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EXPERIMENTAL PAPERS

# The Effect of ACTH/MSH N-Terminal Fragment Analogs on the Anxiety Level, Pain Sensitivity and Levels of Neurotrophic Factors BDNF and VEGF in Primary Neuronal Cultures of Rats

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**Abstract**—ACTH/MSH-like peptides (melanocortins) have a wide range of neurotropic effects, including effects on learning and memory processes, neuroprotection, emotional state and pain sensitivity. Present work is aimed to compare the effects of peptides, the structure of which includes a natural fragment of ACTH and a stabilizing tripeptide PGP. The peptides $ACTH_{4-7}$PGP (Semax), $ACTH_{6-9}$PGP, and $ACTH_{7-10}$PGP were used in the work. The effects of these peptides on the exploratory behavior, anxiety level and pain sensitivity of white rats, as well as on the protein levels of the neurotrophic factors BDNF (brain derived neurotrophic factor) and VEGF (vascular endothelial growth factor) in primary neuron cultures were studied. A comparative study of the effects of analogs of different ACTH/MSH fragments revealed both similarities and differences in their neurotropic activity. The peptides structure of which includes a sequence of $ACTH_{4-7}$ or $ACTH_{6-9}$ have nootropic, anxiolytic and analgesic activity, and also cause an increase in VEGF levels in the culture of hippocampal neurons. The peptide containing the $ACTH_{7-10}$ sequence in the structure exhibits anxiolytic activity, increases exploratory behavior, does not affect pain sensitivity and has a stimulating effect on BDNF and VEGF levels in neuronal cultures. The data obtained indicate that different parts of the N-terminal region of the ACTH molecule are responsible for the manifestation of certain neurotropic effects of melanocortins. The results of the study can be used in the development of therapeutics based on natural melanocortins.

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**Keywords:** melanocortins, ACTH/MSH fragments, synthetic analogs, anxiety, pain sensitivity, neurotrophic factors, rat

## INTRODUCTION

Melanocortins are a family of neuropeptide hormones that includes adrenocorticotropic hormone (ACTH), three different forms of melanocyte-stimulating hormone (α-, β-, and γ-MSH), fragments of these hormones, and their synthetic analogs. Melanocortins (MCs), MC receptors and endogenous antagonists of these receptors form the melanocortin system of the body [1]. The MC system is involved in the regulation of a wide range of physiological functions, including steroidogenesis, pigmentation,

immune system, neuroprotection, memory and attention, emotional state, pain sensitivity, and other functions [2–4]. The MC system plays an important role in modulating a number of pathological processes, including neurodegenerative and neuropsychiatric diseases [4, 5]. Currently, 5 types of MC receptors (MCR1–MCR5) have been characterized, which differ in their distribution in tissues and in their affinity for different ligands [6]. All endogenous MCs contain in their structure the HFRW sequence corresponding to the ACTH/α-MSH$_{6–9}$ fragment [7]. The HFRW tetrapeptide is the minimal fragment that can activate MCR, but this peptide has very low activity and selectivity, indicating the importance of residues outside the common sequence [8, 9].

The N-terminal region of the ACTH/MSH molecule, the ACTH$_{4–10}$ fragment (MEHFRWG) is responsible for the neurotropic effects of MC [7]. ACTH$_{4–10}$ heptapeptide is able to bind to MCR, has nootropic, neuroprotective and analgesic activity, and participates in the regulation of the body's response to stressors [2, 10, 11]. Neurotropic activity is also retained by fragments of natural peptides and their analogs, whose structure contains ACTH$_{4–7}$, ACTH$_{6–9}$ or ACTH$_{7–10}$ sequences [10, 12, 13]. It is suggested that active centers for different neurotropic effects may be located in different loci of the N-terminal region of the ACTH molecule [13, 14]. ACTH/MSH fragments can interact with MC receptors as biased ligands, leading to the activation of different signaling cascades that mediate different effects of the peptides [4, 15]. In addition, MCs can act as allosteric modulators of receptor activity of other neurotransmitter systems [16]. Such properties may determine a variety of behavioral effects of short ACTH/MSH fragments.

The dependence of MC neurotropic effects on structure is currently insufficiently investigated. The study of the structure-activity relationship will make it possible to identify the sequences responsible for the manifestation of certain effects, which is necessary for the development of new MC analogs with desired properties [17].

