ERRATUM

Erratum to the article "Increased 5-Bromo-2'-Deoxyuridine Incorporation in Various Brain Structures Following Passive Avoidance Training in Mice" by O. I. Ivashkina, M. A. Zots, D. V. Bezriadnov, and K. V. Anokhin, Vol. 153, No. 5, pp. 591-593, September, 2012

This article should look like the following:

Increased 5-Bromo-2'-Deoxyuridine Incorporation in Various Brain Structures Following Passive Avoidance Training in Mice

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We studied the effects of training on DNA synthesis intensity in mouse brain. Brain cells where DNA synthesis-associated processes took place under the influence of training were detected by immunohistochemical labeling of DNA molecules with synthetic thymine analogue 5-bromo-2'-deoxyuridine. The number of 5-bromo-2'-deoxyuridine-positive cell increased in various structures of the brain under the influence of training.

Key Words: *DNA synthesis; 5-bromo-2'-deoxyuridine; long-term memory; dentate gyrus*

The key feature of long-term memory is its persistence over the protracted period. The hypothesis of DNA-dependent mechanisms of memory maintenance was put forward in 1970s, since DNA is the only permanent information carrier in eukaryotic cell, which lifetime corresponds to cell lifetime [2,3].

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Autoradiographic method of [methyl-3H]-thymidine detection in brain cells was employed to obtain results concerning the presence of permanent DNA synthesis in the brain, that can be intensified during learning. This hypothesis was confirmed in later studies [1] conducted using immunohistochemical labeling of cell DNA containing nucleoside analogue molecule (*e.g.*, 5-bromo-2'-deoxyuridine, BrdU). BrdU is a synthetic analogue of thymine and can be incorporated into DNA instead of thymine during the synthesis [9].

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In published reports, BrdU incorporation is frequently erroneously qualified as a reflection of proliferation and neurogenesis, notwithstanding that such incorporation occurs in any DNA synthesis, not necessarily associated with cell division [12]. Thus, a number of studies showed the presence of other DNA synthesis-associated processes in the brain (non-homologous recombination [10], retrotranspositions [8]).

The objective of this study was to investigate the effects of passive avoidance training on DNA synthesis in cells within different brain structures at early terms (3 days) following training.

MATERIALS AND METHODS

Experiments were carried out on 16 mature C57Bl/6 male mice. The animals were kept under standard vivarium conditions, 5-6 animals per cage with free access to water and food. The experiments were conducted in accordance with the Order No. 267 Ministry of Health of the 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).

On the day of the experiment, the animals received an intraperitoneal injection of synthetic nucleoside analogue BrdU (100 mg/kg, Calbiochem). One hour after administration, the mice of the experimental group (n=6) were trained for passive avoidance (modification of "step down" model): the animals were placed on a wooden cube in the center of the chamber with electrode floor and the latency of step down was recorded. When the animal put all four paws on the chamber floor, 1-mA footshock was applied for 15 sec. Thereafter, the animal was placed on the cube for 30 sec and returned into the home cage. In the active control group (n=5), sham training was performed 1 h after the injection: the animals were placed on the cube, step down latency was recorded, and the animals were left for 15 sec to explore the experimental chamber; thereafter the animals were placed on the cube again for 30 sec (or until step down) and returned to the home cage. The animals of the passive control group (n=5) were placed into the home cage following BrdU injection.

The animals were decapitated 3 days later and immunohistochemical BrdU detection was carried out on thin cryostat sections. To this end, DNA was denatured and immunohistochemical reaction for BrdU was performed (Exalpha, No. 10926, 1:500) with visualization of primary antibody binding sites using fluorescent secondary antibodies (Alexa Fluor 488 donkey anti-sheep, Molecular probe, 1:400).

Fluorescence of stained sections was recorded using a Turboscan system (Objective Imaging) and an Olympus BX-50 microscope. The number of immunopositive cells was determined using ImagePro 6.0 software (Media Cybernetics). Detection of BrdU-positive cells was carried out in the dorsal hippocampus, several neocortex areas, and basal amygdala.

The results were statistically treated using ANOVA and Statistica 8.0 software. The differences were considered significant at $p<0.05$ (Mann–Whitney test).

RESULTS

Three days following passive avoidance training, BrdU-positive cells were detected not only in proliferative brain area (dentate gyrus), but also in over areas involved in long-term memory formation.

The data on the density of BrdU-positive cells in the dorsal hippocampus dentate gyrus are presented in Figure 1. Nonparametric ANOVA revealed significant differences (p=0.037, median test), thereafter the pair-wise comparisons were performed. The density of BrdU-positive cells in the dorsal hippocampus dentate gyrus of trained animals was maximum and significantly differed from that in passive and active control groups (p=0.004 and p=0.006, respectively, Mann–Whitney test). The density of BrdU-positive cells in the dentate gyrus of active control animals was considerably higher than in passive control animals. This attest to intensification of DNA synthesis processes in response to training, which can be associated with enhanced cell proliferation in this case. No BrdU-positive cells were detected in other (non-proliferating) hippocampal areas (CA1, CA3) in any group.

