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Early endocannabinoid system activation attenuates behavioral impairments induced by initial impact but does not prevent epileptogenesis in lithium–pilocarpine status epilepticus model

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ABSTRACT

Mood and anxiety disorders, as well as memory impairments, are important factors affecting quality of life in patients with epilepsy and can influence the antiepileptic therapy. Clinical studies of psychiatric comorbidities are quite complicated to design and interpret, so animal studies of behavioral impairments associated with seizures can be of use. We investigated the effect of early administration of endocannabinoid receptor agonist WIN-55,212-2 on the development of spontaneous seizures, long-term behavioral and memory impairments, and neurodegeneration in the hippocampus on the lithium–pilocarpine model of status epilepticus (SE). We also studied the role of spontaneous seizures in the development of pathologic consequences of the SE. Our results showed that behavioral impairments found in the elevated plus maze test depended mostly on the consequences of SE itself and not on the development of spontaneous seizures while hyperactivity in the open-field test and light–dark chamber was more prominent in rats with spontaneous seizures. Administration of WIN-55,212-2 decreased emotional behavior in the elevated plus maze but did not affect hyperactive behavior in the open-field test. Spatial memory impairment developed both in the presence or absence of spontaneous seizures and was not affected by administration of WIN-55,212-2. Both administration of endocannabinoid receptor agonist WIN-55,212-2 and the presence of spontaneous seizures affected SE-induced neuronal loss in the hippocampus.

1. Introduction

There is growing evidence of close association between epilepsy and behavioral disorders including mood disorders and learning and memory impairments both in clinical and experimental models [1–6]. Mood and anxiety disorders are common comorbidities in patients with epilepsy and occur 2–3 times and, in some sources, up to 10 times more often in such patients than in general population [7–9]. Cognitive disorders are also a concern in treating patients with epilepsy. It has been reported that cognitive impairments occur in 40% of children and adolescents with epilepsy [10]. Psychiatric comorbidities and cognitive impairment are significant factors decreasing quality of life in patients with epilepsy [5,10]. There is evidence that anxiety is a stronger predictor of poor quality of life than depression despite their similar prevalence in patients with epilepsy [11,12].

Clinical studies of psychiatric comorbidities can be quite complicated to design and interpret, since in patients, especially with uncontrolled seizures, depression and anxiety could be related to the fear of a seizure occurring, socioeconomic impact of their diagnosis, or psychotropic effect of antiepileptic drug (AED) therapy [2]. Hence, animal studies of behavioral impairments associated with seizures can be of some importance. Lithium–pilocarpine rat model is an established model that reproduces the main features of human status epilepticus (SE) with subsequent development of temporal lobe epilepsy with hippocampal sclerosis [13–15]. In this model, lithium–pilocarpine SE plays a role of an initial insult, which is followed by the development of recurrent spontaneous seizures several weeks later [16–18]. This model is characterized by a certain pattern of brain damage involving mainly the hippocampal fields and dentate gyrus, entorhinal and piriform cortex, amygdala, and thalamus [18–20]. Behavioral impairments in rats after SE have been also reported. Rats displayed enhanced intragroup aggression [21], increased locomotor activity [22], reduced anxiety-like behavior [22,23], and depression-like behavior [24–26]. Memory impairments are also commonly found in rats after pilocarpine or lithium–pilocarpine seizures: severe working and reference memory deficits [21], and spatial memory impairments [22,27]. Therefore, rodent models of acquired chronic epilepsy, such as lithium–pilocarpine model, are characterized by a wide range of neurobehavioral impairments, some of which could be equivalents of certain comorbidities of epilepsy in humans.

Endocannabinoid system is known to be a primary regulator of synaptic transmission [28]. Endocannabinoid CB1 receptors are located at the presynaptic membranes and carry out “on-demand” retrograde

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inhibition of neurotransmitter release [29]. Many studies have shown that endocannabinoid receptors play an important role in the termination of seizure activity. Activation of endocannabinoid receptors has anticonvulsant effect [30–33]; endocannabinoid antagonists facilitate seizure activity [34,35]. It was shown that endocannabinoid receptor agonists have a neuroprotective effect in vivo and in vitro [36–39].

Our goal was to investigate the effect of a potent nonselective endocannabinoid receptor agonist WIN-55,212-2 on the development of spontaneous seizures, behavioral and memory impairments, and neurodegeneration in the chronic period after SE. Most existing studies are focused on the behavioral impairments in post-SE epileptic rats, while seizure-free survived after SE animals are excluded from the study. Therefore, we have investigated which factor contributed to the development of behavioral impairments: neuropathology induced by the initial impact (SE) or they emerge secondary to the spontaneous seizures.

2. Materials and methods

2.1. Animals

The study was carried out on adult male Wistar rats weighing 350–370 g at the beginning of the experiments. Animals were kept under the controlled environmental conditions (23–25 °C, 12 h light/dark cycle) with free access to water and standard laboratory food. All animal experiments were carried out according to the EU Directive 2010/63/EU for animal experiments and approved by the local bioethical committee.

2.2. Drugs

The study was carried out on the lithium–pilocarpine model of SE. Rats were treated with 127 mg/kg lithium chloride (Acros Organics, USA) 24 h before the induction of seizures. Status epilepticus was induced by administration of 25 mg/kg pilocarpine hydrochloride (Sigma, USA) and terminated by administration of 25 mg/kg pentobarbital (Ceva Sante Animale, France). A potent agonist of CB1 and CB2 receptors WIN-55,212-2 (Sigma, USA) was administered 4 h after termination of SE. Lithium chloride and pilocarpine were freshly dissolved in 0.9% saline. WIN-55,212-2 was dissolved in 5% dimethyl sulfoxide (DMSO) (Sigma, USA) and 1% Tween-80 (Panreac, Spain). All drugs were injected intraperitoneally.

2.3. Video monitoring

Four months after SE, rats were video-monitored to detect spontaneous motor seizures. The video was recorded using a 4-channel video-monitoring system (Best DVR, Russia) and digital mini video cameras with 6-mm lens placed above the cages with animals. The video was recorded at 25 frames per second and a resolution of 352 × 288 pixels. During the dark hours, recordings were carried out under dim red light. Animals were video-monitored for 7 days. Videos were analyzed manually; clonic and tonic–clonic seizures were detected visually and scored according to the Racine scale [40].

2.4. Behavioral tests

After the video monitoring was completed, a series of behavioral tests was carried out. All behavioral tests took place in a separate room for behavioral testing. Animals were moved there from the vivarium for no less than 1 h before the start of the experiment.

All experiments were video-recorded and analyzed using EthoVision tracking system (Noldus, the Netherlands). Tests were carried out with 24 h intervals. Animals included in the study did not have seizures at least 1 h before the behavioral tests.

