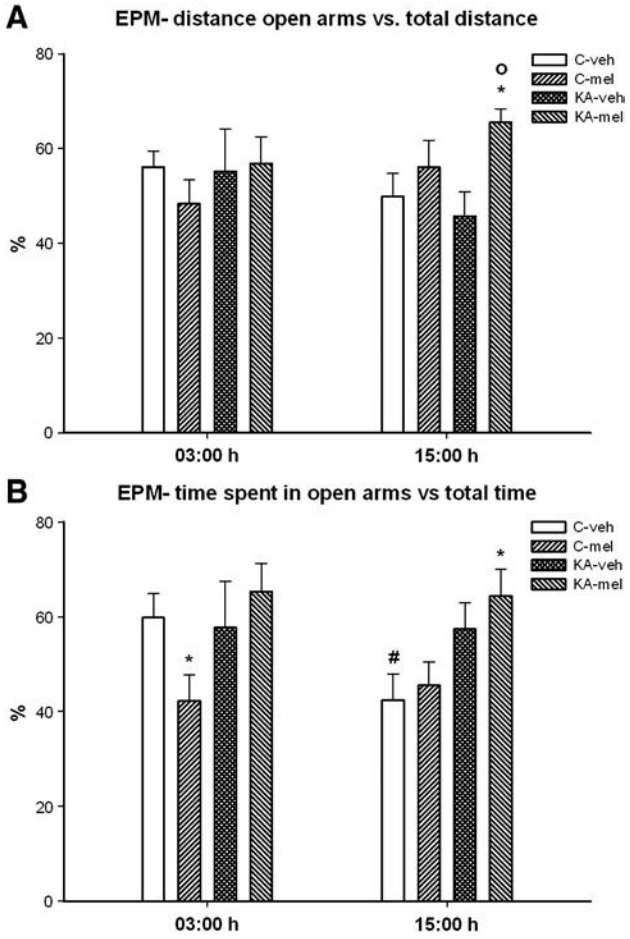

Fig. 7. Diurnal variations of anxiety level in the elevated plus maze test: ratio of distance traveled in open arms vs. total distance traveled (A) and ratio of time spent in open arms vs. total time spent (B). Data are means ± SEM (n = 10); analysis of data by three-way ANOVA indicated a main Epilepsy effect [$F_{1,93} = 8.751$, $p = 0.004$] for the data illustrated in (B). *p < 0.05 vs. C-veh group, °p < 0.05 vs. KA-veh group, #p < 0.05 within a group (15:00 h vs. 03:00 h).

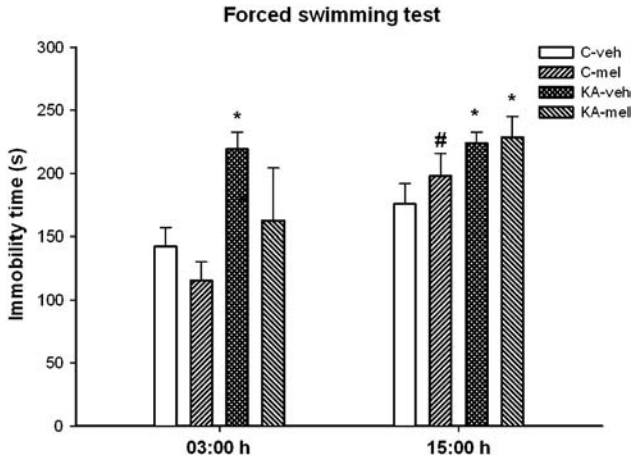

Fig. 8. Diurnal variations of immobility time in forced swimming test. Data are means ± SEM (n = 10); analysis of data by three-way ANOVA indicated a main Epilepsy effect [$F_{1,110} = 14.917$, $p < 0.001$], a Phase effect [$F_{1,110} = 12.629$, $p < 0,001$], as well as a Drug × Phase interaction [$F_{1,110} = 4.439$, $p = 0.038$]. *$p < 0.05$ vs. C-veh, #$p < 0.05$ within a group (15:00 h vs. 03:00 h).

(Fig. 8). The KA-veh group was characterized by increased immobility time without diurnal fluctuations, while the KA-mel group showed a despair-like behavior specifically during the light phase (*$p < 0.05$).

3.5.4. RAM test

Both epilepsy and melatonin treatment increased the working memory error (WME) during the 18-trial session (*°$p < 0.05$) (Fig. 9A). In addition, epilepsy and the melatonin treatment increased the total number of arm entries per session compared with control rats, indicating that animals did not learn the spatial memory task (*$p < 0.05$) (Fig. 9B).

