EFFECTS OF PRAMIPEXOLE ON LEARNING AND MEMORY PROCESSES IN NAÏVE RATS AND RATS WITH HALOPERIDOL-INDUCED DOPAMINERGIC BLOCKADE

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Abstract:

Parkinson's disease is the second most common neurodegenerative disease. It is usually well recognized by its motor symptoms. The non-motor symptoms, including cognitive deficits are object of interest in the last few decades. The **aim** of our study was to assess the effects of the dopamine agonist pramipexole on learning and memory in naïve and haloperidol-challenged rats. **Material and methods:** Male Wistar rats divided into 9 groups (n=8): naïve -saline, pramipexole 0,5; 1 and 3 mg/kg bw; Haloperidol groups- saline, haloperidol, haloperidol + pramipexole 0,5; 1 and 3 mg/kg bw. Step-through passive avoidance test and activity cage were performed. The observed parameters were latency of reaction and vertical and horizontal movements. Statistical analysis was done by SPSS Statistics 19.**Results:** in naïve rats the animals treated with 0,5mg/kg pramipexole significantly increased ($p<0,05$) the latency on 2nd training day, as well during the short and long-term memory tests, compared to the control group. Pramipexole 1 mg/kg did not change significantly the latency during the training session nor during the memory tests. Rats with 3 mg/kg pramipexol prolonged ($p<0,05$) the reaction time on the 1st learning day and the long-term memory test. None of the groups with haloperidol and pramipexole showed significant latency increase during the retention tests when compared to the control groups.**Conclusion:** pramipexole improves learning and memory in naïve rats but not in haloperidol-challenged which indirectly confirms the involvement of dopamine in these processes.

Key words: *learning; memory; pramipexole; dopamine.*

Intoduction:

Parkinson's disease (PD) is one of the most common neurodegenerative diseases following Alzheimer's disease (Fahn, 2004). It affects nearly 1% of the population at age 60 and over (Lin, et al 2017). The diagnosis is usually based on its motor symptoms, such as bradykinesia, loss of postural reflexes, muscle rigidity and rest tremor (Papagno, et al 2017). A good number of clinical studies and experimental findings in animal models helped to recognize PD as something more than just a motor-deficit disease (Yang et al. 2016). Many PD patients suffer not only from motor disabilities but also from non-motor symptoms (NMS) -such as sleep disturbances, autonomic dysfunction, sensory dysfunction and cognitive impairment (Schönberger, et al 2013). All these non-motor disturbances have a significant impact on patient's quality of life (Menza, et al.2010). The cognitive decline can occur at an early stage of the disease and precede the motor symptoms; they also can vary from mild cognitive impairment (PD-MCI) to Parkinson's disease-related dementia (PDD) (Wang, et al. 2015). A growing body of evidence shows that the progression of cognitive impairment is common and fairly quick (Hershey, 2015). The main hallmark in PD pathogensesis is progressive irreversible loss of dopaminergic neurons in substantia nigra pars compacta (SNpc), leading to strial dopamine depletion, and the presence of alfa-synuclein containing bodies, known as Lewy bodies (LB) (Chauhuri, et al 2006). Whereas the motor symptoms are related to the progressive degenartion of dopaminergic neurons in nigrostrial pathway, the pathology of the cognitive disturbances is still unclear (Halliday, et al. 2014). Some clinical studies suggest that the cognitive impairment is linked to the dopaminergic loss in SNpc but also dependent on the prefrontal cortex, amygdala and hippocampus (Ray, 2012). Other clinical researchers have found hippocampal atrophy in PD patients, some of which presented with memory decline (Calabresi et al. 2013). These results are confirmed

by animal experimental models which reveal that the impaired memory and cognition are mediated by brain structures as hippocampus and prefrontal cortex as well as the striatum (Solari,et al. 2013). Both, clinical and preclinical research recognize the association of the hippocampus with emotions, cognition and memory (Castro-Hernández, et al. 2017). The motor symptoms have long been the priority of the pharmaceutical treatment while the management of the non-motor symptoms remains as unmet clinical need (París, et al 2011).