2.4.1. Elevated plus maze

Elevated plus maze (EPM) test was performed in a black plus maze apparatus with two open and two closed arms. The maze was 110 × 110 cm in size and elevated 50 cm above the floor. Animals were put in the center of the maze facing the same open arm. Each rat spent 5 min in the maze. If animals fell from the maze, they were put back immediately. Since it was shown that falls from the plus maze do not affect the results of statistical analysis [23], we decided to not exclude such rats from the experiment.

2.4.2. Light–dark box

An additional anxiety test was carried out in a light–dark box consisting of brightly lit "light" compartment and closed "dark" compartment. Animals were put in the light compartment heading the entrance to the dark compartment entrance. Each experimental session was video-recorded for 5 min.

2.4.3. Open-field test

Locomotor activity of rats was evaluated in a black circular arena 100 cm in diameter and 30 cm high walls. The open field was brightly lit. Animals were put in the center of the arena. The behavior in the open field was video-recorded for 5 min.

2.4.4. Morris water maze

The long-term spatial memory was tested in a circular pool (diameter: 150 cm, height: 60 cm) with black walls filled with water (24 ± 1 °C). Spatial cues were present in the experimental room. A hidden platform made of clear acrylic glass was placed in the southwest quadrant for five days. Each animal was subjected to 4 consecutive trials per day that started from various starting points as described in [41]. Each trial lasted for no more than 60 s; if rats could not find the platform during this time, they were gently guided there by the experimenter. On day 6, the probe trial was carried out to assess the spatial memory formation. For this purpose, the platform was removed, and rats were placed in the pool for 60 s.

2.5. Histology

After the behavioral tests were completed, rats were anesthetized and perfused transcardially with phosphate buffered saline and 4% paraformaldehyde. Brains were removed, fixated in 4% paraformaldehyde, cryoprotected in 20% sucrose, frozen, and stored at −20 °C. Twenty-micrometer coronal slices were obtained, mounted on slides, and stained with Cresyl violet.

Neuronal density was assessed in dorsal and ventral CA1 and CA3 fields of dorsal and ventral hippocampus and in the hilus of dentate gyrus. For this purpose, cells were counted; high-resolution images were obtained using BZ-9000 fluorescence microscope (Keyence, Osaka, Japan). Cell counts were assessed in three 0.023-mm^2 fields of view for three consecutive slices for CA1 and CA3 fields and in seven 0.011-mm^2 fields of view on three consecutive slices for the hilus of the dentate gyrus; and then, cell density per 0.1 mm was calculated. Cell counting was performed using ImageJ software by an observer blinded to the treatment. Only neurons with a cell body larger than 0.01 mm were counted.

2.6. Statistical analysis

The statistical analysis was carried out using Statistica 7.0 and GraphPad Prism 6 software. The samples were tested for normality visually by distribution histograms and using the Kolmogorov–Smirnov test. Two-way analysis of variance (ANOVA) (treatment, presence of spontaneous seizures, and their interaction treatment × seizure factors) and repeated measures ANOVA (for the analysis of the performance in the open field and Morris water maze) with Tukey post hoc test were used to analyze the behavioral data and neuronal counts. Incidence and frequency of seizures were compared using nonparametric

Mann–Whitney U-test and Fisher test. Data were presented as mean ± standard error of the mean (SEM). Differences were considered significant at $p < 0.05$.

3. Results

3.1. Lithium–pilocarpine SE

Rats treated with lithium chloride and pilocarpine developed severe clonic and tonic–clonic convulsions (stages 3 and 4 [40]) that lasted the whole 90 min of the experiment before pentobarbital injection. After pentobarbital injection, rats remained anesthetized for about 4 h. After that, rats awakened and regained posture. Animals were randomly grouped for the treatment; no significant differences in the severity of seizures were found between the groups. The following experimental groups were studied: 1) control rats that did not receive pilocarpine and were not subjected to SE ($n = 10$); 2) vehicle-treated rats that survived after SE and received vehicle (5% DMSO and 1% Tween-80) ($n = 12$); 3) WIN-treated rats that survived SE and received WIN-55,212-2 ($n = 12$).

3.2. Spontaneous seizures

Video monitoring conducted 4 months after SE showed that both WIN-treated and vehicle-treated rats experienced spontaneous seizures. The frequency of seizure episodes did not differ significantly between these two groups and were 0.48 ± 0.17 and 0.88 ± 0.31 seizures per day in the vehicle-treated (SE + vehicle, $n = 12$) and WIN-treated (SE + WIN, $n = 12$) groups respectively ($p = 0.49$, Mann–Whitney U tests). The incidence of seizures also did not change in WIN-treated rats in comparison with vehicle-treated rats: spontaneous seizures appeared in 6 out of 12 (50.00%) and 7 of 12 (58.33%) video-monitored vehicle and WIN-treated rats respectively ($p = 0.34$, Fisher test). Thus, experimental animals were divided into epileptic (with spontaneous recurrent seizures (SRS), SE + SRS, $n = 13$) and nonepileptic (seizure-free, SE + no SRS, $n = 11$). We could, in some cases, conduct the analysis of the cross-effect of chronic seizures and WIN-55,212-2 administration on the behavior and neuronal counts.

3.3. Neurodegeneration in the hippocampus

The analysis of neuronal counts in CA1 and CA3 subfields of dorsal and ventral hippocampus demonstrated a significant reduction in the number of pyramidal neurons in dorsal CA1 and CA3 ($F(1, 34) = 6.771$, $p = 0.014$; $F(1, 34) = 4.614$, $p = 0.040$), ventral CA1 and CA3 ($F(1, 34) = 19.830$, $p < 0.001$; $F(1, 34) = 6.877$, $p = 0.014$), and neurons in the hilus of the dentate gyrus ($F(1, 34) = 36.030$, $p < 0.001$) in post-SE rats in comparison with the control group (no SE).

The factorial ANOVA showed a significant effect of both WIN-55,212-2 administration ($F(2, 20) = 7.550$, $p = 0.013$ and $F(2, 20) = 8.466$, $p = 0.009$ respectively) and the presence of spontaneous seizures ($F(2, 20) = 26.30$, $p < 0.001$ and $F(2, 20) = 8.606$, $p = 0.008$ respectively), as well as their interaction (treatment × seizure) ($F(2, 20) = 6.350$, $p = 0.021$ and $F(2, 20) = 6.880$, $p = 0.016$) on the neuronal counts in the ventral CA3 and dentate hilus (Table 1). The post hoc analysis of the treatment × seizure effect revealed that the number of ventral CA3 pyramids of vehicle-treated epileptic rats was significantly reduced in comparison with nonepileptic rats and WIN-treated epileptic rats ($p < 0.01$, Tukey post hoc test). In the hilus, the number of neurons in vehicle-treated and epileptic WIN-treated rats was significantly lower than in nonepileptic WIN-treated rats ($p < 0.01$, Tukey post hoc test). Epileptic rats generally had lower neuronal counts (Table 1), and the difference between vehicle-treated and WIN-treated rats was significant in the ventral CA3 of epileptic rats and dentate hilus of nonepileptic rats (Table 1). Therefore, the neuroprotective effect of WIN-55,212-2 appeared in selected hippocampal fields depending on the presence of spontaneous seizures.

