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Involvement of D_1/D_2 dopamine antagonists upon open-arms exploratory behaviours induced by intra-nucleus accumbens shell administration of *N*-methyl-*D*-aspartate

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Abstract

Glutamatergic system stimulation in some parts of the brain may affect anxiety-related behaviours, aversive learning and memory. This system retains many interactions with dopaminergic neurotransmission. We have studied the effect of nucleus accumbens (NAc) shell glutamatergic system activation on anxiety-related behaviours as well as aversive learning and memory in adult male Wistar rats using the N-methyl-D-aspartate (NMDA) receptor agonist, NMDA. Furthermore, the possible involvement of the NAc shell dopamine D_1 and D_2 receptors upon NMDA-induced effects was evaluated. The elevated plus-maze task was used to assess the drugs' concomitant effects on anxiety, learning and memory in rats. All drugs were delivered into the NAc shell via bilaterally implanted indwelling cannulae. The NMDA-induced anxiolytic-like behaviours upon retest could possibly be attributed to the further avoidance acquisition impairments. Moreover, the inhibition of dopaminergic system using SCH 23390 and sulpiride induced an anxiolytic-like response and impaired the aversive memory acquisition during retest. However, the concurrent intra-NAc shell microinjection of the subthreshold dose of SCH 23390 and sulpiride (0.125 µg/rat) reversed the anxiolytic-like effect and blocked the aversive memory impairment induced by intra-NAc shell NMDA. Our results suggest a modulatory role of the NAc shell dopaminergic system on NMDA-induced effects in the aversive memory.

Key words: *glutamate, dopamine, anxiety, aversive learning, elevated plus-maze, nucleus accumbens, rat.*

Introduction

Nucleus accumbens (NAc) is one of the main limbic system nuclei receiving rich dopaminergic inputs hence taking an important role in the regulation of many physiological cognitive and non-cognitive behaviours [39]. Anatomical studies have determined at least two main functionally important parts in NAc, i.e. the core and the shell [8,87]. With regard to the dopaminergic system, these two parts seem different. For instance, the dopamine plexus and concentration are richer and higher in the shell than in the core, respectively [20,80]. Evidence has indicated the pivotal role of NAc dopaminergic system

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in the modulation of learning, memory [11,14,43], fear and/or anxiety-like behaviours [50]. Dopamine exerts its effect via two different dopamine subtypes receptors, called the D_1- and D_2-like families [55]. These two dopamine family receptors have a high expression in both parts of NAc while at different distribution patterns. For example, the D_1- and D_2-like dopamine receptor families are higher and lower in the NAc shell than in core, respectively [30]. This may partly explain the different physiological functions of the two parts of NAc [40,42]. Due to the abundant dopaminergic inputs which NAc shell receives from various brain parts and the consequent high contraction level of dopamine in the NAc shell, this part is believed to render a critical role in dopamine-mediated functions [30]. The NAc receives its dopaminergic afferents from the ventral tegmental area (VTA) and the substantia nigra (SN), and the glutamatergic inputs from several brain areas such as prefrontal cortex, amygdala and hippocampus [30].

On the other hand, glutamate is known as one of the most excitatory neurotransmitters modulating learning, memory and anxiety-like behaviours in different parts of the brain [33,94]. With respect to the preferential agonists, at least three subtypes have been identified for glutamate, including *N*-methyl-*D*-aspartate (NMDA), AMPA and kainate [57]. Investigations have revealed that the NMDA receptor plays a critical part in regulation of learning, memory formation (possibly through long-term potentiation and depression) [9,38] and anxiety-related behaviours [26,46,63,73,78]. A plethora of anatomical experiments have demonstrated that there are close relationships between NAc glutamatergic and dopaminergic systems [66,67]. For instance, it has been shown that NMDA receptors are localized on dopamine D_1 receptor-containing neurons in the NAc shell [27].

Emotional states (including, fear and aversion) can be modulated through amplification of impairment in memory formation [51]. Due to possible misinterpretations, the available animal models for learning and memory seem to have a limited ability to detect the effect of drugs on fear and anxiety. Therefore, the proposed test-retest paradigm in the elevated plus-maze (EPM) task is an attempt to concomitantly assess the effects of drugs on anxiety, learning and memory in rodents [6]. The use of EPM in testing anxiety is based on the natural tendency of animals to avoid dangerous situations when they face height and open spaces [91]. In general, animals acquire information with regard to safe and dangerous areas in the maze upon test. Animals retested in the EPM avoid exploring open spaces and displaying a clear enclosed arm preference with a low percentage of entries and time spent in the open arms [59], relative to their respective measures during the test [10,22,44,74,79]. The aversive and fear-inducing nature of the open arms represents a useful tool for the study of aversively motivated learning processes in the EPM [16]. Based on the above, learning and memory are usually studied in the EPM through avoidance to open-arms during the retest session. Given this, the purpose of the current study was to examine the possible involvement of the NAc shell D_1 and/or D_2 dopaminergic receptors on NMDA-induced effects in the aversive memory using the elevated-plus maze (EPM) task.

Material and methods

Animals

Male Wistar rats weighing approximately 250-280 g were provided by the Central Animal House of the Institute for Cognitive Science Studies (ICSS), Tehran, Iran. Prior to the experiments, animals underwent a period of seven days habituation in groups of five in polypropylene home cages (45 × 30 × 15 cm), having access to food and water *ad libitum*, under a light/dark cycle of 12 h (lights on at 06:00) and the temperature ranging between 20°C and 24°C. Animal handling was restricted to the time of home cage cleaning (each 48 h), weighing and drug administration. Each experimental group comprised eight animals. All experimental procedures were conducted in compliance with the recommendations set down by the Institute's Ethics Commission for the use of experimental animals.

Stereotaxic surgery and microinjections

Animals were anaesthetized intraperitoneally using ketamine hydrochloride (50 mg/kg) and xylazine (4 mg/kg) then placed in a Stoelting stereotaxic instrument (Wood Dale, IL, USA). Two stainless steel guide cannulae (22 gauge) were implanted in the right and left of the NAc shell regions according to the atlas of Paxinos and Watson [58]. The stereotaxic coordinates for the NAc regions were as follows: +1.4 mm posterior to bregma, ±0.8 mm lateral to the Midline and –5.5 mm ventral to the dorsal surface of

the skull. The cannulae were fixed to the skull using acrylic dental cement. Rats were allowed 5 days before the test to recover from surgery. The left and right of the NAc areas were infused by means of an internal cannula (27 gauge), terminating 2 mm below the tip of the guides and connected by polyethylene tubing to a 2 µL Hamilton syringe (Bonaduz, GR, Switzerland). A volume of 0.3 µL solution was injected over a 60-second period, in each side. The inner cannulae were left in place for an additional 60 seconds to allow diffusion of the solution and to reduce the possibility of reflux. Intra-NAc shell injections were made just five minutes before testing. Control groups and drug infused groups were surgeried and all of the animals were anaesthetized using a ketamine solution, therefore all of groups were under the same condition and under the same effect of ketamine anaesthesia.

Elevated plus-maze (EPM) apparatus

This plexiglas, plus-shaped apparatus, was set at 50 cm height from the floor. This apparatus was composed of two 50 × 10-cm open arms and two 50 × 10 × 40-cm enclosed arms, each with an open roof. The junction area of the four arms (central platform) measured 10 × 10 cm. The maze was placed at the centre of a quiet and dimly lit room [91,92].

Behavioural testing

Rats were placed in the experimental room at least 1 h before testing. All experiments were done during the light phase of the light/dark cycle between 11 a.m. and 2 p.m. Animals were randomly assigned to treatment conditions and tested in a counter-balanced order. Animals' behaviours were tracked and recorded by an observer who quietly sat 1 m behind one of the closed arms of the maze, using a chronometer. During the five-minute post-drug treatment, rats were individually placed at the centre of the plus maze facing one of the open arms and allowed for 5 min free exploration in EPM (test session) then were taken back to their home cages. In 24 hours, rats were returned to the test room and placed again in the EPM for a new exploration period of 5 min (retest session). The observer measured: 1) time spent in open arms, 2) time spent in closed arms, 3) number of entries into open arms and 4) number of entries into closed arms during the five-minute period both upon test and retest. An entry was defined as 'all four paws in the arm'. Between EPM sessions and after each rat the maze was cleaned with distilled water. The obtained data were used to calculate: a) % OAT (the ratio of time spent in open arms to the time spent in all arms ×100); b) % OAE (the ratio of entries into open arms to total entries ×100) [55,97,98], and c) the total closed and open arm entries were considered as a relatively pure index for the locomotor activity [93,95].