Short ACTH fragments are either unable to activate known MC receptors (ACTH$_{4–7}$ and ACTH$_{7–10}$) or have very low activity (ACTH$_{6–9}$), which may be due to their rapid proteolytic degradation [8, 14]. It was previously shown that the addition of proline-enriched sequences to natural peptides with neurotropic activity leads to an increase in the severity of action and prolongation of their effects [18, 19]. This approach was used in the development of heptapeptide Semax (MEHFPGP), the structure of which includes a fragment of ACTH$_{4–7}$ and stabilizing tripeptide PGP (ACTH$_{4–7}$PGP). Studies have shown that Semax has nootropic, anxiolytic, and analgesic activities [20–22]. In addition, Semax had a neurotrophic effect in in vivo and in vitro experiments—it increased the expression of brain derived neurotrophic factor (BDNF) in primary glial cell culture [23] and in rat brain structures when administered systemically [24]. Subsequently, ACTH$_{6–9}$PGP and ACTH$_{7–10}$PGP peptides (HFRWPGP and FRWGPGP) were synthesized and studied. It was shown that heptapeptide ACTH$_{6–9}$PGP, like Semax, exhibits nootropic activity, but its duration of action is shorter than that of Semax [25]. ACTH$_{6–9}$PGP had an anti-stressor effect in models of acute and chronic stress, showed neuroprotective activity, and also reduced the reaction of animals to painful thermal irritation [3, 26, 27]. The study of the physiological activity of ACTH$_{7–10}$PGP showed that this peptide exhibits anxiolytic activity and is able to improve the learning of rats in tests with negative reinforcement; however, unlike Semax, its administration leads to an increase in the number of erroneous reactions during learning with positive reinforcement [28]. The effect of ACTH$_{7–10}$PGP on pain sensitivity has not been previously studied.

It has now been shown that vascular endothelial growth factor (VEGF), originally considered as an endothelium-specific factor, has a direct effect on neurons and glial cells, regulating their growth and differentiation [29]. Along with the better-studied BDNF, VEGF is an important participant in a number of hippocampus-dependent processes, including those related to the regulation of cognitive function and emotion. VEGF is able to stimulate synaptic plasticity, enhance neurogenesis, and improve learning and emotional state in animals [29, 30]. It has been shown that α-MSH stimulates VEGF expression in the primary culture of rat hippocampal astrocytes, indicating a possible role of this factor in the neuroprotective and cognitive effects of MC [31]. The effect of N-terminal fragments of ACTH/MSH and their analogs on VEGF expression has not been previously studied.

The neurotropic effects of Semax, $ACTH_{6-9}PGP$ and $ACTH_{7-10}PGP$ were studied in different experimental models in vitro and in vivo, using different doses and route of peptide administration, which makes it difficult to compare the results obtained. To compare the activity of the peptides, we studied the neurotrophic, anxiolytic, and analgesic effects of analogs of N-terminal fragments of ACTH under the same experimental conditions.

The aim of the presented work was a comparative study of the effects of heptapeptides Semax, $ACTH_{6-9}PGP$ and $ACTH_{7-10}PGP$ on pain sensitivity and anxiety level of rats, as well as on the levels of neurotrophic factors BDNF and VEGF in primary cultures of rat brain neurons.

## MATERIALS AND METHODS

The following synthetic melanocortins—$ACTH_{4-7}PGP$ (MEHFPGP, Semax), $ACTH_{6-9}PGP$ (HFRWPGP), and $ACTH_{7-10}PGP$ (FRWGPGP) were used in this work. The peptides were synthesized in the Laboratory of Molecular Pharmacology of Peptides at the National Research Centre "Kurchatov Institute."

### *Experiments on primary cultures of hippocampal and cerebral cortex neurons of the rat brain*

Primary cultures of hippocampal and cerebral cortex neurons of embryonic rats (E17) of the Sprague–Dawley strain were used. Tissues were mechanically dissociated, cells were seeded at a density of 150 thousand cells per well into poly-L-lysine-treated 96-well polystyrene plates (Nunc) and cultured at 37°C in 100 μL of standard serum-free medium N2 [32] containing 15 mM HEPES in addition. After culturing for 5 days, sterile solutions of the tested peptides were added to the culture medium up to the indicated concentration. An equivalent volume of solvent was added as a control. 24 h after peptide addition, the culture medium was withdrawn and cold (4°C) extraction buffer was added [33]. After incubation for 15 min at 4°C and four freeze-thaw cycles, cell extracts were centrifuged for 1 h at 15000 *g* (4°C), and the supernatants were withdrawn and stored at –70°C. The levels of BDNF and VEGF in the obtained lysates were assessed by enzyme immunoassay using BDNF Emax (Promega) and Rat VEGF Construction Kit (Antigenix) according to the methods and recommendations of the manufacturers.

### *Animal experiments*

The work was performed on 170 male Wistar rats weighing 220–250 g obtained from the Stolbovaya Breeding Centre (Moscow region, Russia). The animals were kept in standard vivarium conditions with 12-hour light regime and free access to water and standard laboratory food. Before the beginning of the experiment, all rats were subjected to 10-day adaptation—daily handling for 1–2 min.