3.4. Behavioral impairments in the chronic post-SE period

3.4.1. Elevated plus maze

In the elevated plus maze, the following parameters were studied: traveled distance, time spent in the open arms of the maze, the number of entries to the open arms, grooming, rearing, and the number of defecations. Vehicle-treated rats that survived SE spent more time in the open arms ($F(2, 34) = 5518$, $p = 0.009$, Tukey post hoc test); WIN-treated rats were not significantly different from the control group ($p = 0.159$, Tukey post hoc test) (Fig. 1B). The presence of spontaneous seizures did not have a significant effect on time spent in the open arms of a maze.

All rats that survived after SE made more entries to the open arms ($F(2, 34) = 3.664$, $p = 0.037$, Tukey post hoc test) (Fig. 1C) and demonstrated a significantly decreased number of grooming acts ($F(2, 34) = 16{,}370$, $p = 0.0001$, Tukey post hoc test) (Fig. 1F); a decrease in the number of rearings was not statistically significant ($F(2, 34) = 3.136$, $p = 0.058$) (Fig. 1E). Treatment with WIN-55,212-2 or the presence of spontaneous seizures did not significantly affect these parameters.

The number of defecations was significantly increased in the group of vehicle-treated rats ($F(2, 34) = 5.756$, $p = 0.007$) in comparison with the control group (Tukey post hoc test $p = 0.008$) and WIN-treated rats (Tukey post hoc test $p = 0.04$) (Fig. 1G).

No differences in traveled distance and time in the center of the maze were found between the studied groups (Fig. 1A).

3.4.2. Open-field test

The following parameters were analyzed in the open-field test: distance moved, time in the center, number of entries to the center, rearings, grooming acts, and the number of defecations. Rats that survived after SE were significantly more active in comparison with control rats. The analysis of variance showed that distance traveled ($F(2, 34) = 9{,}6$, $p = 0.004$), the number of entries ($F(2, 34) = 3{,}7$, $p = 0.036$), and time spent in the center of the arena ($F(2, 34) = 3{,}5$, $p = 0.041$) significantly ($p < 0.05$) increased in the post-SE animals. The number of

Table 1
Neuronal counts in WIN- or vehicle-treated epileptic and nonepileptic rats (number of neurons per 0.1 mm^2 in dorsal and ventral CA1 and CA3 fields, and per field of view in the hilus). No SE — control rats without SE; SE + vehicle — rats received vehicle 4 h after SE; SE + WIN — rats received 5 mg/kg WIN-55,212-2 4 h after SE; SE + SRS — rats with spontaneous recurrent seizures recorded 4 months after SE; SE + no SRS — rats with no spontaneous recurrent seizures 4 months after SE. Data are shown as mean ± SEM.

Subgroup	Hippocampal field				
	CA1 dorsal	CA3 dorsal	CA1 ventral	CA3 ventral	Hilus
No SE	174.59 ± 6.60	138.57 ± 3.29	228.93 ± 13.67	147.10 ± 7.67	5.06 ± 0.25
SE + vehicle	136.51 ± 16.31	123.21 ± 5.55	148.70 ± 20.57*	100.61 ± 12.32*	2.51 ± 0.17*
SE + WIN	135.93 ± 17.13	117.87 ± 7.74	133.65 ± 15.87*	125.02 ± 8.45#	3.27 ± 0.35*,#
SE + SRS	121.00 ± 16.86*	118.47 ± 6.17	122.57 ± 10.07*	93.37 ± 8.99*,@	2.51 ± 0.16*,@
SE + no SRS	155.99 ± 13.05	122.96 ± 7.39	162.00 ± 18.83*	138.59 ± 7.11	3.34 ± 0.37*

* Significant difference at $p < 0.01$ in comparison with the control group.
\# A significant effect of treatment with WIN-55,212-2 in comparison with respective vehicle-treated group at $p < 0.05$.
@ A significant effect of the presence of spontaneous seizures at $p < 0.05$, two-way ANOVA with Tukey post hoc.

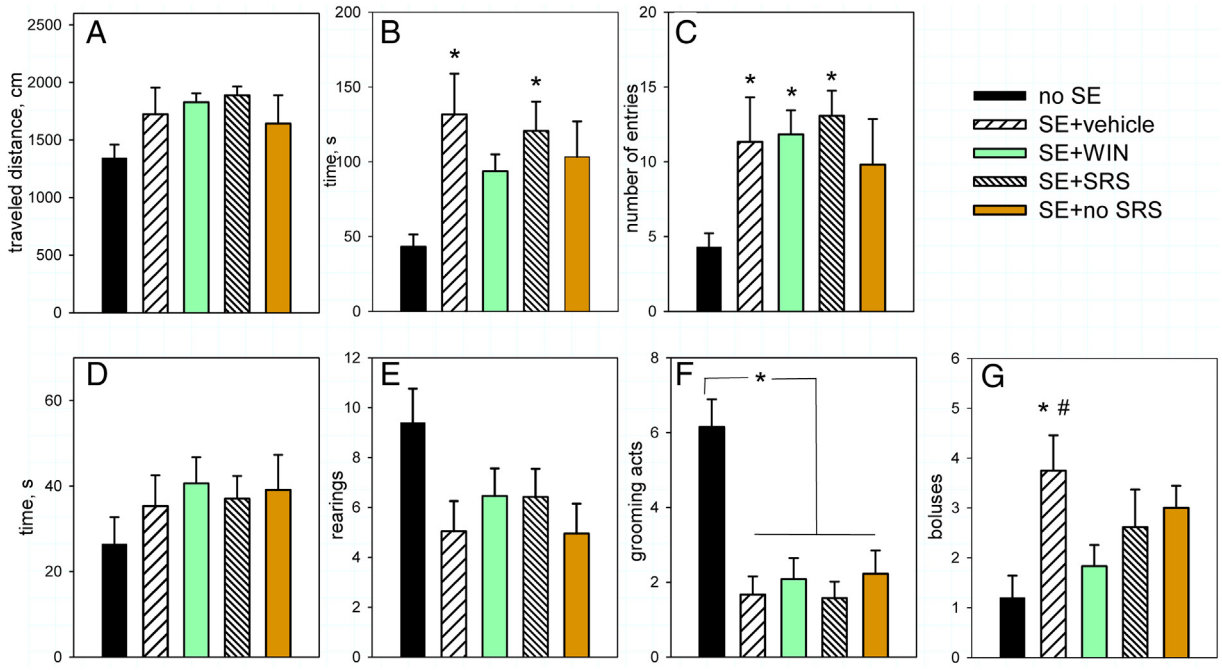

Fig. 1. Behavioral parameters in the elevated plus maze. (A) The distance moved in the maze. Activity per min is shown; (B) time spent in the open arms of the maze, s; (C) number of exits to the open arms; (D) time in the center of the maze, s; (E) number of rearings; (F) number of grooming acts; (G) number of defecations. No SE — control rats without SE; SE + vehicle — rats received vehicle 4 h after SE; SE + WIN — rats received 5 mg/kg WIN-55,212-2 4 h after SE; SE + SRS — rats with spontaneous recurrent seizures recorded 4 months after SE; SE + no SRS — rats with no spontaneous recurrent seizures 4 months after SE. * — significant difference from no SE group at $p < 0.05$; # — a significant difference at $p < 0.05$ between vehicle and WIN-55,212-2-treated rats.