Drugs

The drugs used in the present study were ketamine and xylazine (Alfasan Chemical Co, Woerden, Holland) for animal anaesthesia, NMDA (*N*-methyl-*D*-aspartic acid as NMDA receptor agonist, Tocris Cookson, Bristol, UK), SCH 23390 (as dopamine D_1 receptor antagonist) and sulpiride (as dopamine D_2 receptor antagonist). NMDA and SCH 23390 were dissolved in sterile 0.9% saline while sulpiride was dissolved in vehicle (the vehicle was one drop of glacial acetic acid from Hamilton microsyringe, made up to a volume of 5 ml with sterile 0.9% saline, then diluted to the required volume) just before the experiment. Control animals received either saline or vehicle. NMDA and SCH were administered into the shell of the nucleus accumbens at a volume of 0.3 µL/rat.

Drug treatments

Experiment 1: Effects of NMDA administration on open arms exploratory-like behaviours in the presence or absence of SCH 23390

To substantiate whether the microinjection of drugs involved in anxiety, the drug infusion took place before EPM testing. In this experiment, 12 groups of animals were examined. These were as follows: 1) animals which received intra-NAc shell saline (0.3 µL/rat) or NMDA (0.125, 0.25, and 0.5 µg/rat), 5 min after saline (0.3 µL/rat); 2) animals which received intra-NAc shell saline (0.3 µL/rat) or SCH (0.125, 0.25 and 0.5 µg/rat), 5 min before saline (0.3 µL/rat) and 3) animals which received intra-NAc shell saline (0.3 µL/rat) or the subthreshold dose of SCH (0.0125 µg/rat), 5 min before different doses of NMDA (0.125, 0.25, and 0.5 µg/rat).

To investigate the possible drug carryover effects of aversive learning during test day to aversive memory upon retest, treated groups were retested undrugged in EPM 24 h later.

Experiment 2: Effects of NMDA administration on open-arms exploratory-like behaviours in the presence or absence of sulpiride

To substantiate whether the microinjection of drugs involved in anxiety, the drug infusions were done before EPM testing. In this experiment, 12 groups of animals were examined. These included: 1) animals which received intra-NAc shell saline (0.3 µL/rat) or NMDA (0.125, 0.25, and 0.5 µg/rat), 5 min after saline (0.3 µL/rat); 2) animals which received intra-NAc shell vehicle (0.3 µL/rat) or sulpiride (0.125, 0.25 and 0.5 µg/rat), 5 min before saline (0.3 µL/rat) into NAc shell, and 3) animals which received intra-NAc shell vehicle (0.3 µL/rat) or the subthreshold dose of sulpiride (0.0125 µg/rat), 5 min before different doses of NMDA (0.125, 0.25, and 0.5 µg/rat).

To investigate the possible drug carryover effects of aversive learning during test day to aversive memory upon retest, treated groups were retested undrugged in EPM 24 h later.

Histology

Following the completion of behavioural testing, animals were euthanized using an overdose of chloroform. Ink (0.3 µL of 1% aquatic methylene blue solution) was injected into the guide cannulae using 27-gauge injection cannulae. Brains were then removed and fixed in a 10% formalin solution for 48 hours before sectioning. The brains were sliced using the vibro-slice apparatus in transverse planes (40 µm). Cannula placements were verified based on the corresponding map of Paxinos and Watson atlas of rodents' brain [58]. Data from the animals in which injection sites were located outside the NAc shell were not included in the analyses.

Statistical analysis

Data were expressed as mean ± SEM and analyzed using the repeated measure protocol during test and retest days. In addition to the analysis made to compare test to test or retest to retest, the two-way analysis of variance (ANOVA) was also applied. Where *F*-value was significant, one-way ANOVA and post-hoc analysis (Tukey-test) were performed. Between-groups differences with $p < 0.05$ were considered statistically significant.

Results

Histology

Data from the animals in which injection sites were located outside the NAc shell were not included in the analyses and in the present study we used only data from animals that were cannulated clearly within-NAc-shell. Cannulae were implanted into the NAc shell of a total of 208 rats, however only the data from 192 animals with correct cannulae implants were included in statistical analyses.

Experiment 1 results

Effects of NMDA administration on open arms exploratory-like behaviours

Repeated measure and post hoc analyses demonstrated that NMDA increases the %OAT (at 0.25 and 0.5 µg/rat, as seen in Fig. 1; panel 1A and Fig. 2; panel 1A, respectively), %OAE (at 0.25 and 0.5 µg/rat as seen in Fig. 1; panel 1B and Fig. 2; panel 1B, respectively) and decreases the locomotor activity (non-significantly and significantly as seen in Fig. 1; panel 1C and Fig. 2; panel 1C, respectively), indicating an anxiolytic-like response to NMDA.

Adding to the above, data showed that NMDA increases the %OAT (at 0.25 and 0.5 µg/rat, as shown in Fig. 1; panel 2A and at 0.125 and 0.5 µg/rat in Fig. 2; panel 2A) and %OAE (at 0.25 and 0.5 µg/rat as shown in Fig. 1; panel 2B and at 0.125 and 0.5 µg/rat in Fig. 2; panel 2B). However, this did not alter the locomotor activity (Fig. 1, panel 2C and Fig. 2, panel 2C) upon retest as compared to the control group, indicating an NMDA-induced impairment of the aversive memory acquisition.

According to the above data, NMDA induced anxiolytic-like behaviours. Furthermore, the retest data suggested that the NMDA anxiolytic-like effect may also be linked to the impairment in further avoidance acquisition. The corresponding repeated measure results have been demonstrated in Table I and Table II.

Effects of intra-NAc shell microinjection of SCH 23390 on open arms exploratory-like behaviours

In this experiment two-way ANOVA analysis was done to assess the NMDA treated group dose-response as compared to controls as well as the interac-

