Neurogenesis Enhancer RO 25-6981 Facilitates Repeated Spatial Learning in Adult Rats

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The effects of Ro 25-6981 (selective NMDA receptor blocker) in a dose stimulating neurogenesis on repeated learning, reversal learning, and memory reconsolidation were studied in adult rats in Morris water maze. Ro 25-6981 facilitated repeated learning 13 days after injection, but did not influence reversal learning. The blocker injected directly before reminder did not disturb repeated learning and reversal learning in Morris water maze. These effects of Ro 25-6981 on the dynamics of repeated learning seemed to be due to its effects on neurogenesis processes in adult brain.

Key Words: *memory reactivation; learning; neoneurogenesis; Morris water maze; NMDA receptor antagonist*

New nerve and glial cells involved into the mechanisms of learning and memory are incessantly forming in adult human and animal brain structures (neoneurogenesis) [4,7,12,13]. Substances suppressing or stimulating proliferation, differentiation, and death of new cells in the adult brain, for example, NMDA glutamate receptor antagonists, are widely used in studies of the relationships between neurogenesis and learning. For example, MK-801 (nonselective blocker of NMDA receptors) stimulates proliferation of granular neuronal precursor cells and increases the counts of new neurons in the hippocampal dentate gyrus [15]. Injection of MK-801 under conditions of memory reactivation causes its disorders [2]. Systemic administration of Ro 25-6981 (selective blocker of NMDA receptor NR2B subunit) stimulates neurogenesis in the hippocampus and facilitates the formation of spatial memory in adult rats in Morris water maze [11]. On the other hand, we have shown that behavioral effects of this substance in rats trained under novel conditions depend on the periods of the substance injection and initial learning capacity of animals [1].

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For better understanding of neoneurogenesis contribution into learning and memory mechanisms, we studied the effects of systemic Ro 25-6981 on repeated learning, reversal learning, and long-term memory reconsolidation in adult male rats in Morris water maze.

MATERIALS AND METHODS

The study was carried out on adult male Wistar rats (n=54) from Stolbovaya Breeding Center. All manipulations on animals were carried out in accordance with the regulations on humane handling of experimental animals (EC Directive No. 86/609/EEC of November 24, 1986). During 2-week adaptation to the vivarium conditions and experiments, the animals were kept in cages, 4 per cage, with free access to water and fodder at 21±1°C and 12-h light:dark regimen.

The rats were trained in Morris water maze (basin with gray inner surface, 160 cm in diameter, 60 cm high) filled with water to a height of 40 cm, 23±3°C. Transparent (Plexiglas) round platform 9 cm in diameter was located in the center of one of the squares of the basin 2 cm below the water surface. The basin was placed in a room with numerous situational spatial clues. Initial learning was carried out for 4 days with 24-h intervals between the sessions. During a session,

the animal was put into water from 4 points at random. After the rat reached the platform, it was left there for 15 sec, and then placed into an individual cage for 60 sec. The rats that failed to find the platform in 60 sec were gently guided to it. The time needed to reach the platform was recorded in each test with a timer and videorecords (Samsung VP-D355i digital videocam). The location of situational clues and the platform remained unchanged throughout the initial learning period. Four groups of animals were formed by paired equilibration of the time needed to find the platform on the last day of learning (day 4): 1) injection of Ro 25-6981 before reminder in experimental room (n=14); 2) injection in vivarium without reminder (n=14); 3) saline before reminder in experimental room (n=14); and 4) saline injection in vivarium without reminder (control, n=12). The reminder procedure consisted in swimming in a basin without the platform for 60 sec directly after the substance injection. Ro 25-6981 was injected intraperitoneally in a dose of 5 mg/kg, diluted in 1 ml/kg saline, 24 h after the end of initial learning (on day 5). Animals of groups 3 and 4 were injected with 1 ml/kg saline on the same day. Repeated learning was carried out 11 days after injections (days 16-19 after the beginning of the study) according to the protocol similar to initial learning procedure. Reversal learning with modified position of the platform was started 24 h after the end of repeated learning (on day 20).

The results were processed by the nonparametric Kruskal–Wallis, Mann–Whitney, and Wilcoxon tests using SPSS 17.0 for Windows software (SPSS Inc). The differences were considered significant at $p<0.05$, a trend to differences was evaluated at $0.05<p<0.1$.

RESULTS

The time needed to find the platform decreased significantly from day 1 to day 4 during initial learning in all animals (Wilcoxon test, $p<0.05$; Table 1) and was similar in all groups (Kruskal–Wallis test, χ^2, $p>0.1$).

