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Research Paper

Impact of dihydropyrimidinase-related protein 2 in memory formation on rats and its possible role in neuronal back remodeling

Arif A. Mekhtiev *, Shamsiyya M. Asadova

Academician Abdulla Garayev Institute of Physiology, Ministry of Science and Education, Baku, Azerbaijan

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ABSTRACT

The article concerns the problem of molecular mechanisms of memory formation. In this study the effects of polyclonal antibodies to serotonin-modulating anticonsolidation protein (SMAP) complex and its component dihydropyrimidinase-related protein 2 (DRP2) have been analyzed. Intra-cerebral administration of polyclonal anti-SMAP antibody significantly enhanced elaboration and strengthened memory formation in two complex behavioral conditioned models. At the same time, intra-cerebral administration of anti-SMAP antibody resulted in an increase of the content of nerve growth factor (NGF) in the water-soluble fraction of the hippocampus while intra-cerebral administration of anti-DRP2 antibody caused a decrease in the content of β-III tubulin (a marker of differentiated neurons) in the hippocampus and in the left parietal cortex of untrained rats. The obtained results indicate that DRP2 might participate in regulation of the processes of back remodeling of mature nerve cells of adult organisms, occurring during training of rats in the behavioral paradigm used in this study under the effects of anti-SMAP and anti-DRP2 antibodies. Conclusion is made that back remodeling (dedifferentiation) of mature nerve cells, apparently, is engaged in memory formation.

1. Introduction

The problem of memory formation has still been remaining the subject of multidisciplinary researches and unceasing discussions. Though mounting experimental results on the subject shed significant light on different aspects of memory formation, the problem is still far from its full understanding. One of the main questions of ongoing discussion is how memory is formed at the cellular and molecular level, in other words, what is the underlying mechanism of memory formation.

The discovery of neurogenesis in the adult brain (neoneurogenesis) added novel aspects to the analysis of this problem. In particular, it was proposed and thereafter proven in some experiments that newly formed neurons may be engaged into formation of acquired behavior, i.e. into the process of memory consolidation. The studies, undertaken by different researchers in the recent years, demonstrated direct engagement of newly formed neurons in the brain structures of the adult organisms in the formation of memory traces (Winocur et al., 2006; Gu et al., 2013; Sherstnev et al., 2015).

Earlier conducted studies showed that intra-cerebral administration of SMAP, identified first in the brain cortex and thereafter purified from the whole brains of rats, and related directly to serotonin levels (Mekhtiev, 2000), impairs processes of memory consolidation in the conditioned models with negative and positive reinforcements (Mekhtiev, 2000; Guseinov and Mekhtiev, 2013; Mekhtiev et al., 2015). Proceeding from these studies, we have undertaken an attempt to clarify the molecular mechanisms underlying possible engagement of the precursors of the nerve cells into the formation of long-term memory. For this purpose, we studied the effects of antibody-mediated blockade of SMAP activity on the formation of memory traces in the complex conditioned models of alternative running and two-lever operant differentiation in rats. Besides, we studied the effects of anti-SMAP antibody administration on the levels of nerve growth factor (NGF). Since mass-spectroscopy analysis of SMAP revealed that it is composed of three proteins – dihydropyrimidinase-related protein 2 (DRP2; or collapsin response mediator protein 2, CRMP2 (Nakamura et al., 2020)), actin and tubulin (Garina et al., 2018) we also studied the effects of polyclonal antibody to DRP2 on the levels of a marker of the differentiated neurons – beta III tubulin (Dráberová et al., 1998) – in the hippocampus and in the brain left and right parietal cortex of the rats.

* Correspondence to: Academician Abdulla Garayev Institute of Physiology, 78 Sharif-zadeh St., AZ1100 Baku, Azerbaijan.
E-mail address: arifmekht@yahoo.com (A.A. Mekhtiev).

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2. Material and methods

2.1. Biochemical methods

2.1.1. Proteins purification protocol

SMAP was purified from the cow brains. The brains were homogenized in the extracting buffer containing 0.05 M phosphate buffer (pH 7.2), 0.3 M NaCl, 5 mM EDTA and 0.1% Triton X-100 in a volume ratio of tissue and buffer as 1:4. The main stages of fractionations were as follows: 1) protein partial precipitation by ammonium sulfate under the final concentration of 40%, 2) gel-chromatography on the column (3 ×60 cm) of Sephadex G-150. The process of fractionation and selection of the immune-positive protein fractions was realized under the screening control by the indirect ELISA-test with application of anti-SMAP polyclonal immunoglobulins (Mekhtiev, 2000).