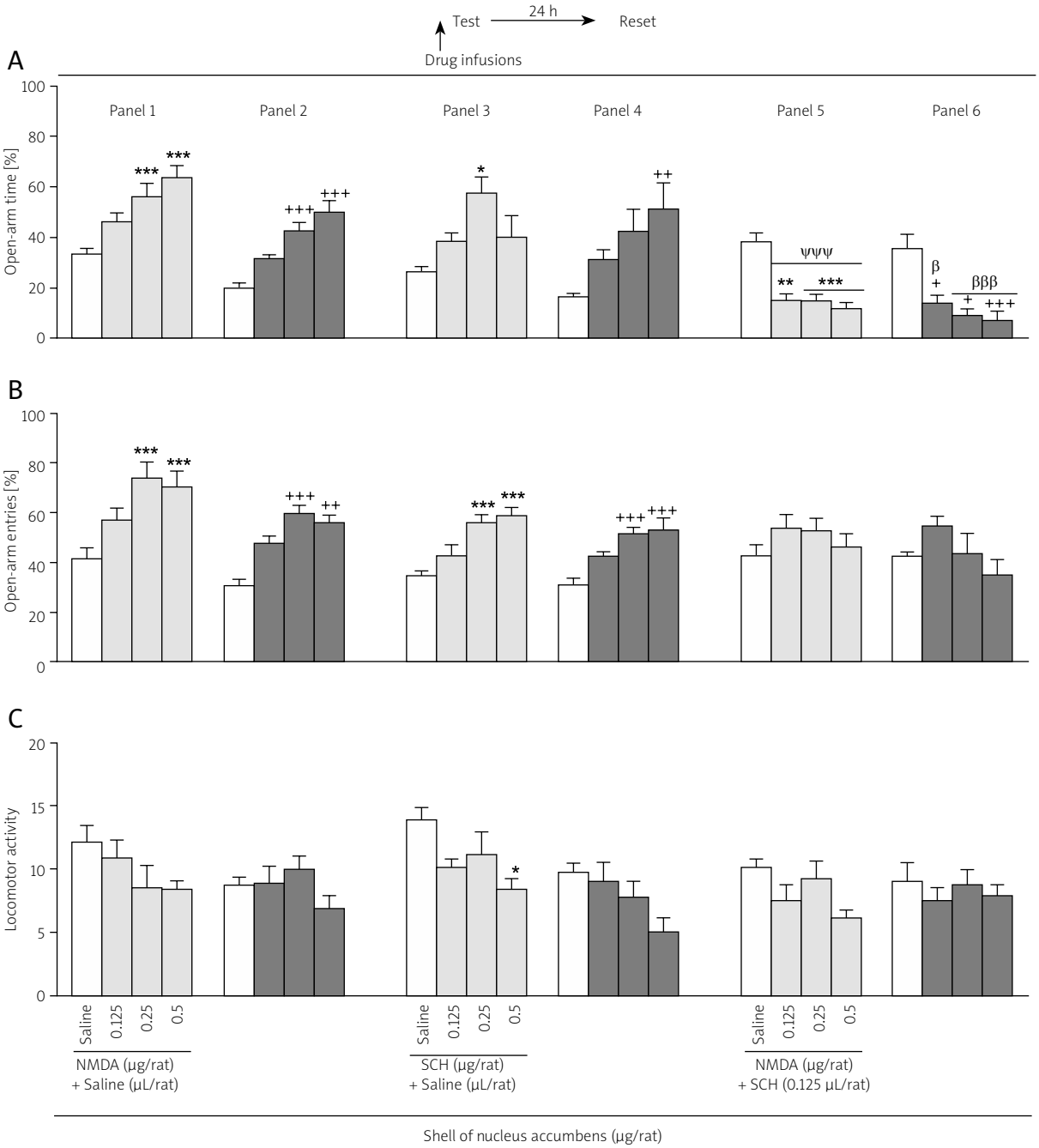

Fig. 1. The effects of NMDA administration on open-arms exploratory-like behaviours in the presence or absence of SCH 23390. Rats were tested in the EPM, 5 min after concurrent microinjection of saline (0.3 μL/rat) or NMDA (0.125, 0.25 and 0.5 μg/rat) or saline (0.3 μL/rat) or SCH 23390 (0.125, 0.25 and 0.5 μg/rat) and the subthreshold dose of SCH 23390 prior to intra-NAc shell NMDA. In 24 h, all groups were retested in the EPM, undrugged. $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ different from the control group on the test day. $^{+}p < 0.05$, $^{++}p < 0.01$ and $^{+++}p < 0.001$ different from control groups on the retest day. $^{\alpha}p < 0.05$, $^{\alpha\alpha}p < 0.01$ and $^{\alpha\alpha\alpha}p < 0.001$ different from the test and retest groups. $^{\Psi}p < 0.05$, $^{\Psi\Psi}p < 0.01$ and $^{\Psi\Psi\Psi}p < 0.001$ different from the test groups. $^{\beta}p < 0.05$, $^{\beta\beta}p < 0.01$ and $^{\beta\beta\beta}p < 0.001$ different from the retest groups.

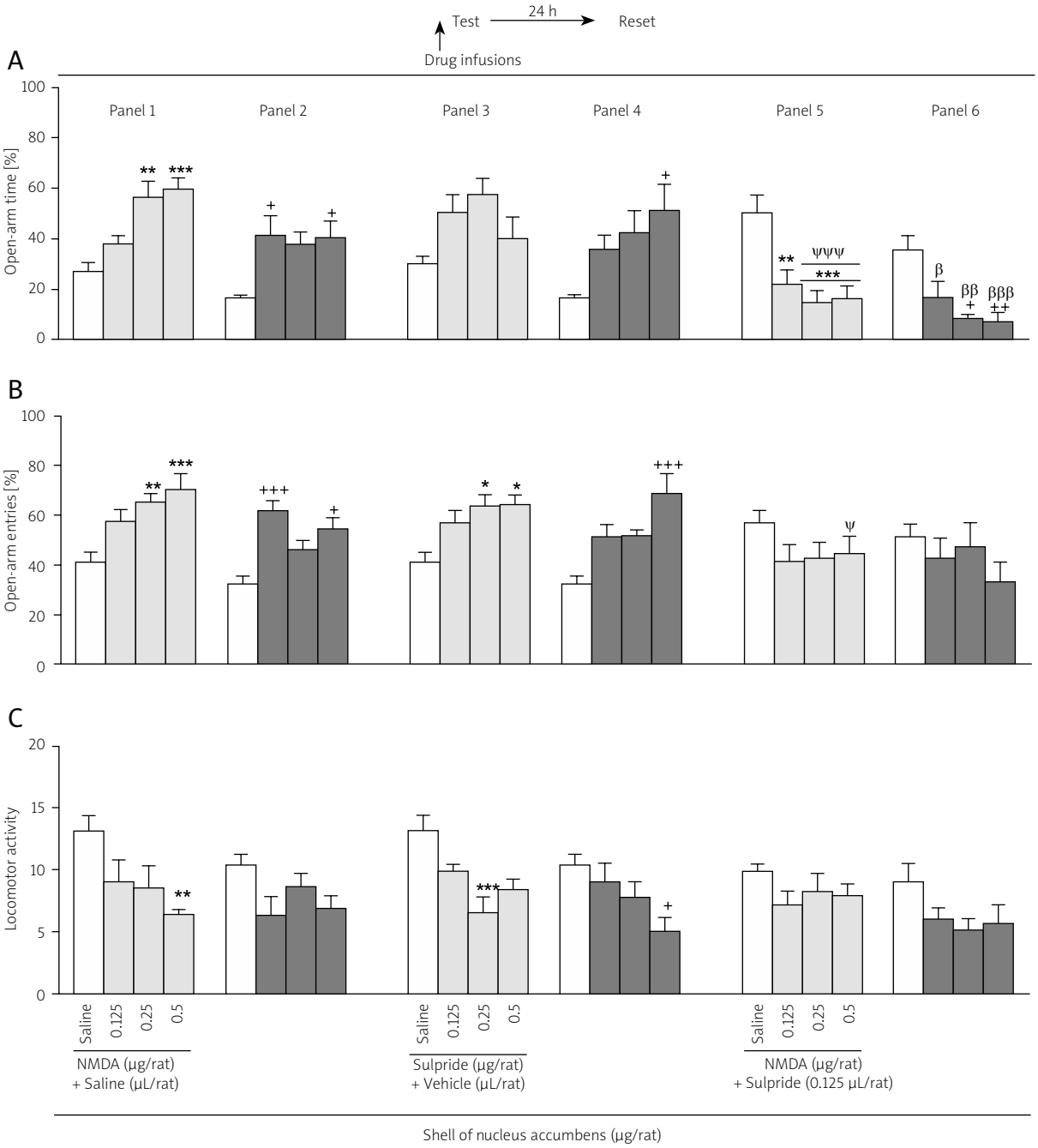

Fig. 2. The effects of NMDA administration on open-arms exploratory-like behaviours in the presence or absence of sulpiride. Rats were tested in the EPM, 5 min following the concurrent microinjection of saline (0.3 μL/rat) or NMDA (0.125, 0.25 and 0.5 μg/rat) or vehicle (0.3 μL/rat) or sulpiride (0.125, 0.25 and 0.5 μg/rat) and the subthreshold dose of sulpiride prior to the intra-NAc shell NMDA. In 24 h, all groups were retested in the EPM, undrugged. $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ different from the control saline group on the test day. $^{+}p < 0.05$, $^{++}p < 0.01$ and $^{+++}p < 0.001$ different from control saline groups on the retest day. $^{\alpha}p < 0.05$, $^{\alpha\alpha}p < 0.01$ and $^{\alpha\alpha\alpha}p < 0.001$ different from the test and retest groups. $^{\Psi}p < 0.05$, $^{\Psi\Psi}p < 0.01$ and $^{\Psi\Psi\Psi}p < 0.001$ different from the test groups. $^{\beta}p < 0.05$, $^{\beta\beta}p < 0.01$ and $^{\beta\beta\beta}p < 0.001$ different from the retest groups.

Table I. Repeated measure and two ANOVA results with *p* values for experiment 1

Experiments	Behaviours	Day		Group		Day and group interaction		Final results/ conclusion of each experiment
Repeated measure results (panel 1 and 2 of Fig. 1)		$F_{(1, 28)}$	*p*	$F_{(3, 28)}$	*p*	$F_{(3, 28)}$	*p*	NMDA at doses of 0.25 and 0.5 µg/rat induced anxiolytic-like behaviour and impaired aversive memory acquisition
	% OAT	30.516	0.000	25.923	0.000	0.012	0.998	
	% OAE	21.514	0.000	14.052	0.000	0.424	0.737	
	Locomotion	3.661	0.066	1.591	0.214	2.141	0.118	
Repeated measure results (panel 3 and 4 of Fig. 1)		$F_{(1, 28)}$	*p*	$F_{(3, 28)}$	*p*	$F_{(3, 28)}$	*p*	SCH at doses of 0.25 and 0.5 µg/rat induced anxiolytic-like behaviour and impaired aversive memory acquisition
	% OAT	1.258	0.272	9.286	0.000	1.506	0.235	
	% OAE	2.954	0.097	18.856	0.000	0.330	0.804	
	Locomotion	17.398	0.000	5.332	0.005	0.816	0.496	
Two ANOVA results (panel 5 and 1 of Fig. 1)		$F_{(1, 56)}$	*p*	$F_{(3, 56)}$	*p*	$F_{(3, 56)}$	*p*	SCH at a dose of 0.125 µg/rat in the NAc decreases the anxiolytic-like behaviours induced by the intra-NAc shell injection of NMDA at doses of 0.125, 0.25 and 0.5 µg/rat and meanwhile improves the aversive memory impairment
	% OAT	149.065	0.000	1.581	0.220	25.770	0.000	
	% OAE	9.469	0.003	5.621	0.002	2.731	0.052	
	Locomotion	4.092	0.048	3.500	0.021	1.062	0.373	
Two ANOVA results (panel 6 and 2 of Fig. 1)		$F_{(1, 56)}$	*p*	$F_{(3, 56)}$	*p*	$F_{(3, 56)}$	*p*	
	% OAT	63.043	0.000	1.074	0.368	26.897	0.000	
	% OAE	1.342	0.252	5.307	0.003	6.047	0.001	
	Locomotion	0.198	0.658	1.265	0.295	0.566	0.640	