When studying the effect of peptides on rat behavior, the substances were administered intranasally (i/n) 15 min before testing. Peptides were administered to awake animals at a dose of 0.05 mg/kg in an aqueous solution in the volume of 0.1 mL/kg body weight. Peptide doses, time and route of administration were chosen on the basis of previously conducted studies of the effect of ACTH fragment analogs on animal behavior [20, 25, 28]. When studying the effect of peptides on pain sensitivity in rats, the substances were administered intraperitoneally (i/p) at a dose of 0.5 mg/kg in an aqueous solution in the volume of 1 mL/kg body weight. Earlier we have shown that Semax when administered i/p has an analgesic effect, with the most effective dose being 0.5 mg/kg. When administered i/n Semax had no effect on pain sensitivity of rats [34]. Based on these data, we selected the doses and method of peptide administration to study their effect on the pain sensitivity of rats. In all experiments, control animals were injected with an equivalent volume of water for injections at appropriate times and in an appropriate manner.

### *The elevated plus maze test*

The elevated plus maze (EPM) test was used to assess exploratory behavior and anxiety level of the animals. The experimental chamber of the maze consists of four arms diverging from the center. Two opposite arms are closed with walls; the other two arms are open. Two series of independent experiments were conducted on different animals. In the first case animals were tested under homogeneous dim illumination (illumination of open arms—45 lx, closed arms—20 lx), in the second case—under contrast illumination of the arms (closed arms darkened—8 lx, open arms—brightly illuminated—450 lx). The rat was placed in the center of the maze

and during 5 min the time spent in the open and closed arms of the maze, the number of open and closed arm entries, as well as the number of rears and head dips from the open arms were recorded.

### *The paw-pressure test*

To assess the pain sensitivity of animals, we used the paw-pressure test, in which the analgesic effect of Semax was previously recorded. In this test, a uniformly increasing pressure on the hind paw serves as a pain stimulus. The measurement was performed with the help of analgesimeter ("Ugo Basile" company, Italy). The level of pain sensitivity was determined by the value of pressure on the paw at the moment of paw withdrawal. The pressure was measured in conventional units of the device (one conventional unit corresponds to an increase in load by 20 g/cm$^2$). The maximum load on the paw was 25 conventional units. Three measurements of baseline pain sensitivity were performed before drug administration. When analyzing the results to calculate baseline pain sensitivity, the initial values were averaged. After injection, 6 measurements of pain sensitivity were made at 15 min intervals. The severity of the analgesic effect was evaluated as a percentage of the maximum possible effect. For this purpose, during statistical analysis of data, the relative change in pain sensitivity for each animal at each measurement was calculated using the formula: $(P_i - P_0) / (P_{max} - P_0) \times 100$, where $P_i$ is the value of pain threshold at measurement, $P_0$ is the baseline pain sensitivity, $P_{max}$ is the maximum load on the paw [35].

### *Statistical analysis*

Statistical analysis of the data was performed using the software packages "Statistica 10" and "GraphPad Prism 8". Analysis of the samples showed compliance of the distribution with the criteria of normal distribution ($p > 0.20$; Kolmogorov–Smirnov and Shapiro–Wilk tests) and homogeneity of variance ($p > 0.10$, Brown–Forsythe test), which allowed us to use analysis of variance (ANOVA). EPM test results and BDNF and VEGF levels were analyzed using one-way ANOVA for between-subjects factor PEPTIDE (control vs. Semax vs. $ACTH_{6–9}PGP$ vs. $ACTH_{7–10}PGP$) followed by post hoc analysis using Dunnett's test. When analyzing the effect of each peptide on changes in pain threshold, a two-way ANOVA with repeated measures (repeated ANOVA) was used for the between-subjects factor PEPTIDE (control vs. peptide) and the within-subjects factor TIME (time after drug administration). In case of significant influence of the above factors or their interaction, post hoc analysis was performed using the Fisher LSD test. Differences were considered statistically significant at $p < 0.05$. Data in the graphs are presented as mean ± standard error of the mean.