Purification of DRP2 in the first two stages was similar to SMAP purification. After elution from a column Sephadex G-150, the protein fraction (SMAP) was exposed to the effect of 40 mM deionized EDTA throughout the night on the end-to-end shaker. In the next morning the mixture of proteins with EDTA was subjected to isoelectric focusing on a flat-bed gel "Ultrogel" (LKB, Sweden) with application of ampholines of narrow pH range (pH 4–6; LKB, Sweden). After ending isoelectric focusing 1 cm width gel strips were collected, pH values were measured in each gel strip and the fraction with pH value that was equal to *pI* value of DRP2, was eluted from the gel and analyzed in SDS electrophoresis with protein standards (Gaisina et al., 2022).

2.1.2. Indirect ELISA-test

Measurements of the levels of SMAP and NGF in the brain samples were carried out by the indirect ELISA-test on the polystyrene plates with moderate level of adsorption (Sigma, Germany) with application of rabbit polyclonal immunoglobulins to SMAP and NGF (2.5 S; Sigma Immunochemicals, Germany). The animals were anesthetized and sacrificed, and the occipital and parietal areas of the cortex and the hippocampus were removed from the brain and frozen at − 70ºC. Prior to the beginning the ELISA-test, the water-soluble proteins were extracted from the brain samples. The proteins were extracted in the extraction buffer, containing 0.05 M phosphate buffer, 0.3 M NaCl, 5 mM EDTA and 0.1% Triton X-100 (pH 7.3), and their concentrations were leveled to 20 μg/mL with 0.1 M Tris-HCl buffer (pH 8.6). Each sample was repeated three times by pouring it into three wells of the polystyrene plate and the average value of three repeats was calculated. The concentrations of the total proteins were measured according to the Bradford method with application of 0.01% solution of Coomassi Brilliant Blue G-250 at the wavelength 595 nm. Immunoglobulins to SMAP, NGF (2.5 S; Sigma Immunochemicals, Germany) and beta III tubulin (Abcam, USA) were used as the primary antibodies, and goat anti-rabbit immunoglobulins with conjugated horseradish peroxidase (Sigma Immunochemicals, Germany) were used as the secondary antibodies. Visualization of the reaction was realized with application of substrate of horseradish peroxidase – 0.05% orthophenilendiamine in 0.05 M citrate-phosphate buffer (pH 4.5). The reaction was stopped 30 min after addition of substrate by adding 50 μL of 3 M NaOH solution into each well. The results of the reaction were recorded in the photometer for the ELISA-test "Spectra Max 250″ (Molecular Devices Co., USA) at the wavelength 492 nm.

2.1.3. Antibody purification

Anti-SMAP and anti-DRP2 polyclonal immunoglobulins were produced through 5–6-month immunization of the male rabbits of Chincilla species by sub-cutaneous administration of 300 μg of the purified protein per animal, always in a mixture with complete Freund adjuvant (Sigma, Germany). The first three injections were done within a time-frame of 14 days; the following injections were done once per month. Ten days after the third and following injections blood samples were taken from the ear vein, serum was separated after blood clotting by centrifugation of blood sample at 4000 g for 10 min and then polyclonal immunoglobulins G were precipitated by adding 100% ammonium sulfate to equal volume of serum (final concentration 50% of ammonium sulfate). Anti-SMAP and anti-DRP2 polyclonal immunoglobulins were analyzed as the primary antibodies in the indirect ELISA-test for the purpose of evaluation of their affinity level toward correspondent antigen used for immunization.

Anti-SMAP polyclonal antibody was purified from the solution of anti-SMAP immunoglobulins through a technique of immune-affinity chromatography carried out on the column (0.5 ×3 cm) of CNBr-Sepharose (Sigma, Germany) with covalently immobilized SMAP (Catty, 1989). After application of anti-SMAP immunoglobulins onto the column at a low speed of 8 mL/h, the column was thoroughly washed with 20 column volumes of 0.01 M phosphate buffer (pH 7.2) and under the control of spectrophotometric extinction at the wavelength 595 nm (Bradford method for measurement of protein concentration) specifically bound anti-SMAP antibody was eluted from the column through application of a chaotropic agent – 3 M KCNS. The eluted antibodies were dialyzed against 0.15 M NaCl, buffered to the value of pH 7.2, and frozen. In a single cycle, up to 12 mg of antibodies were eluted from the affinity column.

Similar to purification scheme of anti-SMAP polyclonal antibody, anti-DRP2 polyclonal antibody was purified from the solution of anti-DRP2 immunoglobulins through a technique of immune-affinity chromatography carried out on the column (0.5 ×3 cm) of CNBr-Sepharose (Sigma, Germany) with covalently immobilized DRP2 (Catty, 1989). In a single cycle, up to 6 mg of antibodies were eluted from the affinity column.

After finalizing purification procedures of anti-SMAP and anti-DRP2 polyclonal antibodies, they were analyzed as the primary antibodies in the indirect ELISA-test for getting information concerning evaluation of an affinity level toward correspondent antigen used for getting anti-SMAP and anti-DRP2 immunoglobulins and for immobilization on CNBr-Sepharose.