tion with SCH 23390 or sulpiride treatment. According to the repeated measure and Post hoc analyses, SCH 23390 increased the %OAT (at 0.25 µg/rat, Fig. 1; panel 3A), %OAE (at 0.25 and 0.5 µg/rat, Fig. 1, panel 3B) and decreased the locomotor activity (at 0.5 µg/rat, Fig. 1; panel 3C) upon test, indicating that SCH 23390 may induce an anxiolytic-like response.

Moreover, the data revealed that SCH 23390 increases the %OAT (at 0.5 µg/rat, Fig. 1; panel 4A), and %OAE (at 0.25 and 0.5 µg/rat, Fig. 1; panel 4B), while does not alter the locomotor activity (Fig. 1; panel 4C) on retest day as compared to the control group, indicating that SCH 23390 possibly impairs the aversive memory retrieval.

In conclusion, the data revealed that SCH 23390 induces an anxiolytic-like response. Besides, the increased %OAT upon retest indicates that SCH 23390-treated rats had their aversive memory to open-arm exploration negatively affected as compared to the control group. The corresponding repeated measure results have been summarized in Table I.

Effects of NAc shell microinjection of SCH 23390 prior to NMDA on open arms exploratory-like behaviours

Two-way ANOVA and post hoc analyses revealed that intra-NAc microinjection of SCH 23390 prior to NMDA causes a significant decrease in %OAT (at 0.125, 0.25 and 0.5 µg/rat, Fig. 1; panel 5A) while exerts no significant change in the %OAE and the locomotor activity (Fig. 1; panel 5B, 5C) on test day as compared to NMDA-treated groups (Fig. 1; panel 1A, 1B and 1C), indicating that SCH 23390 potentially reverses the anxiolytic-like response induced by the intra-NAc shell microinjection of NMDA.

On the other hand, this intervention resulted in a significant decrease in the %OAT (at 0.125, 0.25 and 0.5 µg/rat, Fig. 1; panel 6A) while leading to no significant change in %OAE and locomotor activity (Fig. 1; panel 6B, 6C) on retest day as compared to NMDA-treated groups (Fig. 1; panel 2A, 2B and 2C). The above indicates that SCH 23390 potentially restores the aversive memory impairment already induced by the intra-NAc shell microinjection of NMDA.

Table II. Repeated measure and two ANOVA results with *p* values for experiment 2

Experiments	Behaviours	Day		Group		Day and group interaction		Final results/ conclusion of each experiment
Repeated measure results (Panel 1 and 2 of Fig. 2)		$F_{(1, 28)}$	*p*	$F_{(3, 28)}$	*p*	$F_{(3, 28)}$	*p*	NMDA at doses of 0.25 and 0.5 µg/rat induced anxiolytic-like behaviour and at doses of 0.125 and 0.25 µg/rat impaired aversive memory acquisition
	% OAT	10.031	0.004	11.111	0.000	2.264	0.103	
	% OAE	11.446	0.002	12.033	0.000	3.188	0.039	
	Locomotion	3.740	0.063	4.047	0.016	1.983	0.139	
Repeated measure results (Panel 3 and 4 of Fig. 2)		$F_{(1, 28)}$	*p*	$F_{(3, 28)}$	*p*	$F_{(3, 28)}$	*p*	Sulpiride at doses of 0.25 and 0.5 µg/rat induced anxiolytic-like behaviour and at dose of 0.5 µg/rat impaired aversive memory acquisition
	% OAT	2.466	0.128	6.472	0.002	1.591	0.214	
	% OAE	2.414	0.131	14.298	0.000	1.058	0.383	
	Locomotion	5.324	0.029	6.447	0.002	2.795	0.059	
Two ANOVA results (Panel 5 and 1 of Fig. 2)		$F_{(1, 56)}$	*p*	$F_{(3, 56)}$	*p*	$F_{(3, 56)}$	*p*	Sulpiride at a dose of 0.125 µg/rat in the NAc decreases the anxiolytic-like behaviours induced by the intra-NAc shell injection of NMDA at doses of 0.25 and 0.5 µg/rat, and meanwhile improves the aversive memory impairment by the intra-NAc shell injection of NMDA
	% OAT	28.098	0.000	1.137	0.342	18.014	0.000	
	% OAE	8.996	0.004	0.984	0.407	5.668	0.002	
	Locomotion	1.242	0.270	4.772	0.005	1.394	0.254	
Two ANOVA results (Panel 6 and 2 of Fig. 2)		$F_{(1, 56)}$	*p*	$F_{(3, 56)}$	*p*	$F_{(3, 56)}$	*p*	
	% OAT	21.579	0.000	0.541	0.656	10.833	0.000	
	% OAE	1.517	0.223	1.070	0.369	4.634	0.006	
	Locomotion	3.574	0.064	3.574	0.064	0.657	0.582	

Having mentioned these, we may conclude that the intra-NAc shell injection of the subthreshold dose of SCH 23390 decreases the anxiolytic-like behaviours induced by the intra-NAc shell injection of NMDA, and meanwhile improves the aversive memory impairment. The two-way ANOVA results have been outlined in Table I.

Experiment 2 results

Effects of NAc shell microinjection of sulpiride on open arms exploratory-like behaviours

According to the repeated measure and post hoc analyses, the data showed that sulpiride does not alter the %OAT (Fig. 1; panel 3A) while increases the %OAE (at 0.25 and 0.5 µg/rat, Fig. 2, panel 3B) and decreases the locomotor activity (at 0.25 and 0.5 µg/rat, Fig. 2; panel 3C) upon test, indicating that sulpiride may induce an anxiolytic-like response.

Moreover, the data revealed that sulpiride increases the %OAT (at 0.5 µg/rat, Fig. 2; panel 4A), %OAE (at 0.5 µg/rat, Fig. 2; panel 4B) whereas decreases the locomotor activity (at 0.5 µg/rat, Fig. 2; panel 4C) on retest day as compared to the control group, indicating that sulpiride possibly impairs the aversive memory retrieval.

In conclusion, the data revealed that sulpiride induces an anxiolytic-like response. Besides, the increased %OAT upon retest indicates that sulpiride-treated rats had their aversive memory to open-arm exploration negatively affected as compared to the control group. The corresponding repeated measure results have been summarized in Table II.

The effects of intra-NAc shell microinjection of sulpiride prior to NMDA on open arms exploratory-like behaviours

Two-way ANOVA and post hoc analyses demonstrated that the intra-NAc microinjection of sulpiride prior to NMDA causes a significant decrease in %OAT (at 0.25 and 0.5 µg/rat, Fig. 2; panel 5A) and %OAE

(at 0.5 µg/rat, Fig. 2; panel 5B) while exerts no significant change in locomotor activity (Fig. 2; panel 5C) on test day as compared to NMDA-treated groups (Fig. 2; panel 1A, 1B and 1C). This indicates that sulpiride potentially reverses the anxiolytic-like response induced by intra-NAc shell microinjection of NMDA.

On the other hand, this intervention resulted in a significant decrease in %OAT (at 0.125, 0.25 and 0.5 µg/rat, Fig. 2; panel 6A) while leading to no significant change in %OAE and locomotor activity (Fig. 2; panel 6B, 6C) on retest day as compared to NMDA-treated groups (Fig. 2; panel 2A, 2B and 2C). The above indicates that sulpiride potentially restores the aversive memory impairment already induced by the intra-NAc shell microinjection of NMDA.

