

Subchronic effects of ligands of cannabinoid receptors on learning and memory processes of olfactory bulbectomized rats

Margarita Velikova^{1*}, Dobrinka Doncheva¹ and Roman Tashev²

¹ Department of Physiology and Pathophysiology, Medical University, Varna, Bulgaria,

² Department of Pathophysiology, Medical University of Sofia, Sofia, Bulgaria,

* Email: msvelikova@yahoo.com

The brain endocannabinoid system has been shown to play a role in many physiological processes, including mood, learning and memory. It is also involved in the pathogenesis of anxiety, depression, mood disorders, as well as neurodegenerative disorders, although the exact mechanisms by which cannabinoid receptors interfere in these disorders are not well established. The aim of the present study was to evaluate the effects of cannabinoid ligands HU-210 (CB1 receptor agonist) and SR 141716A (CB1 receptor antagonist) on learning and memory processes of rats with depressive – like state, induced by bilateral olfactory bulbectomy. The bilateral olfactory bulbectomy (OBX) is a validated model of depression, which can be used also as an animal model of Alzheimer's disease. We found that the subchronic treatment of OBX rats with HU 210 and SR 141716A exerted modulatory effect on rat's performance in both active avoidance (shuttle box) and passive avoidance (step through) tests. HU 210 ameliorated the memory deficits of OBX rats; however, the scores of the sham-operated controls had not been reached. SR 141716A modified the avoidance performance in OBX rats and showed a memory enhancing effect in the sham-operated rats. Our findings suggest that CB1 receptors might be involved in avoidance learning and memory acquisition in OBX rats.

Key words: CB1 cannabinoid receptors, learning and memory, olfactory bulbectomy, depression, rat

INTRODUCTION

Endocannabinoid system (ECS) plays role in many physiological processes, including mood, learning and memory. It is also involved in the pathogenesis of anxiety and mood disorders, as well as neurodegenerative disorders (Hill and Gorzalka, 2009; Ranieri et al., 2016). ECS consists of the endogenous cannabinoids (endocannabinoids), cannabinoid receptors and the enzymes that synthesize and degrade endocannabinoids. The cannabinoid receptors (CB1 and CB2) belong to the class of G-protein coupled membrane receptors. CB1 receptors are expressed mainly in the central nervous system with highest density being detected in the basal ganglia, hippocampus, cerebellum, prefrontal cortex, amygdala

(Mackie, 2005). The endogenous cannabinoids act as retrograde modulators of neurotransmission being released on demand from the postsynaptic neurons and binding to the presynaptically located CB1 receptors (Freund et al., 2003; De Petrocellis and Di Marzo, 2009). The manipulation of the endocannabinoid signaling often produces contrasting findings concerning cognition and emotions, which can be related to the fact that CB1 receptors are expressed at stimulatory (glutamatergic) and inhibitory (GABAergic) synapses (Ruehle et al., 2012).

The role of the cannabinoid receptors in learning and memory processes has been intensively studied, but is not yet fully understood. There are numerous reports about in general, memory impairing effects of cannabinoid agonists and memory enhancing effects of cannabinoid antagonists (Zanettini et al., 2011; Kruk-Slomka

et al., 2017). However, this is a simplification, as the effects may differ depending on the dose, behavioral methods used, experimental design, etc. There is an intensive research on modulation of the components of ECS in attempt to develop pharmacological therapy for treatment of anxiety and depressive disorders. Studies also have provided evidence that the ECS has neuro-protective properties and might be a target in neurodegenerative diseases (Ranieri et al., 2016).

The bilateral olfactory bulbectomy (OBX) is a validated model of depression. The removal of bulbi olfactorii produces a syndrome of behavioral, neurochemical, neuroendocrine, immune, etc. alterations, that resembles human depressive disorder (Kelly et al., 1997; Song and Leonard, 2005). OBX causes long lasting neurodegenerative changes in many brain areas and therefore, it is suggested that it might be a valuable tool in the study of neurodegenerative disorders like Alzheimer's disease (AD) (Douma et al., 2011; Borre et al., 2012). OBX can be used as an animal model of AD because it produces some effects similar to AD-related symptoms and pathology (Aleksandrov et al., 2004; Yehuda and Rabinovitz, 2013; Franks et al., 2015). Behavioral abnormalities developed by the OBX rodents include hyperlocomotion, memory disturbances, reduced sexual activity, aggressive behavior, hyperemotionality. OBX rats display deficits in passive-avoidance test (Kelly et al., 1997) and impaired response in the two-way active avoidance task (Gomita et al., 1984; Archer et al., 1984). While the activation of the CB1 receptor by agonists has been found to predominantly improve the depressive-like state in animal models (Segev et al., 2014; Kruk-Slomka et al., 2015; Haj-Mirzaian et al., 2017), the data on the role of CB1 antagonists are contradictory. Previously, we have demonstrated impaired performance of OBX rats in both active and passive avoidance tests (Tashev et al., 2010) and modulatory effects of acutely i.c.v. applied CB1 ligands on the performance of OBX rats in these tests (Marinov et al., 2013).

Based on the results of our acute treatment experiments, the aim on the present study was to evaluate the subchronic effects of CB1 agonist HU 210 and CB1 antagonist SR 141716A applied i.c.v. for 7 days on the background of developed depressive-like state, on learning and memory performance of OBX rats, tested in two-way active avoidance (TWAA) and passive avoidance (PA) paradigms.

METHODS

Subjects

The experiments were carried out on male Wistar rats (200–220 g at the time of surgery). For each behavioral task (locomotor activity, shuttle box, step

through), the animals were randomly divided into 6 major experimental groups (7 animals in each group). Group I – sham-operated (sham) and group II – OBX (controls) received saline; group III and IV (sham) as well as group V and VI (OBX) received HU 210 or SR141716A respectively. The rats were housed in polypropylene boxes with free access to food and water. The animals were maintained in a constant temperature environment ($22 \pm 2^\circ\text{C}$) on a 12 h light/dark cycle (lights on at 6:00 a.m.). The behavioral experiments were carried out between 9:00 a.m. and 1:00 p.m. After the testing procedure, the rats were returned to their home cages.

Ethical statement

The experiments were performed according to the “Principles of laboratory animal care” (NIH publication no. 85-23, revised 1985) and the rules of the Ethics Committee of the Institute of Neurobiology, Bulgarian Academy of Sciences. All efforts were made to minimize animal suffering and reduce the number of animals used in the study.

Surgical procedures

OBX was performed according to the method described by Kelly et al. (1997). Animals were anesthetized with Calyptol (50 mg/kg i.p.) and placed in a stereotaxic apparatus. The surgical procedure involved drilling two burr holes 2 mm in diameter at the points 8 mm anterior to bregma and 2 mm from the midline on its both sides (coordinates of bulbi olfactorii were detected according to the stereotaxic atlas of Pellegrino and Cushman (1967). The bulbs were aspirated with a stainless needle attached to a water pump. The implantation of the cannula into the right lateral ventricle was performed 7 days after OBX surgery, as previously described (Marinov et al., 2013). The sham operation included the same procedures as for the olfactory bulbectomy, without the removal of the olfactory bulbs. During the 7 day recovery period, the rats were handled daily.