2.2. Physiological methods

In the first series of studies the behavioral experiments were carried out on the 5–6-month-old Wistar male rats in a conditioned model of alternative running with a food reinforcement (Semyonova, 1976), which is considered as difficult task for rats to learn. The experimental box was made from organic glass with dimensions of 60 by 60 cm. The platforms of 20 × 10 cm in size were secured to the left and right walls of the box, at the height of 16 cm from the bottom of the box. In this series of studies, the rats were divided into 3 groups: 1) intact group (n = 13); 2) control group – injections with rabbit non-immune γ-globulins (n = 13); 3) experimental group – injections with rabbit anti-SMAP antibody (n = 12). The animals were anesthetized with sodium ethaminal (40 mg per 1 kg of body mass) and administered with 10 μL of preparations at a concentration 1.5 mg/mL in saline, buffered to pH 7.2, into the brain left lateral ventricle. The learning sessions were undertaken 24 h after administration of the preparations until reaching 50% criterion of correct trials by the animals. The learning sessions of this session lasted for 7 days. In this series, the latencies of the first trials towards the platform were measured and a ratio of the correct trials to the total amount of trials in percent was calculated. The animals of the control and experimental groups were food deprived for 24 h before beginning the behavioral sessions. The animals of the experimental group were put onto the start platform at the entrance of the experimental box and their running and climbing onto the left and right platforms were reinforced by advanced placing on them food pellets of 200 mg in mass. The food pellets were prepared from sunflower oil, millet flour, ground corn grains and compound feed. After animal running towards the platform and eating the pellet on one side, it was placed again onto the starting platform and its running to the opposite side was again reinforced by food pellets. The food pellets were placed onto the platforms at the time,

when animal could not see it. The animals of the control group as well as the animals of the experimental group were food deprived for 24 h before experiments and just put into the experimental box for a timeframe equal to the timeframe of the experimental animals without reinforcement of their trials. The animals of the control and experimental groups at the end of experiments were allowed to get unrestricted access to food for 2 h.

In the second series, the experiments were carried out on the conditioned two-lever operant differentiation model. The rats were divided into 3 groups: 1) intact group (n = 13), 2) control group – injections with rabbit non-immune γ-globulins (n = 13) and 3) experimental group – injections anti-SMAP antibody (n = 12). During the learning sessions, the animals were learned to press the right-located lever of the two levers secured to the back wall of the experimental box close to each other to get food pellet as the reinforcement. In this series of experiments, the preparations were administered to the animals the same way and within the same timeframe between injections and beginning of learning sessions as in the second series of studies. The animals of the control and experimental groups were food deprived for 24 h before beginning the behavioral sessions. In this series, the latencies of the first trials towards the platform were measured and a ratio of the pressing the right-located lever to the total amount of trials in percent was calculated. The learning sessions of this series continued for 8 days.

In the third series of experiments, we analyzed the effect of administration of polyclonal anti-SMAP antibody into the brain left lateral ventricle on the levels of NGF in the hippocampus, right and left parietal cortex of the rats. The rats were culled into 3 groups: 1) intact group (n = 7), 2) control group – rabbit non-immune γ-globulins (n = 8), and 3) experimental group – anti-SMAP antibodies (n = 7). The preparations were administered to the animals the same way as in the second series of studies and 72 h later the rats were sacrificed, the hippocampus, right and left parietal cortex were removed from the brain, the water-soluble proteins were extracted and used as antigens in the indirect ELISA-test whose detailed stages are described above.

In the fourth series of experiments, we analyzed the effect of administration of anti-DRP2 polyclonal antibody into the brain left lateral ventricle on the levels of beta III tubulin in the hippocampus, right and left parietal cortex of the rats. The rats were culled into 3 groups: 1) intact group (n = 6), 2) control group – rabbit non-immune γ-globulins (n = 6) and 3) experimental group – anti-DRP2 antibody (n = 6). The preparations were administered to the animals the same way as in the second series of studies and 72 h later the rats were sacrificed, hippocampus, right and left parietal cortices were removed from the brain, the water-soluble proteins were extracted and used as antigens in the indirect ELISA-test.

The results of the all series of studies were grouped and analyzed by Friedman's χ^2-criterion and Student's t-criterion (Rohlf and Sokal, 1995).

All work was performed in accordance with the ethical standards of the relevant institutional and national committees for such matters and the **WMS Declaration of Helsinki** on ethical principles for medical research.

3. Results

3.1. Results of behavioral studies

Before beginning our studies on animals, we have got the results of indirect ELISA-test concerning affinity levels of anti-SMAP and anti-DRP2 polyclonal immunoglobulins and anti-SMAP and anti-DRP2 antibodies. The results showed high affinity levels of immunoglobulins and antibodies to both proteins used for rabbit immunization and antibody purification through immune-affinity chromatography carried out on the column of CNBr-Sepharose (Sigma, Germany).