Based on these, we may conclude that the intra-NAc shell microinjection of the subthreshold dose sulpiride decreases the anxiolytic-like behaviours induced by the intra-NAc shell injection of NMDA, and meanwhile improves the aversive memory impairment. The corresponding two-way ANOVA results are outlined in Table II.

Discussion

In our study, animals were given pretest intracerebral drugs injection followed by no injection upon retest 24 h later. Based on this, drug effects on anxiety-like behaviours and aversive learning with subsequent long-term effects on memory in 24 h were tested. It has been reported that the prior experience of an undrugged EPM testing session may alter the behavioural responses in an undrugged retest session [13,74]. The injury caused by the injection might change the expression of messenger RNAs and proteins. But there is a study that investigated changes in the expression of messenger RNAs for trkA, trkB and trkC in the brain following an injury caused by insertion of a 30-gauge needle into adult rat hippocampus or neocortex. The increased levels of mRNA after the injury returned to control levels a few hours after the injury. Pretreatment of the animals with the ketamine completely prevented the changes of trkB and trkC messenger RNAs, suggesting that the brain injury caused a release of glutamate with subsequent activation of NMDA receptor [54]. In the present study, rats were allowed 5 days before the test to recover from surgery so the changes of mRNA levels after the injury may be returned to control levels after the recovery days.

Effects of intra-NAc shell NMDA administration on anxiolytic-like behaviours and aversive memory formation

Our results indicated that the intra-NAc shell infusion of NMDA receptor agonist at applied doses induces an anxiolytic-like response in EPM. This NMDA-induced anxiolytic-like effect emerges into the retest day. Current findings suggest that NMDA treatment induces impairment in the aversive memory acquisition upon test. There is a body of evidence supporting that NAc shell is an essential brain site regulating emotion, motor activity [17], motivation-related learning, memory [12,29], and anxiety-like behaviours [48,49,61]. On the other hand, the NMDA receptor (as an ionotropic glutamate receptor) plays a critical role in the regulation of glutamate-induced behaviours such as learning and memory formation (possibly through long-term potentiation and depression) [9,38], and anxiety-related behaviours [26,63].

Our results are in agreement with the previous investigation showing that NMDA agonist releases behavioural and anxiolytic-like behaviours indicating the role of NMDA receptors in modulation of anxiety-related behaviours [26,73]. Moreover, there is also an investigation showing that the activation of NMDA receptor induces anxiogenic-like effects in EPM and social interaction tasks [21].

Evidence has suggested the critical role of NAc in regulation of several learning functions which require a flexible use of sensory information [47,64, 65]. It has been postulated that NAc manipulations induce spatial memory deficit in the Morris water maze [64,68] and radial maze [24,70]. In agreement with our results, evidence has demonstrated that the systemic administration of NMDA leads to an impaired dark-avoidance learning in rats [89]. On the other hand, some investigations have postulated that the deactivation of the NAc glutamatergic ionotropic receptors disrupt the working memory [7,35, 41] and spatial responses [71] while other contradictory studies have shown that the NMDA receptor blockade in NAc shell does not alter the spatial learning [47,71]. Furthermore, it appears that the NAc shell is more involved in the regulation of spatial learning and memory as compared to the NAc core since the NAc shell (but not the core) lesions disrupt the spatial learning [35]. The NAc sends a dense GABA pro-

jection to the ventral pallidum (VP), and stimulation of either the NAc or its glutamatergic afferents can inhibit VP neuronal firing [23]. The VP can influence DA neural activity via a direct projection to the ventral tegmental area (VTA) [23]. Dopaminergic neurons projection of the VTA sends back to the NAc. Dopaminergic terminals arising from this area make synaptic contacts with NAc GABAergic neurons. According to the present results, stimulation of the NAc by injection of NMDA in this area would have increased firing of GABAergic projection neurons of the NAc causing a decrease in VP the GABAergic neural activity. The decrease in VP activity would then be expected to cause a reduction of the GABAergic inhibition over the VTA and this could alter an increased release of dopamine in NAc. The present data suggested that maybe glutamate exerts its function by affecting the release of dopamine. Several studies have indicated that dopaminergic system modulates the neuronal activities involved in fear or anxiety-like behaviours [25,60]. Several investigations have substantiated that the mesocortical DA system produces a robust and specific response to stressors [28]. Some investigations have suggested that these high levels of DA released under stress are above the optimal range for working memory and therefore impair this cognitive function [5,88].

Effect of intra-NAc shell dopamine D_1 and D_2 receptors antagonists injection on anxiolytic-like behaviours and aversive memory formation

It has been made clear that the NAc shell plays a critical part in modulation of dopamine-mediated functions as it contains the largest amount of dopaminergic terminals and thus the highest concentration of dopamine [20,27,80]. Therefore, the NAc dopaminergic system has a pivotal role in modulation of learning and memory [11,14,43], fear and/or anxiety [50]. The idea of investigating the role of NAc shell dopamine D_1 and D_2 receptors in NMDA-induced effects using the elevated plus-maze test, appealed to our interest.

Present results indicated that the intra-NAc shell microinjection of SCH 23390 (a dopamine D_1 receptor antagonist) increases both %OAT and %OAE whereas decreases the locomotor activity. On the other hand, inhibition of the dopamine D_2 receptor in NAc shell by sulpiride (a dopamine D_2 receptor antagonist) did not affect the %OAT, however; increased %OAE and decreased the locomotor activity. Several investigations have substantiated that the extensive D1- and D2-like receptors are on the presynaptic varicosities of medium-spiny neurons of nucleus accumbens [84]. Similar to the response seen with SCH 23390 and sulpiride on anxiolytic-like behaviours there is a study showing that dopamine D_1 and D_2 receptors blockade in the basolateral amygdala exerts anxiolytic-like behaviours [96]. Intra-NAc injection of SCH 23390 or sulpiride induced anxiolytic- and did not alter anxiety-like behaviours, respectively [90]. Furthermore, our present data indicated that beside the increased %OAT on the test day, the increased %OAT upon retest in both SCH 23390 and sulpiride show that these drug-induced anxiolytic-like effects emerge on the retest day. Current findings suggest that SCH 23390 and sulpiride treatments impair the aversive memory function upon retest. Moreover, evidence has reemphasized the crucial role of dopaminergic system in the regulation of several neural activities which are involved in learning and memory (see [34], for a review). For instance, it has been shown that the activation or deactivation of dopamine receptors provides a capability to learn and store information [1]. Other studies have demonstrated that the immediate post-training blockade of D_1 and D_2 receptors located within the NAc impairs the performance of spatial learning tasks [52]. Based on some other reports, the intra-dorsal hippocampal [62] and peripheral [2,15] administration of the antagonists impair the one-trial passive avoidance and spatial or non-spatial memories in mice, respectively. However, other investigations indicate that pre-test single administration of SCH 23390 or sulpiride causes no significant change in the step-down latency [56]. One study demonstrated that inhibition of the dopamine exocytosis from pre-synaptic neuron via Ca^{2+}-channel blockade by SKF96365 decreases anxiolytic-like behaviours induced by sulpiride in the NAc shell region indicating the involvement of the pre-synaptic dopamine D_2 receptors in sulpiride induced anxiolytic-like behaviours [3]. However, blockade of pre-synaptic dopamine D_2 receptors increases the presynaptic release of dopamine, which in turn induced anxiolytic-like behaviours and aversive memory acquisition impairment. The anxiolytic-like behaviours and aversive memory acquisition impairment that induced by SCH may be related to its effects on pre-synaptic

dopamine D_1 receptors in shell on NAc which will cause the increase in dopamine release.