Drugs

HU 210 and SR 141716A (Tocris Bioscience) were dissolved ex tempore in a 1:19 solution of dimethyl sulfoxide/0.9% saline. One μl of drug solution HU-210 (0.5%) or SR 141716A (0.3%) with pH=7.4 were infused i.c.v. through an injection cannula connected by poly-

ethylene tubing with a constant rate microsyringe (Hamilton, Reno, NV, USA) over a period of 1 min. The injection cannula was left in place for additional 30 s. After the surgery for cannula implantation, the animals were allowed to recover for 7 days. During the recovery period, the rats were handled daily. HU 210 (5 µg/1 µl), SR 141716A (3 µg/1 µl) or saline (1 µl) were infused i.c.v. for 7 consecutive days to the respective group, starting on the 15th day after OBX procedure.

The behavioral tests were carried out 21 days after the surgery for the removal of the olfactory bulbs. Before each test, the rats were placed for 5 min in the respective test cage for 3 consecutive days to acclimatize to it (Fig. 1).

Behavioral tests

Locomotor activity

Locomotor activity was recorded in an Opto Varimex apparatus (Columbus Instruments, USA). The experimental chamber was 50 cm × 50 cm × 25 cm. The apparatus records the number of photo beam interruptions during the movements of the animal. It provides counting of the number of horizontal (ambulation) and vertical movements (rearing) in arbitrary units (AU). The information obtained was recorded automatically for 30 min. The rats were placed in the central quadrant of the activity monitor, 5 min after the microinjection of the drugs.

Two-way active avoidance test

The animals were trained in a shuttle-box apparatus according to the method of Buresova and Bures (1983) and modified by Petkov et al. (1993). The shuttle box apparatus is a box (50 × 29 × 21 cm) separated into two compartments by a wall with a U-shaped gate. Light (21 W) was used as a conditioned stimulus (CS); the un-

conditioned stimulus (US) was electric non-scrambling current (20–30 V, 0.5 mA, AC, 50 Hz) delivered through the grid floor for 12 s. The CS preceded the onset of the US by 9 s and continued during the action of the US. Prior to avoidance training the rats were given a 6-min session of exposure to light stimuli in the shuttle box (alternating 9 s light and 9 s interval) for adaptation. During the first training session each rat was trained with 50 trials; 24 hours later was carried out the second training session. At the retention test (24 h after the 2nd training session), the light stimulus was applied for 9 s and was followed by a 1 s electric shock.

Each trial began with a 9 s light stimulus, followed by a 12 s shock. One trial lasted 21 s, the inter-trial interval was 9 s. Each trial started in the compartment where the rat was located at the end of the inter-trial interval. If the rat crossed the barrier to the opposite compartment within 9 s after the onset of light stimulus, the stimulus was terminated and no shock was delivered (avoidance response). A crossing response escape during shock terminated the stimuli. If the rat failed to cross during the entire trial (inadequate reaction), the light and the shock was terminated. The data from each shuttle box were fed into an analogue-to-digital converter, coupled to a computer with appropriate software. The number of avoidances was analyzed as a primary measure of learning and memory in the active avoidance task. The drugs were microinjected 5 min prior to the first and second training session.

Passive avoidance test

The Ugo-Bassile avoidance instrument is a cage divided into two compartments. In the training trial, the rat is placed in the brightly lit compartment and it must learn to remain there and not to escape to the preferred dark compartment to avoid a mild foot shock. Once the rat had entered the dark compartment, the door was closed and an electrical shock (0.3–0.35 mA for 3 s) was delivered

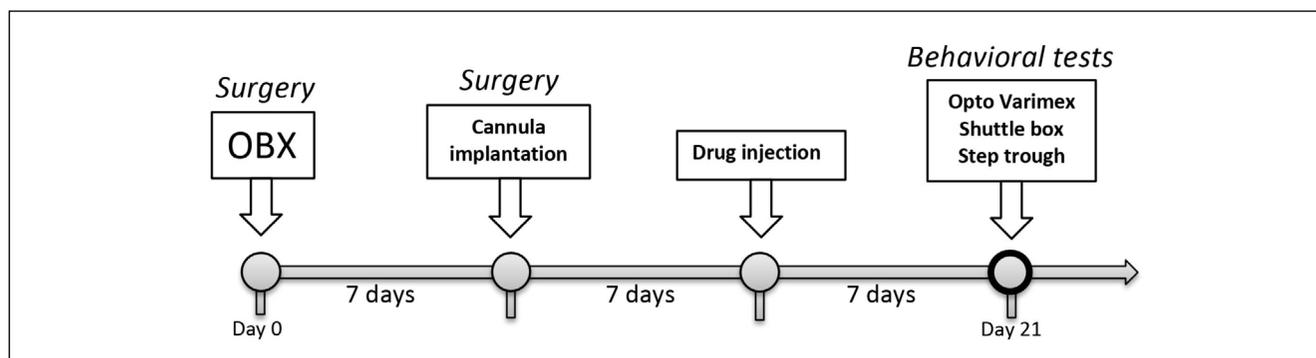


Fig. 1. Outline of the experimental design.

through the grid floor. One training trial and two retention tests were conducted according to the method described by (Gozzani and Izquierdo, 1976). Retention tests (no shocks) were performed 3 h and 24 h after the training trial. The animals were placed in the light compartment, and step-through latency was estimated by measuring the length of time (latent time) for the rat to move to the dark compartment. A maximum latency of 180 seconds was used as a criterion for learning. The drugs were microinjected i.c.v. 5 min prior to the training trial.

Verification

Following termination of the experiments and immediately prior sacrifice, the rats were injected with 1 ml of 2% fast green dye through injection cannula into the right lateral ventricle. The cannula placement was checked visually after dissection. If cannula was successfully placed in a ventricle, the dye was distributed throughout the ventricular system and the liquor was colored. The verification of the olfactory bulbectomy was done by visual inspection. Rats with incomplete (<80%) removal of the olfactory bulbs were excluded from the statistical analysis. Thus, 4 rats were discarded.

Statistical analysis

One-way ANOVA was used to process the data obtained for total number of horizontal and for vertical movements during the whole 30-min period of observation.

Two-way repeated measures ANOVA were used to analyze the data obtained for number of avoidances (shuttle-box) between subject factors: treatment (six levels: sham-saline, sham-HU, sham-SR, OBX-saline, OBX-HU, OBX-SR) and time (three levels: 1st day, 2nd day and 3rd day retention test - 24 h after the 2nd training day).

Two-way repeated measures ANOVA were used to process the data obtained for latent time (step through) between subject factors: treatment (six levels: sham-saline, sham-HU, sham-SR, OBX-saline, OBX-HU and OBX-SR) and time (3rd hour and 24th hour).

ANOVA data were further analysed by *post hoc* Student-Newman-Keuls (SNK) test where appropriate. Analysis of the passive avoidance data (step through) was performed using χ^2 tests.