In the first series of studies, in the course of elaboration of the task, animals demonstrated permanent increase of correct alternative trials to left and right platforms, climbing onto them and consuming food pellets. In the first day of learning sessions in the first 1–2 min after placing the animals into the box, they stayed mostly in one of the corners demonstrating mere signs of anxiety in the form of uninterrupted trembling and freezing. 2–3 min later, they started thorough exploration and sniffing of the box, looking for food and in different timeframes the animals climbed onto one of the platforms and consumed the food pellet. On the second day after putting into the box, the animals during the first minute again demonstrated the signs of anxiety, but thereafter they quickly began thorough exploration of the box. Beginning from the fourth experimental day, the animals very quickly entered the box, without delay came up to the platforms and climbed onto them.

In this series of studies administration of anti-SMAP antibody into the brain lateral ventricle of the rats 24 h prior to learning sessions in the conditioned model of alternative running significantly facilitated elaboration of the task. In particular, if the animals of the intact and control groups reached 50% learning criterion on the seventh experimental day, the animals of the experimental group under the effect of anti-SMAP antibodies reached this criterion on the fourth day ($p < 0.001$ on Friedman's χ^2-criterion; Fig. 1). Besides, to the seventh experimental day the animals of experimental group exceeded significantly 70% level of correct trials ($p < 0.001$ on Friedman's χ^2-criterion).

Furthermore, although the animals of all the studied groups demonstrated dynamics of decreased latencies of the first trial towards the platform, in the experimental group this decrease had much steeper character ($p < 0.001$ on Friedman's χ^2-criterion; Fig. 2). Hence, the obtained data indicate to a stimulating effect of anti-SMAP polyclonal antibody on memory formation and, correspondently, to the negative character of regulation of this process by SMAP.

In the second series of studies intracerebral administration of anti-SMAP polyclonal antibody to the rats, 24 h prior to learning sessions in the complex and time-consuming conditioned two-lever operant differentiation model significantly promoted and strengthened the formation of memory. If the animals of the intact group reached 50% criterion of correct trials on the seventh experimental day and the control animals reached that criterion on the sixth day, the animals of the experimental group reached the level of 50% correct trials on the fourth day ($p < 0.01$ on Friedman's χ^2-criterion; Fig. 3).

Besides the hallmark of the rate of achieving 50% learning criterion, each group had exact value of its upper limit of correct trials (plateau) in percent, no matter how long the learning sessions lasted. Hence, the plateau of correct trials for the animals of the intact group made 50%, the plateau for the control rats – 55%, and the plateau for the rats of the experimental group – 80% on the 8th day of learning sessions (Fig. 3).

As in the second series, the dynamics of the decrease of the latencies of the first trial towards the lever was observed in the all studied groups; however, in the experimental group this downregulation had much steeper character ($p < 0.001$ on Friedman's χ^2-criterion; Fig. 4).

3.2. Results of biochemical studies

After getting the results indicating the role for anti-SMAP antibodies in accelerating and strengthening memory formation, we decided to clarify the underlying mechanisms of such effect. For this purpose in the third series of studies, we evaluated the effect of intra-cerebral administration of anti-SMAP antibodies on the levels of NGF in the water-soluble fraction from the hippocampus, as well as from the right and left parietal cortices. It was shown that 72 h after administration of anti-SMAP antibodies, increased levels of NGF in the water-soluble fraction from the hippocampus ($p < 0.008$ on Friedman's χ^2-criterion and $p < 0.001$ on Student's t-criterion; Fig. 5) and its decrease in the left parietal cortex ($p = 0.008$ on Friedman's χ^2-criterion and $p < 0.001$ on Student's t-criterion; Fig. 5) was observed, whereas in the right parietal cortex the level of NGF did not change (not shown).

