Cent. Eur. J. Med. • 8(4) • 2013 • 443-449
DOI: 10.2478/s11536-013-0165-6

VERSITA

Central European Journal of **Medicine**

The effects of a 6-OHDA induced lesion in murine nuccleus accumbens on memory and oxidative stress status

Research Article

Radu Lefter[1], Alin Ciobica[1,2]*, Lucian Hritcu[1], Bogdan Stoica[3], Dumitru Cojocaru[1], Zenovia Olteanu[1]

1 "Alexandru Ioan Cuza" University, Bd. Carol I, nr. 11, Iasi, 700506, Romania

2 Center of Biomedical Research of the Romanian Academy, Iasi Branch, Romania

3 "Gr. T. Popa" University of Medicine and Pharmacy, 16 Universitatii Street, 700115, Iasi, Romania

Received 21 August 2012; Accepted 15 January 2013

Abstract: Nucleus accumbens (NAcc) are a collection of neurons that form the main part of the ventral striatum, which is a significant dopaminergic structure. Also, NAcc is thought to play an important role in reward, pleasure, laughter, addiction, aggression, fear, and the placebo effect. In the present work we were interested in studying the effects of a 6-OHDA induced lesion in the nucleus accumbens (NAcc), which is known as an important dopaminergic structure, on a specific behavioral task that involves both short term and long term spatial memory (the radial-8-arm-maze task), as well as on the oxidative stress markers (two antioxidant enzymes: superoxide dismutase-SOD and glutathione peroxidase-GPX and a lipid peroxidation marker: malondialdehyde-MDA, as well as the total antioxidant status-TAS) from the temporal lobe, which is considered to be the most vulnerable cortical area to oxygen levels fluctuations and hypoxia. Our results showed some significant effects of this lesion on the reference memory errors and time necessary to finish the test in the radial-8-arm-maze task. Additionally, increased oxidative stress status was demonstrated in the temporal lobe of the lesioned rats, as demonstrated by the high levels of lipid peroxidation and decreased total antioxidant status. Moreover, significant correlations are reported here between the behavioral parameters which we studied in the radial-8-arm-maze task and the aforementioned oxidative stress markers.

Keywords: *Nucleus accumbens • Spatial memory • adial-8-arm-maze • Oxidative stress*

1. Introduction

The nucleus accumbens (NAcc) is an important dopaminergic structure, which besides its implications in the control of locomotor activity [1] has been also reported to exert various effects on learning and memory processes, as studies through various behavioral tasks [2-5].

In this way, it is believed that it is involved mainly in the types of behavior which are associated with the reward and reinforcement processes, and especially those studied in various spatial behavioral task and environments [6]. These also include the appetitive motivation produced by food [1].

The aforementioned aspects are of course connected with the well known actions of the dopaminergic transmission in the reinforcement processes. However, the roles of NAcc in the subtle and complex aspects of behavioral functions associated with the motivational stimuli are not completely understood to this date, considering that many conflicting reports were published in this area of research, stating both negative [1,7,8] or no effects at all [3,6,9] for the NAcc in the mediation of the aforementioned behaviours.

Also, while it was previously demonstrated that the administration of a very well known dopaminergic toxin, 6–OHDA, into the NAcc results in a 60% - 80% decrease DA content of this area [2], in the present study we were interested to see which were the effects of this lesion on a specific behavioral task that involves both spatial memory, as well as food reward: the radial-8-arm-maze task.

* *E-mail: alin.ciobica@uaic.ro*

Additionally, considering the previous experience of our group in studying the relevance of oxidative stress in various neuropsychiatric disorders [10-13], we were also interested to see the effects of this lesion on the oxidative stress status from the temporal lobe, which is considered to be the most vulnerable cortical area to the modifications of the oxidative stress [14], as well as the possible connections that might exists between the main oxidative stress markers we determined in here (two antioxidant enzymes: superoxide dismutase-SOD and glutathione peroxidase-GPX and a lipid peroxidation marker: malondialdehyde-MDA, as well as the total antioxidant status-TAS) and the behavioral parameters which we studied in the radial-8-arm-maze task.