grooming acts and rearings did not significantly change (Fig. 2E, F). The analysis of the change in the locomotor activity during the test with repeated ANOVA showed that all studied groups demonstrated a decrease in the traveled distance during the trial ($p < 0.001$, repeated ANOVA). However, in post-SE rats, it remained increased in comparison with the control group (Fig. 2A,B). The factorial treatment × spontaneous seizure analysis of the results showed that treatment with WIN-55,212-2 did not have a significant effect on any studied behavioral parameters. However, an increase in the traveled distance ($F(2, 20) = 7261$, $p = 0.014$) and center entries ($F(2, 20) = 4{,}8$, $p = 0.040$) depended on the presence of spontaneous seizures: these parameters were significantly increased in epileptic rats while nonepileptic rats were not different from the control (Fig. 2B, D).

3.4.3. Light–dark box

The studied parameters were the number of transitions and time in the light compartment. Rats with spontaneous seizures demonstrated a significant increase in the number of exits to the light compartment ($F(2, 34) = 4.116$, $p = 0.026$, Tukey post hoc test). Rats without spontaneous seizures were not different from controls (Fig. 3A). WIN-55,212-2 administration did not have an effect on any studied behavioral parameters in the light–dark test. No statistically significant differences in time spent in the light compartment were found between the groups (Fig. 3B).

3.4.4. Morris water maze

The analysis of the performance of rats in the Morris water maze showed that the distance swam to the hidden platform significantly decreased in the control rats during learning from day 1 to 5 but did not significantly change in the post-SE rats (repeated measures ANOVA, $p < 0.001$, Tukey post hoc) (Fig. 4A, B). Rats that survived after SE also spent less time in the target quadrant of the water maze in comparison with the control rats ($F = 12{,}52$, $p < 0.001$, ANOVA, Tukey post hoc) (Fig. 4c). Administration of WIN-55,212-2 did not have any significant effect on the performance in the test. Both epileptic and nonepileptic rats demonstrated a prominent disruption of learning in the Morris water maze.

Velocity of movement in the water maze did not differ between the control animals and post-SE animals; and no major sight deficiencies were detected in the test with a visible platform.

4. Discussion

Our main findings include the following: 1) behavioral impairments found in the elevated plus maze test depended mostly on the consequences of SE itself and not on the development of spontaneous seizures; 2) hyperactivity in the open-field test and light–dark box was more prominent in rats with spontaneous seizures; 3) administration of potent endocannabinoid receptor agonist decreased emotional behavior in the elevated plus maze but did not affect hyperactive behavior in the open-field test; 4) spatial memory impairment was developed regardless of the presence of spontaneous seizures in both vehicle- and WIN-55,212-2-treated rats after SE; 5) early administration of endocannabinoid receptor agonist after SE did not prevent the development of spontaneous seizures; 6) both administration of endocannabinoid receptor agonist WIN-55,212-2 and the presence of spontaneous seizures affected neuronal loss in the hippocampus after SE.

In this study, we did not find any long-term antiepileptogenic effect of single post-SE WIN-55,212-2 administration: both WIN-55,212-2-treated and vehicle-treated rats developed spontaneous seizures 4 months after SE, and the occurrence of spontaneous seizures did not differ between these groups. Previous studies have shown early antiepileptogenic effect (for two weeks) in pilocarpine-induced seizures and in kindling models [39,42]. Long-term effects in the chronic period after SE were found for repeated post-SE activation of the endocannabinoid system (7–15 days) [43,44].

Our results indicate that both the presence of spontaneous seizures and treatment with WIN-55,212-2 affected the development of hippocampal neurodegeneration. The largest neuronal loss was found in vehicle-treated epileptic rats; and in the nonepileptic WIN-55,212-2-treated rats, on the contrary, neurodegeneration was the least prominent. The