Analysis of density values of BrdU-positive cells was also carried out for several neocortical areas: the cingulate and prelimbic cortices (Fig. 2). Significant differences were detected for the cingulate cortex using non-parametric ANOVA (p=0.037, median test),

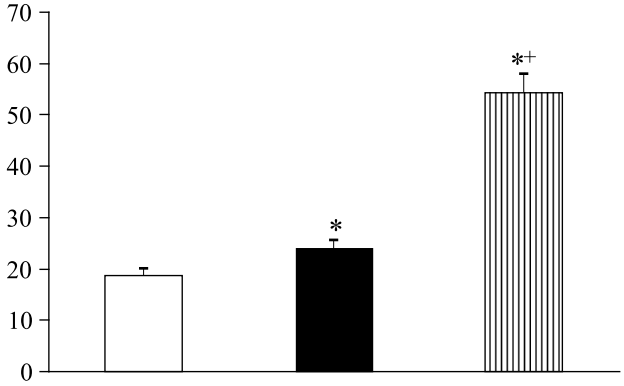

Fig. 1. Density of BrdU-positive cells in the dorsal hippocampus dentate gyrus in three groups of mice. $^{+}p<0.05$ in comparison with active control group. Here and at Figs. 2 and 3: ordinate: number of BrdU-positive cells per 1 mm^2. Light bars: passive control; dark bars: active control; dashed bars: training. $^{*}p<0.05$ in comparison with passive control.

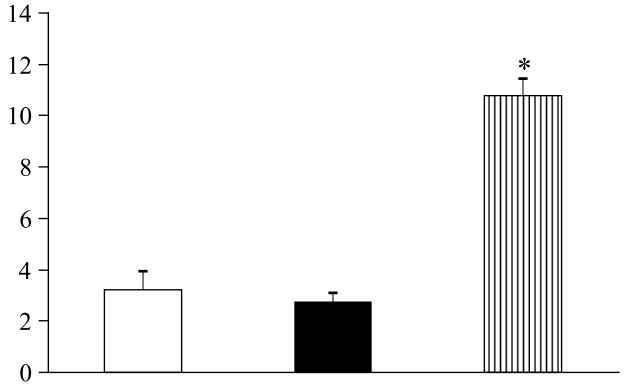

Fig. 2. Density of BrdU-positive cells in the cingulate cortex in three groups of mice.

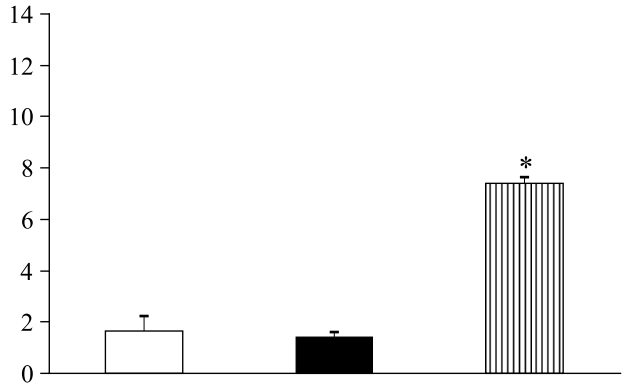

Fig. 3. Density of BrdU-positive cells in the basal amygdala in the three groups of mice.

thereafter pair-wise comparisons between the groups were performed. Maximal density of BrdU-positive cells was observed in the cingulate cortex of trained animals; it was significantly higher than in animals of passive and active control groups (p=0.006 and p=0.006, respectively, Mann–Whitney test). In the prelimbic cortex, there were no differences in the number of BrdU-positive cells between different groups. Thus, we demonstrated increased DNA synthesis in the neocortex, where the possibility for cell proliferation is not commonly accepted [4,6]. Differences between the values for the cingulate and prelimbic cortices may be associated with unequal involvement of these structures into passive avoidance training (inactivation of cingulate cortex [11], but not prelimbic cortex [7] impaires conditioned task performance).

Analysis of BrdU incorporation into the amygdala revealed immunopositive cells only in the basal amygdala (there were no positive cells in the central and lateral amygdala). The data considering density of BrdU-positive cells in basal amygdala are presented at Figure 3. The presence of significant differences within this structure was demonstrated using nonparametric ANOVA (p=0.037, median test) and pairwise comparisons were carried out. The density of BrdU-positive cells in the basal amygdala of trained animals was maximum and significantly differed from that in passive and active control groups (p=0.006 and p=0.006, respectively, Mann–Whitney test). The amygdala also does not belong to classic proliferative brain structures, although the formation of new neurons in this structure in adult animals was reported [3].

Thus, using immunohistochemical detection of nucleoside analogue BrdU we investigated effects of learning on DNA synthesis in the brain. In intact animals DNA synthesiswas detected not only in proliferative zone (dentate gyrus), but also in several areas of the neocortex and amygdala. In trained animals DNA synthesis increased, which was demonstrated by higher number of brain cells incorporated BrdU. The nature of that DNA synthesis remains unclear and requires further investigations.

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