2. Materials and methods

2.1 Animals

12 male Wistar rats weighing 200-250 g and about 20 ±1 weeks of age at the start of the experiment were used. The animals were housed in a temperature and light-controlled room (22°C, a 12-h cycle starting at 08:00 h) and were fed and allowed to drink water ad libitum. Rats were treated in accordance with the guidelines of animal bioethics from the Act on Animal Experimentation and Animal Health and Welfare Act from Romania and all procedures were in compliance with the European Council Directive of 24 November 1986 (86/609/EEC). Also efforts were made to minimize animal suffering and to reduce the number of animals used.

2.2 Neurosurgery

All surgical procedures were conducted under aseptic conditions, under sodium pentobarbital (45 mg/kg b.w., i.p., Sigma) anesthesia. Rats were mounted in the stereotaxic 11° below horizontal zero plane.

Specific right-unilateral lesions of the dopaminergic neurons located in the NAcc were produced with 6-OHDA (SIGMA). Eight micrograms (free base) 6-OHDA, dissolved in 4 µl physiological saline containing 0.1% ascorbic acid was administrated through a Hamilton syringe over 4.50 minutes. The syringe was left in place for 5 minutes after injection before being slowly removed.

The sham-operated rats were injected with saline. The following coordinates were used: 3.5 mm posterior to bregma; 2.0 mm lateral to the midline; 5.3 mm ventral to the surface of the cortex.

The radial-8-arm-maze training was performed 2 weeks after the operations.

2.3 Radial 8 arm-maze task

The radial 8 arm-maze used in the present study consisted of 8 arms, numbered from 1 to 8 (48 x 12 cm), extending radially from a central area (32 cm in diameter). The apparatus was placed 40 cm above the floor, and surrounded by various extramaze visual cues placed at the same position during the study. At the end of each arm, there was a food cup that had a single 50 mg food pellet. Prior to the performance of the maze task, the animals were kept on restricted diet and body weight was maintained at 85% of their free-feeding weight over a week period, with water being available ad libitum.

Before the actual training began, three or four rats were simultaneously pre-trained in the radial maze and allowed to explore for 5 minutes and take food freely. The food was initially available throughout the maze, but was gradually restricted to the food cup. The animals were pre-trained for 3 days to run to the end of the arms and consume the baits. The pre-training trial continued until all the baits had been consumed or until 5 minutes had elapsed. After pre-training, the neurosurgery was performed as previously described.

Also, after 2 weeks of post-operative recovery, all rats were trained with 1 trial per day, for 7 days. Briefly, each animal was placed individually in the center of the maze and subjected to working and reference memory tasks, in which same 5 arms (no. 1, 2, 4, 5 and 7), were baited for each daily training trial. The other 3 arms (no. 3, 6 and 8) were never baited. An arm entry was counted when all four limbs of the rat were within an arm. Measures were made of the number of working memory errors (entering an arm containing food, but previously entered) and reference memory errors (entering an arm that was not baited). Also, the time taken to consume all five baits was also recorded. Reference memory is regarded as a long-term memory for information that remains constant over repeated trials (memory for the positions of baited arms), whereas working memory is considered a short time memory in which the information to be remembered changes in every trial (memory for the positions of arms that had already been visited in each trial) [13].

2.4 Tissue collection

After the behavioral tests, all rats were anesthetized, rapidly decapitated, and the whole brain was removed. The temporal lobes were collected. Each of temporal tissue samples were weighed and homogenized with a Potter Homogenizer coupled with Cole-Parmer Servodyne Mixer in bidistilled water (1 g tissue/10ml bidistilled water). Samples were centrifuged 15 min at 3000 rpm. Following centrifugation, the supernatant was separated and pipetted into tubes.

2.5 Biochemical estimations

2.5.1 Determination of superoxide dismutase

Superoxide dismutase (SOD) activity was measured by the percentage reaction inhibition rate of enzyme with WST-1 substrate (a water soluble tetrazolium dye) and xanthine oxidase using a SOD Assay Kit (Fluka, product number: 19160) according to the manufacturer's instructions. Each endpoint assay was monitored by absorbance at 450 nm (the absorbance wavelength for the colored product of WST-1 reaction with superoxide) after 20 min of reaction time at 37°C. The percent inhibition was normalized by mg protein and presented as SOD activity units.