## RESULTS

### *1. Effect of Semax, $ACTH_{6–9}PGP$ and $ACTH_{7–10}PGP$ on the levels of neurotrophic factors in primary cultures of rat hippocampal and cerebral cortex neurons*

The effect of heptapeptides at a concentration of 0.1 nM on BDNF and VEGF content in neuronal cultures was investigated. The use of one-way ANOVA showed a significant effect of the factor PEPTIDE on BDNF levels in cortical neuronal cultures ($F_{3, 20} = 6.75$; $p < 0.003$). Multiple comparisons showed a statistically significant increase in BDNF levels upon administration of $ACTH_{7–10}PGP$ relative to control values ($p < 0.02$) (Fig. 1). No significant changes in BDNF levels were found in cultured neurons of the rat cerebral hemispheric cortex 24 h after administration of Semax or $ACTH_{6–9}PGP$ at a concentration of 0.1 nM ($p > 0.70$). When evaluating the effects of the drugs on BDNF levels in hippocampal neuronal culture, a statistically significant effect of the factor PEPTIDE was recorded ($F_{3, 12} = 9.08$; $p < 0.002$; ANOVA). Administration of $ACTH_{7–10}PGP$ resulted in a significant increase in BDNF content in culture compared to control ($p = 0.001$). Administration of Semax or $ACTH_{6–9}PGP$ at a concentration of 0.1 nM did not result in a significant change in BDNF levels ($p > 0.30$). Evaluation of the effects of peptides on VEGF levels in hippocampal neuronal culture demonstrated a statistically significant effect of the factor PEPTIDE ($F_{3, 12} = 9.09$; $p < 0.002$; ANOVA). Further analysis showed that administration of all tested peptides at a concentration of 0.1 nM resulted in a significant increase in neurotrophin levels in culture ($p < 0.03$).

### *2. Effects of Semax, $ACTH_{6–9}PGP$ and $ACTH_{7–10}PGP$ on exploratory behavior and anxiety level of animals under different experimental conditions*

The effect of peptides on anxiety level and explor-

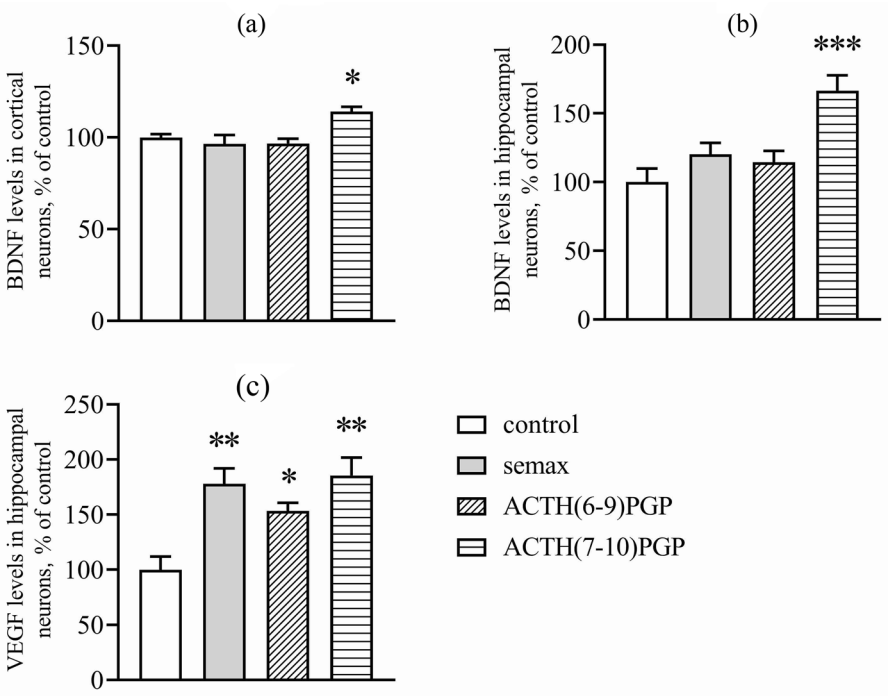


**Fig. 1.** Effects of Semax, $ACTH_{6–9}PGP$ and $ACTH_{7–10}PGP$ on the levels of BDNF and VEGF in cultured fetal rat cortical and hippocampal neurons. (a) BDNF levels in cortical neurons; (b) BDNF levels in hippocampal neurons; (c) VEGF levels in hippocampal neurons. The levels of BDNF and VEGF in protein extracts of cells were measured 24 h after adding peptides at a concentration of 0.1 nM. Each experimental group contains 6 (cortex) or 4 (hippocampus) cell cultures. The mean BDNF levels in the control neuronal cultures from the hippocampus and cerebral cortex were 4.63 and 2.38 pg/100 × $10^3$ plated cells, respectively; the mean VEGF levels in the control neuronal cultures from the hippocampus were 56.21 pg/100 × $10^3$ plated cells. Data are presented as mean ± SEM. * ($p < 0.05$), ** ($p < 0.01$) and *** ($p < 0.001$) represent significant differences vs. control.

atory behavior of rats was evaluated in the EPM test. Peptides were administered i/n, 15 min before the test. Two series of experiments were performed on different animals. In the first series contrast and in the second series even dim lighting conditions of the maze arms was used. There were 4 groups of animals in each series (control group and three groups with peptide administration).

