ORIGINAL ARTICLE

Investigation of the effect of a modified fragment of neuropeptide Y on memory phases and extrapolation escape of animals

Výzkum účinku modifikovaného fragmentu neuropeptidu Y na paměťové fáze a extrapolační únikový test zvířat

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Summary

The article presents a study of the effect of a modified fragment of neuropeptide Y (H-L-Ile-L-Asn-L-Leu-L-Nle-L-Ser-L-Arg-L-Asn-L-Arg-L-Tyr-NH_2) named nonapeptide NP9 on the memory phases and extrapolation escape of animals. The study was performed in the passive avoidance test with intact animals, scopolamine-treated animals, and the extrapolation escape task. NP9 was investigated in the dose range of 0.04–0.4 mg/kg with a single intranasal administration. The comparison drug used peptide nootropic medicine Semax® (Met-Glu-His-Phe-Pro-Gly-Pro) at a dose of 0.1 mg/kg.

Efficiency was assessed by the retention latency, the percentage of animals that have reached the learning criterion, the number of incomplete attempts to enter, the antiamnestic activity index calculated by Butler's formula, and the number of animals that successfully performed the extrapolation escape task. Peptide NP9 was superior to Semax® in most indicators. It demonstrated the ability to improve memorization due to its effect on I phase of memory and facilitated extinction of negative experiences when administered after a stress stimulus. NP9 also increased the cognitive ability of animals in the conditions an aversive environment in the extrapolation escape test. Thus, peptide NP9 is promising for a further study as a potential drug for the treatment of cognitive impairment and therapy of post-traumatic stress disorder.

Key words: modified fragment of neuropeptide Y • passive avoidance test • memory phases • extrapolation escape task

Souhrn

Článek představuje studii účinku modifikovaného fragmentu neuropeptidu Y (HL-Ile-L-Asn-L-Leu-L-Nle-L-Ser-L-Arg-L-Asn-L-Arg-L-Tyr-NH_2) zvaného nonapeptid NP9 na paměťové fáze a extrapolační únikový test zvířat. Studie byla provedena v testu pasivního vyhýbání s intaktními zvířaty a zvířaty ošetřeným skopolaminem a v extrapolačním únikovém testu. NP9 byl zkoumán v rozmezí dávek 0,04–0,4 mg/kg při jednorázovém intranazálním podání. Srovnávaným léčivem byl peptidový nootropní lék Semax® (Met-Glu-His-Phe-Pro-Gly-Pro) v dávce 0,1 mg/kg.

Účinnost byla hodnocena podle retenční latence, procenta zvířat, která dosáhla kritéria učení, počtu neúplných pokusů o vstup, indexu antiamnestické aktivity vypočítaného Butlerovým vzorcem a počtu zvířat, která úspěšně provedla extrapolační únikový test. NP9 u většiny parametrů překonal přípravek Semax®. Ukázal schopnost zlepšit zapamatování ovlivněním I. paměťové fáze, a pokud byl NP9 podán po stresovém stimulu, usnadnil útlum negativních zkušeností. Také zvýšil kognitivní schopnosti zvířat v podmínkách averzního prostředí při extrapolačním únikovém testu. Peptid NP9 je tedy slibným potenciálním léčivem pro léčbu kognitivních poruch a posttraumatické stresové poruchy, vhodným pro další výzkum.

Klíčová slova: modifikovaný fragment neuropeptidu Y • test pasivního vyhýbání • paměťové fáze • extrapolovaný únikový úkol

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Introduction

Neuropeptide Y (NPY) is a biologically active peptide widely distributed in the human body and is responsible for various physiological processes.

It is involved in the control of energy metabolism, the body's responses to stress, emotional behavior, circadian rhythms, etc.[1].

NPY in humans can activate four types of its receptors – Y1, Y2, Y4, Y5. These receptors are found in the central nervous system (CNS) and peripheral organs. The main areas of NPY expression in the CNS are the interneurons of the hippocampus, neocortex, amygdala, striatum, and it is also present in the brainstem, hypothalamus, etc.[2]

NPY is a promising peptide for pharmacological research. The NPY system has been actively studied for almost four decades. Considering its powerful regulatory role, NPY receptor agonists and antagonists are being investigated as potential drugs[3]. The accumulated experience substantiates the therapeutic potential of neuropeptide Y and compounds that activate NPY receptors for the correction of obesity, therapy of depression, stress, cognitive impairment, post-traumatic stress disorder (PTSD), alcohol dependence, neurodegenerative diseases[4].