2.5.2 Determination of glutathione peroxidase

Glutathione peroxidase (GPX) activity was measured using the GPX cellular activity assay kit CGP-1 (Sigma Chemicals). This kit uses an indirect method, based on the oxidation of glutathione (GSH) to oxidized glutathione (GSSG) catalyzed by GPX, which is then coupled with recycling GSSG back to GSH utilizing glutathione reductase (GR) and NADPH. The decrease in NADPH at 340 nm during oxidation of NADPH to NADP is indicative of GPX activity.

2.5.3 Determination of malondialdehyde

Malondialdehyde (MDA) levels were determined by thiobarbituric acid reactive substances (TBARs) assay. 200 µL of temporal lobe homogenate (supernatant) was added and briefly mixed with 1 ml of trichloroacetic acid at 50%, 0.9 ml of TRIS-HCl (pH 7.4) and 1 ml of thiobarbituric acid 0.73%. After vortex mixing, samples were maintained at 100ºC for 20 minutes. Afterwards, samples were centrifuged at 3000 rpm for 10 min and supernatant read at 532 nm. The signal was read against an MDA standard curve, and the results were expressed as nmol/mg protein [10].

Total protein was measured using Bradford dye-binding method, with bovine serum albumin as standard.

2.6 Total antioxidant status

Total antioxidant status (TAS) was assayed with a chemiluminometric method (slightly adapted after Whitehead et al.) with luminol – horseradish peroxidase system (Berthold Lumat 9507 chemiluminometer). In this method, constant light emission results from luminol degradation in the presence of a catalyst (horseradish peroxidase) with an enhancer (p-iodo-phenol) and is kinetically recorded. When a biological fluid is introduced in this system, the level of light emission decreases for a period of time. This is proportional with the total antioxidant capacity. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), a water-soluble alpha-tocopherol analogue, was used as the standard. Calibration was made with Trolox (hydro-soluble vitamin E) and final results are related to Trolox equivalents. The prooxidant system which generate light, was calibrated to five millions relative units of light (RLU) and serum samples was used at a dilution of 1/10.

2.7 Histological control

At the end of the experiment, all rats were killed with an overdose of sodium pentobarbital (100 mg/kg b.w., i.p., SIGMA) followed by a transcardial infusion of 0.9% saline and a 10% formalin solution. The brains were removed and placed in a 30% sucrose/formalin solution. Then they were cut into coronal sections and stained with cresyl violet for verification of the point of the syringe needle. Only experimental data from lesions correctly located in the NAcc were used for statistical analysis.

2.8 Data analysis

The animal's behavior in the radial task was statistically analyzed using one-way analysis of variance (one-way ANOVA). The results are expressed as mean ± SEM. F alues for which $P<0.05$ were regarded as statistically significant. Pearson's correlation coefficient was used to evaluate the connection between the behavioral parameters in radial-8-arm-maze task and the central oxidative stress markers.

3. Results

During the pre-training procedure the data collection showed that rats required the maximum amount of time (300 seconds) in order to eat all the baits.

Regarding the results of the actual behavioral training in the radial-8-arm-maze, we report here some significant effects of the NAcc lesion on the long term memory, as showed by the decrease in the number of the reference memory errors ($F(1,10)=18$, $p= 0.001$), as compared to sham-operated group (Figure 1).

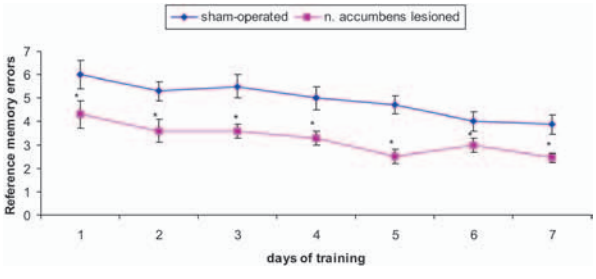

Figure 1. The number of reference memory errors in the rats with 6-OHDA-induced lesion of the NAcc, as studied in the radial-arm-maze task. The values are mean ± SEM (n=6 animals per group). *p<0.05 vs. sham-operated group.

Still, no significant effects (F(1,10)=0.35, p= 0.56) were observed in the case of the working memory errors, between the rats with the NAcc lesion and the sham-operated (Figure 2).