In the fourth series, we studied the effect of intra-cerebral

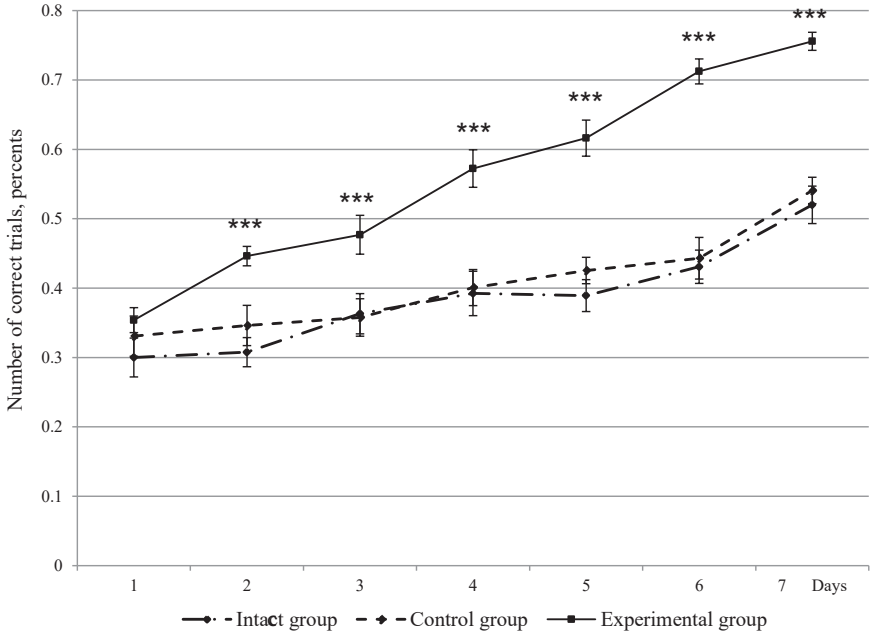

Fig. 1. The effects of anti-SMAP polyclonal antibody on the dynamics of memory formation in the conditioned alternative running model. n = 12; ***- $p < 0.001$ on Friedman's χ^2-criterion.

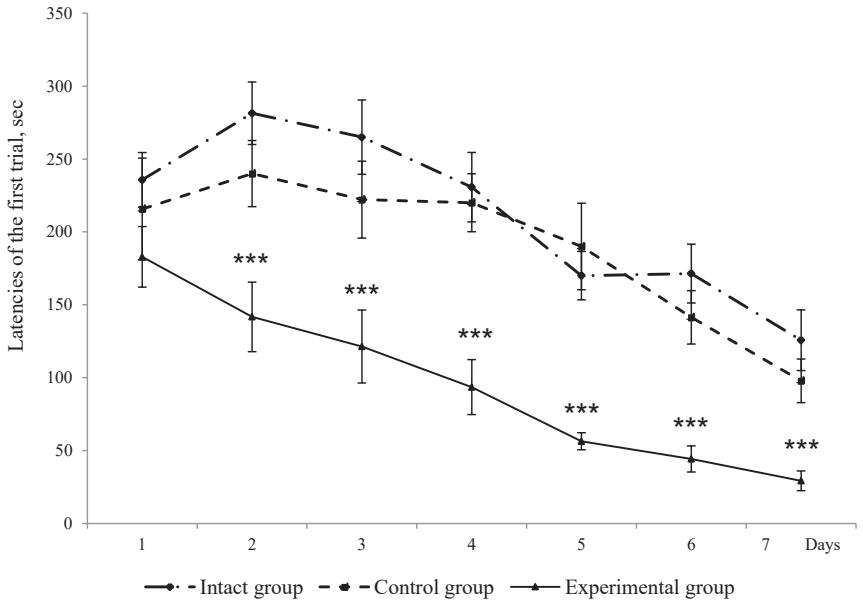

Fig. 2. The effects of anti-SMAP polyclonal antibody on the latencies of the first trial in the conditioned alternative running model. n = 12; ***- $p < 0.001$ on Friedman's χ^2-criterion.

administration of anti-DRP2 polyclonal antibody on the levels of beta III tubulin in the hippocampus, and in the right and left parietal cortex. It was revealed that 72 h after administration of anti-DRP2 antibodies there was a decrease of beta III tubulin content in the water-soluble fraction of the hippocampus of the experimental group rats relative to the control group values ($p < 0.01$ on Friedman's χ^2-criterion and $p < 0.001$ on Student's t-criterion; Fig. 6).

On the whole, the obtained results in these series of experiments indicate to a decrease of SMAP content in the parietal cortex after rats were trained in the conditioned alternative running paradigm. Besides, the results also suggest acceleration and strengthening of memory formation in the rats trained on the complex conditioned models when polyclonal antibody-mediated blockade of SMAP has been performed. Moreover, 72 h after intra-cerebral administration of anti-SMAP antibody, a decrease in the content of NGF was noticed in the water-soluble fraction of the left parietal cortex, while in the hippocampus its content increased. Finally, 72 h after intra-cerebral administration of anti-DRP2 polyclonal antibody a decrease of beta III tubulin in the hippocampus and left parietal cortex was observed.

4. Discussion

The experimental results obtained in the first series and concerning natural downregulation of SMAP in the rat brain cortex as a result of elaboration of a novel conditioned task, indicate to negative regulation of memory formation of complex conditioned tasks by DRP2. This