ANOVA data were further analysed by *post hoc* Student-Newman-Keuls (SNK) test where appropriate. Analysis of the passive avoidance data (step through) was performed using χ^2 tests.

RESULTS

Locomotor activity

Effects of HU-210 and SR 141716A in sham-operated rats

Separate one-way ANOVA analysis on the total number of horizontal or vertical movements for the whole 30-min period of observation showed a significant effect for factor drug ($F_{2,20}=87.597$; $P\leq 0.0001$) for the number of horizontal movements, and respectively for the number of vertical movements: ($F_{2,20}=51.877$; $P\leq 0.0001$). The microinjections of HU 210 significantly decreased the total number of horizontal ($P\leq 0.0001$), and vertical ($P\leq 0.0001$) movements as compared to saline-treated controls, while SR 141716A increased the locomotor activity ($P\leq 0.0001$; $P\leq 0.0001$ respectively) as compared to the controls (Fig. 2A-B).

Effects of HU-210 and SR 141716A in OBX rats

Three weeks after the OBX procedure, OBX rats showed hyperlocomotion, increased number of move-

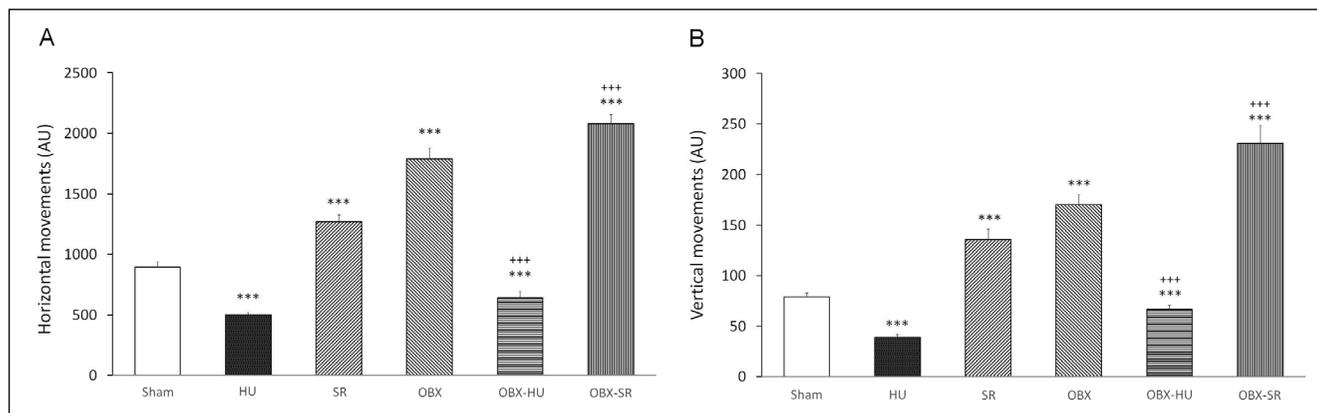


Fig. 2. Effects of HU 210 and SR 141716A administered i.c.v. for 7 days to OBX rats, on the total number of (A) horizontal and (B) vertical movements for 30 min period of observation. AU – arbitrary units. n=7. Means (\pm S.E.M.) are presented. *** $P\leq 0.001$ – comparison vs. sham controls; *** $P\leq 0.001$ – comparison to the OBX rats.

ments in the Opto Varimex apparatus. Separated one way ANOVA on the total number of horizontal and vertical movements of OBX rats demonstrated a significant effect for bulbectomy factor (respectively $F_{1,13}=93.120$, $P\leq 0.00001$; $F_{1,13}=66.305$, $P\leq 0.00001$). *Post hoc* SNK comparisons demonstrated that the total number of horizontal ($P\leq 0.0001$), and vertical ($P\leq 0.0001$) movements was higher as compared to sham-operated controls (Fig. 2A-B) ANOVA of the total number horizontal and vertical movements after infusion of HU 210 and SR 141716A in OBX rats showed a significant effect for factor drug (respectively $F_{2,20}=107.006$, $P\leq 0.00001$; $F_{2,20}=48.923$, $P\leq 0.00001$).

The SNK test showed that HU-210 significantly decreased the number of both horizontal ($P\leq 0.0001$) and vertical ($P\leq 0.0001$) movements as compared to the OBX saline-treated rats. The horizontal activity was not significantly different in comparison to the sham-operated rats (P -NS), the number of vertical movements was lower ($P\leq 0.001$) (Fig. 2A-B).

SR 141716A increased the locomotor activity as compared to both OBX-saline controls ($P\leq 0.001$ for horizontal movements; $P\leq 0.05$ for vertical movements) and sham-operated rats ($P\leq 0.0001$; $P\leq 0.0001$) (Fig. 2A-B).

Avoidance tests

Shuttle box test

Repeated two-way ANOVA analysis on the number of avoidances in the shuttle box test demonstrated a significant effect for factors treatment (sham-saline, sham-HU, sham-SR, OBX-saline, OBX-HU and OBX-SR) ($F_{5,125}=67.707$, $P\leq 0.00001$) and time (1st day, 2nd day and 3rd day) ($F_{2,125}=27.676$, $P\leq 0.00001$). There was a significant interaction between treatment and time ($F_{10,125}=2.013$; $P\leq 0.03$).

Post hoc SNK comparisons showed that HU 210 impaired the performance of sham-operated rats by decreasing the number of avoidances on the 1st day ($P\leq 0.04$); 2nd day ($P\leq 0.01$) and at the retention test ($P\leq 0.006$) as compared to the sham-saline controls (Fig. 3A-C).

SR 141716A microinjected in the sham-operated rats increased significantly the number of avoidances on the 1st day ($P\leq 0.04$), 2nd day ($P\leq 0.03$) and at the retention test ($P\leq 0.04$) as compared to the saline-treated controls (Fig. 3A-C).

The bilateral removal of bulbi olfactorii impaired the performance of OBX rats in the shuttle box. The SNK test showed significantly lower number of avoidances as compared to the sham- controls on the 1st training

day ($P\leq 0.0001$), the 2nd training day ($P\leq 0.0001$) and on the retention test ($P\leq 0.0001$) (Fig. 3A-C).