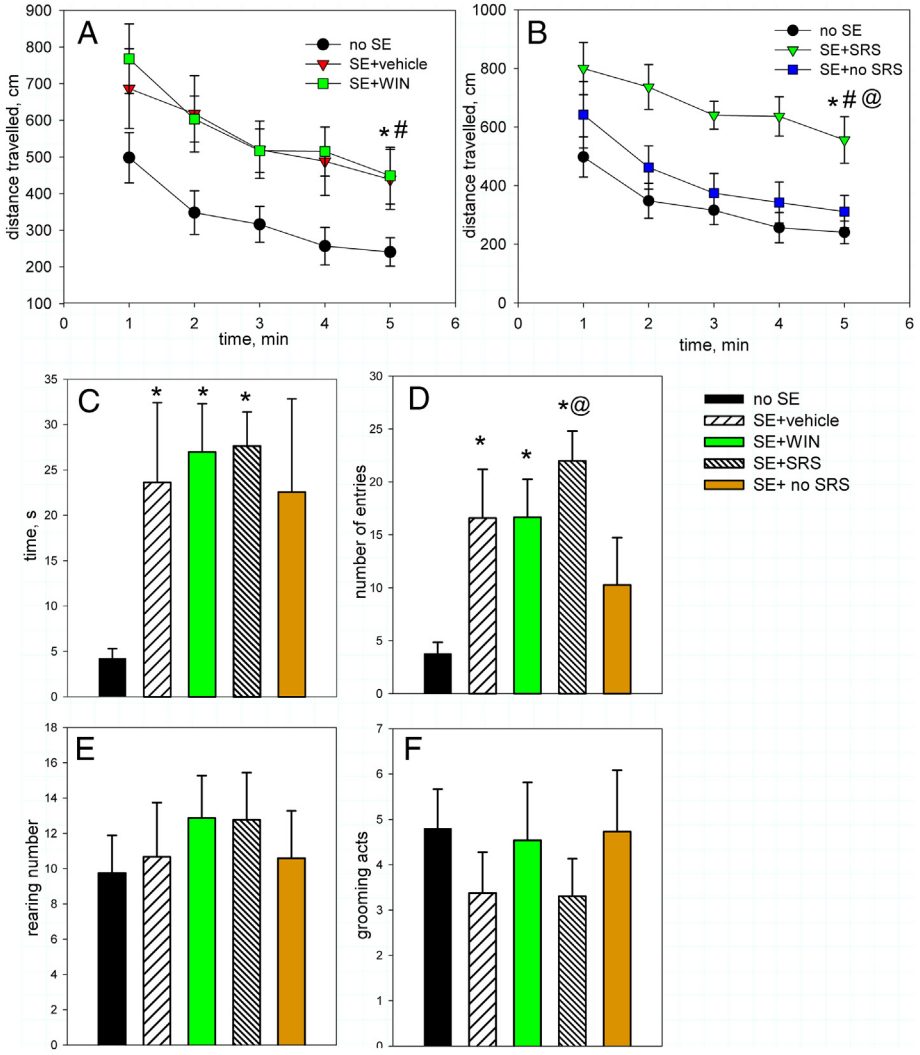

Fig. 2. Behavioral parameters in the open-field test. (A), (B) — distance moved per minute in the open field. X-axis shows time, min, Y axis shows traveled distance, cm; (C) — time spent in the center of the arena, s; (D) — number of exits to the center of the arena; (E) — number of rearings; (F) — number of groomings. No SE — control rats without SE; SE + vehicle — rats received vehicle 4 h after SE; SE + WIN — rats received 5 mg/kg WIN-55,212-2 4 h after SE; SE + SRS — rats with spontaneous recurrent seizures recorded 4 months after SE; SE + no SRS — rats with no spontaneous recurrent seizures 4 months after SE. * — significant difference from no SE group at $p < 0.05$, # — significant difference between the start and the end of the trial at $p < 0.05$, @ — significant difference between epileptic and nonepileptic groups at $p < 0.05$.

partial protective effect of WIN-55,212-2 was found in the ventral CA3. Cells in the hilus of the dentate gyrus in nonepileptic rats were the most sensitive to the effect of WIN-55,212-2, but no neuroprotection was found in this region of epileptic rats. The hilus of dentate gyrus contains mossy fibers, mossy cells, and gamma-aminobutyric acid (GABA)ergic interneurons [45]. Mossy cells are unique for the dentate hilus [46] and are extremely sensitive to excitotoxic damage [47]. Our present findings show that endocannabinoid agonist administration after SE

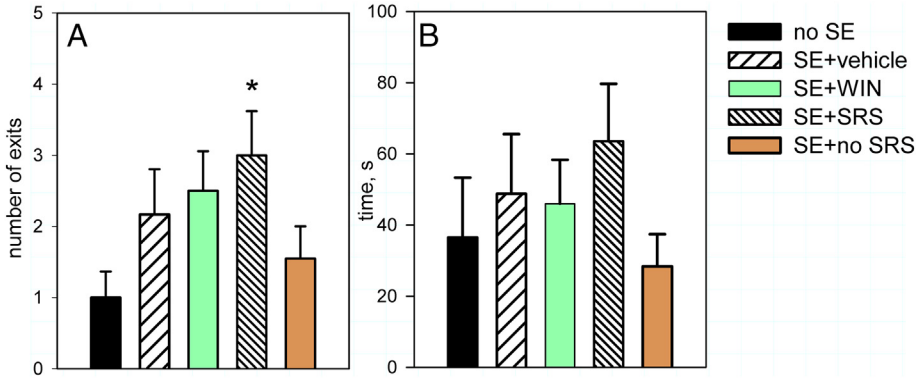

Fig. 3. Behavioral parameters in the light/dark box test. (A) Number of transitions between dark and light compartments; (B) time spent in the light compartment, s. No SE — control rats without SE; SE + vehicle — rats received vehicle 4 h after SE; SE + WIN — rats received 5 mg/kg WIN-55,212-2 4 h after SE; SE + SRS — rats with spontaneous recurrent seizures recorded 4 months after SE; SE + no SRS — rats with no spontaneous recurrent seizures 4 months after SE. * — significant difference from no SE group at $p < 0.05$.

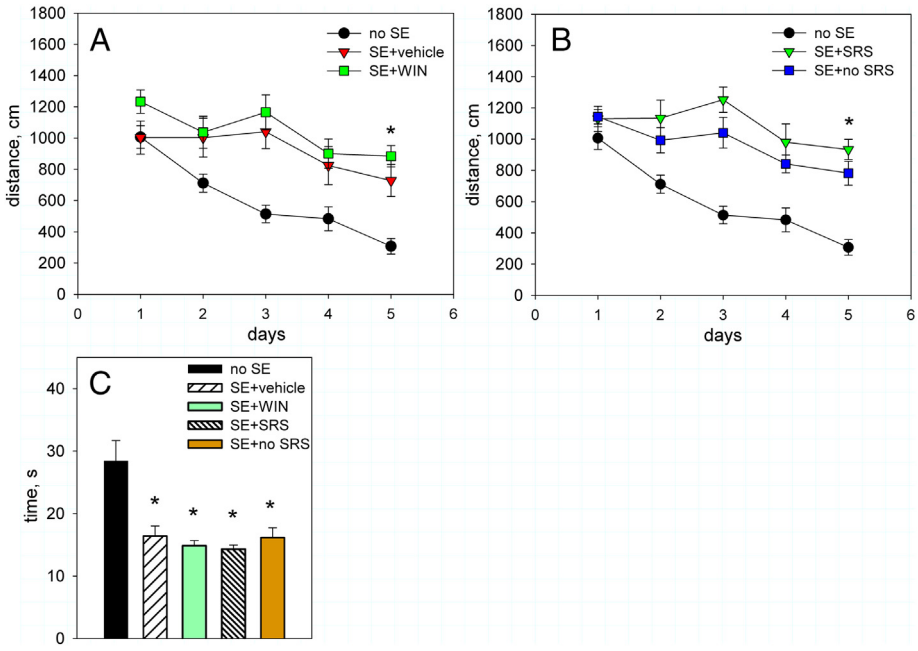

Fig. 4. Morris water maze test. (A), (B) — 5-day learning in the maze, X-axis shows days 1–5 of the experiment, Y-axis shows traveled distance in the maze before finding the hidden platform. (C) — the probe trial on day 6, time spent in the target quadrant of the maze is shown. No SE — control rats without SE; SE + vehicle — rats received vehicle 4 h after SE; SE + WIN — rats received 5 mg/kg WIN-55,212-2 4 h after SE; SE + SRS — rats with spontaneous recurrent seizures recorded 4 months after SE; SE + no SRS — rats with no spontaneous recurrent seizures 4 months after SE. * — significant difference from no SE group at $p < 0.05$.

prevented hilar neuron damage induced by the initial insult in seizure-free rats, but the development of spontaneous seizures could cause further neurodegeneration in this region.