3.6. Histopathological changes and 5-HT levels in the brain

Following the RAM test, histological analysis and HPLC were performed to evaluate the neuronal damage in selected brain structures and the level of 5-HT in the frontal cortex and the hippocampus. The histological examination of Nissl-stained sections from the dorsal hippocampal formation, piriform cortex, and basolateral nucleus of the amygdala (BL) showed that in the intact SHRs, pyramidal neurons in all layers of the hippocampus (Fig. 10A), BL, and piriform cortex (Fig. 10D) were densely arranged and intensely stained. The treatment with KA resulted in severe neuronal loss in each part of the hippocampal formation (Fig. 11B) and in certain parts of the BL and piriform cortex (Fig. 10E). In addition, a higher magnification of the selected areas revealed damaged and dead neurons there. Conversely, the application of melatonin caused retrieval in the cell number and density in all the examined brain regions (Figs. 10C, F) that did not differ significantly from controls. The loss of neurons in the KA-veh group was significant in the CA1, CA2, and CA3 pyramidal neurons, the dentate hilus, granule cell layer of the hippocampus, as well as in the BA and piriform cortex (Figs. 11A, B) (p* < 0.05). In contrast, epileptic rats treated with melatonin did not show morphological differences in the CA1 layer, CA3 layer, and dentate hilus of the hippocampus and piriform cortex from controls without epilepsy.

No significant differences among groups were detected for the 5-HT levels in the frontal cortex (Fig. 12A). In the hippocampus, long-term melatonin treatment significantly decreased the 5-HT levels both in the C-mel and the KA-mel group (Fig. 12B) (*$p < 0.05$).

4. Discussion

The main finding of the present study was that long-term melatonin treatment after SE alleviates seizure activity and brain damage during the chronic epileptic phase in SHRs. However, in contrast to normotensive

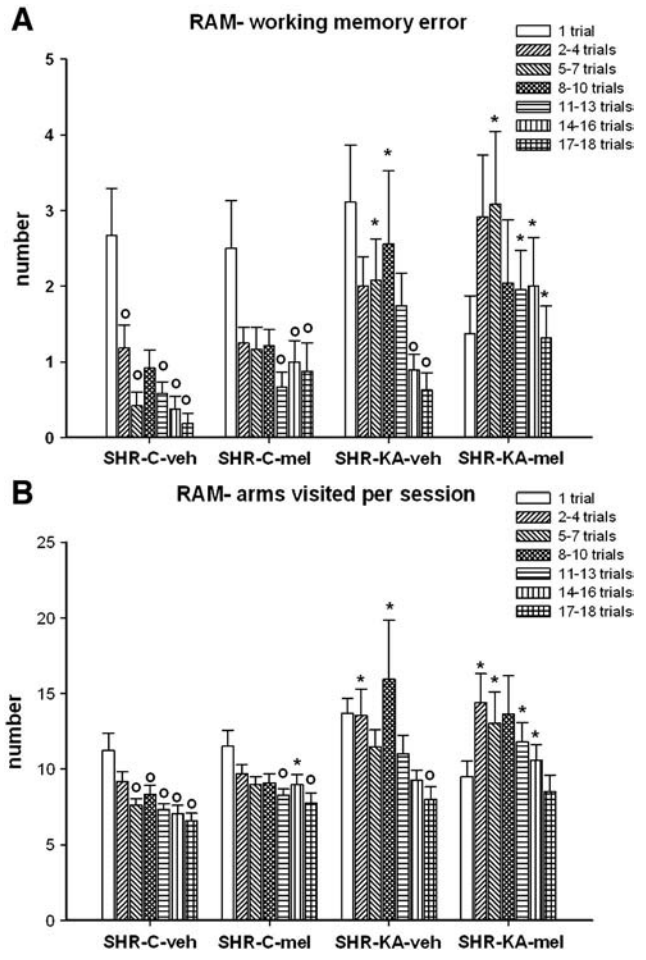

Fig. 9. Working memory error (A) and number of arms visited per session during 18-day trials (B) during 18-day trials in radial arm maze test. Data are means ± SEM (n = 10). Analysis of data by two-way ANOVA indicated a main Drug effect [$F_{1,31} = 36.261$, $p < 0.001$] and an Epilepsy × Drug effect [$F_{1,31} = 63.152$, $p < 0.001$] in (B). *$p < 0.05$ vs. C-veh groups; °$p < 0.05$ vs. KA-veh group. *$p < 0.05$ vs. C-veh, within a group °$p < 0.05$ vs. the 1st session or the 2–4 sessions.

Wistar rats [19], melatonin was unable to restore the disturbed diurnal rhythms, behavioral abnormalities, and spatial memory deficit in epileptic SHRs. Our results support literature data indicating that this hormone has a potent anticonvulsant and neuroprotective effect in different experimental models [17,20,24–26]. To our knowledge, no data have been previously reported on the efficacy of continuous administration of melatonin after a sustained KA-induced SE in a model of comorbidity of hypertension and epilepsy.