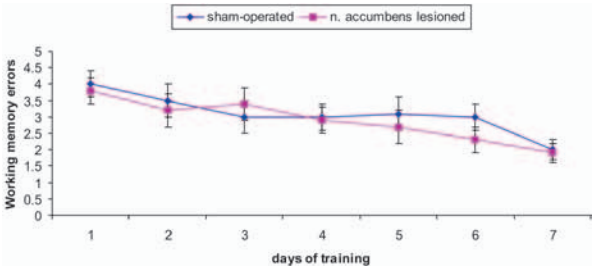

Figure 2. The number of working memory errors in the rats with 6-OHDA-induced lesion of the NAcc, as studied in the radial-arm-maze task. The values are mean ± SEM (n=6 animals per group).

On the other side, when it comes to the time necessary for the finalization of the radial task, we noticed that the rats with the NAcc–induced lesion reached almost all the time the maximum 5 minutes (300 sec) barrier for this test and of course, showed significant differences (F(1,10)=8, p= 0.01) when compared to sham-operated group (Figure 3).

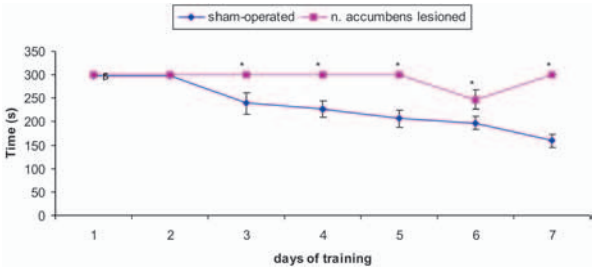

Figure 3. The time taken to consume all five baits in the rats with 6-OHDA-induced lesion of the NAcc, as studied in the radial-arm-maze task. The values are mean ± SEM (n=6 animals per group). *p<0.05 vs. sham-operated group.

Regarding the levels of oxidative stress, we report a significant increase of the lipid peroxidation processes in the NAcc lesioned rats, as demonstrated by the significant increase of the MDA levels from the temporal lobe (F(1,10)=9, p= 0.01), when compared to sham-operated rats (Figure 4).

However, no significant changes were observed in the specific activities of both antioxidant enzymes determined, SOD (F(1,10)=0.66, p= 0.4) and GPX (F(1,10)=0.68, p= 0.42), between the sham-operated rats and those with the 6-OHDA induced lesion of the NAcc (Figure 5 and 6).

Still, the increased oxidative stress status as a result of the 6-OHDA administration into NAcc was also confirmed by the significant decrease (F(1,10)=5, p= 0.04)

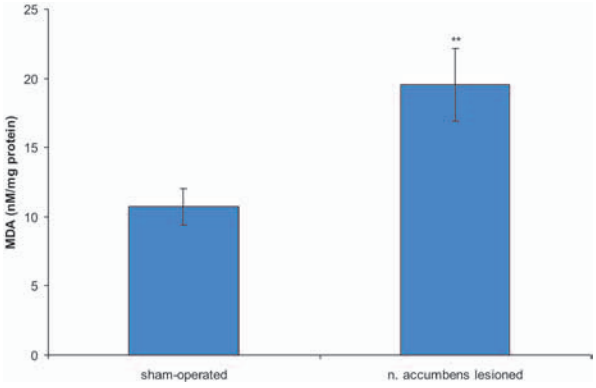

Figure 4. Effects of 6-OHDA-induced lesion of the NAcc on MDA level from the temporal lobe. The values are mean ± SEM (n=6 animals per group). **p=0.01 vs. sham-operated group.

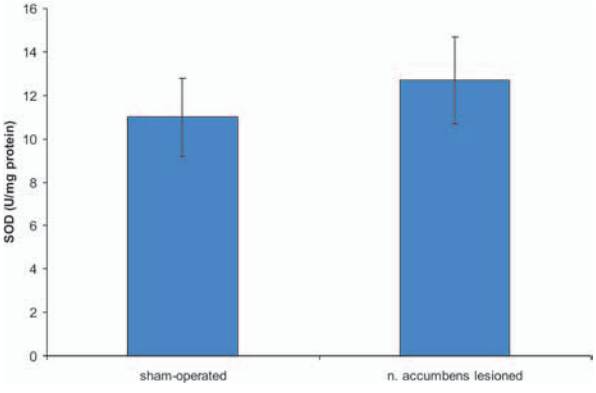

Figure 5. Effects of 6-OHDA-induced lesion of the NAcc on SOD specific activity from the temporal lobe. The values are mean ± SEM (n=6 animals per group).