Under contrast bright lighting conditions of the arms (under high stress load), the use of one-way ANOVA demonstrated a significant effect of the factor PEPTIDE on the time spent in the open arms ($F_{3, 36} = 9.76$; $p < 0.001$), the number of open arms entries ($F_{3, 36} = 5.76$; $p < 0.003$), and the number of head dips ($F_{3, 36} = 7.25$; $p < 0.001$). Further analysis showed that the administration of all investigated peptides resulted in statistically significant increases in the above parameters relative to control values (Fig. 2). No differences between the peptides were detected ($p > 0.20$). No significant effect of the investigated preparations on the number of rears and closed arms entries was observed ($F_{3, 36} < 1.8$; $p > 0.15$, one-way ANOVA).

Using even dim lighting conditions of the arms (under mild stress load), ANOVA showed a statistically significant effect of the factor PEPTIDE on the number of rears ($F_{3, 36} = 6.44$; $p < 0.001$) and the number of closed arms entries ($F_{3, 36} = 4.33$; $p < 0.01$). Further analysis showed that administration of $ACTH_{7–10}PGP$ leads to a significant increase in these indices both relative to control values and compared to groups of rats receiving injections of Semax or $ACTH_{6–9}PGP$ (Fig. 3).

In the groups of rats injected with Semax or $ACTH_{6–9}PGP$, the number of rears and entries into the closed arms of the maze did not differ from control values ($p > 0.17$). No significant effect of the factor PEPTIDE on the time spent in the open arms, the number of head dips and entries into the open arms was recorded in this test modification ($F_{3, 36} < 1.5$; $p > 0.20$).

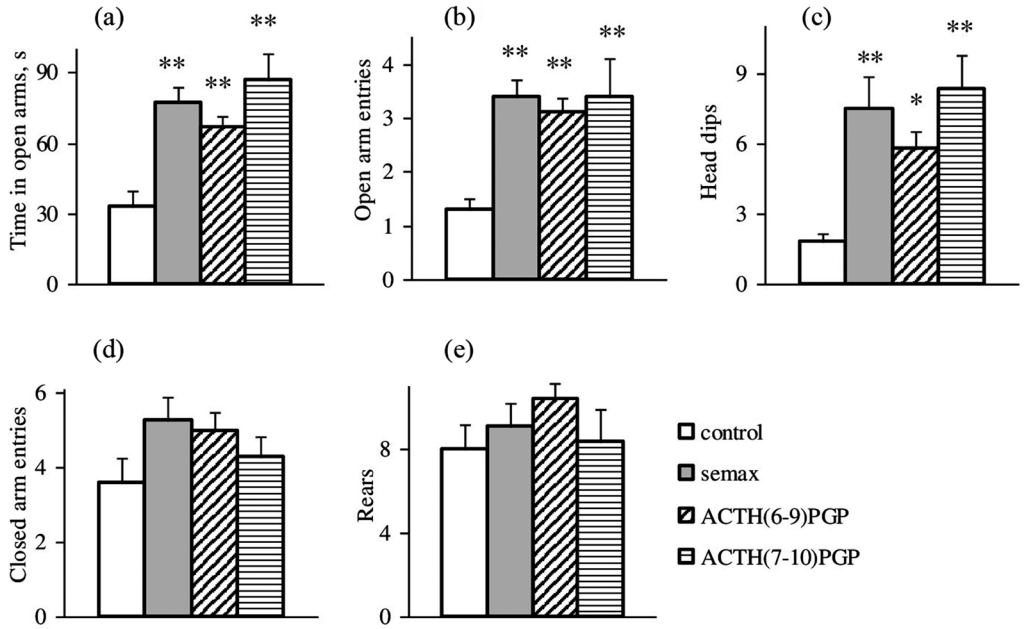


**Fig. 2.** Effects of Semax, $ACTH_{6-9}PGP$ and $ACTH_{7-10}PGP$ on rat behavior in the Elevated plus maze under contrast bright lighting conditions. (a) Time spent in the open arms; (b) the number of entries into open arms; (c) the number of head dips; (d) the number of entries into closed arms; (e) the number of rears. Peptides were injected intranasally, at dose of 0.05 mg/kg, 15 min prior to test. $n = 10$ rat / group. Data are presented as the mean ± *SEM*. * ($p < 0.05$) and ** ($p < 0.01$) represent significant differences vs. control.

### *3. Effect of Semax, $ACTH_{6-9}PGP$ and $ACTH_{7-10}PGP$ on pain sensitivity of rats*

The effect of peptides on pain sensitivity of rats was studied in the "paw-pressure" test. Peptides were administered i/p at a dose of 0.5 mg/kg. The effects of each peptide were studied in a separate experiment. In each experiment there were 2 groups of rats (control group and group with peptide administration).

