# Effects of Systemic Administration of Histone Deacetylase Inhibitor on Memory Formation and Immediate Early Gene Expression in Chick Brain

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 153, No. 5, pp. 703-706, May, 2012
Original article submitted March 5, 2011

We studied the effects of histone deacetylase inhibitor that stimulates transcriptional activity via histone hyperacetylation on memory formation. Sodium butyrate and sodium valproate enhanced memory in chicks following "weak" training with memory transfer into long-term state. Quantitative analysis of c-Fos and ZENK transcriptional factor gene expression in six structures of chick brain revealed induction of these genes in the structures involved in this type of learning. Sodium valproate administration did not increase this induction, but even reduced it. These findings suggest that sodium butyrate and sodium valproate exert cognitive stimulating action in the "weak" memory formation paradigm, and that this effect is not mediated via enhanced expression of transcriptional factors, which are traditionally considered as "molecular switcher" for memory transfer into long-term state.

**Key Words:** *learning; histone deacetylase inhibitors; gene expression*

According to contemporary views, memory consolidation, *i.e.* its transfer from short-term to the long-term state, is provided by two stages of protein synthesis in the brain [1]. Learning is associated with induction of immediate early gene expression in the neurons, particularly for the genes of transcriptional factors, which in turn initiate expression of late genes [2,7]. These processes result in structural changes in synaptic contacts between the neurons, involved in learning [11,12]. Among the mechanisms of gene expression regulation, the long-term chromatin modifications based upon dynamic histone acetylation/deacetylation are of specific interest. These epigenetic processes play the key role in brain development, particularly in cell differentiation. In adult nervous system, the same mechanism are involved in long-term memory maintenance [4,10]. In such a way, histone deacetylase inhibitors enhance memory in adult mice [8,9,13]. Sodium butyrate administration to animals with brain atrophy resulted in recovery of the memory, lost in the consequence of degeneration of nervous elements and connections [5].

The objective of the study was to investigate effects of systemic administration of histone deacetylase inhibitors on weak memory in accepted model of one-trial learning of newborn chicks and to test the hypothesis of c-Fos and ZENK transcription factor gene involvement in the processes that provide memory enhancement when histone deacetylase inhibitors are used.

## MATERIALS AND METHODS

Experiments were carried out in accordance with method of "weak" passive avoidance training in 1-2 day old chicks (*Gallus gallus domesticus*) ($n$=441) [6]. The experiments were conducted in accordance with the Order No. 267 Ministry of Health of Russian Fed-

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eration (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, 03.09.2005). Sodium butyrate (NaBut, 1.2 g/kg; Sigma) and sodium valproate (NaVal, 0.1 g/kg; Sigma) were diluted in saline and administered intraperitoneally in the volume of 0.1 ml at different times before and after training. Control animals were injected with physiological solution.

To train the chicks, they were presented with "aversive" bead, moistened with pungent substance (10% methyl anthranilate solution in ethanol). Chick training in the "weak" model results in the formation of impermanent memory that lasts for 6-12 h after training; the memory decays in most of the animals 24 h later [6], therefore testing was carried out 24 h after and comprised 10 sec presentation of the same bead used during training, but dry, and subsequent presentation of the neutral bead. Selective avoidance of aversive bead was assessed as presence of long-term memory. The percents of the animals demonstrated avoidance reaction in different experimental groups was compared; significance of the differences was assessed using $\chi^2$ test.

Transcriptional activity in the brain was assessed in the following experimental groups: passive control (group 1), training+saline (group 2); training+NaVal 0.1 mg/kg 5 min after training (group 3). Trained animals were decapitated 90 min after the injection. Immunohistochemical detection of c-Fos and ZENK proteins was carried out on cryostat sections (20 μ) using primary rabbit antibodies (Santa Cruz) and secondary anti-rabbit horse antibodies (ImmPress KIT, Vector Labs). Staining was visualized with diaminobenzidine (Sigma) and digitalized using Olympus BX50 microscope and TurboScan system (Objective Imaging Ltd.). Quantitative analysis of the expression pattern was carried out using Image Pro Plus 3.0 software (Media Cybernetics). Density of expression was calculated as the ratio of the number of labeled cells in selected region to the area of this region (in $mm^2$).