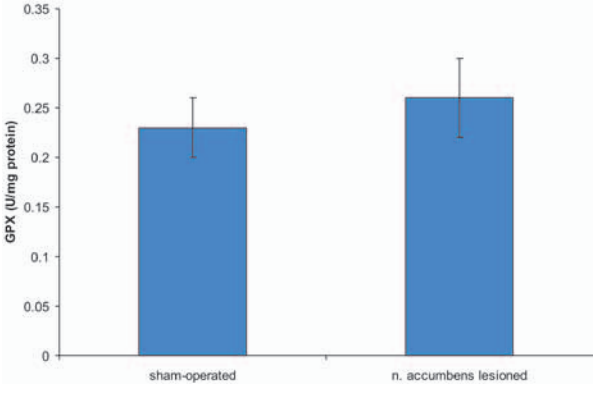

Figure 6. Effects of 6-OHDA-induced lesion of the NAcc on GPX specific activity from the temporal lobe. The values are mean ± SEM (n=6 animals per group).

of the chemiluminometric determined TAS, when compared to sham-operated group of rats (Figure 7).

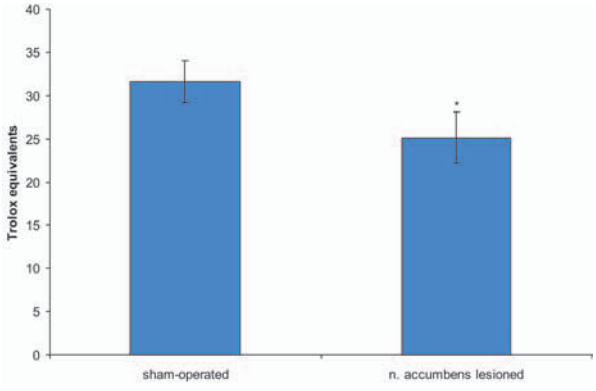

Figure 7. Effects of 6-OHDA-induced lesion of the NAcc on TAS levels from the temporal lobe. The values are mean ± SEM (n=6 animals per group). *p=0.04 vs. sham-operated group.

Additionally, the present study demonstrated some significant correlations between the behavioral parameters we determined in the radial-8-arm-maze task and the oxidative stress markers from the temporal lobe, as follows: time necessary to finish the radial vs. SOD (n=12, r=0.620, p=0.031), time necessary to finish the radial vs. MDA (n=12, r=0.580, p=0.047), number of reference memory errors vs. GPX (n=12, r=-0.595, p=0.041) and number of reference memory errors vs. MDA (n=12, r=-0.653, p=0.021).

4. Discussion

In the present study we confirmed the importance of NAcc in the mediation of spatial behaviour connected with the appetitive motivation produced by food, through the 6-OHDA-induced lesion of the accumbal dopaminergic transmission. Additionally, we demonstrated here an induced oxidative stress as a result of the NAcc lesion, as well as a possible connection between the behavioral parameters which we studied in the radial-8-arm-maze task and the main oxidative stress markers from the temporal lobe, the most vulnerable cortical area to oxidative stress [14].

As mentioned, previous studies regarding the behavioral effects of NAcc-induced lesion showed contradictory results. In this way, behavioral deficits were reported in tasks as latent inhibition, acquisition of discriminated approach, conditioned reward or response allocation in cost/benefit procedure [1,7,8,15-17]. Additionally, recent reports state that the unilateral lesions of the NAcc are sufficient in order to generate memory deficits [4,5]. Also, it was demonstrated that the lesion of NAcc results in an obvious impairment of the discriminated Pavlovian approach [2].