Elevated plus maze is an established test for the assessment level of anxiety in animals [48]. It is a well-known fact that rodents usually avoid open spaces, so they prefer closed arms of the elevated plus maze and stay in the periphery of the open field arena. In our study, this behavior was typical for control rats, which did not sustain SE, but post-SE rats demonstrated an increased time in the open arms and decreased time in the closed arms of the maze in comparison with the control group. Such behavior is typically attributed to the decreased anxiety and was reported in previous studies on the lithium–pilocarpine model of SE [22].

In addition to the elevated plus maze, we investigated the behavior of rats in a light/dark box, which is another test on anxiety in rodents based on their innate aversion to brightly illuminated places [49]. We did not find significant changes in the time spent in the light compartment but found a significant increase in the transitions between compartments in epileptic rats. An increase in the dark–light transitions can indicate an increase in the exploratory activity of rodents in response to mild stress — novel brightly lit environment [49,50]. Anxiolytics are known to increase locomotion and time spent in the light compartment of the test apparatus [51,52]. In studies on kainic acid [53] and diisopropylfluorophosphate [54] SE models, post-SE rats exhibited increased number of transitions to the light compartment of the light/dark apparatus. In the pilocarpine SE model in mice, on the contrary, a decrease in the number of transitions, distance moved, and time in the light compartment was reported [4], but in this case, the contradicting results could be due to the between-species differences. In our study, the light/dark box test showed a significant increase in number of transitions to the light compartment in rats exhibiting spontaneous seizures but not in nonepileptic post-SE rats. This result is similar to the results of the open-field test, where an increase in locomotion in the open-field test was found in rats with chronic epilepsy. Therefore, behavior in the open field and light/dark box tests changed in the same direction and could indicate an abnormal exploratory drive in animals with chronic epilepsy, but not in seizure-free post-SE rats. Interestingly, the results of these two tests are different from the elevated plus-maze (EPM) results, where an anxiolytic-like effect and emotional response to the stressing environment was reduced by WIN-55,212-2 administration early after SE: the agonist did not affect behavioral changes in either open field or light/dark box tests. Some behavioral changes found in EPM (increase in the number of defecations) are not directly related to locomotor hyperactivity unlike the parameters of the open field and light/dark box tests. We assume that some changes underlying behavioral impairment in the EPM could be caused by the initial damage induced by SE while hyperactive behavior in the open field and light/dark box depends mostly on the secondary changes associated with the development of spontaneous seizures. WIN-55,212-2 modulates only changes developing early after SE.

Spatial memory impairment was observed in all rats after SE and was apparently associated with a significant hippocampal neuronal loss typical for the SE models [13,18,55]. The decreased learning ability was found both in rats with spontaneous seizures and seizure-free rats and was not affected by the administration of the endocannabinoid receptor agonist.

Endocannabinoid receptor agonists are reported to have a neuroprotective effect on the models of excitotoxic neuronal damage [56,57], ischemic brain damage [37], and traumatic brain injury [58], which is in agreement with our results. It was shown that CB1 receptors are primarily located on the cholecystokinin-containing (CCK) GABAergic interneurons [59] and control neurotransmitter release from these neurons [60], but neuroprotective effect of endocannabinoid receptor agonists is reported to depend on less abundant subpopulation of CB1 receptors located on glutamatergic receptors [56]. In our previous study, it was shown that a single administration of the agonist of endocannabinoid receptors WIN-55,212-2 4 h after lithium–pilocarpine SE suppressed spontaneous epileptiform activity in the rat cortex and hippocampus that was observed within two weeks after the status [39]. Thus, a single administration of an endocannabinoid receptor agonist may have a sufficiently long anticonvulsant effect, which could reduce the excitotoxic neuronal damage in selected hippocampal areas. However, this effect was not enough to prevent the development of behavioral and spatial memory impairments and spontaneous seizures.

5. Conclusion

The results of our study demonstrate that behavioral impairments occurring in the chronic period after SE can result from the initial impact of SE or accompany the development of epilepsy, suggesting that different mechanisms can underlie even seemingly close types of behavioral comorbidities. Early post-SE administration of endocannabinoid agonist WIN-55,212-2 attenuated behavioral changes in the elevated plus maze and reduced neurodegeneration in the hippocampus but did not prevent the development of spontaneous seizures, hyperactivity, and spatial memory in the chronic period after SE.

Conflict of interest

We have no conflict of interest to declare.