Statistical treatment was carried out using Statistica 6.0 software (Kruskal–Wallis test and Mann–Whitney test).

## RESULTS

NaBut was administered 3 min after training and NaVal 30 min before training. Memory testing 24 h after training revealed significant increase in avoidance level in groups receiving NaBut (Fig. 1) or NaVal (Fig. 2) in comparison with control groups. Thus, both histone deacetylase inhibitors produced similar potentiating influence on "weak" memory. In addition, NaBut was administered after training, what excludes influence of training process *per se* and suggests involvement in proper memory consolidation.

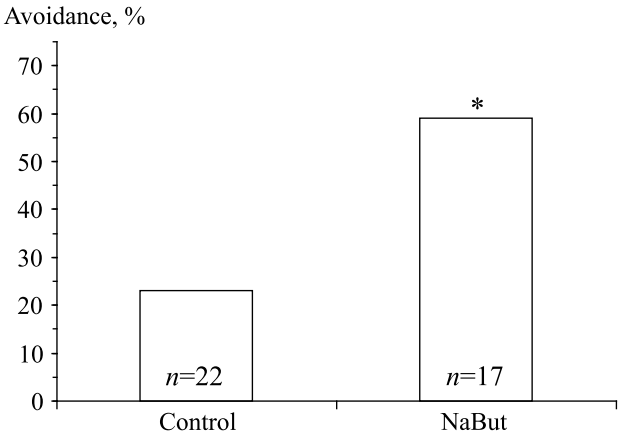


**Fig. 1.** Effects of systemic administration of NaBut 3 min after "weak" training (1.2 g/kg intraperitoneally, 0.1 ml). Here and in Fig. 2, 3: the test was performed 24 h after training. *n*: number of animals. **p*<0.05 in comparison with the control.

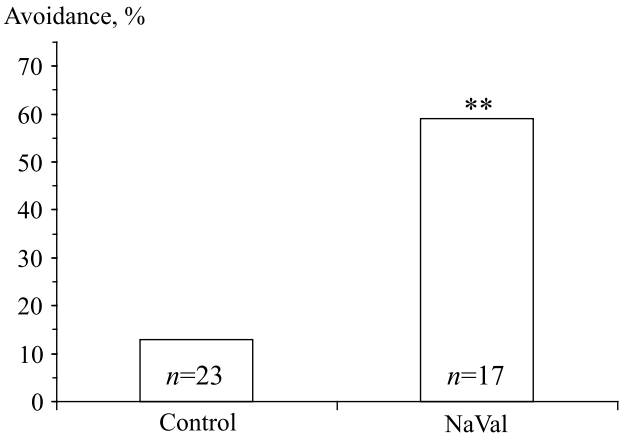


**Fig. 2.** Effects of NaVal system administration 30 min before "weak" training (0.1 g/kg intraperitoneally, 0.1 ml). ***p*<0.01 in comparison with the control.

Long-term memory formation is provided by two successive phases of gene expression [1]. New experimental series to detect time window for NaVal effects was performed in order to establish which stage of learning-induced expression can be affected by histone deacetylase inhibitors. NaVal was administered at different times before and after training and reproduction of learned behavior was tested 24 h later. Separate control groups with saline administration were created for each time point. The test revealed significant increase in avoidance level only when NaVal was administered 30 min before or 5 min after training (Fig. 3).