Dopamine receptor agonists, such as Pramipexole, are beneficial in ameliorating the motor symptoms but their effect on the cognition remains fairly obscure (Relja, 2006). Pramipexole is a second generation, non-ergot alkaloid dopamine agonist with high affinity for D_3/D_2 receptors however it displays higher selectivity for D_3 than D_2 and D_4 subtypes (Tokunaga, et al. 2012). As dopamine agonist, pramipexole may be valuable in the treatment of cognitive disturbances since they increase the dopaminergic neurotransmission in different brain areas. Dopamine and its regulation most probably play crucial role in variety of hippocampal functions, including learning and memory (Castro-Hernández et al. 2017).

Aim:

The aim of our study was to assess the effect of the dopamine agonist pramipexole on learning and memory in naïve rats and rats with haloperidol-induced dopaminergic blockade.

Materials and Methods:

Ethical statement: All experimental procedures were carried out in accordance with the European Convention for protection of Vertebrate Animals used for Experimental and Other Scientific Purposes. For this study we obtained permission from the Ethics Committee at Medical University of Plovdiv, protocol № 2/19.04.2018 and the Bulgarian Food Safety Agency, permit № 4/09.12.2015.

Drugs: Pramipexole (PMX) and haloperidol (HP) purchased from Sigma-Aldrich.

Animals: Ault male rats of Wistar strain (200 ± 20 gr body weight) were used. They were housed in standard cages under controlled laboratory conditions (08:00-20:00 light-dark cycle, temperature 22 ± 2 ºC, humidity 55 ± 5%). The animals had access to food and water *ad libitum*. Overall the laboratory room was well ventilated, quiet and with no vibrations.

Design: To evaluate the effect of PMX on learning and memory in naïve rats they were divided randomly into 4 groups (n=8) as follows: 1st group – (control) saline 0,1 ml/100 gr body weight; 2nd group - PMX 0,5mg/kg bw; 3rd group - PMX 1 mg/kg bw; 4th group - PMX – 3 mg/kg bw; To evaluate the effect of PMX on learning and memory in rats with haloperidol-induced dopaminergic blockade the animals were randomly divided into 5 groups (n=8) as follows: 1st group – (control) saline 0,1 ml/100 gr body weight; 2nd group – (control) haloperidol 1mg/kg bw; 3rd group - HP + PMX 0,5 mg/kg bw; 4th group - HP + PMX 1 mg/kg bw; 5th group - HP + PMX 3 mg/kg bw.PMX was given orally (p.o.) while haloperidol was administered intraperitoneally (i.p.). All animals were pretreated with PMX for 7 days. HP was administered only during the testing days 60 minutes before the tests. PMX was administered 60 minutes before HP.

Haloperidol challenge: HP acts as a of D_2/D_1 receptors antagonist. Usually, it is administered intraperitoneally in dose 1 mg/kg bw and the dopamine strial depletion is manifested with muscle rigidity and catalepsy within 1 hour of the injection (Kulkarni, et al. 2009).

Behavioral tests

One-way step-through inhibitory "passive" avoidance test: we used a two-compartment set up (UgoBasile, Italy), with one light and one dark compartment divided by a sliding automatic door. The learning session was performed in 2 consequtive days. The rat is placed in the light box,

following a door delay of 7 second it gets access to the dark compartment. When entering the dark chamber (training latency) the door shuts down and the rat is subjected to a brief aversive stimulus (UC, electrical foot shock for 9 sec with the intensity of 0.4 mA). The latent time for reaction was used as a criterion for learning and retention. The animals that remained in the light chamber for more 178 sec were considered as trained. Short-memory test was carried out 24 hour later (on the 3rd day) and the long-term memory retention was tested on the 10th day. Both, learning and memory retention sessions consisted of 3 trials. The memory retention test was performed without the electrical shock.

Loco-motor activity (activity cage) test: an automatic apparatus (multiple activity cage, UgoBasile, Italy) was used to assess horizontal and vertical spontaneous movements of the animals. The set-up comprises an electronic unit and an Infra-Red Beam cage complete with two sets of sensor arrays for horizontal and vertical activity. The animal is placed into the plastic cage for 5 minutes. The movement it makes inside the cage interrupts one or more infra-red beam(s). The beam interruptions are counted and recorded by the electronic device.

Statistics: Statistical analysis was performed by using IBM SPSS Statistics 19.0. Data was expressed as mean ± SEM of values for memory tests and initial latency. Shapiro-Wilk test showed normal distribution between the groups. Comparison between groups was performed with Independent Sample T test. A value of $p<0,05$ was considered as a significant difference.