The *post hoc* test of the effects of the CB1 ligands on the number of avoidances showed that HU 210 ameliorated the memory deficits of OBX rats, demonstrated by an increased number of avoidances on the 1st day ($P\leq 0.02$); 2nd day $P\leq 0.005$ and the retention test ($P\leq 0.003$)

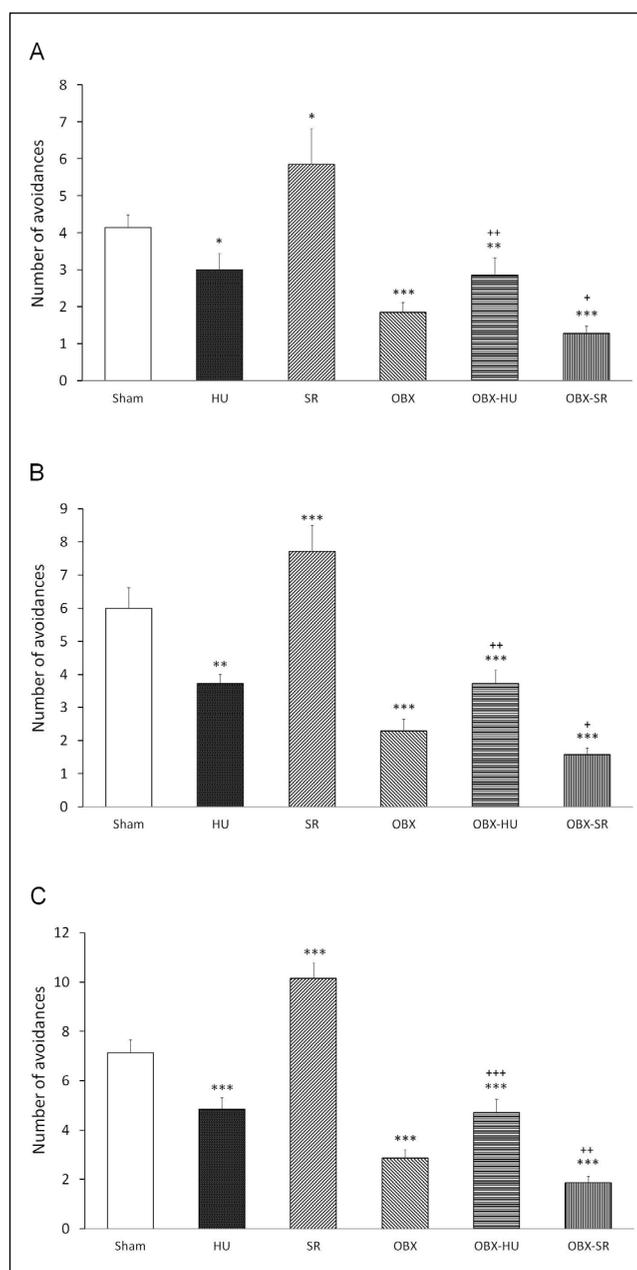


Fig. 3. Effects of HU 210 (5 μ g/1 μ l) and SR 141716A (3 μ g/1 μ l) administered i.c.v. for 7 days to OBX rats on the number of avoidances (shuttle box). (A) 1st training day; (B) 2nd training day; (C) retention test. n=7. Means (\pm S.E.M.) are presented. * $P\leq 0.05$; ** $P\leq 0.01$; *** $P\leq 0.001$ – comparison vs. sham controls; + $P\leq 0.05$; ** $P\leq 0.01$; *** $P\leq 0.001$ – significance vs. OBX rats *** $P\leq 0.001$.

as compared to the OBX-saline controls. However, the number of avoidances on the two training trials and at the retention test remained lower (respectively $P \leq 0.02$; $P \leq 0.005$, $P \leq 0.003$) as compared to the sham-operated rats (Fig. 3A-C).

SR 141716A revealed a memory deteriorating effect in OBX rats by worsening the memory deficits, induced by the bullectomy. The CB1 antagonist decreased the number of avoidances in the TWAA test on the 1st, 2nd training day and at the retention test as compared to both OBX saline-treated controls ($P \leq 0.05$; $P \leq 0.05$; $P \leq 0.01$) and sham-operated rats ($P \leq 0.0001$; $P \leq 0.0001$; $P \leq 0.0001$) (Fig. 3A-C).

Step-through test

Two-way repeated ANOVA on the latent time in the passive avoidance test showed a significant effects for factors treatment (sham-saline, sham-HU, sham-SR, OBX-saline, OBX-HU and OBX-SR) ($F_{5,83}=44.809$, $P \leq 0.0001$) and time (3rd and 24th hour) ($F_{1,83}=19.010$, $P \leq 0.001$) and no significant interaction between treatment and time ($F_{5,83}=1.102$, $P=NS$).

HU 210 microinjected in the sham-operated rats reduced the latent time at 3rd hour ($P \leq 0.01$) and 24th hour ($P \leq 0.006$) as compared to the sham-operated rats and decreased the percentage of rats, reaching the learning criteria at the retention tests – on 3rd hour ($\chi^2=2.800$, $P \leq 0.05$) and 24th hour ($\chi^2=4.667$, $P \leq 0.02$) (Fig. 4A-B; Table I). SR 141716A increased the latent time of the sham-operated rats on the 3rd hour only ($P \leq 0.05$), with no effect on the 24th hour ($P=NS$). As compared to the sham-saline rats, where 57% reached learning criteria on the 3rd hour), SR 141716A increased the percent-

age of rats to 71%, while on the 24th hour there was no significant difference (the percentage remained 71%, same as the one for the saline-treated controls) (Fig. 4A-B; Table I).

The bilateral removal of rat bulbi olfactorii produced a significant decrease in the latent time of OBX rats on 3rd h ($P \leq 0.0001$) and on 24th h after training ($P \leq 0.0001$) as compared to the sham-operated controls. The percentage of OBX rats, that did not reach the learning criteria diminished to 0% in both retention tests ($P \leq 0.001$) and was lower on the 3rd h ($\chi^2=4.000$; $P \leq 0.05$) and 24th h ($\chi^2=6.002$; $P \leq 0.02$) as compared to the sham controls (Fig. 4A-B; Table I).

The *post hoc* test of the effects of HU 210 revealed an increased latent time of the OBX rats on the 3rd hour ($P \leq 0.03$) and the 24th h ($P \leq 0.04$). The number of OBX rats reaching the learning criteria on 24th hour ($\chi^2=2.333$, $P \leq 0.05$) was higher as compared to the OBX-saline controls. HU 210-treated OBX rats had a decreased latent time on 3rd and 24th hour ($P \leq 0.005$ and $P \leq 0.01$ respectively); the number of rats reaching learning criteria also was lower on 3rd ($\chi^2=2.800$, $P=NS$) and 24th hour ($\chi^2=2.571$, $P \leq 0.05$) as compared to the sham-operated rats. (Fig. 4A-B; Table I).

SR 141716A decreased the latent time of OBX rats on 3rd hour ($P \leq 0.05$); on 24th h ($P \leq 0.01$) increased it, and did not affect the number of rats, reaching the learning criteria at the 3rd and 24th hour as compared to the OBX-saline controls. The comparison of the effects of SR 141716A in OBX rats to the sham-saline group showed a decrease of the latent time on 3rd hour ($P \leq 0.001$), 24th hour ($P \leq 0.001$) and a decrease of the number of rats, reaching learning criteria on 3rd ($\chi^2=5.600$, $P \leq 0.02$) and 24th hour ($\chi^2=7.778$, $P \leq 0.01$) (Fig. 4A-B; Table I).