In agreement with literature and our previous studies on epileptic normotensive Wistar rats, epileptic SHRs exhibited circadian fluctuations in the appearance of spontaneous seizures in the KA model [1,23,27,28]. We have found that melatonin treatment after SE not only decreases seizure frequency during the stable chronic phase of epilepsy in SHRs but also attenuates the circadian rhythm of seizure activity, which is probably related to its decreasing effect mostly on seizure frequency during the light phase. However, our results are not sufficient to uncover the effect of circadian rhythms on seizure activity in SHRs because we did not measure the plasma levels of melatonin and its effect on seizure distribution. Further research is needed in constant conditions to confirm the hypothesis about the close relationship between circadian rhythmicity, melatonin synthesis, and seizure onset in experimental animals.

Like epileptic Wistar rats [19], SHRs were characterized with neuronal loss in the hippocampus, the amygdala, and the piriform cortex. These brain areas are known to be vulnerable to the neurotoxic effect

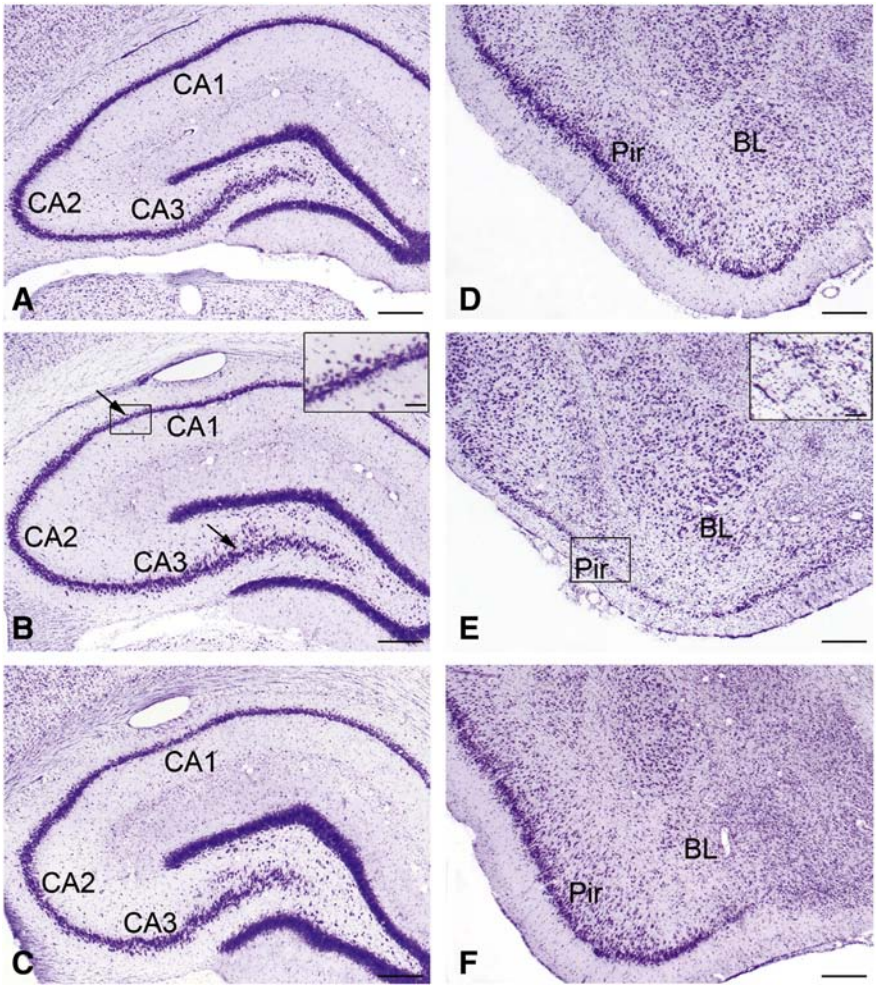

Fig. 10. Representative Nissl-stained coronal sections of the hippocampal formation, the piriform cortex (Pir), and the basolateral amygdaloid nucleus (BL) of a control rat (A, D), and a rat with epilepsy that was treated with a vehicle after SE (B,E) and a rat with epilepsy that was treated with melatonin after SE (C,F). The vehicle-treated epileptic rats showed severe neuronal loss in parts of the hippocampal formation (arrows) reminiscent of hippocampal sclerosis in mesial temporal lobe epilepsy. Please note neuronal death in CA1 area of the hippocampus and piriform cortex (insets in B and E). In contrast, the melatonin-treated epileptic rats did not obviously differ from controls. Scale bars = 200 μm (A–F); 50 μm in higher magnification insets (in B and E).

