Delayed Effects of NMDA Receptor Antagonist MK-801 on Storage and Reconsolidation of Spatial Memory in Rats

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We studied the effects of NMDA receptor antagonist MK-801 on the storage and reconsolidation of spatial memory in Morris water maze in adult rats. MK-801 (50 μg/kg) administered 24 h after the completion of training was shown to improve the resistance of spatial memory to spontaneous extinction, while reminder against the background of MK-801 suppresses its ameliorating effect on memory storage. The detected behavioral effects of MK-801 persisted over 60 days after administration and can be associated with its influence on coupled neurogenesis/apoptosis processes induced during memory trace formation in adult animals.

Key Words: *glutamate NMDA glutamat receptors; MK-801; learning; spatial memory; rats*

Evaluation of the role of NMDA glutamate receptors in the mechanisms of memory and learning is a pressing theoretical and practical problem in modern neurobiology. Large body of evidence demonstrates impairment of consolidation and reconsolidation, as well as extinction of different types of memory following administration of NMDA receptor antagonists; however, under pathological conditions (stroke, trauma) and during aging, these compounds can restore impaired cognitive functions [1-3,6,9,13]. Experiments showed that NMDA receptor antagonists in doses of 0.05-0.15 mg/kg can stimulate learning and memory [14]. The diversity of the effects of NMDA receptor antagonists is considered to be associated with multiplicity of behavioral mechanisms of their effects at the neurochemical and morphological levels, particularly with ambiguous effects on adult neurogenesis. Thus, low doses of MK-801 (0.05-0.10 mg/kg) were shown to stimulate neurogenesis in adults [12]. In this regard, there is particular interest in investigation of MK-801 effects on the formation, storage, and reconsolidation of long-term memory within the periods comparable with the time of integration of newly formed cells into neuroglial assemblies in adult brain, *i.e.* 2-8 weeks after administration. However, such studies have never been conducted before.

Here we studied the effects of NMDA receptor antagonist dizocilpine maleate (MK-801) on storage and reconsolidation of spatial memory in adult rats at different terms after its administration.

MATERIALS AND METHODS

Experiments were carried out on adult male Wistar rats (n=144, body weight 280-350 g) obtained from Stolbovaya laboratory animal nursery. The experiments were conducted in accordance with Council of the European Communities Rules (Directive 86/609/EEC dated November 24 1986). During 2-week adaptation to the vivarium condition and throughout the experiment, the animals were kept at 21±1°C and 12 daylight cycle with free access to food and water.

The animals were trained in Morris water maze: a gray pool 160 cm in diameter and 60 cm high filled with water up to 40 cm. Transparent round Plexiglas platform was placed in the center of one of the quadrants 2 cm below the water surface. The maze was situated in a room with numerous contextual spatial cues: stands, calendars, furniture, *etc*. Initial training was carried out for 4 days with 24-h interval be-

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tween the sessions. The rat was placed into the water from four different randomly chosen points. When rat reached the platform, it was left there for 15 sec and then placed into an individual cage for 60 sec. The rats that did not reach the platform were gently guided to it. The time to platform was registered in each trial. During initial training, the disposition of spatial cues and platform was constant.

NMDA blocker MK-801 (Sigma) was administered intraperitoneally in a dose of 50 μg/kg in saline (1 ml/kg).

According to the time of finding the platform in last training trial, four groups equal in terms of behavioral skills were formed (36 rats in each) and exposed to the following agents 24 h after the accomplishment of training: group 1 (control) received 1 ml/kg saline in the home cage; group 2 received saline with subsequent reminder procedure in Morris water maze (swimming in the pool for 60 sec without the platform); group 3 received MK-801 (50 μg/kg) in the home cage; group 4 received MK-801 with subsequent reminder procedure in Morris water maze. Reminder procedure was conducted immediately after the injection. Morris water maze performance was tested 11, 30, and 60 days after the injection (12 rats per point).

The results were statistically processed by nonparametric Mann–Whitney test and Wilcoxon test using Statistica 6.0 software.

