Irreversible Amnesia in Rats and Edible Snails under Conditions of Associative Memory Reconsolidation Disturbance Caused by NMDA-Glutamate Receptor Antagonist

Z. I. Storozheva, S. V. Solntseva, V. P. Nikitin, A. T. Proshin, and V. V. Sherstnev

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The effect of MK-801, an antagonist to NMDA-glutamate receptors, on reconsolidation of olfactory discrimination task in rats and taste discrimination in edible snails was examined. Twenty-four hours after conditioning, the animals received a single systemic injection of MK-801 followed by a reminding conditional stimulus. Disturbances in retrieval of the acquired task were observed 10 days after injection followed by a reminding procedure. Repeated conditioning of these animals did not restore the task. Injection of MK-801 without reminding stimulation had no effect on task retention. Thus, disturbances of NMDA-dependent reconsolidation of the associative memory in animals of different taxonomic groups irreversibly eliminated long-term memory.

Key Words: *memory reconsolidation; amnesia; NMDA-glutamate receptors; rat; edible snail*

Recent behavioral studies especially focus on storage (consolidation) and retrieval of the memory trace. It was established that presentation of one of the learning element (reminding procedure) at various terms after consolidation, reactivates long-term memory and it again becomes labile and responsive to various damaging stimuli [3,7,10,14]. The studies carried out on various species (mammals and mollusks included) with different learning paradigms showed that disturbance of reconsolidation of the long-term memory by biologically active substances such as NMDA-glutamate receptor antagonists can provoke amnesia that cannot be eliminated either spontaneously or by presentation of the reminding stimuli [1,6,10,11,13,14]. However, it is not clear whether the induced amnesia is principally irreversible due to "deletion" of the engram or death of neural and glial cells. Repeated learning is an experimental tool for the study of the amnesia mechanisms (specifically, for evaluation of the degree of retention of the disturbed memory trace and its availability for retrieval).

Our aim was to use repeated learning procedure in the study of rehabilitation mechanisms of the long-term memory disturbed by NMDA-glutamate receptor antagonists during reconsolidation of the long-term associative discriminative food aversion tasks in edible snails and similar olfactory discrimination in rats.

MATERIALS AND METHODS

Edible snails *Helix lucorum* were trained for conditioned food aversion according to the method described elsewhere [1,4]. The snails starved for 3 days before initial and repeated conditioning. Banana and boiled carrot were used as the conditional (aversive) and differential (appetitive) food stimuli, respective-

P. K. Anokhin Research Institute of Normal Physiology, Russian Academy of Medical Sciences, Moscow, Russia. ***Address for correspondence:*** nikitin.vp@mail.ru. V. P. Nikitin

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ly. Alternating electric current (300 msec, 1.2 mA, 50 Hz) was employed as a reinforcing stimulus. The current was passed through the food and snail body during the first consumptional reactions (scraper motions with radula across the food). If the snails did not start to consume the food (for 120 sec), the conditioning procedure was discontinued. The paired presentation of food and electrical stimulus were repeated every 15-20 min. The snails were conditioned for 3 days. The aversive conditional stimulus was presented 12-16 times, while the differential stimulus was presented 8-10 times.

Twenty-four hours after conditioning session, (+)-MK-801 hydrogen maleate (MK-801, Sigma, a specific antagonist to NMDA-glutamate receptors) dissolved in physiological saline (0.5 ml per snail) was injected into the mantle cavity of the experimental snails and the reminding procedure was performed as follows. The snails were placed into the conditioning environment (on plastic balls); conditional food was presented 3 times 10-15-min intervals, but the reinforcing stimulus (electric current) was not used. Control snails were similarly injected with MK-801 and placed into the conditioning environment, but were not presented reminding stimuli.

Retention of the taste discriminative task was tested on postinjection days 10 and 13. To this end, the snails were placed into the conditioning environment for 30 min, thereafter conditional and differential foods were presented at intervals of 10-15 min. The latent time to the onset of food consumption was measured during the observation period of 120 sec. If the snails started to consume food during this period, the test was discontinued without presentation of the reinforcing stimulus. Immediately after the test for retention of the memory trace (performed on postinjection day 10 combined with the reminding procedure), the taste discriminative task was repeatedly conditioned using the same method as in the first learning.