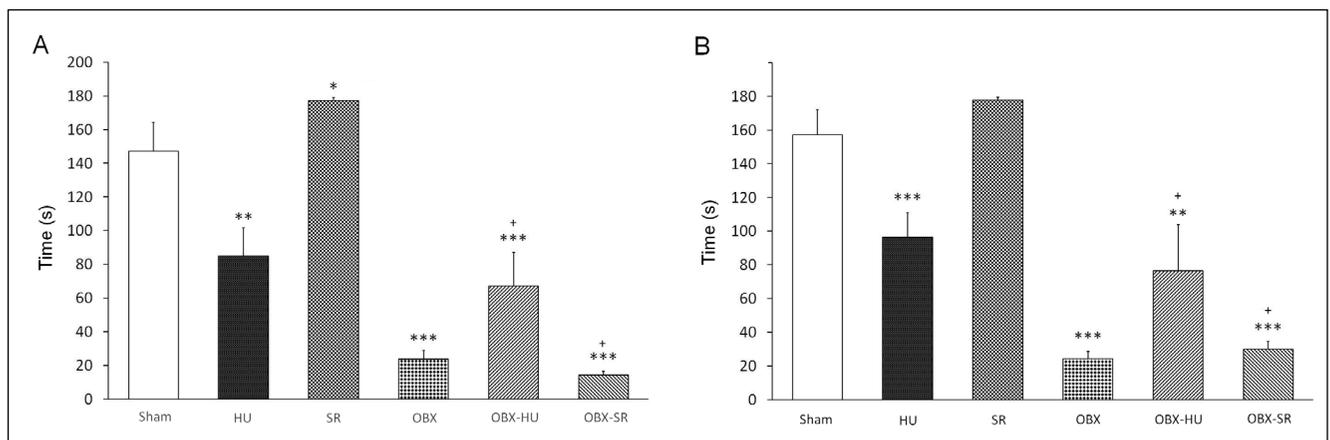


Fig. 4. Effects of HU 210 and SR 141716A administered i.c.v. for 7 days to OBX rats on the latent time (step through). (A) 3rd hour; (B) 24th hour. $n=7$. Means (\pm S.E.M.) are presented. * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$ – comparison vs. sham controls; + $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.01$ – significance vs. OBX rats *** $P \leq 0.001$.

Table I. Effects of HU 210 and SR 141716A administered i.c.v. for 7 days to OBX rats on retention tests (step through). * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$ significance vs. sham operated controls; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$ significance vs. OBX controls.

Groups	Retention test					
	3 h			24 h		
	Latent time X \pm S.E.M.	% rats	Number of rats reaching learning criteria	Latent time X \pm S.E.M.	% rats	Number of rats reaching learning criteria
Sham (n=7)	147.14 \pm 17.00	57	(4/7)	157.14 \pm 14.91	71	(5/7)
Sham + HU-210 (n=7)	85.00 \pm 16.69**	14	(1/7)*	96.43 \pm 14.5**	14	(1/7)*
Sham + SR 141716A (n=7)	177.57 \pm 2.04*	71	(5/7)	177.71 \pm 1.71	71	(5/7)
OBX (n=7)	23.86 \pm 5.17***	0	(0/7)***	24.29 \pm 1.76***	0	(0/7)***
OBX + HU 210 (n=7)	67.14 \pm 19.82***	14	(1/7)	76.43 \pm 27.47**	29	(2/7)*
OBX + SR 141716A (n=7)	14.29 \pm 1.13***	0	(0/7)*	30.00 \pm 1.35***	0	(0/7)**

DISCUSSION

The olfactory bulbectomy is a validated model of depression. Apart from being used primarily as a model for examining the effects of new antidepressant drugs, OBX is also considered a suitable model to study neurodegenerative disorders, such as Alzheimer's disease. OBX model can be used to evaluate the relationship between the abnormal neuronal signaling and the induced cognitive changes. The removal of olfactory bulbs in rodents induces behavioral changes including increased locomotor activity in novel environment, hyperemotionality, memory deficits, etc. (Leonard and Tuite, 1981, Kelly et al., 1997).

In the present study we aimed to investigate the role of CB1 receptors in avoidance learning and memory of rats with OBX model by using CB1 receptor agonist HU 210 and CB1 receptor antagonist SR 141716A. We revealed modulatory effects of the CB1 ligands, administered i.c.v. for 7 consecutive days to OBX rats with developed depressive-like state, on *active and passive* avoidance behavior. HU 210 improved partially the learning and memory deficits of OBX rats in both tasks, demonstrated by an increased number of avoidances (shuttle box), prolonged latent time and increased number of rats, reaching learning criteria (step through). However, the scores of the sham-operated controls had not been reached. The CB1 antagonist SR 141716A impaired the performance of OBX rats in the shuttle box test only, and showed inconsistent effects in the passive avoidance test. In the sham-operated group, HU 210 induced significant learning and memory disturbances, while the SR 141716A administration enhanced avoidance retention in both tasks.

The locomotor activity of rats was evaluated in an Opto Varimex apparatus. HU 210 normalized locomo-

tor hyperactivity of the OBX rats, while SR 141716A exacerbated the depressive-like symptoms by further increasing the locomotor activity. In the sham-operated rats the CB1 ligands showed the same modulatory effects: HU 210 inhibited locomotion, while SR 141716A increased it.

As far as drug- or lesion-induced alterations in locomotor activity are suggested to confound mostly passive avoidance response (Ögren and Stiedl, 2015), we used active and passive avoidance paradigms for evaluation of the effects of CB1 ligands. In the passive-avoidance task (step through) the performance depends on the suppression of the innate drive to enter a dark compartment and rats with decreased locomotor activity would stay longer time over the illuminated platform before moving to the dark compartment (extended latent time).

An interesting finding of our study was that the CB1 ligands produced opposite effects on acquisition and retention of memory in sham and OBX rats, while the locomotor effects remained unchanged in both groups. Thus, HU 210 inhibited locomotion (sham and OBX rats), deteriorated memory (sham) and ameliorated memory deficits (OBX), while SR 141716A increased locomotor activity (sham and OBX rats), enhanced memory (sham), and worsened performance in the active avoidance test (OBX).

The fact that CB1 ligands exerted unidirectional locomotor effects in both sham and OBX rats but oppositely affected the avoidance behavior of the two groups, allows for the assumption that the altered locomotor activity does not interfere significantly with the observed learning and memory effects.

The two avoidance tests, used by us, share similarities, concerning the involvement of major structures of the limbic system and some cortical and brainstem

areas. In addition, each of the two tasks is associated with specific brain areas. Lesion studies have shown that passive avoidance test in rodents requires an intact corticohippocampal circuit (Burwell et al., 2014), while active avoidance is a conditioned task which includes amygdala and requires intact amygdala projections to the ventral striatum (Ramirez et al., 2015). Passive avoidance is mostly fear-motivated, inhibitory conditioning, while active avoidance is an example of operant conditioning. The deficit in passive avoidance task is a consistent finding in OBX model (Sieck, 1972; Kelly et al., 1997), which has been interpreted as an abnormal defensive freezing capacity of the OBX rat (Primeaux and Holmes, 1999). However, the data about the rat's performance in the active avoidance task are contradictory, no significant changes, improved or impaired performance have been observed (Gomita et al., 1984; Archer et al., 1984, Grecksch et al., 1997; Tashev et al., 2010). Yamamoto et al. (1997) suggested that attention deficit is involved in the memory impairment of OBX rats tested in 3-lever operant task.