Effect of intra-NAc shell microinjection of D_1 and D_2 receptors antagonists on anxiolytic-like behaviours and aversive memory deficits induced by the intra-NAc shell NMDA

Data indicated that the intra-NAc shell administration of the subthreshold dose (0.125 µg/rat) of SCH 23390 or sulpiride together with different doses of NMDA, reduce the anxiolytic-like response and improve the aversive memory impairment already induced by the intra-NAc shell infusion of NMDA. These results may suggest the involvement of the dopamine transmission through D_1 and D_2 receptors of the NAc. Several investigations have suggested the possible dopaminergic and glutamatergic systems interaction in the NAc [66,67] based on which the NAc glutamate transmission is modulated by the dopamine system [37,53,82]. With respect to the interaction of these systems, there is a study showing that the NMDA receptors are localized on the NAc shell neurons which abundantly contain dopamine D_1 receptors [81]. Some evidence has also indicated the modulation of dopamine function through NMDA and AMPA receptors [30]. In an interesting report, Kalivas *et al*. declared that SCH 23390 and sulpiride restore the amphetamine-induced glutamate level decrease in NAc suggesting the involvement of presynaptic dopamine receptors in this phenomenon [36]. Furthermore, Dalia *et al*. indicated that dopamine increases the extracellular glutamate levels in the NAc [18]. Meanwhile, activation of glutamate receptor increases the dopamine release in the NAc [31]. We also know from the literature that NAc dopamine and glutamate signalling interactions are crucially required in behavioural reinforcement and habit formation and dopamine can modulate excitatory glutamatergic projection from the PFC [82]. The elevation of extracellular dopamine and glutamate levels in the striatum might disrupt Ca^{2+} homeostasis leading to the endoplasmic reticulum (ER) stress response [4,72]. The stimulation of dopamine D_1 receptors in the mouse neostriatum activates the cAMP/protein kinase A (PKA) pathway [76]. PKA activated increases in the levels of nitric oxide (NO) in the dorsal striatum [45]. NO in turn produces peroxynitrite which can regulate poly (ADPribose) polymerase-1 (PARP-1) activation [86]. Activation of three subtypes of glutamatergic ionotropic receptors leads to calcium influx, nitric oxide (NO) and reactive oxygen species (ROS) generation. Superoxide can combine with NO forming peroxynitrite ($ONOO^-$). Excessive production of peroxynitrite and other free radicals induces chromosomal DNA nicks and breaks resulting in PARP-1 activation [19,83]. This enzyme is the nuclear target for different types of stress and signalling pathways. PARP-1 plays a crucial role in regulation of many transcription factors and nuclear proteins. Under physiological conditions, this enzyme is involved in memory formation and it should not be inhibited [32]. However, under massive stress and other pathological conditions it could be over-activated and involved in cell death by different mechanisms including activation of pro-inflammatory gene expression or modulation of NMDA and cholinergic receptor signalling [75,77]. Thus, there are three possible mechanisms for PARP-1 activation: first, dopamine D_1 receptor-dependent cAMP/PKA pathway is able to activate PARP-1; second, group I mGluRs and NMDA receptors interact with each other to increase PAPR-1 activation; and third, crosstalk between dopamine and glutamate receptors may provide another means of interaction [85]. Another study demonstrated that systemic inflammation evoked by an intraperitoneal injection of lipopolysaccharide induces morphological and biochemical changes in the brain including alterations of PARP-1 activity and expression of several genes [32]. PARP-1 inhibitor protects against LPS-evoked recognition impairment and significantly improves spatial memory in LPS-treated mice. PARP-1 inhibitor in control mice decreased memory function because under physiological conditions this enzyme is involved in memory formation and it should not be inhibited [32].

In conclusion, the stimulation of NMDA receptors in the shell of NAc and the effects of dopamine antagonists on presynaptic receptors might cause the increase in dopamine levels in the striatum and disrupt Ca^{2+} homeostasis leading to the endoplasmic reticulum (ER) stress response [4,72]. In addition, stimulating dopamine and glutamate receptors increases nitric oxide efflux which activates PARP-1 in the central nervous system [69,86]. Under physiological conditions, this enzyme is involved in memory formation and it should not be inhibited [32]. Activation of PARP-1 by elevation of extracellular dopamine and glutamate levels in the striatum might disrupt memory function and induce anxiolyt-

ic-like behaviours. Finally, our results suggest a modulatory role of the NAc shell dopaminergic system on NMDA-induced effects in the aversive memory.

Disclosure

Authors report no conflict of interest.

References

1. Adriani W, Felici A, Sargolini F, Roullet P, Usiello A, Oliverio A, Mele A. N-methyl-D-aspartate and dopamine receptor involvement in the modulation of locomotor activity and memory processes. Exp Brain Res 1998; 123: 52-59.
2. Adriani W, Sargolini F, Coccurello R, Oliverio A, Mele A. Role of dopaminergic system in reactivity to spatial and non-spatial changes in mice. Psychopharmacology (Berl) 2000; 150: 67-76.
3. Ahmadi H, Nasehi M, Rostami P, Zarrindast MR. Involvement of the nucleus accumbens shell dopaminergic system in prelimbic NMDA-induced anxiolytic-like behaviors. Neuropharmacology 2013; 71: 112-123.
4. Ahn SM, Kim SW, Choe ES. Cocaine increases immunoglobulin heavy chain binding protein and caspase-12 expression in the rat dorsal striatum. Psychopharmacology (Berl) 2007; 195: 407-414.
5. Arnsten AF. Catecholamine modulation of prefrontal cortical cognitive function. Trends Cogn Sci 1998; 2: 436-447.
6. Asth L, Lobao-Soares B, Andre E, Soares Vde P, Gavioli EC. The elevated T-maze task as an animal model to simultaneously investigate the effects of drugs on long-term memory and anxiety in mice. Brain Res Bull 2012; 87: 526-533.
7. Baiardi G, Ruiz AM, Beling A, Borgonovo J, Martinez G, Landa AI, Sosa MA, Gargiulo PA. Glutamatergic ionotropic blockade within accumbens disrupts working memory and might alter the endocytic machinery in rat accumbens and prefrontal cortex. J Neural Transm 2007; 114: 1519-1528.
8. Bardo MT, Hammer RP, Jr. Autoradiographic localization of dopamine D_1 and D_2 receptors in rat nucleus accumbens: resistance to differential rearing conditions. Neuroscience 1991; 45: 281-290.
9. Barkus C, McHugh SB, Sprengel R, Seeburg PH, Rawlins JN, Bannerman DM. Hippocampal NMDA receptors and anxiety: at the interface between cognition and emotion. Eur J Pharmacol 2010; 626: 49-56.
10. Bertoglio LJ, Carobrez AP. Previous maze experience required to increase open arms avoidance in rats submitted to the elevated plus-maze model of anxiety. Behav Brain Res 2000; 108: 197-203.
11. Bossert JM, Poles GC, Wihbey KA, Koya E, Shaham Y. Differential effects of blockade of dopamine D1-family receptors in nucleus accumbens core or shell on reinstatement of heroin seeking induced by contextual and discrete cues. J Neurosci 2007; 27: 12655-12663.
12. Cardinal RN, Everitt BJ. Neural and psychological mechanisms underlying appetitive learning: links to drug addiction. Curr Opin Neurobiol 2004; 14: 156-162.
13. Carvalho MC, Albrechet-Souza L, Masson S, Brandao ML. Changes in the biogenic amine content of the prefrontal cortex, amygdala, dorsal hippocampus, and nucleus accumbens of rats submitted to single and repeated sessions of the elevated plus-maze test. Braz J Med Biol Res 2005; 38: 1857-1866.
14. Cheer JF, Wassum KM, Sombers LA, Heien ML, Ariansen JL, Aragona BJ, Phillips PE, Wightman RM. Phasic dopamine release evoked by abused substances requires cannabinoid receptor activation. J Neurosci 2007; 27: 791-795.
15. Coccurello R, Adriani W, Oliverio A, Mele A. Effect of intra-accumbens dopamine receptor agents on reactivity to spatial and non-spatial changes in mice. Psychopharmacology (Berl) 2000; 152: 189-199.
16. Da Cunha IC, Jose RF, Orlandi Pereira L, Pimenta JA, Oliveira de Souza IA, Reiser R, Moreno H, Jr., Marino Neto J, Paschoalini MA, Faria MS. The role of nitric oxide in the emotional learning of rats in the plus-maze. Physiol Behav 2005; 84: 351-358.
17. da Cunha IC, Lopes AP, Steffens SM, Ferraz A, Vargas JC, de Lima TC, Marino Neto J, Paschoalini MA, Faria MS. The microinjection of AMPA receptor antagonist into the accumbens shell, but not into the accumbens core, induces anxiolysis in an animal model of anxiety. Behav Brain Res 2008; 188: 91-99.
18. Dalia A, Uretsky NJ, Wallace LJ. Dopaminergic agonists administered into the nucleus accumbens: effects on extracellular glutamate and on locomotor activity. Brain Res 1998; 788: 111-117.
19. Dawson VL, Dawson TM. Deadly conversations: nuclear-mitochondrial cross-talk. J Bioenerg Biomembr 2004; 36: 287-294.
20. Deutch AY, Cameron DS. Pharmacological characterization of dopamine systems in the nucleus accumbens core and shell. Neuroscience 1992; 46: 49-56.
21. Dunn RW, Corbett R, Fielding S. Effects of 5-HT1A receptor agonists and NMDA receptor antagonists in the social interaction test and the elevated plus maze. Eur J Pharmacol 1989; 169: 1-10.
22. Fernandes C, File SE. The influence of open arm ledges and maze experience in the elevated plus-maze. Pharmacol Biochem Behav 1996; 54: 31-40.
23. Floresco SB, Todd CL, Grace AA. Glutamatergic afferents from the hippocampus to the nucleus accumbens regulate activity of ventral tegmental area dopamine neurons. J Neurosci 2001; 21: 4915-4922.
24. Gal G, Joel D, Gusak O, Feldon J, Weiner I. The effects of electrolytic lesion to the shell subterritory of the nucleus accumbens on delayed non-matching-to-sample and four-arm baited eight-arm radial-maze tasks. Behav Neurosci 1997; 111: 92-103.
25. Garpenstrand H, Annas P, Ekblom J, Oreland L, Fredrikson M. Human fear conditioning is related to dopaminergic and serotonergic biological markers. Behav Neurosci 2001; 115: 358-364.
26. Guimaraes FS, Carobrez AP, De Aguiar JC, Graeff FG. Anxiolytic effect in the elevated plus-maze of the NMDA receptor antagonist AP7 microinjected into the dorsal periaqueductal grey. Psychopharmacology (Berl) 1991; 103: 91-94.
27. Hara Y, Pickel VM. Overlapping intracellular and differential synaptic distributions of dopamine D1 and glutamate N-methyl-D-aspartate receptors in rat nucleus accumbens. J Comp Neurol 2005; 492: 442-455.