of the KA [29]. Chen and Buckmaster [30] provided evidence that the KA-treated epileptic Sprague–Dawley rats tend to display extensive neuronal damage mostly in the amygdala and the olfactory cortex. These findings suggest that there are strain-specific differences in brain damage induced by SE. Chung and Han [17] reported that a cumulative dose of 10 mg/kg melatonin attenuates KA-induced neuronal death and reduces the number of DNA breaks. On the other hand, the lack of neuroprotective efficacy of melatonin in the study of Kelso et al. [31] raised several critical issues, which might be the reason for its inconsistency with other reports. The time of administration of melatonin, the duration of treatment, and the age of testing were also considered critical for drug efficacy [32]. Although we delivered melatonin through tap water during the light phase, we measured and compared the total water consumption during a twenty-four-hour period. The prevalence of consumption during the active period suggests that the drug effect was evident mostly during the dark phase. Consistent with our data on normotensive Wistar rats [19], melatonin showed significant neuroprotection in the CA1 hippocampal area and the piriform cortex. The observed effect was associated with a significant delay in the onset of the first spontaneous seizure and a decrease in seizure frequency during the treatment and after its discontinuation. Daily administration of melatonin at a dosage of 10 mg/kg for 60 days exerted a more efficient attenuation of seizure activity in SHRs than in Wistar rats [19] because it suppressed the seizure activity after discontinuation of melatonin exposure. Nevertheless, all melatonin-treated rats developed epilepsy and exhibited SRSs. We can assume that this strain-dependent difference in the efficacy of melatonin in the KA model of TLE might be related to the simultaneous decrease of blood pressure detected in epileptic SHRs. Both experimental and clinical studies, contributing to our understanding of ictal cardiovascular events, suggested correlation of the temporal lobe partial epileptic activity and the cardiovascular phenomena [33,34].

Spontaneously hypertensive rats demonstrated divergence in learning performance, depending on the methodology used. While some authors reported impaired spatial learning of SHRs compared with either Sprague–Dawley rats [35–37] or normotensive Wistar rats [38,39], others revealed a better performance of SHRs compared with normotensive strains [40]. Moreover, anxiety level and motor activity in memory performance were considered critical indices in SHRs [41]. These data suggest that altered anxiety level could be responsible for the outcome in memory responses, which might explain the controversial results found in memory studies for SHRs. In this regard, although the reported abnormalities in the hippocampus of SHRs suggest cognitive impairments, a previous study has demonstrated that young male SHRs outperform young male Sprague–Dawley rats in an eight-arm radial maze task [42]. Calzavara et al. [41] showed that the low anxiety level of adult SHRs has an important role in the performance of the plus-maze discriminative avoidance task and that the anxiety level should be taken into account in studies where SHRs are used. In the present work, we performed behavioral and memory tests to find out