Thus, the test histone deacetylase inhibitors effectively enhanced "weak" memory when administered before training or immediately after it. Such time window for agent effects suggests that they exert their influence on early stages of memory consolidation. If

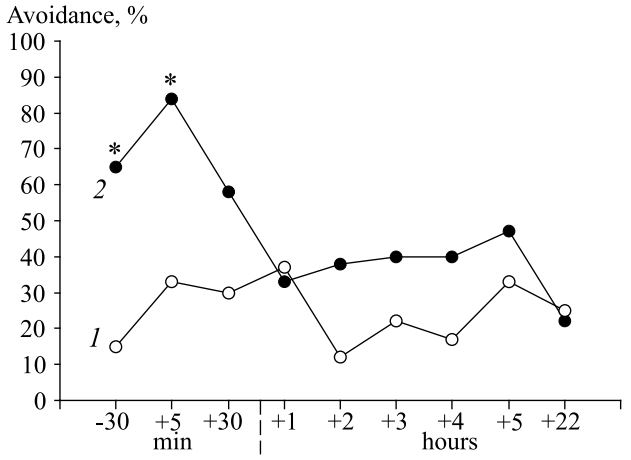


**Fig. 3.** Effects of NaVal system administration (0.1 g/kg intraperitoneally, 0.1 ml) at different times before and after "weak" passive avoidance training. Along the abscise axis – time of agent administration in relation to the time of training. Separate control group of animals corresponds to every time point ($n$=17-21). *1*) control; *2*) NaVal. *$p$<0.05 in comparison with the control.

the effects of inhibitors are based on nuclear chromatin modifications and on increased gene expression, the observed dynamics suggests that these agents affect immediate early gene expression. To test this assumption we performed quantitative analysis of c-Fos and ZENK transcriptional factor expression following "weak" training and following NaVal system administration within six structures of chick brain: medial striatum (MSt, subpallidal motor area); associative areas – hyperpallium densocellulare (HD), acropalium intermediate (Ai), hippocampus (Hpc); higher integrative areas – intermediate mesopallium (IMM), dorsocaudal nidopallium (Ndc). Expression density data for the structures where statistically significant intergroup differences were observed are presented in the Table 1.

Induction of ZENK expression in HD, Ai and IMM as well as c-Fos induction in Ai were observed in brains from trained animals, administered with saline, in comparison with passive control animals. Involvement of some of mentioned structures in passive avoidance training was previously demonstrated for "strong" training [3]; our findings demonstrated that formation of "weak" memory also induces protein synthesis in these areas. At the same time, NaVal induced no increase in c-Fos and ZENK expression, what rejects the hypothesis that histone acetylase effect can be mediated by induction of these transcriptional factors.

It has been shown previously that effects of histone deacetylase inhibitors on synaptic plasticity in mouse brain are mediated by constitutive transcription factor CREB (cAMP response element-binding protein), that regulates *c-fos* and ZENK gene expression [14]. At the meantime, no induction of *c-fos* and *egr-1* (analogue of ZENK in mammals) expression was observed in this study following administration of histone deacetylase inhibitors and it has been shown that among 12 genes regulated by CREB and involved in long-term memory formation, inhibitors increased expression of only two of them (*Nr4a1* and *Nr4a2*) [14]. Our data indicated that cognition stimulating NaVal effects in chick brains are also realized not by induction of c-Fos and ZENK transcriptional factors; in addition, its inhibiting effect on their induction may be associated, for example, with redistribution of transcriptional factor CREB activity.

The study was supported by Russian Foundation for Basic Research (grant No. 09-04-12283 ofi-m).

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**TABLE 1.** Expression of Transcriptional Factors c-Fos and ZENK in Chick Brain Structures

| Protein | Brain region | Experimental groups | | |
|---|---|---|---|---|
| | | passive control ($n$=6) | "weak" training+saline ($n$=6) | "weak" training+NaVal ($n$=6) |
| c-Fos | Ai | 156.8±35.5 | 317.4±50.9* | 197.1±40.4 |
| ZENK | Ai | 70.6±12.0 | 117.8±16.3* | 98.7±27.8 |
| ZENK | IMM | 64.3±24.5 | 156.7±22.3* | 167.3±60.6 |
| ZENK | HD | 130.4±42.2 | 256.9±19.3* | 191.4±57.1 |

**Note.** *$p$<0.05 in comparison with passive control group. Mean values for expression density are presented (the number of immunopositive cells per mm$^2$)±standard error of the mean.

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