When examining the effects of Semax, a two-way ANOVA revealed a statistically significant effect of the factor PEPTIDE ($F_{1, 28} = 14.42$; $p < 0.001$), as well as a significant interaction between the PEPTIDE and TIME factors ($F_{8, 224} = 4.20$; $p < 0.0001$) for the value of pain threshold. Further analysis showed a statistically significant increase in this index in the group of rats receiving Semax injection from the 15th to 75th min after peptide administration relative to control values. Moreover, from the 15th to the 60th min after injection the value of pain threshold in this group of rats statistically significantly exceeded the baseline values. No significant differences from the baseline values were observed in the control group of animals ($p > 0.16$) (Fig. 4).

In examining the effects of $ACTH_{6-9}PGP$, a two-way ANOVA revealed a statistically significant effect of the factors PEPTIDE ($F_{1, 28} = 14.42$; $p < 0.001$) and TIME ($F_{8, 224} = 5.71$; $p < 0.001$), as well as a significant interaction of these factors ($F_{8, 224} = 2.70$; $p < 0.01$) for the magnitude of pain threshold. Post hoc analysis showed a statistically significant increase in pain threshold in the group of rats injected with $ACTH_{6-9}PGP$ from the 15th to 45th min after injection compared to the control. The value of this index at 15 and 45 min after the peptide injection was statistically significantly higher than the baseline values, and after 30 min the increase relative to the baseline values was at the level of trend ($p < 0.06$). In the control group of rats, no significant differences from baseline values were observed ($p > 0.11$) (Fig. 4).

In examining the effect of $ACTH_{7-10}PGP$ on the pain sensitivity of rats, ANOVA revealed no significant effect of the factors PEPTIDE and TIME and the interaction of these factors for the magnitude of the pain threshold of rats in the paw-pressure test ($F < 1.6$; $p > 0.15$) (Fig. 4).

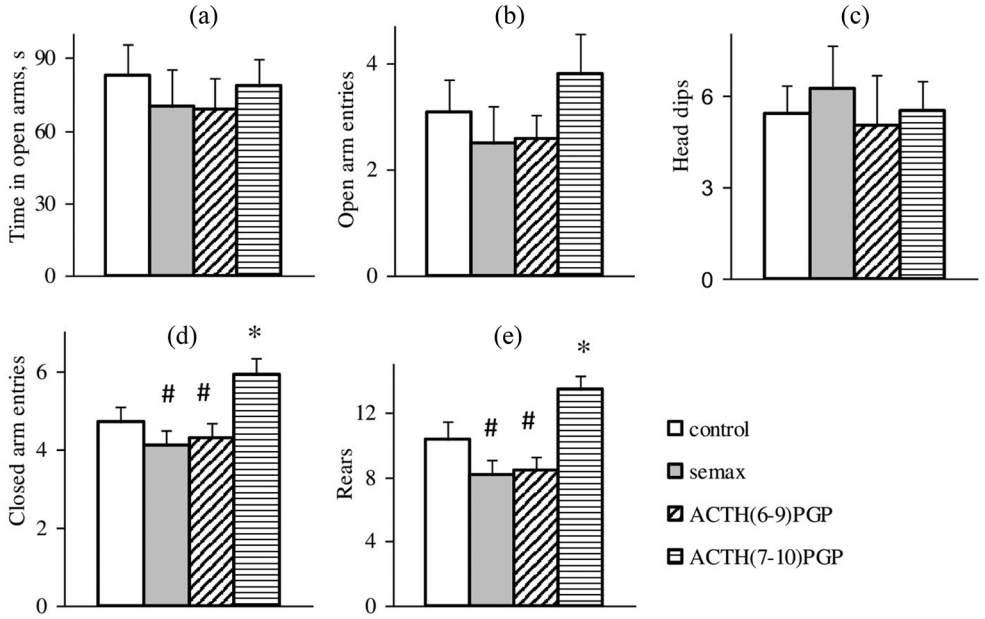


**Fig. 3.** Effects of Semax, $ACTH_{6–9}PGP$ and $ACTH_{7–10}PGP$ on rat behavior in the Elevated plus maze under even dim lighting conditions. (a) Time spent in the open arms; (b) the number of entries into open arms; (c) the number of head dips; (d) the number of entries into closed arms; (e) the number of rears. Peptides were injected intranasally, at dose of 0.05 mg/kg, 15 min prior to test. N = 10 rat / group. Data are presented as the mean ± *SEM*. * ($p < 0.05$) represent significant differences vs. control, # ($p < 0.01$) and ## ($p < 0.01$)—vs. group $ACTH_{7–10}PGP$.