RESULTS

Analysis of the time to platform in four training sessions revealed no statistically significant differences between the groups. Statistical analysis of the sample showed that the parameter distribution significantly differed from the normal one: symmetry coefficient was 1.71±0.29, *i.e.* significantly differed from zero. In addition, comparison of the data obtained during testing on day 11 after saline administration to group 1 rats showed that dispersion of the time to platform values was significantly higher than during the last training day ($F_{(2,23)}$=4.98, p<0.05). It is indicative of substantial variability of spatial memory trace stability. Taking into account the sample heterogeneity in terms of both initial learning capacity and spatial memory stability, the analysis was carried out separately for good learners and poor learners as reflected by mean time to platform during the last training day (median value in the total sample served as the reference value, 10.25 sec).

Comparison of individual times to platform in the last training session (Med4) and in test session (Medt) detected the following regularities: good learners (Med4<median) demonstrated spontaneous memory extinction during the period between training and testing (Medt values were statistically significantly higher than Med4 values, Fig. 1). At the same time, poor learners (Med4>median) demonstrated spontaneous strengthening of spatial skill, *i.e.* Medt in these animals was significantly shorter than Med4 (Fig. 1). Spatial memory retrieval in rats exposed to reminder against the background of saline administration was similar to the control animals. MK-801 administration in the home cage enhanced spatial memory stability in good learners: the mean time to platform during testing was close to that on the last training day in that group (8.56±1.64 sec *vs.* 7.94±0.56). Reminder immediately after MK-801 administration eliminated its ameliorating effect on spatial memory stability in good learners: Medt in group 4 rats were similar to those in groups 2 and 3 and the rats reached the platform significantly slower than on the last training day (Fig. 1). No substantial effects of MK-801, reminder, or their combination on spatial memory preservation were observed in poor learners (Fig. 1). Similar pattern was observed when the test was conducted 30 days after the injection (Fig. 2).

Testing in 60 days after MK-801 administration (day 61 after the end of training) revealed memory extinction in both good and poor learners (Fig. 3). In addition, MK-801 administration in the home cage improved skill retention in both good learners and poor learners (Fig. 3). It should be noted that the reminder against the background of MK-801 administration did not completely abolish the ameliorating effect of this compound on spatial skill retention in poor learners (but not in good learners) in 60 days after administration (Fig. 3).

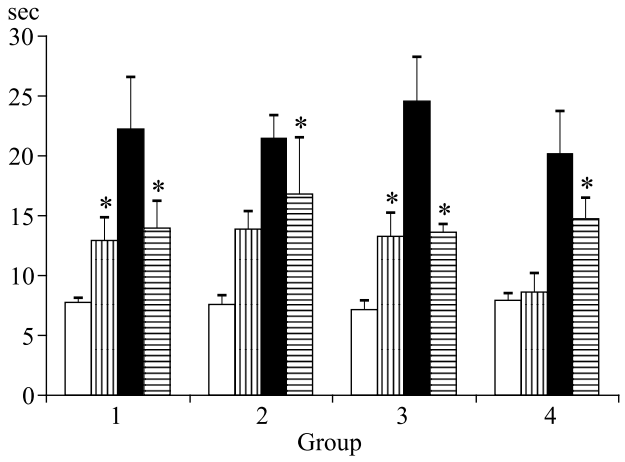

Fig. 1. Effects of MK-801 alone and in combination with reminder on representation of spatial skill in adult rats when tested 11 days after administration. Here and in Figs. 2 and 3: ordinate: time to platform, mean value for the session. Light bars: good learners, day 4 of training; vertical shading: good learners, test session; dark bars: poor learners, day 4 of training, horizontal shading: poor learners, test session. *p<0.05 in comparison with day 4 of training.