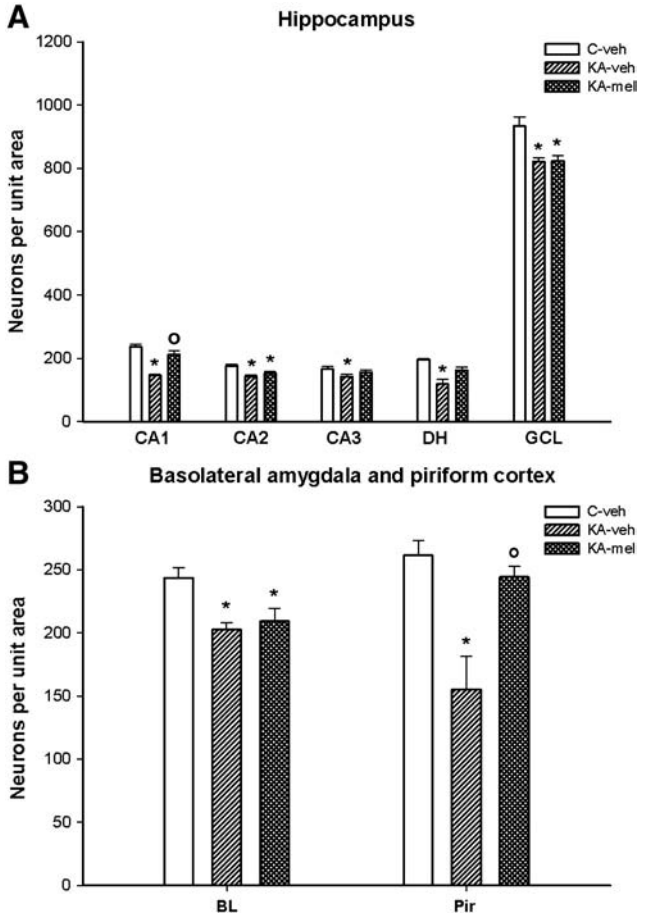

Fig. 11. Effect of chronic melatonin treatment (10 mg/kg/day in drinking water, 8 weeks) on KA-induced temporal lobe epilepsy on the histology scores (Nissl staining). A/Neuronal damage in the hippocampus — CA1, CA2, and CA3 pyramidal cell layers, dentate hilus (DH), and granule cell layer (GCL); B/Neuronal damage in the piriform cortex and the basolateral amygdala (BL). Analysis of data by two-way ANOVA indicated a main Epilepsy effect [$F_{1,17} = 51.086$, $p < 0.001$] and Drug effect [$F_{1,17} = 27.684$, $p < 0.001$] in CA1; a main Epilepsy effect [$F_{1,17} = 19.520$, $p < 0.001$] in CA2; a main Epilepsy effect [$F_{1,17} = 5.178$, $p < 0.040$] in CA3; a main Epilepsy effect [$F_{1,17} = 12.859$, $p < 0.003$] in BL; a main Epilepsy effect [$F_{1,17} = 19.079$, $p < 0.001$] and a Drug effect [$F_{1,17} = 13.452$, $p < 0.002$] in the piriform cortex; *$p < 0.05$ vs. C-veh groups; °$p < 0.05$ vs. KA-veh group.

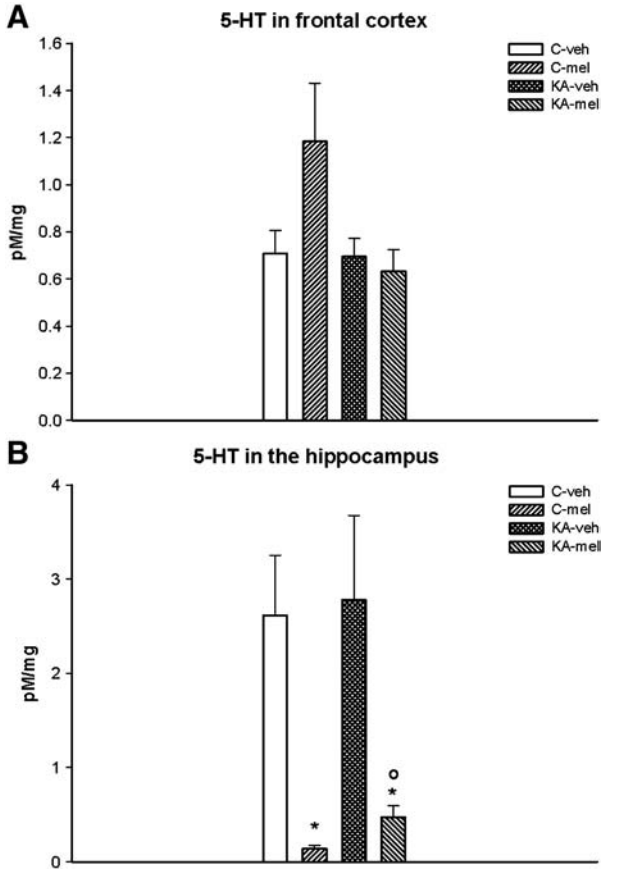

Fig. 12. Concentrations of 5-HT in the frontal cortex (A) and the hippocampus (B) in pM/mg of wet tissue as measured by the HPLC method. Data are means ± SEM ($n = 9–10$); Analysis of data by two-way ANOVA indicated a main Drug effect [$F_{1,42} = 12.30$, $p < 0.001$] in (B). *$p < 0.05$ vs. C-veh group.

whether long-term administration of melatonin exerts a disease-modifying effect on these comorbidities of the epileptic state in SHRs, which should be associated with a detected neuroprotective and seizure-decreasing efficacy of the hormone. However, unlike Wistar rats [19] where melatonin showed a disease-attenuating efficacy on some epilepsy-associated behavioral abnormalities, including depressive-like comorbidity, both intact and melatonin-treated epileptic SHRs exhibited a low anxiety level in the EPM test, depressive-like behavior, and impairment of hippocampus-dependent spatial working memory. Although melatonin failed to affect the low anxiety level in the EPM test, which is characteristic for rats with epilepsy, it alleviated hyperlocomotion. Furthermore, we found that melatonin-treated epileptic SHRs were characterized with shorter distance traveled and less time spent in the central aversive zone of the OF. These results are also similar to the effect of melatonin in epileptic Wistar rats. The divergence in the melatonin effects demonstrated in the two tests of anxiety, i.e., OF and EPM, might be specific for the procedure applied.