The mature male Wistar rats were conditioned to olfactory discrimination with drinking reinforcement (the operant conditioning for water drinking). The odors of mint and lemon were employed as the conditional (aversive) and differential stimuli, respectively. The rats were deprived of water 48 hours before initial and repeated learning. Conditioning was performed in Y-maze, where round drinking dishes (0.5 ml) were attached to the wall 1 cm above the floor. These dishes were connected to Eppendorf tubes having orifice in the lid and filled with 0.2 ml aromatic oil (Vivasan). The dishes with mint oil contained also 0.1% quinine solution, while dishes with lemon oil were filled with pure water (total volume of the fluid was 0.4 ml). Learning was performed for 2 days. On the first day, each rat was placed into the maze 9 times. Two dishes with water and quinine were randomly placed into the arms of the maze. Location of the dishes was changed before the next learning session. In each session, the number of efficacious actions (terminated with consumption of water or quinine solution) was counted. Duration of a single session was 3 min, while the interval between the sessions was 5 min. On day 2 of learning, 3 dishes with water and 3 dishes with quinine were randomly placed in the maze. The rats were thrice placed into the maze, and the number of efficacious approaches to the dishes of either type was recorded. Thirty minutes after each session, the rats were provided with free access to water in their home cages for 1 h.

Twenty-four hours after conditioning procedure, half of the rats (n=8, the experimental group) were intraperitoneally injected with MK-801 (50 μg/kg) followed with the reminding procedure: the rats were placed into the maze without dishes but with the tubes fixed to the walls. Three tubes contained mint oil, while other 3 tubes were filled with lemon oil. The rats were allowed to examine the maze for two times during 3 min, thereafter they were returned to home cages. The control rats (n=8) were injected with MK-801 without subsequent reminding stimulation. On postinjection day 10, both groups of rats were tested for retention of the acquired task. Immediately after this testing, the repeated conditioning procedure (described above) was carried out for 2 days. The task was tested 24 h after repeated learning.

The data were processed statistically using nonparametric Mann–Whitney U test. All experimental procedures were carried out in accordance to humanitarian principles specified in European Economic Community Directive 86/609/EEC.

RESULTS

In unconditioned snails, the latency of consumption response to presentation of banana or boiled carrot was 20-30 sec. On conditioning day 3, the latency of consumption response to the last presentation of conditioned stimulus (banana) increased to 115±5 sec (n=26), which greatly surpassed the latency to differential stimulus of 43±11 sec (n=30, p<0.001).

Examination of control snails (injected with MK-801, but not subjected to the reminding procedure, n=12) on postinjection days 10 and 13 showed that the latency of masticatory movements to presentation of the conditional food was far greater than that to presentation of the differential stimulus remaining about 100 sec (p<0.0001, Fig. 1). Thus, the conditioning environment *per se* (not accompanied by the reminding procedure) produced no effect on retention of the discriminative task in the control snails, although

similar to the experimental rats, they were injected with MK-801. In contrast, the latency to conditional stimulus in the experimental group placed into the conditioning environment and subjected to the reminding procedure (n=14) dramatically decreased to 27±8 sec and became far below the control value (106±11 sec, p<0.001). Moreover, it did not significantly differ from the latency of the response to presentation of the differential stimulus (21±5 sec, p>0.05; Fig. 1). Thus, the reminding procedure performed in the snails treated with MK-801 (which disturbed retrieval of the acquired aversive task to certain food) resulted in amnesia at least for the following 10 days.

Immediately after examination of the experimental snails, they were repeatedly conditioned to discriminate banana (the aversive stimulus, which was also employed in the first conditioning procedure). Twenty-four hours after this repeated 3-day conditioning, the latency to aversive stimulus was pronouncedly shorter than in the control (25±7 and 114±8 sec, respectively; p<0.0001, Fig. 1). Moreover, this latency did not differ from that to presentation of differential food (22±3 sec, p>0.05) despite the fact that the number of combined presentation of the conditional and reinforcing stimuli during repeated associative learning was greater than during the first conditioning session (12.3±1.0 and 8.4±0.7, p<0.05). Thus, the experimental snails, which lost the discriminative task after the reminding procedure, could not restore it during the repeated conditioning.