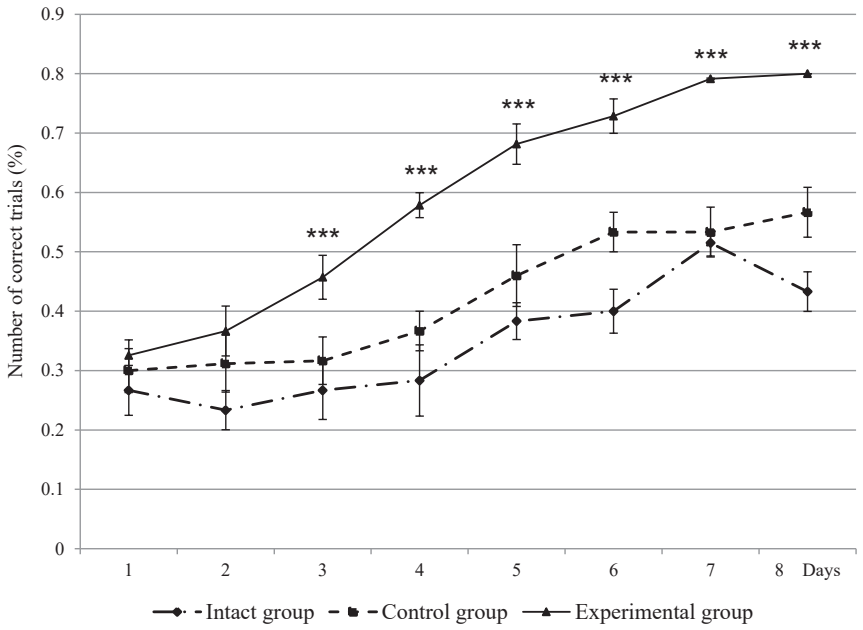

Fig. 3. The effects of anti-SMAP polyclonal antibody on the dynamics of memory formation in the conditioned model of two-lever operant differentiation. n = 12; ***- $p < 0.001$ on Friedman's χ^2-criterion.

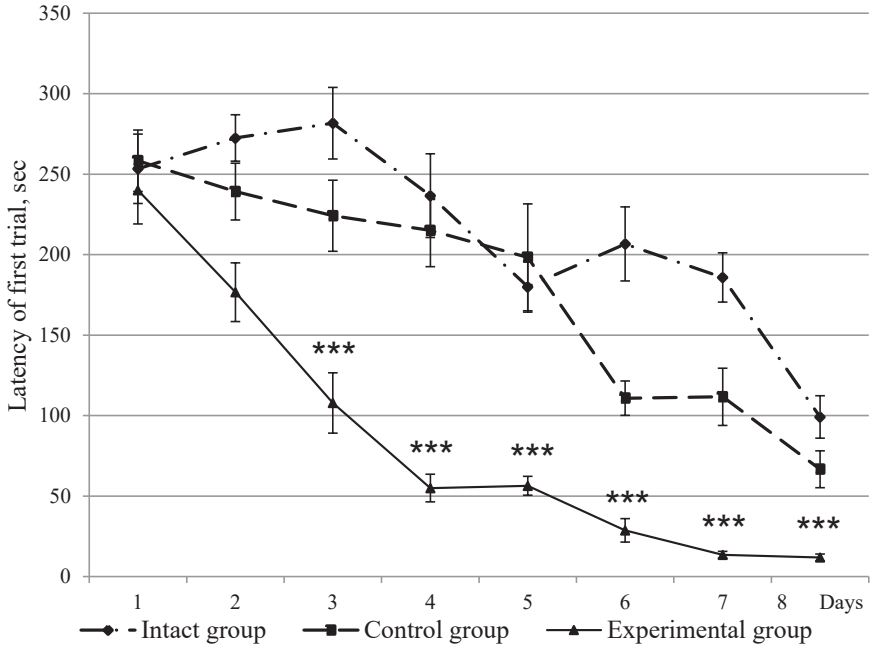

Fig. 4. The effects of anti-SMAP polyclonal antibody on the latencies of the first trial in the conditioned model of two-lever operant differentiation. n = 12; ***- $p = 0.001$ on Friedman's χ^2-criterion.

conclusion is made basing on the fact that other two component proteins of SMAP complex – tubulin and actin – both belong to a group of cellular structural proteins, deprived of any regulatory activity, and for this reason they cannot be attributed for realization of such kind of regulation. At the same time, another fact that supports this conclusion is that SMAP inactivation with polyclonal antibody promotes memory formation from standpoint of the less timeframe relative to the controls required to its formation and the sharp elevation of the values of the maximal level of correct trials in both types of complex conditioning models used in our studies (up to 70% and 80% of correct trials, respectively).

Results of the third series of the experiments have revealed prominent increase of NGF content in the water-soluble fraction of the hippocampus and its decrease in the left parietal cortex 72 h after intracerebral administration of anti-SMAP polyclonal antibody to the rats. At the same time, in the fourth series of experiments, a significant decrease of beta III tubulin, the marker of differentiated neurons (Dráberová et al., 1998), was observed in the water-soluble extract of the hippocampus within the same timeframe between administration of the anti-DRP2 polyclonal antibody and their sampling as in the third series of experiments. Selection of the timeframe of 72 h between administration of the antibody and sampling the brain tissues for the analysis in the fourth and fifth series of experiments was not occasional and was based on the fact that the most prominent increase of the number of