The time needed to find the platform on the first and last days (days 16 and 19 of experiment) of repeated learning after 12-day interval virtually did not differ in the control rats (Wilcoxon test, Z=-1.844, p=0.065). Rats of the rest groups found the platform much sooner (Wilcoxon test, p<0.05). The habit was significantly worse reproduced after 12-day interval by rats injected with saline before reminder (Wilcoxon test, Z=-2.229, p=0.026; Table 1).

Comparative analysis showed that Ro 25-6981 facilitated repeated learning 13 days after injection in vivarium in comparison with rats injected with the substance before reminder (Mann–Whitney test, U=1045.5, Z=-3.076, p=0.002) and in comparison with controls (Mann–Whitney test, U=195.0, Z=-1.935), p=0.053).

Reversal learning on day 20 of experiments showed that animals of all groups found the platform significantly slower than on the previous day (Wilcoxon test, $p<0.01$). Ro 25-6981 injected without reminder or before it did not affect reversal learning of animals (Mann–Whitney test, $p>0.1$; Table 1).

Hence, Ro 25-6981 facilitated repeated learning of animals 13 days after its injection. Previous studies showed that Ro 25-6981 injected in a dose of 5 mg/kg disturbed reversal learning (with the platform position changed and clues unchanged) 1 and 2 days after 6 daily injections [6] and reversal learning in Morris maze (with modified position of the platform and clues) 13 and 14 days after the beginning of treatment [1]. Injection of the blocker in a dose of 6 mg/kg disturbed reversal instrumental behavior learning (lever pressing) [5]. On the other hand, Ro 25-6981 improved the formation of a new spatial habit only 31-34 days, but not 1-6 days after 2 injections in a dose of 5 mg/kg [11]. Importantly that Ro 25-6981 effects on spatial learning manifested in the majority of cases 8-30 days after its injection [1,6,11]. Other NMDA receptor NR2B subunit antagonists (Ro 63-1908 in a dose of 1-10 mg/kg, CP-101,606 in a dose of 60 mg/kg) injected 30 or 60 min before learning had no effect on learning of animals in Morris water maze [8,10]. The available data suggest that these effects of Ro 25-6981 could be explained by different number of newly formed nerve cells involved in learning and memory mechanisms.

It is noteworthy that the age of new neurons, which could be involved in the formation and storage of long-term spatial memory under conditions of our experiment, was 11-13 days. The hippocampal neurons of this age formed in adult brain were characterized by the morphology and functions allowing their involvement in the spatial memory formation [3].

For several reasons, Ro 25-6981, in contrast to MK-801, injected under conditions of memory reactivation caused no amnesia in rats in our experiments [2]. First, MK-801 and Ro 25-6981 belonged to different classes of NMDA glutamate receptor antagonists and differently modulated the behavior [9]. Second, the behavioral effects of these antagonists depended on the type of the task [14]. Third, the results of the study depended on the reminder, blocker dose, and time of memory retention testing. Interestingly, Ro 25-6981 in a dose of 5 mg/kg in this study did not affect reversal learning in Morris water maze 15 days after injection, while in a previous study 2 injections of the blocker in a dose of 5 mg/kg suppressed reversal learning 17 days after the first injection [1]. This disagreement could be attributed to the dose-dependent effects of Ro 25-6981 [9] and/or different protocols of experiments: in this study, the platform position was changed once

TABLE 1. Time Course of the Initial and Repeated Learning and Reversal Learning in Rats Injected with Ro 25-6981, Glutamate NMDA Receptor NR2B Subunit Blocker

Day of testing	Ro 25-6981, reminder (n=14)	Saline, reminder (n=14)	Ro 25-6981, vivarium (n=14)	Saline, vivarium (n=12)
Initial learning				
1	43.4±2.8 (60; 36.8)	33.6±3.0 (29^{+}; 49.8)	40.4±3.0 (56; 45.3)	39.1±3.3 (43.5; 45.3)
2	16.9±1.8 (12; 14.5)	20.3±2.4 (12; 19.5)	18.9±2.4 (10.5; 16.8)	16.7±2.6 (9; 13.3)
3	11.9±1.6 (7; 10.8)	15.1±2.0 (9.5; 9.5)	13.8±1.7 (9; 13.8)	11.5±1.5 (7.5; 10.8)
4	9.63±1.50* (6; 6.8)	10.6±1.3* (7; 9)	11.4±1.5* (7; 9.8)	9.7±1.4* (7; 6.5)
Repeated learning				
16	13.1±1.8 (8; 8.8)	16.57±1.95^{o} (11; 18)	13.82±1.58 (9; 12.5)	11.23±1.10 (9; 9.8)
17	10.2±1.2 (8; 6)	11.0±1.5 (7.5; 8.5)	7.9±0.8 (6; 6)	9.8±1.3 (7.5; 8)
18	8.2±1.0 (6; 6)	7.6±0.8 (5.5; 5)	5.2±0.6 (4^{+}; 2.8)	7.3±1.0 (5; 5.8)
19	6.0±0.5^{x} (5; 4)	7.4±0.7^{x} (6; 5)	6.1±0.6^{x} (4; 4.8)	8.3±0.9 (6; 6)
Reversal learning				
20	34.2±3.3$^{\#}$ (36.5; 46.8)	29.1±3 (3$^{\#}$; 42.5)	27.0±3.2$^{\#}$ (17; 50)	31.0±3.5$^{\#}$ (22.5; 51.8)