28. Horger BA, Roth RH. The role of mesoprefrontal dopamine neurons in stress. Crit Rev Neurobiol 1996; 10: 395-418.
29. Huang YH, Ishikawa M, Lee BR, Nakanishi N, Schluter OM, Dong Y. Searching for presynaptic NMDA receptors in the nucleus accumbens. J Neurosci 2011; 31: 18453-18463.
30. Ikeda H, Kamei J, Koshikawa N, Cools AR. Nucleus accumbens and dopamine-mediated turning behavior of the rat: role of accumbal non-dopaminergic receptors. J Pharmacol Sci 2012; 120: 152-164.
31. Imperato A, Honore T, Jensen LH. Dopamine release in the nucleus caudatus and in the nucleus accumbens is under glutamatergic control through non-NMDA receptors: a study in freely-moving rats. Brain Res 1990; 530: 223-228.
32. Jacewicz M, Czapski GA, Katkowska I, Strosznajder RP. Systemic administration of lipopolysaccharide impairs glutathione redox state and object recognition in male mice. The effect of PARP-1 inhibitor. Folia Neuropathol 2009; 47: 321-328.
33. Jamali-Raeufy N, Nasehi M, Zarrindast MR. Influence of N-methyl D-aspartate receptor mechanism on WIN55,212-2-induced amnesia in rat dorsal hippocampus. Behav Pharmacol 2011; 22: 645-654.
34. Jay TM. Dopamine: a potential substrate for synaptic plasticity and memory mechanisms. Prog Neurobiol 2003; 69: 375-390.
35. Jongen-Relo AL, Kaufmann S, Feldon J. A differential involvement of the shell and core subterritories of the nucleus accumbens of rats in memory processes. Behav Neurosci 2003; 117: 150-168.
36. Kalivas PW, Duffy P. Dopamine regulation of extracellular glutamate in the nucleus accumbens. Brain Res 1997; 761: 173-177.
37. Kalivas PW, Duffy P, Barrow J. Regulation of the mesocorticolimbic dopamine system by glutamic acid receptor subtypes. J Pharmacol Exp Ther 1989; 251: 378-387.
38. Kew JN, Kemp JA. Ionotropic and metabotropic glutamate receptor structure and pharmacology. Psychopharmacology (Berl) 2005; 179: 4-29.
39. Kienast T, Heinz A. Dopamine and the diseased brain. CNS Neurol Disord Drug Targets 2006; 5: 109-131.
40. Kitamura M, Koshikawa N, Yoneshige N, Cools AR. Behavioural and neurochemical effects of cholinergic and dopaminergic agonists administered into the accumbal core and shell in rats. Neuropharmacology 1999; 38: 1397-1407.
41. Klein S, Hadamitzky M, Koch M, Schwabe K. Role of glutamate receptors in nucleus accumbens core and shell in spatial behaviour of rats. Neuroscience 2004; 128: 229-238.
42. Koshikawa N, Kitamura M, Kobayashi M, Cools AR. Contralateral turning elicited by unilateral stimulation of dopamine D2 and D1 receptors in the nucleus accumbens of rats is due to stimulation of these receptors in the shell, but not the core, of this nucleus. Psychopharmacology (Berl) 1996; 126: 185-190.
43. Kuo YM, Liang KC, Chen HH, Cherng CG, Lee HT, Lin Y, Huang AM, Liao RM, Yu L. Cocaine-but not methamphetamine-associated memory requires de novo protein synthesis. Neurobiol Learn Mem 2007; 87: 93-100.
44. Lee C, Rodgers RJ. Antinociceptive effects of elevated plus-maze exposure: influence of opiate receptor manipulations. Psychopharmacology (Berl) 1990; 102: 507-513.
45. Lee DK, Koh WC, Shim YB, Shim I, Choe ES. Repeated cocaine administration increases nitric oxide efflux in the rat dorsal striatum. Psychopharmacology (Berl) 2010; 208: 245-256.
46. Liu JL, Li M, Dang XR, Wang ZH, Rao ZR, Wu SX, Li YQ, Wang W. A NMDA receptor antagonist, MK-801 impairs consolidating extinction of auditory conditioned fear responses in a Pavlovian model. PLoS One 2009; 4: e7548.
47. Maldonado-Irizarry CS, Kelley AE. Excitatory amino acid receptors within nucleus accumbens subregions differentially mediate spatial learning in the rat. Behav Pharmacol 1995; 6: 527-539.
48. Martinez G, Ropero C, Funes A, Flores E, Blotta C, Landa AI, Gargiulo PA. Effects of selective NMDA and non-NMDA blockade in the nucleus accumbens on the plus-maze test. Physiol Behav 2002; 76: 219-224.
49. Martinez G, Ropero C, Funes A, Flores E, Landa AI, Gargiulo PA. AP-7 into the nucleus accumbens disrupts acquisition but does not affect consolidation in a passive avoidance task. Physiol Behav 2002; 76: 205-212.
50. Martinez RC, Oliveira AR, Macedo CE, Molina VA, Brandao ML. Involvement of dopaminergic mechanisms in the nucleus accumbens core and shell subregions in the expression of fear conditioning. Neurosci Lett 2008; 446: 112-116.
51. McGaugh JL. The amygdala modulates the consolidation of memories of emotionally arousing experiences. Annu Rev Neurosci 2004; 27: 1-28.
52. Mele A, Avena M, Roullet P, De Leonibus E, Mandillo S, Sargolini F, Coccurello R, Oliverio A. Nucleus accumbens dopamine receptors in the consolidation of spatial memory. Behav Pharmacol 2004; 15: 423-431.
53. Morari M, Marti M, Sbrenna S, Fuxe K, Bianchi C, Beani L. Reciprocal dopamine-glutamate modulation of release in the basal ganglia. Neurochem Int 1998; 33: 383-397.
54. Mudo G, Persson H, Timmusk T, Funakoshi H, Bindoni M, Belluardo N. Increased expression of trkB and trkC messenger RNAs in the rat forebrain after focal mechanical injury. Neuroscience 1993; 57: 901-912.
55. Nasehi M, Mafi F, Oryan S, Nasri S, Zarrindast MR. The effects of dopaminergic drugs in the dorsal hippocampus of mice in the nicotine-induced anxiogenic-like response. Pharmacol Biochem Behav 2011; 98: 468-473.
56. Nasehi M, Piri M, Nouri M, Farzin D, Nayer-Nouri T, Zarrindast MR. Involvement of dopamine D1/D2 receptors on harmane-induced amnesia in the step-down passive avoidance test. Eur J Pharmacol 2010; 634: 77-83.
57. Ozawa S, Kamiya H, Tsuzuki K. Glutamate receptors in the mammalian central nervous system. Prog Neurobiol 1998; 54: 581-618.
58. Paxinos G, Watson C. The Rat Brain in Stereotaxic Coordinates. 6rd ed. Academic Press, London 2007.
59. 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: 149-167.
60. Pezze MA, Feldon J. Mesolimbic dopaminergic pathways in fear conditioning. Prog Neurobiol 2004; 74: 301-320.
61. Plaznik A, Palejko W, Nazar M, Jessa M. Effects of antagonists at the NMDA receptor complex in two models of anxiety. Eur Neuropsychopharmacol 1994; 4: 503-512.