Unlike the study of González-Burgos et al. [43] where melatonin was reported to reduce pyramidal neuronal death in the hippocampus in parallel with prevention of the spatial memory impairments as a sequence of global ischemia in rats, in the present study, melatonin treatment protocol failed to restore the disturbed spatial memory function in SHRs. Furthermore, vehicle-treated controls showed responses similar to epileptic vehicle-treated SHRs in RAM. Previously, we have found that intact and epileptic SHRs exhibit similar anxiety levels, which was associated with low levels of 5-HT and dopamine in the hippocampus [1]. Moreover, the intact SHRs showed depressive-like responses in the sucrose preference test during the light phase compared with intact Wistar rats [1]. We can suggest that naive SHRs demonstrate abnormal behavioral responses and biochemical parameters, which are also characteristic for epileptic rats. In literature, substantial data exist on hippocampal neuropathology, including neuronal loss, mostly in the CA_1 subfield, and astrocyte reactivity of intact adult SHRs [44,45]. Similarity in the EEG spectral profiles of SHRs suffering from congenital hypertension and in the KA-treated normotensive rats was reported earlier [46]. In the present study, both melatonin-treated control and epileptic SHRs were characterized with decreased hippocampal levels of 5-HT compared with intact SHRs. In vivo evidence showed clear coupling between norepinephrine release and melatonin secretion [47] suggesting that the daily rhythm of melatonin synthesis depends on the activity of sympathetic fibers by the SCN. Spontaneously hypertensive rats are characterized with a disturbed noradrenergic neurotransmission, suggesting changes in circadian rhythms of endogenous melatonin release. On the other hand, 5-HT neurotransmission is known to play a crucial role in depression, while an antidepressant effect of melatonin was suggested to be mediated by 5-HT2A receptors as the hormone has been shown to act as a 5-HT2A antagonist [48,49]. It has been shown that melatonin per se regulates both spontaneous efflux and evokes release of 5-HT in the hippocampus, which is a major target of serotoninergic antidepressants [50]. Unlike in epileptic Wistar rats, where the exogenously applied melatonin was able to restore the disturbed 5-HT balance [19], melatonin was ineffective presumably

because serotoninergic neurotransmission was already disturbed in intact SHRs.

In conclusion, although melatonin treatment after SE was unable to prevent the development of the chronic epileptic state, the drug exerted neuroprotection and alleviated the seizure activity in epileptic SHRs. However, melatonin did not restore the disturbed diurnal behavioral rhythms and was ineffective in reducing the deleterious consequences of SE on the behavioral and memory deficits in epileptic SHRs. This result might be explained with similar behavioral and biochemical parameters of intact and epileptic SHRs. Therefore, long-term treatment with melatonin after SE did not prevent epileptogenesis but was effective in decreasing seizure activity and brain damage in a model of comorbidity of hypertension and epilepsy.

Acknowledgments

This work was supported by the National Science Fund (research grant # DTK 02/56 2009-1012), Medical Science Council, Medical University, Sofia, Bulgaria, contract No. 2/2010, and World Federation of Science.

Disclosure/conflicts of interest

Each author has no conflict of interest concerning this manuscript.