On the other side, there are authors reporting that a 6-OHDA lesions of dopaminergic terminals in the NAcc does not results in any significant impairment of acquisition or working memory, as tested in the Morris water maze task [9]. Similar reports regarding the lack of any behavioral effects in the case of NAcc lesion were also stated by other research groups [6]. Also, the normal acquisition and delayed long-term extinction of fear conditioning did not suffer any modifications after a 6-OHDA induced lesion of the NAcc [3].

The aforementioned contrasting reports could be perhaps explained by the usage of different behavioral techniques, different number and frequency of training sessions, type of memory evaluated, as well as the animal strains used. In this way, Grigoryan et al., stated even from 1996 that water maze could not be the most appropriate behavioral task in order to study the effects of a NAcc lesion on memory processes [9]. As mentioned, in the present experiment we decided to use the radial-8-arm-maze, a behavioral tasks which involves both spatial memory, as well as food reward.

Our results showed a significant decrease on the number of reference memory errors, as compared to sham-operated rats, while the number of working memory errors did not suffer any significant changes. On the other side, the time in which the animals finished the test was significantly increased in the NAcc-lesioned animals when compared to sham-operated.

To our knowledge this is one of the first times when the effects of a 6-OHDA-induced lesion in NAcc were tested through the radial-8-arm-maze task. There is a previous work of Schacter et al. in 1989, but the mentioned authors only studied the effects of glutamatergic innervations blockade from the hippocampus, medial prefrontal cortex and amygdala to the NAcc in the radial arm maze, which were expressed through impairments in reference memory, but not working memory errors [18].

Actually, it has been suggested that NAcc could be a mediator between limbic areas involved in motivation/behavioral activation and central areas controlling the motor activities. Additionally, it was demonstrated that besides the aforementioned interactions between NAcc and the glutamatergic transmission [18], some other neurotransmissions, such as the one connected to brain angiotensins [12] could influence its activity, considering that the dopaminergic transmission of the NAcc could mediate the memory-enhancing effect of angiotensins in rats [19].

Regarding the effects of the 6-OHDA-induced NAcc lesion on oxidative stress status, there are also very few studies. In this way, we only found a previous re-

port studying the effects of a 6-OHDA-induced lesion in the nigro-striatal pathway on the levels of alfasynuclein mRNA in the nucleus accumbens [20].

Concerning our results, we showed here a significant increase in the concentration of MDA, as a lipid peroxidation marker, in the temporal lobe, after the NAcc lesion, suggesting an increase in the oxidative stress status. However, no significant changes were observed in the case of the specific activity for both antioxidant enzymes determined: SOD and GPX. On the other side, the increased oxidative stress status was confirmed by the significant decrease of TAS in the rats with the 6-OHDA induced lesion of the NAcc.

In this way, it is possible that the lack of modification we observed for the both antioxidant enzymes we determined here, to be caused by a compensatory mechanism, as a direct result of the increased oxidative stress status.

The increased levels of oxidative stress are also sustained by the various studies, including some of our group [13], which previously demonstrated that the central administration of 6-OHDA results in increased oxidative stress levels, mainly caused through its oxidation by molecular oxygen and/or monoamine oxidase and resulting in the generation of H_2O_2 (while this could be further transformed into more toxic hydroxyl radicals).

In conclusion our study demonstrates that a 6-OHDA-induced lesion of the NAcc results in significant modifications for most of the behavioral parameters determined in the radial-8-arm-maze task, as well as in increased oxidative stress status in the temporal lobe. Also, a possible connection between the behavioral parameters and the main oxidative stress markers was found.

References

[1] Cousins MS, Salamone JD. Nucleus accumbens dopamine depletions in rats affect relative response allocation in a novel cost/benefit procedure. Pharmacol Biochem Behav 1994; 49: 85-91

[2] Parkinson JA, Dalley JW, Cardinal RN, Bamford A, Fehnert B, Lachenal G, Rudarakanchana N, Halkerston KM, Robbins TW, Everitt BJ. Nucleus accumbens dopamine depletion impairs both acquisition and performance of appetitive Pavlovian approach behaviour: implications for mesoaccumbens dopamine function. Behav Brain Res 2002; 137: 149-163

[3] Fernandez Espejo E. Prefrontocortical dopamine loss in rats delays long-term extinction of contextual conditioned fear, and reduces social interaction without affecting short-term social interaction memory. Neuropsychopharmacology 2003; 28: 490-498