In experiments on rats, they were conditioned to olfactory discrimination with drinking reinforcement. As a result, the rats decreased the number of efficacious approaches to dishes with mint odor and quinine ingredient by 3-4 times and increased the number of such approaches to dishes with lemon odor containing pure water by 2 times (Figs. 2, 3). During testing the rats on day 10 after the combined action of MK-801 and the reminding procedure, there was a significantly greater number of efficacious approaches to the mint-odor dishes (containing quinine) in comparison with the control group (2.75±0.37 and 1.33±0.21, respectively; p<0.05). In addition, the number of efficacious actions with mint dishes in the experimental group was larger than that attained to the end of the first conditioning session (2.75±0.37 and 1.00±0.27, respectively; p<0.05). In control group, the number of completed approaches to the mint dishes tested on day 10 did not differ from that attained to the end of the first conditioning session (p>0.05, Fig. 2). In the experimental group, the number of efficacious actions with lemon-odor dishes (filled with water) differed neither from the control value, nor form the level attained to the end of conditioning session (p<0.05, Fig. 3). Thus, disturbance of reconsolidation of the olfactory discrimination task in rats resulted in amnesia for at least 10 days.

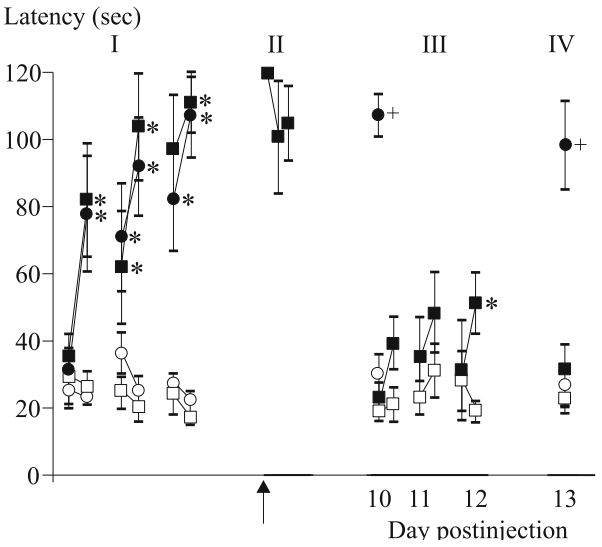

Fig. 1. Latency to conditional (closed symbols) and differential (open symbols) stimuli in control (circles: MK-801 without reminding procedure) and experimental (squares: MK-801+reminding procedure) conditioned snails with discriminative taste. Mean latencies during the first and last learning days are presented. The arrow marks injection of MK-801. *p<0.05 relates to significant difference in latency between to aversive and differential stimuli; +p<0.0001 corresponds to significant difference in latency to presentation of aversive stimulus in control and experimental snails. The stages of experiments here and in Figs. 2, 3: the first learning (*I*), reminding procedure (*II*), repeated learning (*III*), and examination (*IV*).

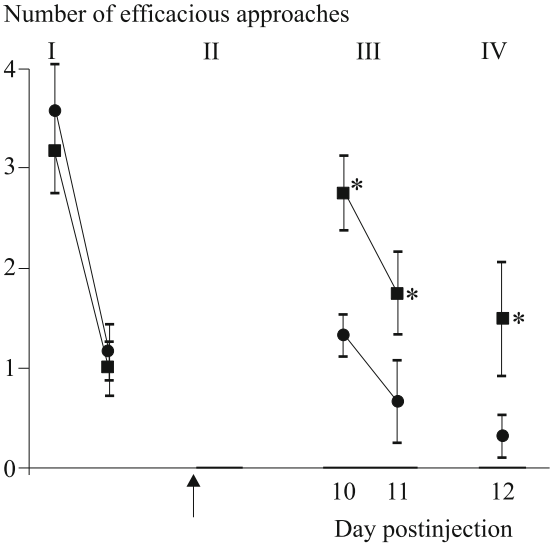

Fig. 2. Efficacious approaches to the dishes filled with 0.1% quinine water solution and connected to the tubes with mint oil (the conditional stimulus). The mean numbers of the efficacious approaches during the learning day are shown.

