Recovery of Impaired Memory and *c-fos* Gene Expression in Brains of Amnestic Animals in Response to Reminder Stimulation

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 153, No. 5, pp. 698-702, May, 2012
Original article submitted March 2, 2012

Possible mechanisms of recovery of the memory impaired during consolidation process were investigated. In mice, amnesia was induced by intraperitoneal cycloheximide (100 mg/kg) administration 20 min before exposure to tone signal combined with footshock (2 sec, 0.5 mA). Reminder by the footshock (2 sec, 0.5 mA) 24 h after the learning procedure resulted in recovery of impaired memory in amnestic animals up to the level of control animals. Analysis of c-Fos expression in response to reminder indicated increased number of c-Fos-positive cells in prelimbic cortex in the animals with unaffected memory in comparison with corresponding parameter in amnestic animals. These findings are indicative of impairment in prelimbic cortex activity in experimental amnesia as well as for reminder ability to recover the memory impaired in that way.

Key Words: *amnesia; memory recovery; c-Fos; memory; consolidation*

According to classical concepts, memory is stabilized gradually following acquisition with the transfer from short-term state to the long-term state [9]. This process was called consolidation, and the hypothesis was confirmed in numerous studies where role of intracellular processes in mechanisms of sustained memory formation was demonstrated [4-6]. Interruption of consolidation at any stage was considered for a long time to result in irreversible memory loss. However, small amount of investigations have shown that under certain condition impaired memory can be recovered using remaindering procedure, *i.e.* presentation of one of the learning procedure components to the animals [3,10,11]. At the present moment, the nature and mechanisms of memory recovery phenomenon are obscure, however findings concerning this potential are indicative of the fact that behavioral manifestation of amnesia in many instances is associated not with memory collapse, but with failure to retrieve acquired experience.

In this study, we investigated the possibility for recovery of the memory impaired by protein synthesis inhibition in fear-conditioning paradigm in mice. This paradigm is well established; neuronal basis and molecular mechanisms underlying this type of learning are well described [2,7]. Keeping in mind this data we stated the objective to describe the brain condition using functional neuromapping in terms of expression of the immediate early gene *c-fos* protein [1,12] in animals with impaired, but recoverable memory.

MATERIALS AND METHODS

Experiments were carried out on 3-month-old male C57Bl/6 mice. The animals (n=55) were kept in Plexiglas cages, 8-10 animals in each, with free access to

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food and water. All the experiments were carried out at daylight time from 11 a.m. to 16 p.m. The experiments were conducted in accordance with Order No. 267 Ministry of Health of Russian Federation (19.06.2003) and Rules of Studies on Experimental Animals (approved by the Ethics Committee of the P. K. Anokhin Institute of Normal Physiology; protocol No. 1, 3.09.2005).

The animals were trained in experimental chamber A (20×30×20 cm) with two transparent and two nontransparent walls. Chamber floor consisted of iron rods (1.5 mm diameter, 8 mm distance between the rods). The walls and floor were cleaned with 70% ethanol before each animal was placed into the chamber. Illumination was provided by daylight electric lamp. The tone stimulus was supplied via audio speakers F&D SPS-608. Reminder procedure was carried out in chamber B with walls made of two nontransparent rounded plastic inserts (42.5×28.5 см). Thus, chamber B substantially differed from chamber A by shape and size of the interior space. The walls and floor were cleaned with juniper-aromatized 40% ethanol before each animal was placed into the chamber. Illumination was provided by blue incandescent lamp. The animals were tested in the third chamber (chamber C, 40.5×40.5×32.0 cm) with transparent plastic walls. The floor was covered with sawdust. The walls were cleaned with 1% acetic acid before each animal was placed into the chamber. Chamber C was illuminated by red incandescent lamp during the testing. The tone was supplied via audio speakers F&D SPS-608.

The animals from the experimental group received protein synthesis inhibitor cycloheximide (Sigma) 100 mg/kg intraperitoneally 20 min before training. Control animals received saline ntraperitoneally (Sal; 0.9% NaCl solution) in the equivalent volume (0.1 ml/10 g body weight).

Training was carried out in chamber A. The animals were presented a single combination of conditioned tone (10 kHz, 70 dB, 30 sec) and unconditioned stimulus footshock (0.5 mA, 2 sec) applied during the last two seconds of the tone.

Reminder was carried out in chamber B 1 day after the training. Animal was placed into the chamber with rod floor, where 0.5 mA current was applied; the mouse received footshock immediately after placement on the rod floor. Reminder procedure (being in the chamber B accompanied with the footshock) lasted 2 sec for each animal. Animals were returned to the home cages following completion of the reminder procedure.