Results:

Effects of pramipexole on one-way step-through inhibitory "passive" avoidance test in naïve rats: the animals treated with the lowest dose of PMX (0,5 mg/kg bw) significantly increased the latency on the 2nd training day ($p<0,05$) and during the two tests for short and long-term memory ($p<0,05$), compared to the respective day control group. The rats given PMX in dose 1 mg/kg bw did not show significant increase of the latent period during the learning phase, nor the memory retention tests, when compared to the control rats. The animals treated with the highest dose of PMX (3 mg/kg bw) significantly increased the time for reaction during the long-term memory test on the 10th($p<0,05$), compared to the control group for the respective day. Fig. 1

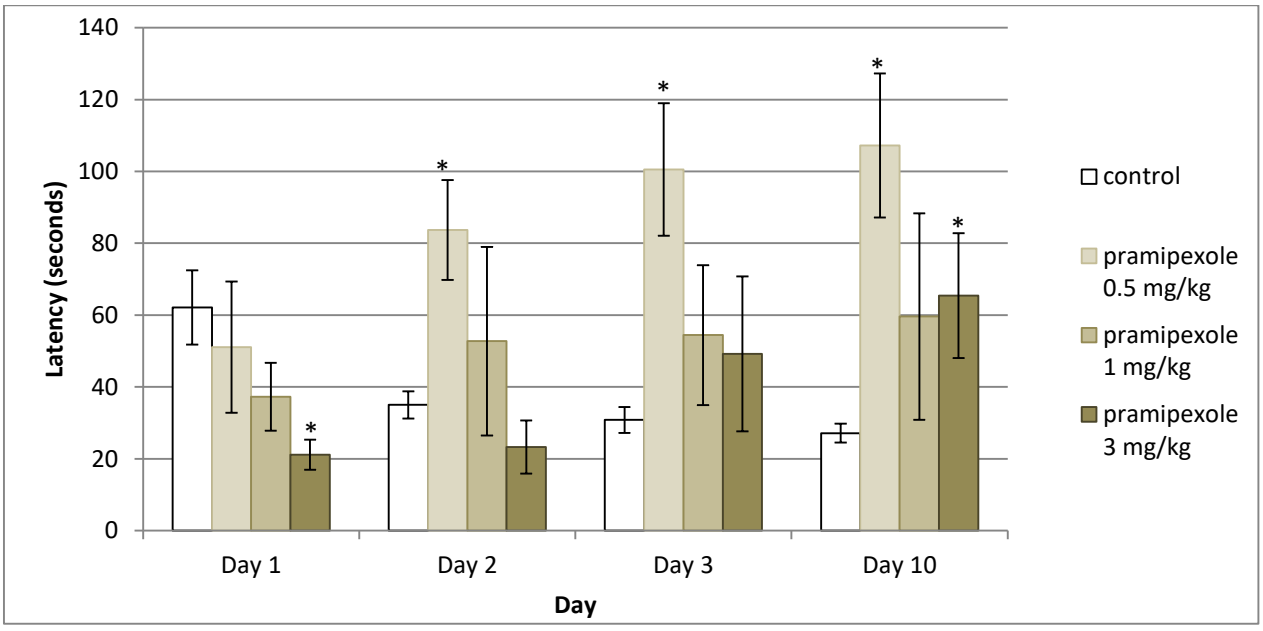

Fig. 1 Effects of pramipexole on the latency in step-through test in naïve rats.

**p<0,05 versus the saline control group*

Effects of pramipexole on Loco- motor activity test in naïve rats: the three experimental groups significantly increased the number of relative units on horizontal (p<0,0001) and vertical (p<0,0001) movements, compared to the saline group. The animals treated with the highest dose of pramipexole are more active in both planes (p<0,05) than the animals treated with the lower doses of pramipexole. Fig. 2

