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Analysis of *NAPA* Gene Expression in Brain Structures of Wistar Rats during the Formation of Long-Term Spatial Memory and Physical Activity under Stress Situation

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In the cerebellum, hippocampus, and prefrontal cortex of mature male Wistar rats with trained spatial navigational skill in the Morris water maze, the transcriptional activity the *NAPA* gene that regulates the transport and secretion of synaptic vesicles, release of neurotransmitters, and protein degradation was determined by real-time PCR. Animals subjected to forced swimming in a time-matched regime (active control) and naïve rats were used as the comparison groups. Suppression of *NAPA* gene activity was found in the hippocampus and cerebellum of the active control group, while navigation skill training led to a significant increase in gene expression in all brain structures under study. The findings suggest the existence of specific mechanisms regulating *NAPA* gene activity during the formation of spatial memory and adaptive behavior under stress conditions.

Spatial memory is a complex cognitive process providing navigation in the physical environment and interaction of the body with the outside world. The neurobiological basis of this process involves multiple plastic rearrangements in different brain areas [1]. Modulation of genome activity is one of the key mechanisms of neuronal plasticity [2], and the genes that regulate the efficiency of synaptic transmission and processes of synaptogenesis play a special role [3]. The *NAPA* gene that encodes the soluble protein α -SNAP is one of the principal genes involved in these processes. It is generally accepted that the interaction of α -SNAP with NSF ATPase (N-ethylmaleimide sensitive factor, vesicle fusing ATPase) and the SNARE membrane protein complex ensures docking and fusion of the synaptic vesicles with their target membranes. This mechanism is crucial for various cellular processes, including the transport and secretion of vesicles, neurotransmitter release, and protein degradation [4]. In addition, there is evidence that α -SNAP can affect energy metabolism via an NSF ATPase-independent pathway. It is assumed that this effect of α -SNAP is due to its participation in the regulation of 5'-AMP-dependent protein kinase activity and mitochondrial neogenesis, thus providing functionally significant links between membrane fusion events, neurotransmitter secretion, and metabolism [5]. An augmented level of *NAPA* expression in rat limbic structures has been confirmed not only during the formation of long-term memory in models of spatial navigation [3], but also during conditioned reflex freezing [6]. However, changes in gene expression in other brain structures during learning have not been studied. In addition, studies using the Morris maze model [3] did not assess the influence of motor load or emotional stress, which, taking into account the specific activity profile of α -SNAP, can make a significant contribution to the dynamics of gene expression encoding the protein. This is especially true for the effects of motor activity associated with task performance in the Morris maze, because there is

evidence that the SNARE complex is involved in ensuring an optimal ratio of tonic and phasic activity of motor neurons preventing motor fatigue [7]. Such a study is appropriate for increased understanding of the participatory mechanisms of the genetic apparatus in the integrative activity of the CNS, as well as for a comparative analysis of the mechanisms of the adaptive process under conditions of unavoidable stress exposure and cognitive processes that occur during learning in stressful situations. The aim of this study was to determine *NAPA* gene expression in the spatial memory-relevant brain structures, hippocampus, cerebellum and prefrontal cortex of mature Wistar rats trained in the navigational skill in the Morris water maze and in animals after forced swimming in a time matched regime