References

[1] Tchekalarova J, Pechlivanova D, Atanasova Ts, Markova P, Lozanov V, Stoynev A. Diurnal variations of depressive-like behavior of Wistar and spontaneously hypertensive rats in kainate model of temporal lobe epilepsy. Epilepsy Behav 2011;20:277–85.
[2] Mazarati A, Siddarth P, Baldwin RA, Don S, Rochelle C, Raman S. Depression after status epilepticus: behavioural and biochemical deficits and effects of fluoxetine. Brain 2008;131:2071–83.
[3] Fountoulakis KN. Circadian rhythms: strong evidence on how to approach depression. Ann Gen Psychiatry 2010;9(Suppl. 1):48.
[4] Quera Salva MA, Hartley S. Mood disorders, circadian rhythms, melatonin and melatonin agonists. J Cent Nerv Syst Dis 2012;4:15–26.
[5] Pevet P, Challet E. Melatonin: both master clock output and internal time-giver in the circadian clock network. J Physiol Paris 2011;105:170–82.
[6] Bazil CW, Short D, Crispin D, Zheng W. Patients with intractable epilepsy have low melatonin, which increases following seizures. Neurology 2000;55:1746–8.
[7] Rufo-Campos M. Melatonin and epilepsy. Rev Neurol Suppl 2002;1:51–8.
[8] Stoynev AG, Ikonomov OC, Minkova NK, Zacharieva SZ, Stoyanovsky VG. Circadian rhythms of arterial pressure: basic regulatory mechanisms and clinical value. Acta Physiol Pharmacol Bulg 1999;24:43–51.
[9] van den Buuse M. Circadian rhythms of blood pressure, heart rate, and locomotor activity in spontaneously hypertensive rats as measured with radio-telemetry. Physiol Behav 1994;55:783–7.
[10] Amenta F, Di Tullio MA, Tomassoni D. Arterial hypertension and brain damage-evidence from animal models (review). Clin Exp Hypertens 2003;25:359–80.
[11] Greenwood SR, Meeker R, Sullivan H, Hayward JN. Kindling in spontaneous hypertensive rats. Brain Res 1989;495:58–65.
[12] Scorza FA, Arida RM, Cysneiros RM, Scorza CA, de Albuquerque M, Cavalheiro EA. Qualitative study of hippocampal formation in hypertensive rats with epilepsy. Arq Neuropsiquiatr 2005;63:283–8.
[13] Zeman M, Dulková K, Bada V, Herichová I. Plasma melatonin concentrations in hypertensive patients with the dipping and non-dipping blood pressure profile. Life Sci 2005;76:1795–803.
[14] Oxenkrug GF, Summergrad P. Ramelteon attenuates age-associated hypertension and weight gain in spontaneously hypertensive rats. Ann N Y Acad Sci 2010;1199:114–20.
[15] Pechánová O, Zicha J, Paulis L, Zenebe W, Dobesová Z, Kojsová S, et al. The effect of N-acetylcysteine and melatonin in adult spontaneously hypertensive rats with established hypertension. Eur J Pharmacol 2007;30:129–36.
[16] Banach M, Gurdziel E, Jêdrych M, Borowicz K. Melatonin in experimental seizures and epilepsy. Pharmacol Rep 2011;63:1–11.
[17] Chung SY, Han SH. Melatonin attenuates kainic acid-induced hippocampal neurodegeneration and oxidative stress through microglial inhibition. J Pineal Res 2003;34:95–102.
[18] de Lima E, Soares JM, del Carmen Sanabria Y, Valente S, Priel MR, Chada E, et al. Effects of pinealectomy and the treatment with melatonin on the temporal lobe epilepsy in rats. Brain Res 2005;10:24–31.
[19] Tchekalarova J, Petkova Z, Pechlivanova D, Moyanova S, Kortenska L, Mitreva R, et al. Stoynev Al. Prophylactic treatment with melatonin after status epilepticus: effects on epileptogenesis, neuronal damage and behavioral changes in kainate model of temporal lobe epilepsy. Epilepsy Behav 2013;27:174–87.
[20] Guisti P, Lipartiti M, Franceschini D, Schiavo N, Floream M, Manev H. Neuroprotection by melatonin from kainate-induced excitotoxicity in rats. FASEB J 1996;10:891–6.
[21] Hellier JL, Partylo PR, Buckmaster PS, Dudek FE. Recurrent spontaneous motor seizures after repeated low-dose systemic treatment with kainate: assessment of a rat model of temporal lobe epilepsy. Epilepsy Res 1998;31:73–84.
[22] Racine RJ. Modification of seizure activity by electrical stimulation. II. Motor seizure. Electroencephalogr Clin Neurophysiol 1972;32:281–94.
[23] Tchekalarova J, Pechlivanova D, Itzev D, Lazarov N, Markova P, Stoynev A. Diurnal rhythms of spontaneous recurrent seizures and behavioural alterations of Wistar and spontaneously hypertensive rats in kainate model of epilepsy. Epilepsy Behav 2010;17:23–32.
[24] Yehuda S, Mostofsky DI. Circadian effects of h-endorphin, melatonin, DSIP, and amphetamine on pentylentetrazole-induced seizures. Peptides 1993;14:203–5.
[25] Lapin IP, Mirzaev SM, Ryzon IV, Oxenkrug GF. Anticonvulsant activity of melatonin against seizures induced by quinolinate, kainate, glutamate, NMDA, and pentylenetetrazole in mice. J Pineal Res 1998;24:215–8.