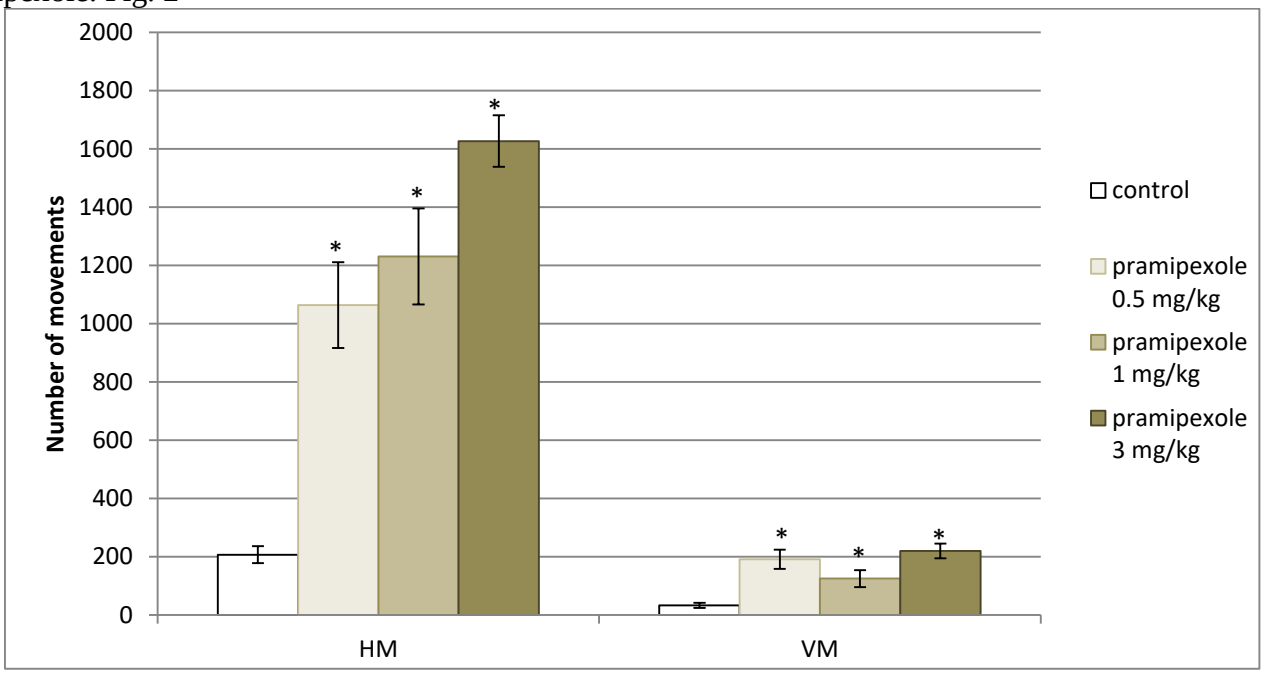

Fig. 2 Effects of pramipexole on motor activity in naïve rats.
**p<0,001 versus the saline control group*

Effects of pramipexole on one-way step-through inhibitory "passive" avoidance test in haloperidol-challenged rats: the animals treated with HP significantly decreased the latency on the 2nd training day, as well as on the 3rd day during the short-term memory test (p<0,05), in comparison with the saline control group for the respective day. The rats given HP and 0,5 mg/kg bw PMX significantly increased the latent period during the two days of learning (p<0,05), when compared with both control groups. The group with HP and PMX in dose 1 mg/kg bw significantly increased the latency only on the 2nd day of the training session (p<0,05), compared two both control groups. The animals treated with HP and the highest dose of PMX significantly decreased the time spent in the light chamber on the 1st training day (p<0,05) when compared to both control groups. However, none of the experimental groups showed retention of memory traces during the short and long-term memory tests. Fig. 3.