The effects of the CB1 agonist on the avoidance behavior are similar to the ones previously reported upon an acute i.c.v injection, where HU 210 improved the scores of the OBX rats in both avoidance tasks and impaired the performance of the sham group (Marinov et al., 2013). Unlike the acute treatment, where SR 141716A enhanced the avoidance learning of sham rats in TWAA only, the subchronic treatment enhanced memory of the sham group (both tasks) and showed a tendency to exacerbate memory deficits of the OBX rats in TWAA.

Data are contradictory regarding possible influence of the rat's anxiety-like behavior on the avoidance responses. Two strains of mice with different level of anxiety were tested in a PA paradigm and the C57BL/6J strain with lower anxiety showed longer retention latencies than the more anxious DBA/2J mice (Baarendse et al., 2008). In a previous study we demonstrated that acutely i.c.v. injected HU 210 alleviated the OBX-induced anxiety, while SR 141716A failed to produce significant effects (Marinov et al., 2019). Therefore, we cannot exclude the possibility that the anxiety-modulating effects of the CB1 ligands might affect the avoidance performance.

The abnormalities seen in the OBX rat are considered to be a result of neurodegenerative changes and disrupted neuronal connections between the olfactory bulbs and other brain regions. Olfactory bulbectomy causes neuronal degeneration and dysfunction in the projection areas to the cortex, hippocampus, amygdala, locus coeruleus (Kelly et al., 1997, Morales Medina et al., 2017). Abnormalities in many neurotransmitter systems in the brain have been reported such as nor-

adrenergic, serotonergic, cholinergic, glutamate, etc. (Song and Leonard, 2005).

Significant alterations of the ECS in several rat brain structures have been detected in the OBX model. The levels of endogenous cannabinoids were changed in prefrontal cortex, hippocampus, striatum and nucleus accumbens; the CB1 receptor expression was lower in the hippocampus, dorsal striatum, and nucleus accumbens and the CB2 receptor expression decreased in the prefrontal cortex and hippocampus (Smaga et al., 2017). Eisenstein et al. (2009) provided evidence for dysfunction of ECS in olfactory bulbectomized rats which may have an impact on the functional activity of other neurotransmitter systems. Hyperlocomotion is a major OBX-induced depressive-like behavioral symptom (van Riezen and Leonard, 1990), which has been attributed to the hyperdopaminergic transmission (Masini et al., 2004). Findings suggest that dysregulation in the ECS (inability to efficiently modulate brain dopaminergic neurotransmission) is implicated in the hyperactive locomotor response induced by OBX (Eisenstein et al., 2010).

Our results support the accumulating data about the antidepressant and memory improving effects due to enhanced activity of endocannabinoid system in different experimental animal models accompanied by deficits in cognitive processes (McLaughlin and Gobbi, 2012, Segev et al., 2014; Kruk-Slomka et al., 2015; Haj-Mirzaian et al., 2017). The effects of the subchronically i.c.v applied CB1 ligands in the PA task are in accordance with the reported involvement of CB1 receptors in memory-related processes in the inhibitory avoidance test in mice. Acutely injected oleamide, a CB1 receptor agonist, showed antidepressant-like effect of in the forced swim test, while AM 251, a CB1 receptor antagonist, did not provoke any effect in this test (Kruk-Slomka and Biala, 2016).

The neuroprotective properties of the cannabinoids in models of neurodegenerative disorders have been confirmed by numerous studies during last decade and may account for the improved performance in the avoidance tests upon subchronic treatment with HU 210 observed by us. The neuroprotective potential involves ECS and its numerous targets by activation of CB1 and CB2 receptors in different types of neurons and glial cells (Fernández-Ruiz et al., 2015, 2017).

Experimental data suggest that fear may be involved in the maintenance of avoidance, although fear and avoidance may not be always correlated (Rachman and Hodgson, 1974; Kryptos et al., 2015). Passive avoidance is a hippocampal dependent task, based on associative (emotional) learning similar to contextual fear conditioning (Ögren and Stiedl, 2010; Burwell et al., 2014). Regarding the effects of the CB1

ligands in the sham-operated rats, our findings are in line with the reports about the established role of hippocampal CB1 receptors in the memory disruptive effects of systemically administered cannabinoids. It was demonstrated that the injection of CB1/CB2 receptor agonist WIN55, 212-2 and the inhibitor of endocannabinoid reuptake and breakdown AM404 in the hippocampal CA1 area facilitated the extinction of fear related inhibitory avoidance, while the CB1 receptor antagonist AM251 impaired it (Abush and Akirav, 2010). Wise et al. (2009) reported that the intrahippocampal administration of rimonabant (CB1 receptor antagonist), prevented the memory disruptive effects of systemically administered Δ^9 -THC in the radial arm maze. In addition, we support the findings that manipulation of CB1 transmission affects classically conditioned fear (Chhatwal et al., 2005; Pamplona et al., 2008).

In our study we used SR 141716A, which is selective CB1 antagonist that shows properties of inverse agonist (Pertwee, 2005) that can exhibit agonist activity on constitutively active receptors. SR 141716A affected negatively the performance of OBX rats in the TWAA only, and improved learning and long-term memory of sham rats in both tasks. Active avoidance is a type of Pavlovian fear conditioning paradigm. The amygdala and its projection to the ventral striatum are essential for aversively motivated activity (Kapp et al., 1984; Ramirez et al., 2015). Discrete lesions of the amygdala produced deficits in active avoidance (Werka et al., 1978, Choi et al., 2010). It could be suggested that the effects of CB1 ligands on the acquisition and memory retention in TWAA are linked to the facilitated extinction of aversive memories through the selective inhibitory effects of endocannabinoids on local inhibitory networks in the amygdala (Marsicano et al., 2002). In this regard, CB1 inverse agonist, but not neutral antagonist has been shown to enhance the retention of contextual fear conditioning in rats (Sink et al., 2010).

Recently, a meta-analysis was conducted on the effects of CB1 receptor agonists, antagonists, and negative allosteric modulators on memory (Borgan et al., 2019). It revealed that CB1 receptor agonists when administered acutely, but not chronically, impair spatial and non-spatial memory and the effects were inversely associated with dose. No effects of acutely administered CB1R antagonists have been reported and there were no data about chronic administration of the drugs. Our data confirm the established detrimental effect of CB1 ligands on memory, and can contribute to the understanding how pharmacological manipulation of CB1 receptors influences cognitive performance.

Strengths and limitations

A strength of the study was that we examined the subchronic effects of CB1 receptor agonist and antagonist, on avoidance performance in rats with a OBX model. Data are lacking about the effects of subchronic and chronic administration of CB1 antagonists on non-spatial memory in rodents.