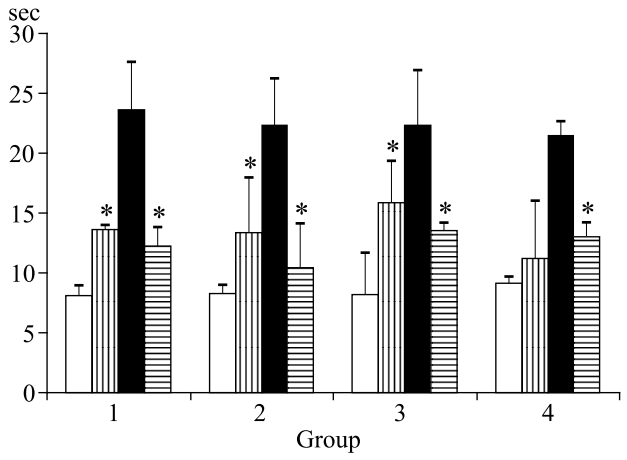

Fig. 2. Effects of MK-801 alone and in combination with reminder on spatial performance in adult rats in 30 days after administration.

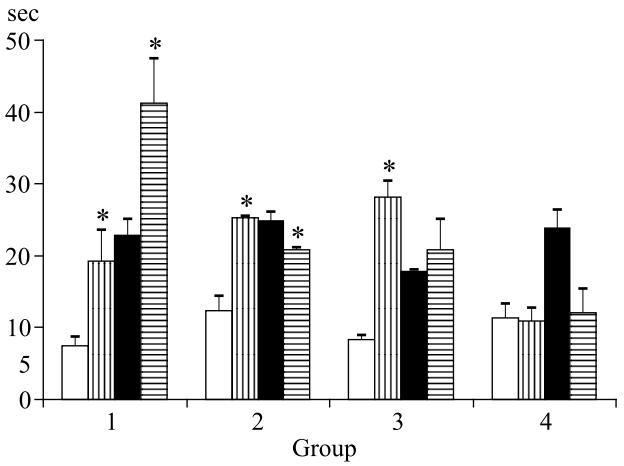

Fig. 3. Effects of MK-801 alone and in combination with reminder on spatial performance in adult rats in 60 days after administration.

All these findings demonstrated that MK-801 administration 24 h after completion of spatial skill training in Morris water maze enhanced memory trace retention by increasing its resistance to spontaneous extinction. This effect persisted for 60 days after administration of the antagonist. In addition, the reminder procedure carried out immediately after MK-801 injection diminished its effects on storage of the spatial skill.

Impairment of memory extinction under the effect of MK-801 has been demonstrated in various studies; however, most of the studies employed active extinction (*i.e.* repeated presentation of conditioned stimulus without reinforcement) [4,5,8,13]. Moreover, the effects of NMDA receptor antagonists on memory trace extinction depend on the experimental conditions, particularly, on the duration of training and extinction procedures [8], preliminary adaptation to conditioned stimulus [4], and learning capacity of experimental animals [13]. We found that MK-801 also enhances memory trace resistance to spontaneous passive extinction. This effect appeared to be protracted, up to 60 days, in comparison with 7-10 days for the effects demonstrated in other studies. Our findings suggest that the effects of MK-801 on extinction can be mediated via modulation of coupled neurogenesis/apoptosis processes in the adult brain. We also showed that the effects of MK-801 on memory trace preservation could be reduced or even eliminated when antagonist administration is combined with the reminder procedure. There are published reports that memory trace extinction may be both NMDA dependent and NMDA independent [5,7]. It is also known that activation of various mechanisms of memory formation and extinction, particularly, mechanisms associated with NMDA receptors, depends on a number of peptide and hormonal factors; the levels of these factors in turn vary under different training conditions [10]. One may assume that reminder procedure in our experiments resulted in altered spontaneous extinction of spatial memory by reduction of its dependence on NMDA receptors. In addition, many hormones involved in switch between NMDA dependent and NMDA independent memory mechanisms, specifically fibroblast growth factor and steroid hormones are also involved in neurogenesis/apoptosis regulation in the adult brain [11,15].

Thus, present knowledge indicates that NMDA receptor involvement in long-term memory mechanisms, particularly in storage and retrieval of memory trace, can be determined by their role in the regulation of proliferation, differentiation, and survival of new cells in the adult brain, which are necessary for learning and memory. This hypothesis can be verified by direct quantitative and qualitative assessment of neurogenesis/apoptosis processes in animal brain using the experimental model presented in current study.

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