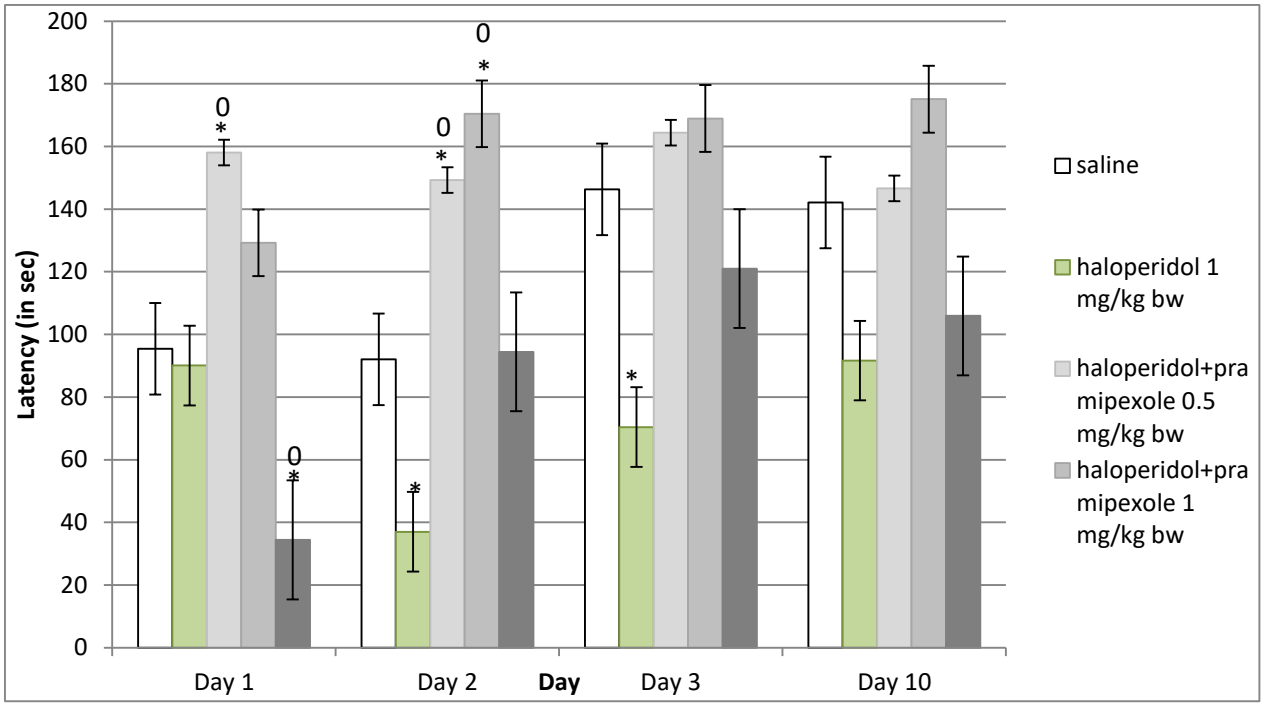

Fig. 3 Effects of pramipexole on the latency in step-through test in haloperidol-challenged rats.
**p<0,05 versus the saline control group*
^{0}p<0,05 versus the haloperidol control group

Effects of pramipexole on activity-cage test in haloperidol-challenged rats: the group treated with HP did not significantly decrease the number of horizontal and vertical movements when compared to the saline control group**.** The rats treated with HP and PMX in dose 0,5 mg/kg bw did not show significant increase in motor activity compared to both control groups. The animals treated with HP and 1 mg/kg and 3 mg/kg bw PMX significantly increased the number of relative units on horizontal and vertical movements, compared to the animals with saline ($p<0,05$) and those with HP ($p<0,0001$). The animals treated with HP and PMX in dose 1 and 3 mg/kg bw significantly increased the number of relative units on horizontal and vertical movements ($p<0,05$), compared to the animals with HP and the lowest dose of PMX. Fig. 4