Our research has some limitations which have to be considered. We used a single dose of the drugs and it was applied prior to task. The experimentation with different drug doses, route of administration, or treatment protocols (i.e. injection of drugs after training) would give more light to the modulatory effects of the CB1 receptor ligands on learning and memory processes in depressive-like states. The comparison of the effects of CB1 antagonist/inverse agonist to a CB1 neutral antagonist may be beneficial for evaluation of the effects of CB1 receptor blockade. Studies with chronic administration of CB1 agonist are also needed, as it looks promising to bring full recovery of the memory disturbances in OBX rats.

CONCLUSION

Our study demonstrated that the subchronic i.c.v. administration of CB1 antagonist HU 210 attenuated the depressive-like state in an OBX rat model by normalizing the locomotor activity and partially improving the memory disturbances, induced by the olfactory bulbectomy. The CB1 antagonist SR 141716A modified the avoidance performance in OBX rats and showed a significant memory enhancing effect in the sham-operated rats. The results point to a possible involvement of CB1 receptors in the learning and memory deficits of OBX rats, tested in active and passive avoidance paradigms. Future studies would add to our understanding of the contribution of the CB1 receptors to the mechanisms of memory impairment in depression and Alzheimer's disease.

ACKNOWLEDGEMENTS

This study was supported by MU-Varna, Grant N° AD-008-1/06.04.2012.

REFERENCES

- Abush H, Akirav I (2010) Cannabinoids modulate hippocampal memory and plasticity. *Hippocampus* 20: 1126–1138.
- Aleksandrov IY, Kuvichkin VV, Kashparov IA, Medvinskaya NI, Nesterova IV, Lunin SM, Samokhin AN, Bobkova NV (2004) Increased level of β -amyloid in the brain of bulbectomized mice. *Biochemistry* 69: 176–180.

- Archer T, Söderberg U, Mohammed AK, Fredriksson A (1984) Active and passive avoidance performances following bilateral olfactory bulbectomy. *Scand J Psychol* 25: 179–188.
- Baarendse PJJ, van Groothedest G, Jansen RF, Pieneman AW, Ögren SO, Verhage M, et al., (2008) Differential involvement of the dorsal hippocampus in passive avoidance in C57BL/6J and DBA/2J mice. *Hippocampus* 18: 11–19.
- Borgan F, Beck K, Butler E, McCutcheon R, Veronese M, Vernon A, Howes OD (2019) The effects of cannabinoid 1 receptor compounds on memory: a meta-analysis and systematic review across species. *Psychopharmacology* 236: 3257–3270.
- Borre Y, Lemstra S, Westphal KG, Morgan ME, Olivier B, Oosting RS (2012) Celecoxib delays cognitive decline in an animal model of neurodegeneration. *Behav Brain Res* 234: 285–291.
- Buresova O, Bures J (1983) Techniques and basic experiments for the study of brain and behavior. Elsevier, Sci Publ, Amsterdam, New York.
- Burwell R, Sadoris M, Bucci D, Wiig K (2014) Corticohippocampal contributions to spatial and contextual learning. *J Neurosci* 24: 3826–3836.
- Chhatwal JP, Davis M, Maguschak KA, Ressler KJ (2005) Enhancing cannabinoid neurotransmission augments the extinction of conditioned fear. *Neuropsychopharmacology* 30: 516–524.
- Choi JS, Cain CK, LeDoux JE (2010) The role of amygdala nuclei in the expression of auditory signaled two-way active avoidance in rats. *Learn Mem* 17: 139–147.
- De Petrocellis L, Di Marzo V (2009) An introduction to the endocannabinoid system: from the early to the latest concepts. *Best Pract Res Clin Endocrinol Metab* 23: 1–15.
- Douma TN, Borre Y, Hendriksen H, Olivier B, Oosting RS (2011) Simvastatin improves learning and memory in control but not in olfactory bulbectomized rats. *Psychopharmacology* 216: 537–544.
- Eisenstein SA, Clapper JR, Holmes PV, Piomelli D, Hohmann AG (2010) A role for 2-arachidonoylglycerol and endocannabinoid signaling in the locomotor response to novelty induced by olfactory bulbectomy. *Pharmacol Res* 61: 419–429.
- Eisenstein SA, Holmes PV, Hohmann AG (2009) Endocannabinoid modulation of amphetamine sensitization is disrupted in a rodent model of lesion-induced dopamine dysregulation. *Synapse* 63: 941–950.
- Fernández-Ruiz J, Gómez-Ruiz M, García C, Hernández M, Ramos JA (2017) Modeling neurodegenerative disorders for developing cannabinoid-based neuroprotective therapies. *Methods Enzymol* 593: 175–198.
- Fernandez-Ruiz J, Moro MA, Martinez-Orgado J (2015) Cannabinoids in neurodegenerative disorders and stroke/brain trauma: from preclinical models to clinical applications. *Neurotherapeutics* 12: 793–806.
- Franks KH, Chuah MI, King AE, Vickers JC (2015) Connectivity of Pathology: The olfactory system as a model for network-driven mechanisms of Alzheimer's disease pathogenesis. *Front Aging Neurosci* 7: 234.
- Freund TF, Katona I, Piomelli D (2003) Role of endogenous cannabinoids in synaptic signaling. *Physiol Rev* 83: 1017–1066.
- Gomita Y, Ogawa N, Ueki S (1984) Effect of bilateral olfactory bulbectomy on discrimination avoidance conditioning in rats. *Physiol Behav* 32: 1011–1016.
- Gozzani I, Isquierdo I (1976) Possible peripheral adrenergic and central dopaminergic influences in memory consolidation. *Psychopharmacology* 49: 109–111.
- Grecksch G, Zhou D, Franke C, Schröder U, Sabel B, Becker A, Huether G (1997) Influence of olfactory bulbectomy and subsequent imipramine treatment on 5-hydroxytryptaminergic presynapses in the rat frontal cortex: behavioural correlates. *Br J Pharmacol* 122: 1725–1731.
- Haj-Mirzaian A, Amini-Khoei H, Haj-Mirzaian A, Amiri S, Ghesmati M, Zahir M, Shafaroodi H, Dehpour AR (2017) Activation of cannabinoid receptors elicits antidepressant-like effects in a mouse model of social isolation stress. *Brain Res Bull* 130: 200–210.
- Hill MN, Gorzalka BB (2009) Impairments in endocannabinoid signaling and depressive illness. *JAMA* 301: 1165–1166.
- Kapp BS, Pascoe JP, Bixler MA (1984) The amygdala: a neuroanatomical systems approach to its contributions to aversive conditioning. In: *Neuropsychology of Memory* (Buttlers N, Squire LR Eds). Guilford: New York, NY, USA, p. 473–488.
- Kelly JP, Wrynn AS, Leonard BE (1997) The olfactory bulbectomized rat as a model of depression: an update. *Pharmacol Ther* 74: 299–316.
- Kruk-Slomka M, Biala G (2016) CB1 receptors in the formation of the different phases of memory-related processes in the inhibitory avoidance test in mice. *Behav Brain Res* 301: 84–95.
- Kruk-Slomka M, Dzik A, Budzynska B, Biala G (2017) Endocannabinoid system: the direct and indirect involvement in the memory and learning processes – a short review. *Mol Neurobiol* 54: 8332–8347.
- Kruk-Slomka M, Michalak A, Biala G (2015) Antidepressant-like effects of the cannabinoid receptor ligands in the forced swimming test in mice: Mechanism of action and possible interactions with cholinergic system. *Behav Brain Res* 284: 24–36.
- Kryptos A, Eftting M, Kindt M, Beckers T (2015) Avoidance learning: a review of theoretical models and recent developments. *Front Behav Neurosci* 9: 189.
- Leonard BE, Tuite M (1981) Anatomical, physiological, and behavioral aspects of olfactory bulbectomy in the rat. *Int Rev Neurobiol* 22: 251–86.
- Mackie K (2005) Cannabinoids. In: *Handbook of Experimental Pharmacology* (Pertwee RG, Ed), Springer, Berlin, Heidelberg, p. 299–325.
- Marinov M, Ivanova M, Belcheva S, Belcheva I, Tashev R (2013) Effects of acutely applied cannabinoid CB1 ligands on learning and memory in rats with a model of depression. *CR Acad Bulg Sci* 66: 1331–1338.
- Marinov MD, Velikova MS, Tashev RE, Doncheva DK (2019) Modulatory effect of cannabinoid ligands on the anxiety-like behavior of bulbectomized rats. *J IMAB. Ann Proceeding Scientific Papers* 25: 2544–2548.
- Marsicano G, Wotjak CT, Azad SC, Bisogno T, Rammes G, Cascio MG, Hermann H, Tang J, Hofmann C, Zieglerberger W, Di Marzo V, Lutz B (2002) The endogenous cannabinoid system controls extinction of aversive memories. *Nature* 418: 530–534.
- Masini CV, Holmes PV, Freeman KG, Maki AC, Edwards GL (2004) Dopamine overflow is increased in olfactory bulbectomized rats: An *in vivo* microdialysis study. *Physiol Behav* 81: 111–119.
- McLaughlin RJ, Gobbi G (2012) Cannabinoids and emotionality: a neuro-anatomical perspective. *Neuroscience* 204: 134–144.
- Morales-Medina JC, Iannitti T, Freeman A, Caldwell HK (2017) The olfactory bulbectomized rat as a model of depression: The hippocampal pathway. *Behav Brain Res* 317: 562–575.
- Ögren SO, Stiedl O (2010) Passive avoidance. In: *Encyclopedia of Psychopharmacology* (Stolerman IP, Ed), Berlin: Springer p. 960–967.
- Ögren SO, Stiedl O (2015) Passive avoidance. *Encyclopedia of Psychopharmacology* (Stolerman IP, Lawrence HP Eds). Berlin: Springer p. 1221–1222.
- Pamplona FA, Bitencourt RM, Takahashi RN (2008) Short- and long-term effects of cannabinoids on the extinction of contextual fear memory in rats. *Neurobiol Learn Mem* 90: 290–293.
- Pellegrino LJ, Cushman AJ (1967) A stereotaxic atlas of the rat brain. New York: Appleton.
- Pertwee RG (2005) Inverse agonism and neutral antagonism at cannabinoid CB1 receptors. *Life Sci* 76: 1307–1324.
- Petkov VD, Kehayov R, Belcheva S, Konstantinova E, Petkov VV, Getova D, Markovska V (1993) Memory effects of standardized extracts of Panax ginseng (G115), Ginkgo biloba (GK501) and their combination Gincosan (PHL-00701). *Planta Med* 59: 106–114.
- Primeaux SD, Holmes PV (1999) Role of aversively motivated behavior in the olfactory bulbectomy syndrome. *Physiol Behav* 67: 41–47.
- Rachman S, Hodgson RI (1974) Synchrony and desynchrony in fear and avoidance. *Behav Res Ther* 12: 311–318.
- Ramirez F, Moscarello J, Ledoux J, Sears RM (2015) Active avoidance requires a serial basal amygdala to nucleus accumbens shell circuit. *J Neurosci* 35: 3470–3477.