Note. The mean times of finding the hidden platform by rats receiving injection of Ro 25-6981 or saline directly before reminder or the same without reminder are presented. All values are presented as the means and standard errors, median and interquartile range (in seconds). p<0.01 *vs.* $^{+}$the group injected with Ro 25-6981 before reminder (Mann–Whitney test), *day 1 of initial learning (Wilcoxon test), ^{x}day 1 of repeated learning (day 16 of experiments; Wilcoxon test), $^{\#}$day 4 of repeated learning (day 19 of experiments; Wilcoxon test); ^{o}p<0.05 *vs.* day 4 of initial learning (Wilcoxon test).

after 8 days of learning, while in a previous study the platform position was changed twice and the animals had to be retrained twice.

Hence, glutamate NMDA receptor blocker Ro 25-6981 stimulating neurogenesis in adult animals facilitated repeated spatial learning of rats in Morris water maze 13 days after its injection and did not affect reversal learning. Been injected directly before memory reactivation, the blocker did not disturb repeated learning and reversal learning of animals. Presumably, these behavioral effects of Ro 25-6981 were caused by stimulation of the formation and survival of new nerve cells involved in the learning processes.

REFERENCES

1. O. A. Soloviova, Z. I. Storozheva, A. T. Proshin, and V. V. Sherstnev, *Ros. Fiziol. Zh.*, **97**, No. 2, 146-154 (2011).
2. Z. I. Storozheva, S. V. Solntseva, V. P. Nikitin, *et al.*, *Byull. Eksp. Biol. Med.*, **150**, No. 9, 253-257 (2010).
3. I. E. Aasebo, S. Blankvoort, and A. Tashiro, *Eur. J. Neurosci.*, **33**, No. 6, 1094-1100 (2011).
4. C. Dalla, D. A. Bangasser, C. Edgecomb, and T. J. Shors, *Neurobiol. Learn. Mem.*, **88**, No. 1, 143-148 (2007).
5. G. L. Dalton, L. M. Ma, A. G. Phillips, and S. B. Floresco, *Psychopharmacology*, **216**, No. 4, 525-535 (2011).
6. S. Duffy, V. Labrie, and J. C. Roder, *Neuropsychopharmacology*, **33**, No. 5, 1004-1018 (2008).
7. E. Gould, A. Beylin, P. Tanapat, *et al.*, *Nat. Neurosci.*, **2**, No. 3, 260-265 (1999).
8. M. R. Guscott, H. F. Clarkeb, F. Murray, *et al.*, *Eur. J. Pharmacol.*, **476**, No. 3, 193-199 (2003).
9. J. Haller, R. Nagy, M. Toth, *et al.*, *Behav. Pharmacol.*, **22**, No. 2, 113-121 (2011).
10. G. A. Higgins, T. M. Ballard, J. Huwyler, *et al.*, *Neuropharmacology*, **44**, No. 3, 324-341 (2003).
11. M. Hu, Y. J. Sun, Q. G. Zhou, *et al.*, *J. Neurochem.*, **106**, No. 4, 1900-1913 (2008).
12. G. Kempermann, *J. Neurosci.*, **22**, No. 3, 635-638 (2002).
13. B. Leuner, E. Gould, and T. J. Shors, *Hippocampus*, **16**, No. 3, 316-224 (2006).
14. C. Mondadori, L. Weiskrantz, H. Buerki, *et al.*, *Exp. Brain Res.*, **75**, No. 3, 449-456 (1989).
15. D. S. Petrus, K. Fabel, G. Kronenberg, *et al.*, *Eur. J. Neurosci.*, **29**, No. 2, 244-252 (2009).