Long-term fear memory test for the tone was carried out 24 h after the reminder session. Cued test in novel environment (chamber C) lasted for 6 min. First 3 min of free exploration were followed with 3 min of tone presentation. Duration of freezing episodes (total immobility of the animal; expressed as the % from the test time) was registered separately during two testing periods: without tone and with tone (3 min each).

Some animals was killed 90 min after the reminder session by cervical dislocation and decapitated; the brains were removed and frozen in liquid nitrogen vapors. Immunohistochemical detection of c-Fos-positive cells was carried out on frontal brain sections according to protocol for avidin-biotin-peroxidase immunohistochemistry with ImmPRESS kit (Vector Laboratories) using polyclonal rabbit anti-c-Fos antibodies (Calbiochem, Ab-5 Cat. No. PC38).

The animals from the control groups (Groups 1, 2, and 3) received Sal injection 20 min before training. Thereafter, Group 1 animals (n=4) received no footshock during the training, but were exposed to footshock 24 h later as a reminder and were decapitated after 30 min. Group 2 animals (n=6) were not subjected to reminder stimulus 24 h after the training. Some of them were decapitated simultaneously with group 1 animals subjected to the reminder, while others were tested 48 h after training. Group 3 animals (n=12) were reminded 24 h after training. Some of them were decapitated 90 min after the reminder session, while others were tested 24 h after the reminder session. Animals from groups 4 and 5 were injected with cycloheximide 20 min before training. Group 4 animals (n=17) trained under the influence of this compound were not subjected to reminder stimulus 24 h after the training. Some of them were decapitated simultaneously with groups 1 and 3; others were tested 48 h after the training. Group 5 animals (n=16) were reminded 24 h after the training. Some of them were decapitated 90 min after; others were tested 24 h after the reminder session.

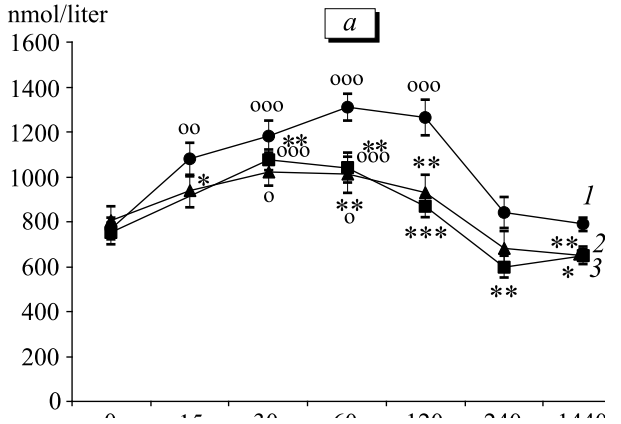

Fig. 1. Results of animal testing to the tone 24 h after reminder session. *1*) group 2; *2*) group 3; *3*) group 4; *4*) group 5. *p<0.05, **p<0.01 (post-hoc analysis, Fisher's test) in comparison with group 4.

RESULTS

Animal training in cued fear conditioning paradigm resulted in formation of long-term memory in relation to conditioned tone. Cycloheximide administration before the training impaired long-term memory what was demonstrated by significantly decreased freezing duration in Group 4 in comparison with Group 2 (15.02±4.30 and 35.36±10.40%, respectively, $p<0.05$; Fig. 1). Reminder procedure had almost no effect on freezing period in Group 3: this parameter had no significant differences from that in Group 2 (44.33±6.40 and 35.36±10.40%, respectively, $p=0.4$; Fig. 1). However, reminder appeared to be effective for memory recovery in Group 5, what was demonstrated by increased freezing level in that group up to the level observed in animals with normal memory (42.64±5.60 and 44.33±6.40% in Group 3; $p=0.83$) and by significant increase of this value compared to mice with impaired memory from Group 4 ($p<0.001$). Thus, reminder session resulted in increased freezing duration in amnestic animals in the cued test, what is indicative of memory recovery.

Analysis of transcription factor c-Fos expression in mouse brain was performed after the reminder session. In the basal amygdala, the number of c-Fos-expressing cells had no significant differences between groups; only the trend was evident concerning increased number of such neurons in mice subjected to footshock as a reminder (Groups 1, 3, and 5; Fig. 2, *a*). There were no differences between animals with reminder and without reminder in the number of c-Fos-positive cells in the lateral amygdala, where the signals converge when conditioned and unconditioned stimuli are coupled (Fig. 2, *c*). In the central amygdala, there were no significant differences between groups in the number of c-Fos-positive cells (Fig. 2, *b*). Thus, it can be concluded that brief reminder with the footshock had no significant effect on amygdala activity. c-Fos expression in the amygdala nuclei had no dependence on the integrity of memory trace in mice treated with cycloheximide. Reminder presentation also had no effect on it.