## DISCUSSION

MCs have a wide range of neurotropic effects, including effects on learning and memory, neuroprotection, emotional state, and pain sensitivity [2–4]. The site responsible for the neurotropic activity of MC is a fragment of $ACTH_{4–10}$ [7]. The results of structure-function studies suggest that the sequence of this fragment includes several different, possibly overlapping sites that are capable of inducing different effects by acting on receptors and activating different signaling pathways [4]. This work was devoted to the comparison of the effects of peptides whose structure included the natural fragment ACTH—$ACTH_{4–7}$, $ACTH_{6–9}$ or $ACTH_{7–10}$—and the stabilizing tripeptide PGP.

Previously, we showed that Semax ($ACTH_{4–7}PGP$) and $ACTH_{6–9}PGP$ when administered i/n improved learning in rats in models with positive (food) and negative (pain) reinforcement [25, 28]. Similar administration of $ACTH_{7–10}PGP$ improved the retention of the passive avoidance task, but negatively affected the acquisition of the food-motivated maze task [28].

In this work, to evaluate the effect of peptides on rat behavior, we used the EPM test in two modifications (under conditions of low and high stressor load), which allowed us to assess the dependence of peptide effects on test conditions. Experimental conditions (including differences in the illumination of the setup) determine the baseline level of exploratory activity and anxiety and, as a consequence, can influence the severity of anxiolytic/anxiogenic effects of various drugs and exposures [36, 37]. Comparison of the behavior of control rats in the EPM test under different conditions showed that increasing the illumination of open arms leads to a decrease in the time spent in the open arms, the number of head dips and entering the open arms. At the same time, the indicators related to exploratory activity, i.e., the number of rears and entrances into closed arms, did not change (Figs. 2, 3). Such changes indicate an increase in the animals' anxiety and fear reactions when the lighting level of the experimental setup is changed [38].

Evaluation of the effect of the studied drugs on the emotional state of animals showed that Semax and $ACTH_{6–9}PGP$ did not affect the behavior of rats

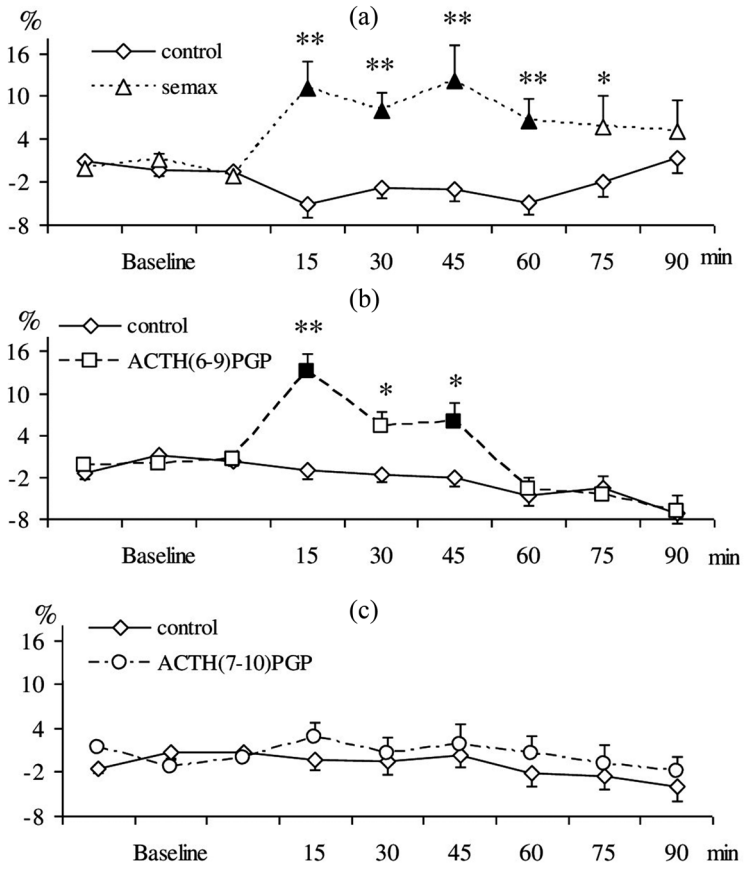


**Fig. 4.** Effects of Semax, $ACTH_{6–9}PGP$ and $ACTH_{7–10}PGP$ on the pain sensitivity of rats in the paw-pressure test. Peptides were injected intraperitoneally, at dose of 0.5 mg/kg. *X*-axis: time after peptide administration (min), *Y*-axis: pain threshold in percent to the maximum possible effect. $n = 15$ rat/group. Data are presented as the mean ± *SEM*. * ($p < 0.05$) and ** ($p < 0.01$) represent significant differences vs. control. Significant differences vs. baseline values are marked with shaded symbols ($p < 0.01$).

under conditions of mild stress load, however, under conditions provoking anxiety and fear reactions, they had anxiolytic effect. Administration of $ACTH_{7–10}PGP$ also resulted in a decrease in the anxiety of animals under stressfull conditions. However, this peptide, unlike Semax and $ACTH_{6–9}PGP$, stimulated exploratory activity of rats under conditions of mild stress load.