MATERIALS AND METHODS

The experiments were performed on 3-month-old male Wistar rats ($n=36$, body weight 250 ± 15 g) maintained under standard conditions at 12/12-h light/dark cycle with free access to water and food in compliance with the requirements of the Directive 2010/63/EU of the European Parliament and of the Council (September 22, 2010; On the Protection of Animals Used for Scientific Purposes). The experiments were also conducted in accordance with the rules approved by the Commission on Bioethics of the P. K. Anokhin Research Institute of Normal Physiology (Protocol No. 1, February 1, 2023). Animal behavior was studied using the Morris spatial water maze (Columbus Instruments) according to the protocol described earlier [8,9]. The rats for behavioral and genetic experiments were divided into 3 groups (12 rats per group): group 1 consisted of naïve animals, group 2 included rats subjected to the procedure of forced swimming without an escape platform (active control), and group 3 comprised animals trained in the Morris water maze with an escape platform. The experimental protocol was designed in such a way that the time of forced swimming in the active control group corresponded to the time spent in the water by the trained animals in the water maze, i.e., each trained rat according to the swimming duration pattern corresponded to a specific control rat. Twenty-four hours after the end of the 5-day behavioral testing protocol, all animals were decapitated and the hippocampus, prefrontal cortex, and cerebellum were isolated on ice-cold plates (4°C) for studying *NAPA* gene expression by real-time PCR according to a previously described protocol [8,9]. The β -actin gene was used as a reference gene for calculation of the *NAPA* gene expression level using the $2^{-\Delta\Delta C_t}$ method [10]. The level of *NAPA* gene expression in groups 2 and 3 was calculated relative to *NAPA* gene expression data in naïve animals. The results were statistically processed using Statistica 10.0 software (StatSoft, Inc.). The significance of differences between the variational series of indicators was assessed using the nonparametric Mann-Whitney U test, with a correction for the multiple comparison false discovery rate [11]. Correlation analysis was performed using Spearman's rank correlation coefficient (r). The data are presented as $M\pm SE$. The differences were considered statistically significant at $p<0.05$.

RESULTS

Analysis of behavioral experiments on the formation of long-term spatial memory showed that the mean latency of reaching the escape platform in rats trained in the Morris water maze during the second and all subsequent training sessions was significantly lower ($p<0.05-0.01$) than during the first day; by the end of training (day 4), the mean platform escape latencies reached a plateau and were <10 sec ($p<0.01$), which attested to the formation of a stable long-term

spatial memory, as we described in detail earlier [8,9]. Analysis of the transcriptional activity of the *NAPA* gene revealed significant differences in the level of its expression in the hippocampus, cerebellum, and prefrontal cortex between the active control and trained animals and significant differences of both experimental groups from naïve animals. Thus, in animals subjected to forced swimming for 4 days (active control), *NAPA* gene activity was suppressed by 90% ($p<0.01$) in the hippocampus and by 95% in the cerebellum ($p<0.01$), but no significant differences from naïve control were revealed in the cerebral cortex (Fig. 1). In all three brain structures, *NAPA* activity in trained animals was considerably greater than in the active control group (in the hippocampus by 1000%, in the cerebellum by 1130%, and in the prefrontal cortex by 1700%) (Fig. 2). Comparison of individual spatial task performance indicators and *NAPA* gene activity in trained animals revealed a negative correlation between platform latency on the fourth day of training and the *NAPA* gene expression in the prefrontal cortex ($r=-0.942857$, $p=0.005$).

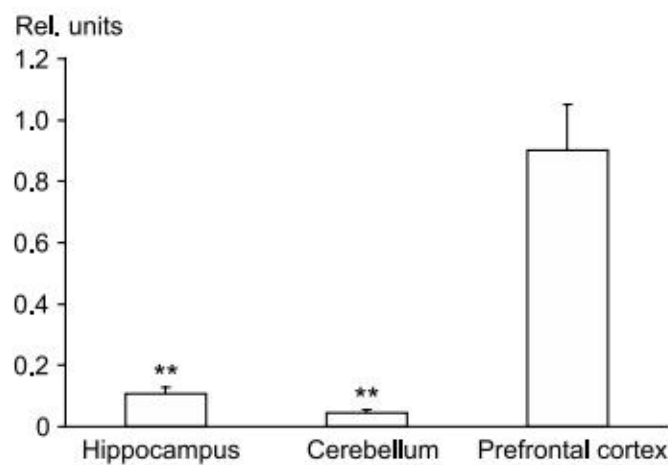


Fig. 1. Expression of *NAPA* gene in the hippocampus, cerebellum, and prefrontal cortex of active control group rats in the Morris water maze. Here and in Fig. 2: ** $p<0.01$ in comparison with intact animals.