62. Rezayof A, Motevasseli T, Rassouli Y, Zarrindast MR. Dorsal hippocampal dopamine receptors are involved in mediating ethanol state-dependent memory. Life Sci 2007; 80: 285-292.
63. Riaza Bermudo-Soriano C, Perez-Rodriguez MM, Vaquero-Lorenzo C, Baca-Garcia E. New perspectives in glutamate and anxiety. Pharmacol Biochem Behav 2012; 100: 752-774.
64. Sargolini F, Florian C, Oliverio A, Mele A, Roullet P. Differential involvement of NMDA and AMPA receptors within the nucleus accumbens in consolidation of information necessary for place navigation and guidance strategy of mice. Learn Mem 2003; 10: 285-292.
65. Schacter GB, Yang CR, Innis NK, Mogenson GJ. The role of the hippocampal-nucleus accumbens pathway in radial-arm maze performance. Brain Res 1989; 494: 339-349.
66. Sesack SR, Pickel VM. In the rat medial nucleus accumbens, hippocampal and catecholaminergic terminals converge on spiny neurons and are in apposition to each other. Brain Res 1990; 527: 266-279.
67. Sesack SR, Pickel VM. Prefrontal cortical efferents in the rat synapse on unlabeled neuronal targets of catecholamine terminals in the nucleus accumbens septi and on dopamine neurons in the ventral tegmental area. J Comp Neurol 1992; 320: 145-160.
68. Setlow B, McGaugh JL. Sulpiride infused into the nucleus accumbens posttraining impairs memory of spatial water maze training. Behav Neurosci 1998; 112: 603-610.
69. Shin EH, Bian S, Shim YB, Rahman MA, Chung KT, Kim JY, Wang JQ, Choe ES. Cocaine increases endoplasmic reticulum stress protein expression in striatal neurons. Neuroscience 2007; 145: 621-630.
70. Smith-Roe SL, Kelley AE. Coincident activation of NMDA and dopamine D1 receptors within the nucleus accumbens core is required for appetitive instrumental learning. J Neurosci 2000; 20: 7737-7742.
71. Smith-Roe SL, Sadeghian K, Kelley AE. Spatial learning and performance in the radial arm maze is impaired after N-methyl-D-aspartate (NMDA) receptor blockade in striatal subregions. Behav Neurosci 1999; 113: 703-717.
72. Smith JA, Mo Q, Guo H, Kunko PM, Robinson SE. Cocaine increases extraneuronal levels of aspartate and glutamate in the nucleus accumbens. Brain Res 1995; 683: 264-269.
73. Solati J. Dorsal hippocampal N-methyl-D-aspartate glutamatergic and delta-opioidergic systems modulate anxiety behaviors in rats in a noninteractive manner. Kaohsiung J Med Sci 2011; 27: 485-493.
74. Stern CA, Do Monte FH, Gazarini L, Carobrez AP, Bertoglio LJ. Activity in prelimbic cortex is required for adjusting the anxiety response level during the elevated plus-maze retest. Neuroscience 2010; 170: 214-222.
75. Strosznajder JB, Jesko H, Strosznajder RP. Age-related alteration of poly(ADP-ribose) polymerase activity in different parts of the brain. Acta Biochim Pol 2000; 47: 331-337.
76. Strosznajder RP, Jesko H, Adamczyk A. Effect of aging and oxidative/genotoxic stress on poly(ADP-ribose) polymerase-1 activity in rat brain. Acta Biochim Pol 2005; 52: 909-914.
77. Strosznajder RP, Jesko H, Zambrzycka A. Poly(ADP-ribose) polymerase: the nuclear target in signal transduction and its role in brain ischemia-reperfusion injury. Mol Neurobiol 2005; 31: 149-167.
78. Tonetto LL, Terzian AL, Del Bel EA, Guimaraes FS, Resstel LB. Inhibition of the NMDA receptor/Nitric Oxide pathway in the dorsolateral periaqueductal gray causes anxiolytic-like effects in rats submitted to the Vogel conflict test. Behav Brain Funct 2009; 5: 40.
79. Treit D, Menard J, Royan C. Anxiogenic stimuli in the elevated plus-maze. Pharmacol Biochem Behav 1993; 44: 463-469.
80. Voorn P, Jorritsma-Byham B, Van Dijk C, Buijs RM. The dopaminergic innervation of the ventral striatum in the rat: a light- and electron-microscopical study with antibodies against dopamine. J Comp Neurol 1986; 251: 84-99.
81. Walaas I. Biochemical evidence for overlapping neocortical and allocortical glutamate projections to the nucleus accumbens and rostral caudatoputamen in the rat brain. Neuroscience 1981; 6: 399-405.
82. Wang W, Dever D, Lowe J, Storey GP, Bhansali A, Eck EK, Nitulescu I, Weimer J, Bamford NS. Regulation of prefrontal excitatory neurotransmission by dopamine in the nucleus accumbens core. J Physiol 2012; 590: 3743-3769.
83. Wang Y, Dawson VL, Dawson TM. Poly(ADP-ribose) signals to mitochondrial AIF: a key event in parthanatos. Exp Neurol 2009; 218: 193-202.
84. Wong AC, Shetreat ME, Clarke JO, Rayport S. D1- and D2-like dopamine receptors are co-localized on the presynaptic varicosities of striatal and nucleus accumbens neurons in vitro. Neuroscience 1999; 89: 221-233.
85. Yang JH, Choe ES. Repeated cocaine administration increases cleaved poly(ADP-ribose) polymerase-1 expression in the rat dorsal striatum. Neurosci Lett 2010; 471: 58-61.
86. Yu SW, Wang H, Dawson TM, Dawson VL. Poly(ADP-ribose) polymerase-1 and apoptosis inducing factor in neurotoxicity. Neurobiol Dis 2003; 14: 303-317.
87. Zahm DS, Heimer L. Specificity in the efferent projections of the nucleus accumbens in the rat: comparison of the rostral pole projection patterns with those of the core and shell. J Comp Neurol 1993; 327: 220-232.
88. Zahrt J, Taylor JR, Mathew RG, Arnsten AF. Supranormal stimulation of D1 dopamine receptors in the rodent prefrontal cortex impairs spatial working memory performance. J Neurosci 1997; 17: 8528-8535.
89. Zajaczkowski W, Frankiewicz T, Parsons CG, Danysz W. Uncompetitive NMDA receptor antagonists attenuate NMDA-induced impairment of passive avoidance learning and LTP. Neuropharmacology 1997; 36: 961-971.
90. Zarrindast MR, Khalifeh S, Rezayof A, Rostami P, Aghamohammadi Sereshki A, Zahmatkesh M. Involvement of rat dopaminergic system of nucleus accumbens in nicotine-induced anxiogenic-like behaviors. Brain Res 2012; 1460: 25-32.
91. Zarrindast MR, Naghdi-Sedeh N, Nasehi M, Sahraei H, Bahrami F, Asadi F. The effects of dopaminergic drugs in the ventral hippocampus of rats in the nicotine-induced anxiogenic-like response. Neurosci Lett 2010; 475: 156-160.
92. Zarrindast MR, Nasehi M, Khansari M, Bananej M. Influence of nitric oxide agents in the rat amygdala on anxiogenic-like effect induced by histamine. Neurosci Lett 2010; 489: 38-42.
93. Zarrindast MR, Nasehi M, Khansari M, Bananej M. Influence of nitric oxide agents in the rat amygdala on anxiogenic-like effect induced by histamine. Neurosci Lett 2011; 489: 38-42.

94. Zarrindast MR, Nasehi M, Pournaghshband M, Ghorbani Yekta B. Dopaminergic system in CA1 modulates MK-801 induced anxiolytic-like responses. Pharmacol Biochem Behav 2012; 103: 102-110.
95. Zarrindast MR, Solati J, Oryan S, Parivar K. Effect of intra-amygdala injection of nicotine and GABA receptor agents on anxiety-like behaviour in rats. Pharmacology 2008; 82: 276-284.
96. Zarrindast MR, Sroushi A, Bananej M, Vousooghi N, Hamidkhaniha S. Involvement of the dopaminergic receptors of the rat basolateral amygdala in anxiolytic-like effects of the cholinergic system. Eur J Pharmacol 2011; 672: 106-112.
97. Zarrindast MR, Torabi M, Rostami P, Fazli-Tabaei S. The effects of histaminergic agents in the dorsal hippocampus of rats in the elevated plus-maze test of anxiety. Pharmacol Biochem Behav 2006; 85: 500-506.
98. Zarrindast MR, Valizadegan F, Rostami P, Rezayof A. Histaminergic system of the lateral septum in the modulation of anxiety-like behaviour in rats. Eur J Pharmacol 2008; 583: 108-114.