[4] Schildein S, Huston JP, Schwarting RK. Open field habituation learning is improved by nicotine and attenuated by mecamylamine administered posttrial into the nucleus accumbens. Neurobiol Learn Mem 2002; 77: 277-290

[5] Schildein S, Huston JP, Schwarting RK. Injections of tacrine and scopolamine into the nucleus accumbens: opposing effects of immediate vs delayed posttrial treatment on memory of an open field. Neurobiol Learn Mem 2000; 73: 21-30

[6] Hagan JJ, Alpert JE, Morris RG, Iversen SD. The effects of central catecholamine depletions on spatial learning in rats. Behav Brain Res 1983; 9: 83-104

[7] Dalley JW, Chudasama Y, Theobald DE, Pettifer CL, Fletcher CM, Robbins TW. Nucleus accumbens dopamine and discriminated approach learning: interactive effects of 6-hydroxydopamine lesions and systemic apomorphine administration. Psychopharmacology (Berl) 2002; 161: 425-433

[8] Wolterink G, Phillips G, Cador M, Donselaar-Wolterink I, Robbins TW, Everitt BJ. Relative roles of ventral striatal D1 and D2 dopamine receptors in responding with conditioned reinforcement. Psychopharmacology (Berl) 1993; 110: 355-364

[9] Grigoryan G, Hodges H, Mitchell S, Sinden JD, Gray JA. 6-OHDA lesions of the nucleus accumbens accentuate memory deficits in animals with lesions to the forebrain cholinergic projection system: effects of nicotine administration on learning and memory in the water maze. Neurobiol Learn Mem 1996; 65: 135-153

[10] Stefanescu C, Ciobica A. The relevance of oxidative stress status in first episode and recurrent depression. J Affect Disord 2012, doi 10.1016/j.jad.2012.05.022

[11] Ciobica A, Padurariu M, Dobrin I, Stefanescu C, Dobrin R. Oxidative stress in schizophrenia - focusing on the main markers. Psychiatr Danub 2011; 23: 237-245

[12] Ciobica A, Veronica Bild, Lucian Hritcu, Manuela Padurariu, Walther Bild. Effects of angiotensin II receptor antagonists on anxiety and some oxidative stress markers in rat. Central European Journal of Medicine 2011; 6: 331-340

[13] Ciobica A, Olteanu Z, Padurariu M, Hritcu L. The effects of pergolide on memory and oxidative stress in a rat model of Parkinson's disease. J Physiol Biochem 2012; 68: 59-69

[14] Karelson E, Bogdanovic N, Garlind A, Winblad B, Zilmer K, Kullisaar T, Vihalemm T, Kairane C, Zilmer M. The cerebrocortical areas in normal brain aging and in Alzheimer's disease: noticeable differences in the lipid peroxidation level and in antioxidant defense. Neurochem Res 2001; 26: 353–361

[15] Reading PJ, Dunnett SB. The effects of excitotoxic lesions of the nucleus accumbens on a matching to position task. Behav Brain Res 1991; 46: 17-29

[16] Miller R, Wickens JR, Beninger RJ. Dopamine D-1 and D-2 receptors in relation to reward and performance: a case for the D-1 receptor as a primary site of therapeutic action of neuroleptic drugs. Prog Neurobiol 1990; 34:143–183

[17] Weiner I. Shofel A, Feldon J. Disruption of latent inhibition by low dose of amphetamine is antagonized by haloperidol and apomorphine. Journal of Psychopharmacology 1990; 4: 255

[18] Schacter GB., Yang CR., Innis NK., Mogenson GJ. The role of the hippocampal–nucleus accumbens pathway in radial-arm maze performance. Brain Research 1989; 494: 339–349

[19] Winnicka MM. Dopaminergic projection to the nucleus accumbens mediates the memory-enhancing effect of angiotensins in rats. Pharmacol Biochem Behav 1999;62: 625-630

[20] Zeng BY, Dass B, Owen A, Rose S, Cannizzaro C, Tel BC, Jenner P. 6-Hydroxydopamine lesioning differentially affects alpha-synuclein mRNA expression in the nucleus accumbens, striatum and substantia nigra of adult rats. Neurosci Lett. 2002; 322: 33-36