[26] Mevissen M, Ebert U. Anticonvulsant effects of melatonin in amygdala-kindled rats. Neurosci Lett 1998;257:13–6.
[27] Arida RM, Scorza FA, Peres CA, Cavalheiro EA. The course of untreated seizures in the pilocarpine model of epilepsy. Epilepsy Res 1999;34:99–107.
[28] Stewart LS, Leung LS. Temporal lobe seizures alter the amplitude and timing of rat behavioral rhythms. Epilepsy Behav 2003;4:153–60.
[29] Stafstrom CE, Chronopoulosm A, Thurberm S, Thompson JL, Holmes GL. Age-dependent cognitive and behavioral deficits after kainic acid seizures. Epilepsia 1993;4:420–32.
[30] Chen S, Buckmaster PS. Stereological analysis of forebrain regions in kainate-treated epileptic rats. Brain Res 2005;28:141–52.
[31] Kelso ML, Scheff NN, Scheff SW, Pauly JR. Melatonin and minocycline for combinatorial therapy to improve functional and histopathological deficits following traumatic brain injury. Neurosci Lett 2011;488:60–4.
[32] Costa-Lotufo LV, Fonteles MM, Lima IS, de Oliveira AA, Nascimento VS, de Bruin VM, et al. Attenuating effects of melatonin on pilocarpine-induced seizures in rats. Comp Biochem Physiol C Toxicol Pharmacol 2002;131:521–9.
[33] Hilz M, Devinsky O, Doyle W, Maueuer A, Dütsch M. Decrease of cardiovascular modulation after temporal lobe epilepsy surgery. Brain 2002;125:985–95.
[34] Devinsky O. Effects of seizures on autonomic and cardiovascular function. Epilepsy Curr 2004;4:43–6.
[35] Gattu M, Pauly JR, Boss KL, Summers JB, Buccafusco JJ. Cognitive impairment in spontaneously hypertensive rats: role of central nicotinic receptors. Part I. Brain Res 1997;771:89–103.
[36] Diana G. Does hypertension alone lead to cognitive decline in spontaneously hypertensive rats? Behav Brain Res 2002;21:113–21.
[37] De Bruin NMWJ, Kiliaan AJ, De Wilde MC, Broersen LM. Combined uridine and choline administration improves cognitive deficits in spontaneously hypertensive rats. Neurobiol Learn Mem 2003;80:63–79.
[38] Nakamura-Palacios EM, Caldas CK, Fiorini A, Chagas KD, Chagas KN, Vasquez EC. Deficits of spatial learning and working memory in spontaneously hypertensive rats. Behav Brain Res 1996;74:217–27.
[39] Hernandez CM, Hoifodt H, Terry AV. Spontaneously hypertensive rats: further evaluation of age-related memory performance and cholinergic marker expression. J Psychiatry Neurosci 2003;28:197–209.
[40] Lukaszewska I, Niewiadomska G. The differences in learning abilities between spontaneously hypertensive (SHR) and Wistar normotensive rats are cue dependent. Neurobiol Learn Mem 1995;63:43–53.
[41] Calzavara MB, Lopez GB, Abílio VC, Silva RH, Frussa-Filho R. Role of anxiety levels in memory performance of spontaneously hypertensive rats. Behav Pharmacol 2004;15:545–53.
[42] Wyss JM, Fisk G, van Groen T. Impaired learning and memory in mature spontaneously hypertensive rats. Brain Res 1992;592:135–40.
[43] González-Burgos I, Letechipía-Vallejo G, López-Loeza E, Moralí G, Cervantes M. Long-term study of dendritic spines from hippocampal CA1 pyramidal cells, after neuroprotective melatonin treatment following global cerebral ischemia in rats. Neurosci Lett 2007;16:162–6.
[44] Sabbatini M, Catalani A, Consoli C, Marletta N, Tomassoni D, Avola R. The hippocampus in spontaneously hypertensive rats: an animal model of vascular dementia? Mech Ageing Dev 2002;123:547–59.
[45] Pietranera L, Saravia F, Gonzalez Deniselle MS, Roig P, Lima A, De Nicola AF. Abnormalities of the hippocampus are similar in deoxycorticosterone acetate-salt hypertensive rats and spontaneously hypertensive rats. J Neuroendocrinol 2006;18:466–74.
[46] Vorobyov V, Schibaev N, Kaptsov V, Kovalev G, Sengpiel F. Cortical and hippocampal EEG effects of neurotransmitter agonists in spontaneously hypertensive vs. kainate-treated rats. Brain Res 2011;1383:154–68.
[47] Drijfhout WJ, van der Linde AG, de Vries JB, Grol CJ, Westerink BH. Microdialysis reveals dynamics of coupling between noradrenaline release and melatonin secretion in conscious rats. Neurosci Lett 1996;202:185–8.
[48] Gorzalka BB, Brotto LA, Hong JJ. Corticosterone regulation of 5-HT2A receptor-mediated behaviors: attenuation by melatonin. Physiol Behav 1999;67:439–42.
[49] Raghavendra V, Kulkarni SK. Melatonin reversal of DOI-induced hypophagia in rats: possible mechanism by suppressing 5-HT(2A) receptor-mediated activation of the HPA axis. Brain Res 2000;860:112–8.
[50] Monnet FP. Melatonin modulates serotonin release in the rat hippocampus: effects of circadian rhythm. J Neuroendocrinol 2002;14:194–9.
[51] Olton DS, Samuelson RJ. Remembrance of places passed: spatial memory in rats. J Exp Psychol 1996;2:97–116.