- Ranieri R, Laezza C, Bifulco M, Marasco D, Malfitano AM (2016) Endocannabinoid system in neurological disorders. *Recent Pat CNS Drug Discov* 10: 90–112.
- Ruehle S, Rey AA, Remmers F, Lutz B (2012) The endocannabinoid system in anxiety, fear memory and habituation. *J Psychopharmacol* 26: 23–39.
- Segev A, Rubin AS, Abush H, Richter-Levin G, Akirav I (2014) Cannabinoid receptor activation prevents the effects of chronic mild stress on emotional learning and LTP in a rat model of depression. *Neuropsychopharmacology* 39: 919–933.
- Sieck MH (1972) The role of the olfactory system in avoidance learning and activity. *Physiol Behav* 8: 705–710.
- Sink KS, Segovia KN, Collins LE, Markus EJ, Vemuri VK, Makriyannis A, Salamone JD (2010) The CB1 inverse agonist AM251, but not the CB1 antagonist AM4113, enhances retention of contextual fear conditioning in rats. *Pharmacol Biochem Behav* 95: 479–484.
- Smaga I, Jastrzębska J, Zaniewska M, Bystrowska B, Gawliński D, Faron-Górecka A, Broniowska Ż, Miszkiewicz J, Filip M (2017) changes in the brain endocannabinoid system in rat models of depression. *Neurotox Res* 31: 421–435.
- Song C, Leonard B (2005) The olfactory bulbectomized rat as a model of depression. *Neurosci Biobehav Rev* 29: 627–647.
- Tashev R, Ivanova M, Toromanov T, Marinov M, Belcheva S, Belcheva I (2010) Olfactory bulbectomy impairs active and passive avoidance learning in rats. *CR Acad Bulg Sci* 63: 617–622.
- van Riesen H, Leonard BE (1990) Effects of psychotropic drugs on the behavior and neurochemistry of olfactory bulbectomized rats. *Pharmacol Ther* 47: 21–34.
- Werka T, Skår J, Ursin H (1978) Exploration and avoidance in rats with lesions in amygdala and piriform cortex. *J Comp Physiol Psychol* 92: 672–681.
- Wise LE, Thorpe AJ, Lichtman AH (2009) Hippocampal CB1 receptors mediate the memory impairing effects of Δ^9 -tetrahydrocannabinol. *Neuropsychopharmacology* 34: 2072–2080.
- Yamamoto T, Jin J, Watanabe S (1997) Characteristics of memory dysfunction in olfactory bulbectomized rats and the effects of cholinergic drugs. *Behav Brain Res* 83: 57–62.
- Yehuda S, Rabinovitz S (2013) Olfactory bulbectomy as a putative model for Alzheimer's: the protective role of essential fatty acids. *Pharma Nutrition* 2: 12–18.
- Zanettini C, Panlilio LV, Alicki M., Goldberg SR, Haller J, Yasar S (2011) Effects of endocannabinoid system modulation on cognitive and emotional behavior. *Front Behav Neurosci* 5: 57.