It is also known that NPY-sensitive receptors are expressed in areas involved in learning and memory, such as the hippocampus, amygdala, cingulate cortex, thalamus, hypothalamus, and cerebral cortex[5]. Therefore, the study of the impact of NPY on learning and memory attracts attention. NPY as a modulator of neuroplasticity, synaptic transmission, and several types of memory has both inhibitory and stimulatory effects depending on the phase of memory (acquisition, consolidation, retention, and retrieval), the time of administration, dose applied, receptor subtype, and brain area[6]. In one of the first works to study the effect of NPY on the processes of memory consolidation and retention[7], the peptide was administrated into different areas of the brain, and animals were tested on memory retention for the T-maze foot shock avoidance test. NPY improved retention when injected into the septum or rostral hippocampus but impaired memory when injected into the caudal hippocampus or amygdala. It had no effect when injected into the striatum or thalamus. When NPY was administered 24 hours after training, it did not affect retention[7].

Kornhuber et al. showed that NPY increases non-social (object) memory retention time by acting only on Y1 receptors without interacting with Y2 receptors and does not affect social memory[5]. In addition, by acting on Y1 receptors, NPY impairs the assimilation of negative emotional experiences by animals, acting as a resistance factor against excessive fear response after stress[5, 6]. The role of the NPY system in these experiments was further confirmed by the fact that Y1 and Y2 receptor antagonists impaired the consolidation of non-social memory. Both Y1 and Y2 receptor-mediated NPY neurotransmission were not necessary for the acquisition of non-social and social memory but were required to consolidate non-social memory and retrieval of both non-social and social memory[5].

NPY also blocked the amnestic effect of anisomycin, scopolamine[8], dizocilpine (MK-801)[9], and colchicine[10]. The results of experiments by Bouchard et al. indicate that peptides from the NPY family can indirectly interact in vivo with sigma receptors and thus modulate cognitive processes associated with the activity of NMDA receptors[9].

The C-terminal site is a functionally active fragment of NPY important for binding to receptors[11]. Considering the important role of this region, we proposed the amino acid sequence H-L-Ile-L-Asn--L-Leu-L-Nle-L-Ser-L-Arg-L-Asn-L-Arg-L-Tyr-NH_2 as a modified terminal fragment of NPY with the code name nonapeptide NP9. Unlike the original NPY, this sequence consists of 9 amino acid residues instead of 36. NP9 amino acid residues at positions 4, 5, and 7 are replaced by those which do not break the tertiary structure and, therefore, the affinity to receptors NPY, and should reduce the rate of proteolysis of the peptide[12]. The relatively short amino acid chain makes NP9 easier for chemical synthesis, quality control, cheaper, and therefore more appropriate for versatile research and further implementation.

Theoretically, the peptide NP9 should, at least partially, exhibit the biological activity of NPY. At the same time, it is possible that, as an original compound, NP9 may have mechanisms of action not associated with the NPY system. In previous studies, NP9 has shown anxiolytic properties[13]. Considering the significant effect of NPY on the processes of learning and memory formation, we aimed to assess the impact of its derivative (NP9) on cognitive functions, particularly on memory and its phases, and on the ability to extrapolation escape.

The intranasal (IN) route of administration of NP9 was chosen, which avoids the adverse effects of the peptide on peripheral organs and prevents rapid destruction by proteases of the stomach, blood serum, etc. It also allows the peptide to directly enter the central nervous system, bypassing the blood-brain barrier, which causes high cerebral bioavailability[14].

Experimental part

Materials

Based on our order, nonapeptide NP9 was synthesized by Shanghai Apeptide Co., Ltd. (China). A solution of nonapeptide NP9 was prepared immediately before the experiment by dissolving the substance in 0.9% NaCl solution. The solutions were prepared in 4 concentrations of nonapeptide NP9, which provided administration at a dose of 0.04 mg/kg, 0.2 mg/kg, 0.4 mg/kg to mice and 0.02 mg/kg to rats. The last dose was selected based on interspecies dose conversion[15]. For IN injection, which was performed in a volume of 0.01 ml, a syringe with a blunt needle was used.

Semax® (Peptogen, Russia) at a dose of 0.1 mg/kg IN[16] was chosen as the reference drug. This heptapeptide (Met-Glu-His-Phe-Pro-Gly-Pro) is a synthetic analog of adrenocorticotropic hormone ($ACTH_{4–10}$). Three criteria were taken into account when selecting a reference drug:

1. oligopeptide structure, as in the studied peptide NP9
2. the same (IN) route of administration
3. presence of nootropic properties of Semax®[16–18]

Amnesia in mice was induced in animals using scopolamine hydrobromide trihydrate (Sigma, USA), 1.5 mg/kg intraperitoneal (IP).

Methods

Study design

The basic test for studying the effect of compounds on learning and memory is the passive avoidance task (PAT)[19]. Our research was performed in two stages. We performed screening studies of nonapeptide NP9 at different doses in intact animals in the PAT at the first stage. At the second stage, the lowest effective dose of peptide NP9 (0.04 mg/kg), which showed a positive effect on memory in the previous stage (Table 1), was investigated in a model of scopolamine-induced amnesia.