Thus, the studied analogs of ACTH fragments under conditions provoking anxiety and fear reactions (during training with pain reinforcement or in the situation of bright contrast lighting of EPM arms) have positive nootropic and anxiolytic effects. Under conditions of mild stress load (during acquisition of food-motivated maze task or in the situation of even dim lighting of EPM arms), Semax and $ACTH_{6–9}PGP$ peptides improve learning and do not affect the emotional state of animals. Under such conditions, the $ACTH_{7–10}PGP$ peptide stimulates exploratory behavior, which may lead to an increase in erroneous reactions during maze learning.

ACTH-like peptides are known to play an important role in the processes of pain perception. Depending on the structure, dose, and route of administration, both decreased and increased pain sensitivity can be observed [39]. Previously, we have shown that $ACTH_{4–10}$ fragment and Semax when administered i/p have an analgesic effect in the "paw-pressure" test [11, 34]. In the present study, i/p injection of Semax caused an increase in pain threshold in rats in this test, which is consistent with the previously obtained data. Injection of $ACTH_{6–9}PGP$ also led to a decrease in pain sensitivity, but the duration of the effect of this peptide was shorter than that of Semax. $ACTH_{7–10}PGP$ peptide had no effect on the pain sensitivity of rats. Consequently, the presence of $ACTH_{4–7}$ or $ACTH_{6–9}$ sequence in the structure of the peptide is necessary to maintain analgesic activity.

The study of the effect of analogs of ACTH fragments on the content of neurotrophic factors in neuronal culture showed that only $ACTH_{7–10}$PGP at the concentration used (0.1 nM) was able to stimulate BDNF levels in primary cultures of hippocampal and cerebral cortex neurons of the rat brain. Administration of Semax or $ACTH_{6–9}$PGP at the concentration used did not affect the content of this neurotrophin in the cultures. All three studied peptides had a stimulating effect on VEGF levels in hippocampal neuron cultures.

BDNF of the brain, in particular, of the cerebral cortex, is considered to be an important participant in the regulation of exploratory activity [40, 41]. Our data on the stimulation of exploratory activity by the peptide $ACTH_{7–10}$PGP, but not by Semax and $ACTH_{6–9}$PGP, are consistent with the ability of $ACTH_{7–10}$PGP to increase BDNF levels. A peptide containing the $ACTH_{7–10}$ sequence in its structure is able to exert a stimulatory effect on both BDNF and VEGF content in neuronal culture. Peptides containing $ACTH_{4–7}$ or $ACTH_{6–9}$ sequences were less active under the experimental conditions used and had an effect only on the level of VEGF in the hippocampal neuron culture. It has now been shown that VEGF has neuroprotective and neurotrophic effects and plays an important role in the regulation of cognitive functions and emotions [29, 42]. Increased VEGF expression leads to enhanced neurogenesis, improves hippocampus-dependent learning and normalizes anxiety and depression levels [30, 43]. One of the possible mechanisms of neuroprotective and cognitive effects of natural MCs and their analogs may be the regulation of neurotrophic factors production by brain cells [31].

Thus, a comparative study of the effects of synthetic MC analogs of various fragments of the N-terminal region of ACTH/MSH revealed both similarities and differences in their neurotropic activity. Peptides containing a fragment of $ACTH_{4–7}$ or $ACTH_{6–9}$ have nootropic, anxiolytic and analgesic activity, and also cause an increase in VEGF levels in hippocampal neuron cultures. The peptide containing the sequence of $ACTH_{7–10}$ in its structure exhibits anxiolytic activity, increases exploratory behavior, does not affect pain sensitivity, and has a stimulatory effect on BDNF and VEGF levels in neuronal cultures.

The obtained data indicate that different parts of the N-terminal region of the ACTH molecule are responsible for the manifestation of certain neurotropic effects of MC. The results of the study can be used in the development of drugs based on natural melanocortins.

## AUTHORS' CONTRIBUTION

Idea of the work and planning of experiments (N.F.M., I.A.G., O.V.D., N.G.L.) data collection (N.Yu.G., E.A.S., D.M.M., L.A.A.), data processing (N.Yu.G., E.A.S., O.V.D., N.G.L.), manuscript writing and editing (I.A.G., O.V.D., N.G.L.).

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## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Experiments with animals were conducted in accordance with international recommendations for biomedical research with laboratory animals and were approved by the Bioethics Commission of Lomonosov Moscow State University (Protocol 97-zh-3 of December 26, 2022 and no. 12.4-sod of November 16, 2023).

## CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

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