Retrieval of previous experience from the memory by the reminder session in animals treated with Sal before the training resulted in significant increase in c-Fos expression in the prelimbic cortex in comparison with other experimental groups (Fig. 3, *a*). Memory recovery in group 5 was not reflected by the number of c-Fos-expressing cells in this cortex area. No differences were observed in the number of c-Fos-positive cells in the cingulate cortex regardless of integrity of

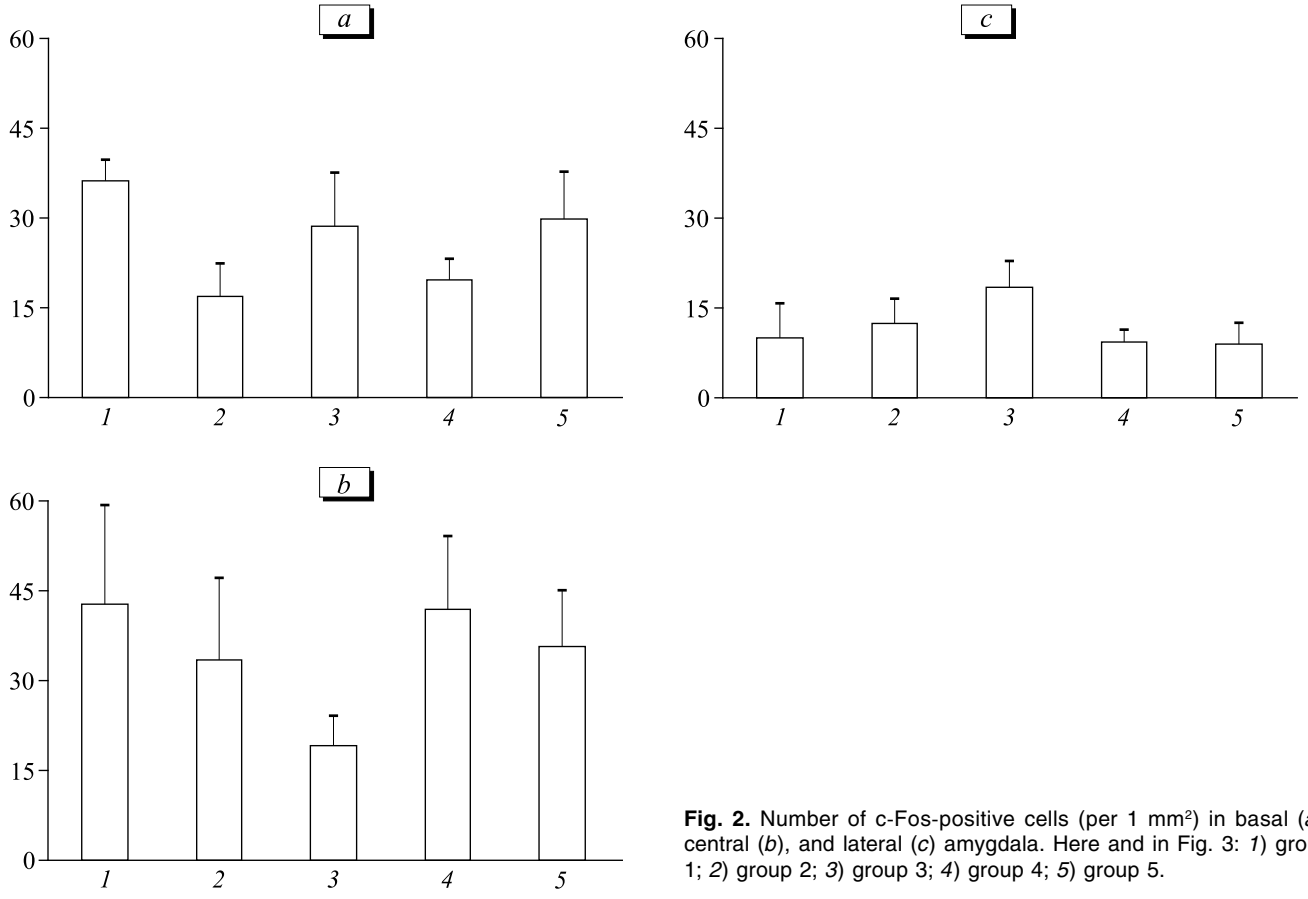

Fig. 2. Number of c-Fos-positive cells (per 1 mm^2) in basal (*a*), central (*b*), and lateral (*c*) amygdala. Here and in Fig. 3: *1*) group 1; *2*) group 2; *3*) group 3; *4*) group 4; *5*) group 5.