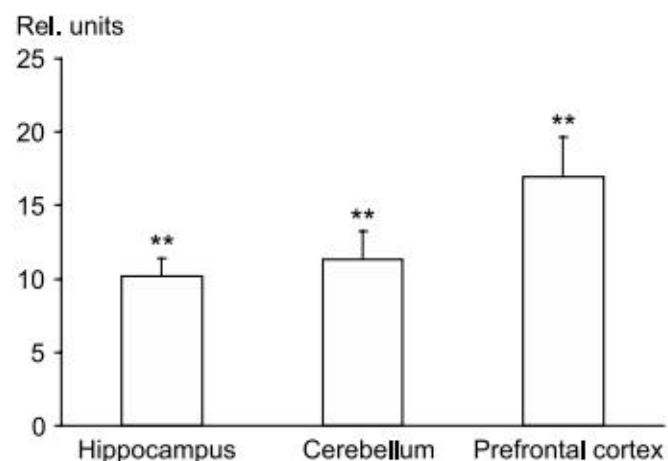


Fig. 2. Expression of *NAPA* gene in the hippocampus, cerebellum, and prefrontal cortex of trained rats in the Morris water maze.

Our data on increased *NAPA* expression in the hippocampus after training are consistent with the results of other researchers who used a similar training protocol [3] and with the data on positive modulation of the expression of this gene in the limbic region after formation of a conditioned freezing reflex [6]. Moreover, our study demonstrated for the first time that the effectiveness of long-term memory mechanisms ensuring stabilization of a spatial skill during the later stages of its formation (platform latency on the fourth day of training) is associated with increased *NAPA* activity in the prefrontal cortex and cerebellum. These data are consistent with the concepts regarding the role of the prefrontal cortex in mechanisms of executive control, the effectiveness of which becomes decisive at the later stages of skill formation [1]. At the same time, the hippocampus and cerebellum are predominantly involved in such spatial memory mechanisms as space mapping and integration of sensory information with information about directional movement, which are dominant in the initial stages of skill formation [12,13]. Hence, *NAPA* expression was increased in all three brain structures of rats with established spatial memory, however, its maximum intensity alongside the correlation with task performance, was present only in the prefrontal cortex. We also found a decrease in *NAPA* expression in the cerebellum and hippocampus of animals of the active control group, i.e. changes were the opposite to those detected in trained rats. Stress and exploratory and physical activity can be the factors influencing gene expression in the active control group. However, in a contextual fear conditioning model [6], no changes in limbic *NAPA* expression were documented relative to that in naïve animals from two active groups allowed either free exploratory activity without reinforcement, or given electrocutaneous stimulation not associated with conditioned stimuli. Thus, it seems most likely that the decrease in *NAPA* activity observed in our active controls was not just due to stress, but also to forced physical activity caused by unavoidable stressful effects. There are published reports on the influence of physical training on activity of the hippocampal genetic apparatus and a decrease in the expression of some genes [14]. Inhibition of *NAPA* expression during physical activity caused by the need to overcome a stressful situation can be associated with the participation of α -SNAP protein in the regulation of cell energy metabolism. In this context, α -SNAP can suppress the activity of 5'-AMP-dependent protein kinase stimulating energy metabolism and mitochondrial formation [5]; hence, a decrease in *NAPA* expression can boost the energy potential of the cell. There are reasons to believe that this mechanism is implemented independently of the mechanisms of α -SNAP involvement in the regulation of the SNARE complex [5]. Furthermore, a decrease in the activity of the SNARE complex that includes α -SNAP and depends on the expression of *NAPA* gene can affect activity ratio of phasic and tonic motor neurons, enhancing resistance to synaptic fatigue thus impacting exercise [7]. At the same time, brain regional *NAPA* expression in trained rats did not decrease, but increased. Thus, reinforcement (the possibility of reaching the escape platform) and associated memory trace formation positively switches *NAPA* expression in the opposite direction compared to that observed in the active control. It is possible that such a reversal leads to a change in the pattern of activity of signaling pathways associated with α -SNAP from nonspecific activation of the energy metabolism and tonic release of mediators in active controls to activation of phasic neurotransmitter release underlying spatial memory formation. Revealingly, the decrease in *NAPA* activity in the active control group is not observed in the prefrontal cortex, a region that is characterized by the highest degree of specificity in terms of participation in cognitive processes. A possible mechanism associated with features of *NAPA* activity in various

functional states may be selective activation of various types of receptors, especially glutamate receptors, which largely control the expression of this gene [3,15].

Overall, further study of the mechanisms that ensure the dynamics of *NAPA* gene expression during learning and stress-induced physical activity is of contributory value towards understanding the mechanisms of neuronal plasticity and possible approaches to correcting any linked disorders.

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