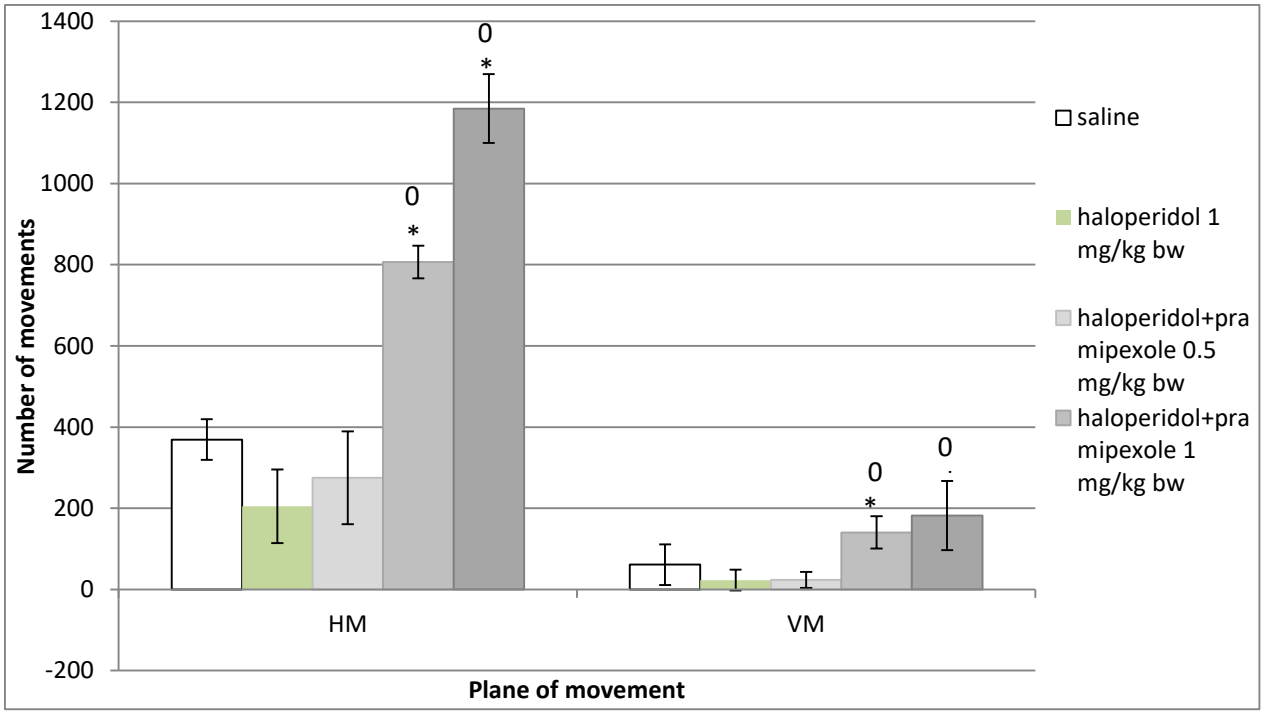

Fig. 4 Effects of pramipexole on motor activity in haloperidol-challenged rats.
**p<0,05 versus the saline control group*
^{0}p<0,0001 versus the haloperidol control group

Discussion:

Our study showed that in naïve rats pramipexole in the lowest experimental dose (0,5 mg/kg bw) significantly improved both types of memory. The highest dose of pramipexole enhanced only the long-term memory. All studied doses improved the loco-motor activity of the animals. Both effects are probably due to pramipexole's effect on dopaminergic neurotransmission but in different brain areas. In haloperidol-challenged rats the animals treated with pramipexole in dose 0,5 and 1 mg/kg bw did not retain memory traces during the memory consolidation tests. The loco-motor activity was ameliorated (raised) in the animals with HP and the two higher doses of PMX.

The hippocampus is a complex structure that belongs to the limbic system and plays very impoprtant role in learning and memory processes. It is known that the hippocampus has two parts - ventral and dorsal. Bagot et al. suggested that the ventral part is related to stress reactions, depression and emotions whereas the dorsal hippocampus is responsible for cognition and different types of memory (Bagot, et al. 2015). Various studies focusing on hippocampus-dependent learning and memory help us to assume that dopamine is one of the major modulators of these processes as reviewed by Edelmann and Lessmann (Edelmann, 2018). The major mechanisms implicated in the processes of learning and memoryare neurogenesis and long-term neuronal potentiation (LTP) which stimulate the synaptic transmission (Noble, 2014). Castro-Hernandez J et al. proved that pramipexole potentiates the LTP like other D3 agonists (Castro-Hernández J, 2017). Our results confirm that pramipexole has beneficial effect on LTP and memory consolidation in *in vivo* experiments. The passive avoidance tests used in our study are dependent on the hippocampal integrity (Izquierdo et al.2006).

In order to establish the probable mechanism of the observed effects we conducted an experiment with haloperidol-challenged rats. Haloperidol is a dopamine receptor antagonist with higher affinity for dopamine D2 receptors than D3 receptors (D2/D3 Ki ratio, 0,195) (Hashimoto T., 2018). In our study it decreased the effect of PMX on learning and memory. Significant improvement was detected only during the learning phase but not the memory retention tests probably due to the antagonizing effect of haloperidol on pramipexole efficiency. That indirectly confirms the dopamine involvement in learning and memory processes.

Conclusion:

PMX improved learning and memory in naïve rats probably by hippocampal dependent mechanism and dopaminergic modulation plays an essential role in the observed effect.

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