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References

[1] Müller CJ, Gröticke I, Bankstahl M, Löscher W. Behavioral and cognitive alterations, spontaneous seizures, and neuropathology developing after a pilocarpine-induced status epilepticus in C57BL/6 mice. Exp Neurol 2009;219:284–97. https://doi.org/10.1016/j.expneurol.2009.05.035.
[2] Josephson CB, Jetté N. Psychiatric comorbidities in epilepsy. Int Rev Psychiatry 2017;29:409–24. https://doi.org/10.1080/09540261.2017.1302412.
[3] Fisher PL, Noble AJ. Anxiety and depression in people with epilepsy: the contribution of metacognitive beliefs. Seizure 2017;50:153–9. https://doi.org/10.1016/j.seizure.2017.06.012.
[4] Gröticke I, Hoffmann K, Löscher W. Behavioral alterations in the pilocarpine model of temporal lobe epilepsy in mice. Exp Neurol 2007;207:329–49. https://doi.org/10.1016/j.expneurol.2007.06.021.
[5] Kanner AM, Barry JJ, Gilliam F, Hermann B, Meador KJ. Anxiety disorders, subsyndromic depressive episodes, and major depressive episodes: do they differ on their impact on the quality of life of patients with epilepsy? Epilepsia 2010;51:1152–8. https://doi.org/10.1111/j.1528-1167.2010.02582.x.
[6] Aguilar BL, Malkova L, N'Gouemo P, Forcelli PA. Genetically epilepsy-prone rats display anxiety-like behaviors and neuropsychiatric comorbidities of epilepsy. Front Neurol 2018;9:476. https://doi.org/10.3389/fneur.2018.00476.
[7] Lambert MV, Robertson MM. Depression in epilepsy: etiology, phenomenology, and treatment. Epilepsia 1999;40:s21–47. https://doi.org/10.1111/j.1528-1157.1999.tb00884.x.
[8] Harden CL. The co-morbidity of depression and epilepsy: epidemiology, etiology, and treatment. Neurology 2002;59:S48–55. https://doi.org/10.1212/WNL.59.6_suppl_4.S48.
[9] Kanner AM, Balabanov A. Depression and epilepsy: how closely related are they? Neurology 2002;58:S27–39.
[10] Mula M, Cock HR. More than seizures: improving the lives of people with refractory epilepsy. Eur J Neurol 2015;22:24–30. https://doi.org/10.1111/ene.12603.
[11] Munger Clary HM, Snively BM, Hamberger MJ. Anxiety is common and independently associated with clinical features of epilepsy. Epilepsy Behav 2018;85:64–71. https://doi.org/10.1016/j.yebeh.2018.05.024.
[12] Kanner AM. Anxiety disorders in epilepsy: the forgotten psychiatric comorbidity. Epilepsy Curr 2011;11:90–1. https://doi.org/10.5698/1535-7511-11.3.90.
[13] Jope RS, Morrisett RA, Snead OC. Characterization of lithium potentiation of pilocarpine-induced status epilepticus in rats. Exp Neurol 1986;91:471–80.
[14] Leite JP, Garcia-Cairasco N, Cavalheiro EA. New insights from the use of pilocarpine and kainate models. Epilepsy Res 2002;50:93–103 [doi:S0920121102000724 [pii]].
[15] Löscher W. Critical review of current animal models of seizures and epilepsy used in the discovery and development of new antiepileptic drugs. Seizure 2011;20:359–68. https://doi.org/10.1016/j.seizure.2011.01.003.
[16] Pitkänen A, Nissinen J, Nairismägi J, Lukasiuk K, Gröhn OHJ, Miettinen R, et al. Progression of neuronal damage after status epilepticus and during spontaneous seizures in a rat model of temporal lobe epilepsy. Prog Brain Res 2002;135:67–83. https://doi.org/10.1016/S0079-6123(02)35008-8.
[17] Andre V, Dube C, Francois J, Leroy C, Rigoulot M-AA, Roch C, et al. Pathogenesis and pharmacology of epilepsy in the lithium–pilocarpine model. Epilepsia 2007;48(Suppl. 5):41–7. https://doi.org/10.1111/j.1528-1167.2007.01288.x [doi:EPI1288 [pii]].
[18] Cavalheiro EA, Leite JP, Bortolotto ZA, Turski WA, Ikonomidou C, Turski L. Long-term effects of pilocarpine in rats: structural damage of the brain triggers kindling and spontaneous recurrent seizures. Epilepsia 1991;32:778–82.
[19] Nairismagi J, Grohn OH, Kettunen MI, Nissinen J, Kauppinen RA, Pitkanen A, et al. Progression of brain damage after status epilepticus and its association with epileptogenesis: a quantitative MRI study in a rat model of temporal lobe epilepsy. Epilepsia 2004;45:1024–34. https://doi.org/10.1111/j.0013-9580.2004.08904.xEPI08904 [pii].
[20] Clifford DB, Olney JW, Maniotis A, Collins RC, Zorumski CF. The functional anatomy and pathology of lithium–pilocarpine and high-dose pilocarpine seizures. Neuroscience 1987;23:953–68.
[21] Persinger MA, Bureau YRJ, Kostakos M, Peredery O, Falter H. Behaviors of rats with insidious, multifocal brain damage induced by seizures following single peripheral injections of lithium and pilocarpine. Physiol Behav 1993;53:849–66. https://doi.org/10.1016/0031-9384(93)90261-D.
[22] Detour J, Schroeder H, Desor D, Nehlig A. A 5-month period of epilepsy impairs spatial memory, decreases anxiety, but spares object recognition in the lithium–pilocarpine model in adult rats. Epilepsia 2005;46:499–508. https://doi.org/10.1111/j.0013-9580.2005.38704.x.
[23] Faure JB, Akimana G, Carneiro JEM, Cosquer B, Ferrandon A, Geiger K, et al. A comprehensive behavioral evaluation in the lithium–pilocarpine model in rats: effects of carisbamate administration during status epilepticus. Epilepsia 2013;54:1203–13. https://doi.org/10.1111/epi.12219.
[24] Pineda E, Shin D, Sankar R, Mazarati AM. Comorbidity between epilepsy and depression: experimental evidence for the involvement of serotonergic, glucocorticoid, and neuroinflammatory mechanisms. Epilepsia 2010;51(Suppl. 3):110–4. https://doi.org/10.1111/j.1528-1167.2010.02623.x.
[25] Mazarati A, Siddarth P, Baldwin RA, Shin D, Caplan R, Sankar R. Depression after status epilepticus: behavioural and biochemical deficits and effects of fluoxetine. Brain 2008;131:2071–83. https://doi.org/10.1093/brain/awn117.
[26] Zanirati G, Azevedo PN, Venturin GT, Greggio S, Alcará AM, Zimmer ER, et al. Depression comorbidity in epileptic rats is related to brain glucose hypometabolism and hypersynchronicity in the metabolic network architecture. Epilepsia 2018;59:923–34. https://doi.org/10.1111/epi.14057.
[27] Leite JP, Nakamura EM, Lemos T, Masur J, Cavalheiro EA. Learning impairment in chronic epileptic rats following pilocarpine-induced status epilepticus. Braz J Med Biol Res 1990;23:681–3.
[28] Katona I, Freund TF. Endocannabinoid signaling as a synaptic circuit breaker in neurological disease. Nat Med 2008;14:923–30. https://doi.org/10.1038/nm.f.1869.
[29] Kano M, Ohno-Shosaku T, Hashimotodani Y, Uchigashima M. Endocannabinoid-mediated control of synaptic transmission; 2009; 309–80. https://doi.org/10.1152/physrev.00019.2008.
[30] Wallace MJ, Wiley JL, Martin BR, Delorenzo RJ. Assessment of the role of CB1 receptors in cannabinoid anticonvulsant effects. Eur J Pharmacol 2001;428:51–7. https://doi.org/10.1016/S0014-2999(01)01243-2.
[31] Kozan R, Ayyildiz M, Agar E. The effects of intracerebroventricular AM-251, a CB1-receptor antagonist, and ACEA, a CB1-receptor agonist, on penicillin-induced epileptiform activity in rats. Epilepsia 2009;50:1760–7. https://doi.org/10.1111/j.1528-1167.2009.02098.x.
[32] Blair RE, Deshpande LS, Sombati S, Falenski KW, Martin BR, Delorenzo RJ. Activation of the cannabinoid type-1 receptor mediates the anticonvulsant properties of cannabinoids in the hippocampal neuronal culture models of acquired epilepsy and status epilepticus. J Pharmacol Exp Ther 2006;317:1072–8. https://doi.org/10.1124/jpet.105.100354.
[33] von Rüden EL, Jafari M, Bogdanovic RM, Wotjak CT, Potschka H. Analysis in conditional cannabinoid 1 receptor-knockout mice reveals neuronal subpopulation-specific effects on epileptogenesis in the kindling paradigm. Neurobiol Dis 2014. https://doi.org/10.1016/j.nbd.2014.08.001.
[34] Vinogradova LV, Shatskova AB, Van Rijn CM. Pro-epileptic effects of the cannabinoid receptor antagonist SR141716 in a model of audiogenic epilepsy. Epilepsy Res 2011;96:250–6. https://doi.org/10.1016/j.eplepsyres.2011.06.007.
[35] Deshpande LS, Sombati S, Blair RE, Carter DS, Martin BR, Delorenzo RJ. Cannabinoid CB1 receptor antagonists cause status epilepticus-like activity in the hippocampal neuronal culture model of acquired epilepsy. Neurosci Lett 2007;411:11–6. https://doi.org/10.1016/j.neulet.2006.09.046.
[36] Zogopoulos P, Vasileiou I, Patsouris E, Theocharis S. The neuroprotective role of endocannabinoids against chemical-induced injury and other adverse effects. J Appl Toxicol 2013;33:246–64. https://doi.org/10.1002/jat.2828.
[37] Schmidt W, Schäfer F, Striggow V, Fröhlich K, Striggow F. Cannabinoid receptor subtypes 1 and 2 mediate long-lasting neuroprotection and improve motor behavior deficits after transient focal cerebral ischemia. Neuroscience 2012;227:313–26. https://doi.org/10.1016/j.neuroscience.2012.09.080.
[38] Nagayama T, Sinor AD, Simon RP, Chen J, Graham SH, Jin K, et al. Cannabinoids and neuroprotection in global and focal cerebral ischemia and in neuronal cultures. J Neurosci 1999;19:2987–95.
[39] Suleymanova EM, Shangaraeva VA, van Rijn CM, Vinogradova LV. The cannabinoid receptor agonist WIN55.212 reduces consequences of status epilepticus in rats. Neuroscience 2016;334:191–200. https://doi.org/10.1016/j.neuroscience.2016.08.004.
[40] Racine RJ. Modification of seizure activity by electrical stimulation: II. Motor seizure. Electroencephalogr Clin Neurophysiol 1972;32:281–94. https://doi.org/10.1016/0013-4694(72)90177-0.
[41] Vorhees CV, Williams MT. Morris water maze: procedures for assessing spatial and related forms of learning and memory. Nat Protoc 2006;1:848–58. https://doi.org/10.1038/nprot.2006.116.
[42] Vinogradova LV, van Rijn CM. Long-term disease-modifying effect of the endocannabinoid agonist WIN55,212-2 in a rat model of audiogenic epilepsy. Pharmacol Rep 2015;67:501–3. https://doi.org/10.1016/j.pharep.2014.12.002.
[43] Ma L, Wang L, Yang F, Meng X-D, Wu C, Ma H, et al. Disease-modifying effects of RHC80267 and JZL184 in a pilocarpine mouse model of temporal lobe epilepsy. CNS Neurosci Ther 2014. https://doi.org/10.1111/cns.12302.