The experiment was performed in the PAT for two days. On the first day, mice were injected IN with saline, peptide NP9 solution, and a reference drug, after which passive avoidance training was performed (foot shock after the entry into the dark compartment). On the second day, 24 hours after training, retention was tested. The duration of the tests was 3 minutes. The transfer latencies to the dark compartment were recorded. The number (percentage) of animals that have reached the learning criterion (did not enter the dark compartment on the second day) was determined. Also, for the model of scopolamine amnesia, the number of incomplete attempts to transfer (NIAT) was determined, namely the animals peeking into the dark compartment without full body entry.

To determine the effect of NP9 on memory phases in mice, anterograde amnesia was modeled by scopolamine, 1.5 mg/kg (IP) 25 minutes before the passive avoidance training[19]. The effect of NP9 on the process of acquisition (I phase of memory) was determined by administration of the nonapeptide 35 minutes before training; consolidation and retention of memory engram (II phase of memory) – NP9 was injected after training; and retrieval of memory engram (III phase of memory) – by administration the peptide NP9 at the next day, 35 minutes before the retention test.

For the second stage, the antiamnestic activity index (AA) was calculated according to the modified Buttler formula[20]:

$$AA = (\Delta TL_D - \Delta TL_{CA}) / (\Delta TL_{IC} - \Delta TL_{CA}) \times 100\ (\%)$$

ΔTL_D – the change in latency of the training and retention test for the drug-treated group (nonapeptide or Semax®), ΔTL_{CA} – the change in latency of the training and retention test for the scopolamine-treated group (amnesia control group), ΔTL_{IC} – the change in latency of the training and retention test for the vehicle control group.

Another aspect of the effect on cognitive functions under acute stress in an aversive environment was investigated in the extrapolation escape task (EET) test in rats[19–22]. This species was used because the test was designed specifically for rats. We used a dose of peptide NP9 of 0.02 mg/kg, which was obtained by interspecies dose conversion[15] based on the lowest effective dose for mice in the PAT test (0.04 mg/kg). Previous studies of the peptide in rats showed psychotropic (anxiolytic) properties of the peptide NP9 at this dose[13]. The test allows you to assess individual differences in the cognitive style of task solving. The experimental equipment consists of a transparent plastic inner cylinder (d = 10 cm) mounted in an external container. The container was filled with water (t = 22–24 °C) so that the cylinder was immersed in water by 2 cm. The rats were carefully placed into the cylinder, and their behavior was

Table 1. The effect of nonapeptide NP9 on passive avoidance test in intact mice (M ± SEM, n = 39)

Group, n		**Transfer latency, sec**		**% of animals that have reached the learning criterion**
		training (1st day)	retention test (in 24 hours)	
Vehicle control (VC), n = 8		36.5 ± 8.4	152.5 ± 18.5§	50.0
Semax®, 0.1 mg/kg, n = 8		12.5 ± 1.9*	146.3 ± 18.6§	62.5
Peptide NP9	0.04 mg/kg, n = 8	27.4 ± 9.8	180.0 ± 0.0§	100.0**#
	0.2 mg/kg, n = 7	49.9 ± 19.9#	170.5 ± 9.5§	85.7*
	0.4 mg/kg, n = 8	44.0 ± 13.2#	180.0 ± 0.0§	100.0**#

Significant relative to the vehicle control group: *p ≤ 0.05, **p ≤ 0.01
Significant relative to the Semax® group: #p ≤ 0.01
Significant relative training latency with Wilcoxon signed-rank test: §p ≤ 0.05
n – number of animals in the experiment

observed for 3 minutes, recording the time of diving under the edge of the cylinder (escape time). The escape time and the number of animals that escaped from the cylinder by diving were determined.

Experimental animals and grouping

The animals were obtained from the vivarium of the Central Research Laboratory (National University of Pharmacy, Kharkiv, Ukraine). Experiments were performed in accordance with „Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes". All experimental protocols were approved by the Bioethics Commission of the National University of Pharmacy (Protocol No. 3 of 20 March 2019). The animals were housed in standard polypropylene cages and kept at 20–26 °C and 50% humidity in a well-ventilated room with a 12 h light/dark cycle with free access to food and water.