During repeated conditioning of the control rats, the number of erroneous free-choice trials significantly

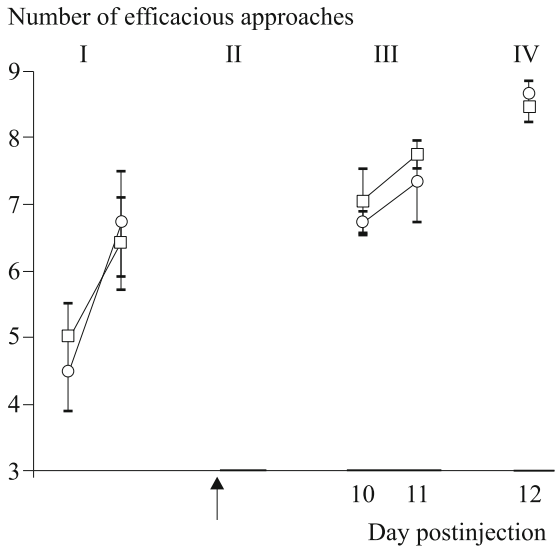

Fig. 3. Efficacious approaches to dishes filled with pure water and connected to the tubes with lemon oil (the differential stimulus).

decreased ($p<0.05$), while the experimental rats demonstrated only a trend to a decrease the number of approaches to the mint dishes in 2 days of the repeated training ($p>0.1$, Fig. 2). At the same time, the number of efficacious approaches to the lemon-odor dishes on day 2 of the repeated conditioning increased in both groups. Testing 24 h after the repeated conditioning showed that the number of efficacious approaches to the mint-odor dishes was greater in the experimental rats in comparison with the controls (1.50±0.56 and 0.33±0.21, respectively). Therefore, disturbance of memory reconsolidation by MK-801 combined with reminding procedure resulted in amnesia that could not be eliminated during repeated conditioning.

Thus, control snails and rats injected with NMDA-glutamate receptor antagonist MK-801 but not subjected to the reminding procedure after this injection retained the task for more than 13 days. In contrast, presentation of the reminding stimuli during the action of MK-801 induced amnesia for at least 10 days. Consolidation of the task during repeated conditioning of the "amnestic" snails was suppressed completely despite the greater number of paired presentation of the conditioning and reinforcing stimuli during the repeated learning in comparison with that during the first conditioning session. Similar results were obtained in the experiments with rats demonstrating disturbance of the conditioned taste discrimination task against mint odor: after the combined action of MK-801 and reminding procedure, the repeated conditioning for aversion against this odor was no more possible.

The development of irreversible amnesia after the disturbance of reconsolidation processes characterized with suppression of conditioning during the repeated learning is a novel fact unknown in literature. The mechanisms of disturbance of the learning potency are still unclear and left for further studies. One of the reason of the persistent amnesia can be "destruction" of the morphologic "carriers" of the engram caused by the death of neural and glial cells or elimination of the cell-cell synaptic connections functionally necessary to maintain the memory trace. The experimental substantiation of this hypothetic development of irreversible amnesia are the data indicating involvement of neurogliogenesis and apoptosis into the long-term memory formation and storage [2,5], as well as the facts of MK-801 inhibitory action on proliferation, differentiation, and apoptotic death of the cell in the mature brain [9,15]. Another reason underlying degradation of memory consolidation during repeated conditioning can be disturbance of the molecular mechanisms of the long-term synaptic plasticity in neurons involved in the formation and storage of the engram. These processes can include persistent repression of transcription of the genes that control plastic properties of neurons, epigenetic mechanisms similar to chromatin modification during DNA methylation, prion-like self-conversion, and other alterations in biochemical processes [8,12,14].

Our data attest to principal similarity between the mechanisms of the development of NMDA-dependent amnesia during disturbance of memory reconsolidation in mammals and mollusks. It can be hypothesized that irreversibility of amnesia provoked by disturbance of the associative tasks is the phenomenon characteristic of at least some memory forms both in the vertebrates and in the invertebrates.

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