[44] Di Maio R, Cannon JR, Timothy Greenamyre J. Post-status epilepticus treatment with the cannabinoid agonist WIN 55,212-2 prevents chronic epileptic hippocampal damage in rats. Neurobiol Dis 2014;73C:356–65. https://doi.org/10.1016/j.nbd.2014.10.018.
[45] Amaral DG, Scharfman HE, Lavenex P. The dentate gyrus: fundamental neuroanatomical organization (dentate gyrus for dummies). Prog Brain Res 2007;163:3–790. https://doi.org/10.1016/S0079-6123(07)63001-5.
[46] Scharfman HE. The enigmatic mossy cell of the dentate gyrus. Nat Rev Neurosci 2016;17:562–75. https://doi.org/10.1038/nrn.2016.87.
[47] Magloczky Z, Freund TF. Selective neuronal death in the contralateral hippocampus following unilateral kainate injections into the CA3 subfield. Neuroscience 1993;56:317–36. https://doi.org/10.1016/0306-4522(93)90334-C.
[48] Pellow S, Chopin P, File SE, Briley M. Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J Neurosci Methods 1985;14:149–67.
[49] Bourin M, Hascoët M. The mouse light/dark box test. Eur J Pharmacol 2003;463:55–65. https://doi.org/10.1016/S0014-2999(03)01274-3.
[50] Crawley J, Goodwin FK. Preliminary report of a simple animal behavior model for the anxiolytic effects of benzodiazepines. Pharmacol Biochem Behav 1980;13:167–70.
[51] Chaouloff F, Durand M, Mormède P. Anxiety- and activity-related effects of diazepam and chlordiazepoxide in the rat light/dark and dark/light tests. Behav Brain Res 1997;85:27–35. https://doi.org/10.1016/S0166-4328(96)00160-X.
[52] Imaizumi M, Suzuki T, Machida H, Onodera K. A fully automated apparatus for a light/dark test measuring anxiolytic or anxiogenic effects of drugs in mice. Nihon Shinkei Seishin Yakurigaku Zasshi 1994;14:83–91.
[53] Tchekalarova J, Atanasova D, Nenchovska Z, Atanasova M, Kortenska L, Gesheva R, et al. Agomelatine protects against neuronal damage without preventing epileptogenesis in the kainate model of temporal lobe epilepsy. Neurobiol Dis 2017;104:1–14. https://doi.org/10.1016/j.nbd.2017.04.017.
[54] Rojas A, Ganesh T, Manji Z, O'neill T, Dingledine R. Inhibition of the prostaglandin E2 receptor EP2 prevents status epilepticus-induced deficits in the novel object recognition task in rats. Neuropharmacology 2016;110:419–30. https://doi.org/10.1016/j.neuropharm.2016.07.028.
[55] Covolan L, Mello LE. Assessment of the progressive nature of cell damage in the pilocarpine model of epilepsy. Braz J Med Biol Res 2006;39:915–24 [doi:S0100-879X2006000700010 [pii].
[56] Chiarlone A, Bellocchio L, Blázquez C, Resel E, Soria-Gómez E, Cannich A, et al. A restricted population of CB1 cannabinoid receptors with neuroprotective activity. Proc Natl Acad Sci U S A 2014;111:8257–62. https://doi.org/10.1073/pnas.1400988111.
[57] Xu C, Hermes DJ, Nwanguma B, Jacobs IR, Mackie K, Mukhopadhyay S, et al. Endocannabinoids exert CB1 receptor-mediated neuroprotective effects in models of neuronal damage induced by HIV-1 Tat protein. Mol Cell Neurosci 2017;83:92–102. https://doi.org/10.1016/j.mcn.2017.07.003.
[58] Panikashvili D, Simeonidou C, Ben-Shabat S, Hanuš L, Breuer A, Mechoulam R, et al. An endogenous cannabinoid (2-AG) is neuroprotective after brain injury. Nature 2001;413:527–31. https://doi.org/10.1038/35097089.
[59] Tsou K, Mackie K, Sañudo-Peña MC, Walker JM. Cannabinoid CB1 receptors are localized primarily on cholecystokinin-containing GABAergic interneurons in the rat hippocampal formation. Neuroscience 1999;93:969–75.
[60] Katona I, Sperlágh B, Sík A, Käfalvi A, Vizi ES, Mackie K, et al. Presynaptically located CB1 cannabinoid receptors regulate GABA release from axon terminals of specific hippocampal interneurons. J Neurosci 1999;19:4544–58.