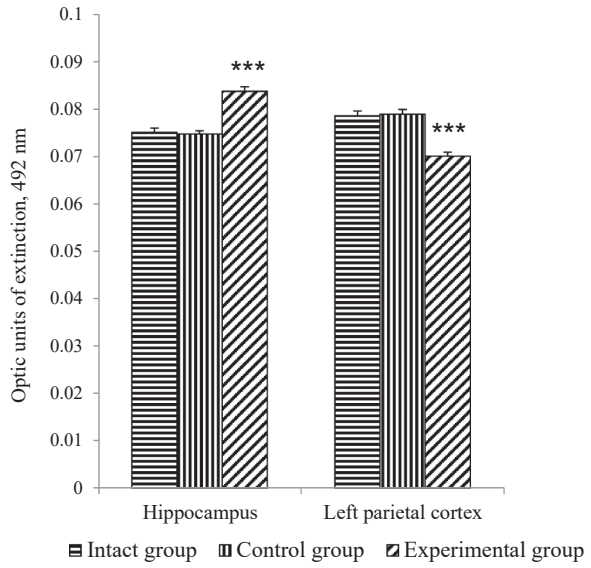

Fig. 5. The effects of anti-SMAP polyclonal antibody on the level of NGF in the hippocampus and left parietal cortex 72 h after antibody administration. n = 7; $p = 0.008$ on Friedman's χ^2-criterion and ***- $p < 0.001$ on Student's t-criterion.

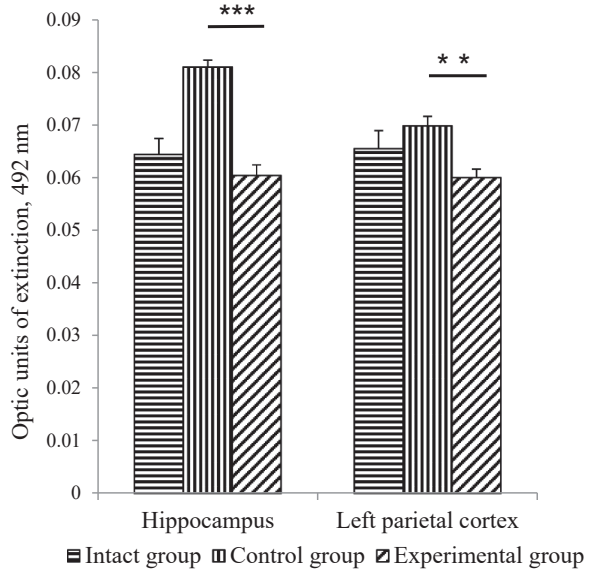

Fig. 6. The effects of anti-DRP2 polyclonal antibody on the level of beta III tubulin in the hippocampus and left parietal cortex 72 h after antibody administration. n = 6; $p < 0.01$ on Friedman's χ^2-criterion and ** - $p < 0.01$, ***- $p < 0.001$ on Student's t-criterion.

correct trials, reflecting positive effects of the antibody on the processes of memory formation, we observed 72 h after antibody administration.

As anti-SMAP and anti-DRP2 antibodies accelerated and strengthened memory formation in the difficult-to-learn conditioned models, the underlying mechanisms of these effects require adequate and significant changes of the amount of the memory storage substrates. Furthermore, the decrease of beta III tubulin content in the water-soluble fraction in the hippocampus after antibody-mediated inactivation of DRP2 might indicate to some loss of this marker of differentiated neurons (Dráberová et al., 1998). This result may be due to significant increase of a number of the newly formed neurons in the hippocampus still deprived of beta III tubulin marker's presentation due to their immaturity. There are mounting amount of literature data concerning adult neurogenesis during acquisition of conditioned behavior (Winocur et al., 2006; Gu et al., 2013; Sherstnev et al., 2015). However, there is serious incongruity between timeframe required for the stem cells differentiation into the mature neurons and duration of long-term memory formation (consolidation). In particular, the process of differentiation of stem cells into mature neurons lasts for at least 5 weeks (Motamed et al., 2019). Such prolonged timeframe is needed for the newly formed neurons to pass through all stages of phenotypic specialization, including molecular specialization and engagement into the earlier formed neural circuits. On the other hand, memory consolidation process comprises much shorter timeframe – according to the studies of different researchers, from 24 h (Seybert et al., 1979) till 48 h (Tse et al., 2007) and 53–56 h (Smith and MacNeill, 1993). Consequently, due to mismatch of the timeframes of these two processes, the newly formed neurons cannot be in any way directly engaged into memory consolidation processes of novel information. From this standpoint, the antibodies-mediated strengthening of memory formation up to 70–80% of correct trials revealed in the experimental group in opposite to 50% in the control and intact groups in the both used complex conditioned models in the rats should be based on quite another mechanism promoting such memory strengthening.

Furthermore, we observed an increase of NGF in the water-soluble fraction of the hippocampus after administration of anti-SMAP polyclonal antibody. According to literature data, upregulation of NGF can affect neuronal cell fate in terms of switching on neurons' back remodeling process through the activation of tyrosine kinase A, phosphatidylinositol 3 kinase/protein kinase B and extracellular signal regulated kinase 1/2 (Zhang et al., 2019). Hence, upregulation of NGF together with the decrease of the content of mature neurons' marker beta III tubulin might reflect actively going the process of neurons' back remodeling in this adult brain structure, i.e. the process of dedifferentiation of the mature neurons under the effect of anti-SMAP and anti-DRP2 antibodies.