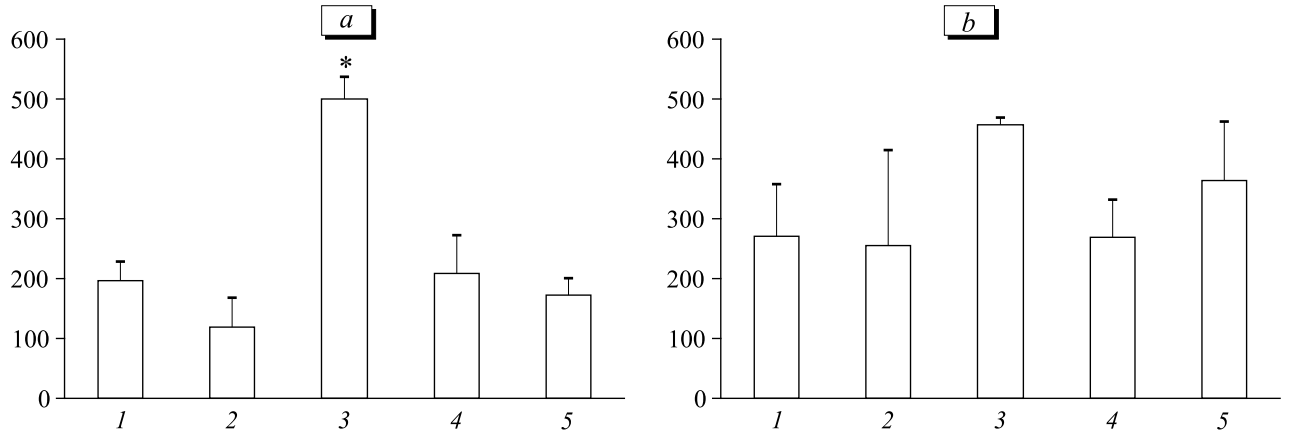

Fig. 3. Number of c-Fos-positive cells (per 1 mm^2) in prelimbic (*a*) and cingulate (*b*) cortex. *$p<0.05$ in comparison with other groups (post-hoc analysis, Fisher's test).

memory trace and presence/absence of reminder session for its retrieval (Fig. 3, *b*).

Thus, we demonstrated possibility for recovery of impaired memory in behaviorally amnestic animals by the reminder 24 h after the training. It indicates that protein synthesis inhibitor does not impair memory formation in fear conditioning paradigm. Presumably, possibility for memory recovery in amnestic animals is indicative of disturbances in memory retrieval mechanisms. Findings obtained by functional brain activity mapping in amnestic animals give evidence of disturbances in prelimbic cortex activity, which can be associated with the block of memory retrieval processes.

The study was supported by the Russian Foundation for Humanities (grant No. 09-06-00383a).

REFERENCES

1. K. V. Anokhin, *Zh. Vyssh. Nerv. Deyat.*, **47**, No. 2, 261-279 (1997).
2. S. V. Zworykina and K. V. Anokhin, *Zh. Vyssh. Nerv. Deyat.*, **53**, No. 4, 518-522 (2003).
3. K. A. Radyushkin and K. V. Anokhin, *Ros. Fiziol. Zh.*, **83**, 11-18 (1997).
4. T. Abel and K. M. Lattal, *Curr. Opin. Neurobiol.*, **11**, No. 2, 180-187 (2001).
5. C. M. Alberini, *Neurobiol. Learn. Mem.*, **89**, No. 3, 234-246 (2008).
6. H. P. Davis and L. R. Squire, *Psychol. Bull.*, **96**, No. 3, 518-559 (1984).
7. M. S. Fanselow and J. E. LeDoux, *Neuron*, **23**, No. 2, 229-232 (1999).
8. S. J. Martin, P. D. Grimwood, and R. G. Morris, *Ann. Rev. Neurosci.*, **23**, 649-711 (2000).
9. J. L. McGaugh, *Science*, **287**, 248-251 (2000).
10. R. R. Miller and A. D. Springer, *Physiol. Behav.*, **8**, No. 4, 645-651 (1972).
11. D. Quartermain, B. S. McEwen, and E. C. Jr. Azmitia, *Science*, **169**, 683-686 (1970).
12. J. Radulovic, J. Kammermeier, and J. Spiess, *J. Neurosci.*, **18**, No. 18, 7452-7461 (1998).
13. W. Tischmeyer and R. Grimm, *Cell. Mol. Life Sci.*, **55**, No. 4, 564-574 (1999).