As showed our earlier studies, training of the rats in the paradigms of conditioned shuttle box and alternative running results in the decrease of the content of the SMAP complex in their parietal cortex (Guseinov and Mekhtiev, 2013; Mekhtiev et al., 2015). Taking into account these data, we propose that this event might be underlain by the same mechanism, which is involved in the processes caused by the antibody-mediated inactivation of the DRP2, representing a regulatory active component of the SMAP protein complex (Garina et al., 2018). The antibody's effect is directed towards SMAP inactivation after highly specific binding to it, which, apparently, is similar to SMAP level reduction in the rat parietal cortex observed in the learning sessions in the conditioned shuttle box and alternative running models (Guseinov and Mekhtiev, 2013; Mekhtiev et al., 2015). On our opinion, no matter which way SMAP activity is diminished in the brain: through the downregulation of its level or through the inactivation of its functional activity via application of anti-SMAP antibody. The final effect of the insufficiency of SMAP activity, apparently, will be the same in the both envisaged cases.

The process of back remodeling of the newly formed neurons is underlain, apparently, by epigenetic mechanisms that are capable of switching off certain groups of the genes defining neurons' differentiation and, conversely, switching on the genes enhancing return of the newly formed neurons into the undifferentiated state that is capable thereafter of tuning up synthesis of their protein markers toward phenotypic challenges related to memory formation. In the process of back remodeling there is no need for the newly formed neurons to get back into the original state of pluripotency, but they can undergo the so-called phenomenon of trans-differentiation (Kelaini et al., 2014), in which they loss specific markers, but in sense of differentiation they do not get too far backward from the mature neuronal state and, therefore, they will not need prolonged timeframe for the second-order differentiation.

As it is already mentioned in the Introduction, mass-spectroscopic

analysis of SMAP revealed its composition of three proteins – dihydropyrimidinase-related protein 2 (DRP2; or collapsin response mediator protein 2, CRMP2 (Nakamura et al., 2020)), actin and tubulin, which are bound tightly to each other by calcium-mediated bonds (Garina et al., 2018) and are resistant to the effect of even 5 M urine solution. The calcium-dependent nature of these bonds was shown by their sensitivity to the effects of 40 mM EDTA, which causes disruption of the SMAP complex into its composing proteins (Gaisina et al., 2022). The existence of such strong bonds between these proteins clarifies the observed behavior of SMAP as a single protein in protein fractionation by gel-chromatography (it is eluted as a single peak from the Sephadex G-150 column), electrophoresis under non-denaturating conditions and western-blotting. As actin and tubulin are referred to structural proteins of the cells, they, apparently, do not have regulatory activity and the memory-promoting and memory-strengthening activity observed in our experiments with application of anti-SMAP polyclonal antibody as well as their effect on the level of NGF in the hippocampus and in the left parietal cortex, obviously, might be attributed to inactivation of DRP2 which can be recognized by anti-SMAP antibody.

On the whole, the results of our experiments, especially the increase of NGF content in the water-soluble protein fraction of the hippocampus and the decrease of beta III tubulin in the analogous extracts from the hippocampus after administration of polyclonal anti-SMAP or anti-DRP2 antibodies to animals, revealed within the same timeframe, when memory formation in the complex conditioned tasks was observed, indicate that DRP2 might participate in regulation of the process of neuronal dedifferentiation, apparently, occurring during memory formation in the behavioral paradigm used in this study, increasing this way a pool of neuronal precursor cells that can further differentiate purposefully for recording of acquired behavior.

5. Conclusion

The results presented in this article give grounds to assuming the mechanism underlying memory formation through back remodeling of the mature newly formed neurons (neurogenesis) in adult organism. Neuron's precursors (stem cells) after their formation cannot stay in stemness state for a long time. For this reason, the neuron's precursors pass through all stages of differentiation up to formation of the mature neurons, covering prolonged timeframe for at least 5 weeks (Motamed et al., 2019). In the proposed mechanism, the process of phenotypic specialization on the first stage occurs, apparently, in the form of back remodeling of the newly formed differentiated neurons covering relative short timeframe. Thereafter through mechanism of trans-differentiation (Kelaini et al., 2014) the process of tuning of molecular specialization to the challenges of the neurons, engaged in memory formation, starts.

The question may arise if the newly formed neurons are not directly engaged into memory formation, then what they are destined for. From our standpoint, the newly formed neurons should replenish the pool of differentiated neurons whose amount has been consumed partly during the back remodeling process presumably underlying memory formation.

Conflicts of Interest

The authors declare that they do not have any competing interests regarding intellectual property of the data used in the article.

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