A cohort of 95 adult random-bred female albino mice weighing 22–26 g were used for the study in PAT. At the first stage of the study, the animals were randomly divided into 5 groups:

group 1 – vehicle control (animals receiving saline IN, n = 8)
group 2 – animals receiving reference drug Semax® 0.1 mg/kg IN (n = 8)
group 3 – animals receiving solution of peptide NP9 0.04 mg/kg IN (n = 8)
group 4 – animals receiving solution of peptide NP9 0.2 mg/kg IN (n = 7)
group 5 – animals receiving solution of peptide NP9 0.4 mg/kg IN (n = 8)

For the second stage of the study (scopolamine amnesia), animals were randomized into 8 equal groups (n = 7):

group 1 – vehicle control (animals receiving saline IN 10 minutes before saline administration IP and training 25 minutes after)
group 2 – animals with a model of amnesia caused by scopolamine 1.5 mg/kg IP 10 minutes after saline administration IN and 25 minutes before the passive avoidance training (control of amnesia);
group 3 – animals treated with NP9 0.04 mg/kg IN 10 minutes before amnesia (scopolamine administration 25 minutes before training)
group 4 – animals treated with Semax® 0.1 mg/kg IN 10 minutes before scopolamine administration
group 5 – animals with amnesia, treated with NP9 0.04 mg/kg IN immediately after passive avoidance training
group 6 – animals with amnesia, treated with Semax® 0.1 mg/kg IN immediately after passive avoidance training
group 7 – animals with amnesia, treated with NP9 0.04 mg/kg IN 30–35 minutes before retention test
group 8 – animals with amnesia, treated with Semax® 0.1 mg/kg IN 30–35 minutes before retention test

Considering the presence of sex differences in the processes of learning and fear conditioning[23], other experiments were performed in animals of the same sex (females) as in the previous studies. Therefore 24 adult random-bred female albino rats weighing 190–220 g were used in the EET test. The animals were divided into 3 equal groups (n = 8):

group 1 – vehicle control (animals that received saline IN)
group 2 – animals treated with Semax® 0.1 mg/kg IN
group 3 – animals treated with a solution of NP9 0.02 mg/kg IN

Test substances and vehicle were administered 30 minutes before testing.

Statistical analysis

The results were processed using descriptive statistics and presented as the mean ± standard error of the mean (M ± SEM) or percentage terms. The statistical difference between the groups was determined using Fisher's exact test, Mann-Whitney U test, Wilcoxon signed-rank test, and also calculated Spearman's rank correlation. The utilized computer software included MS Excel 2016 and STATISTICA v.12. The differences were considered statistically significant at $p \leq 0.05$.

Results

The study results have demonstrated that in mice the peptide NP9 dose-independent increases the main integral index – the percentage of animals that have reached the learning criterion (Table 1). For doses of NP9 0.04 mg/kg and 0.4 mg/kg, it were 100% of animals ($p \leq 0.01$), and for doses of 0.2 mg/kg – 85% ($p \leq 0.05$). The percentage of animals that reached the learning criterion for receiving NP9 peptide in all three doses exceeded Semax® at a dose of 0.1 mg/kg.

As we see in Table 1, the training latency in the groups NP9 0.2 mg/kg and NP9 0.4 mg/kg is significantly more by 4.1 and 3.5 times, respectively ($p \leq 0.01$) than in animals treated with Semax® 0.1 mg/kg, and as a trend more than in animals of the vehicle control group by 34% and 20%, respectively. These data are in good agreement with the psychopharmacological light-dark box test results, which characterizes the anxiety of animals[24]. Light-dark box test and PAT are performed using an equipment similar in design. Similarly, the behavioral principle of avoidance of illuminated spaces is used in both of these tests. Taking this into account, the longer training latency of NP9 in comparison with Semax® can be explained by the lower anxiety of animals due to the anxiolytic properties of the peptide NP9 at these doses[13] and state an increase in the anxiety of animals receiving

Semax® at a dose of 0.1 mg/kg. This indicates the advantage of the nonapeptide in this parameter.

The data (Table 2) show that in the study of phase I of memory, Semax® significantly increases the retention latency compared to the amnesia control group by 2.4 times ($p \leq 0.01$) and 57.1% of animals which have reached the learning criterion against 0% in the amnesia control group ($p \leq 0.01$). The antiamnestic activity of Semax® in the study of phase I of memory is 83.1%. Nonapeptide NP9 in the study of phase I of memory increases the retention latency by two times ($p = 0.072$), and % of animals that have reached the learning criterion was 71.4% against 0% in the group of amnesia control ($p \leq 0.01$), slightly exceeding Semax® (57.1%). The retention latency of animals under the influence of nonapeptide and Semax® significantly increased against the training latency by 5.3 and 3.3 times, respectively ($p \leq 0.05$). Still, the antiamnestic activity practically did not differ – 78.9% vs. 83.1%.

Other patterns were identified in the study of phase II of memory. Semax® increased the retention latency versus the training latency by 2.3 times as a trend, while for nonapeptide, it almost did not change and was less by 5 times than the vehicle control ($p \leq 0.05$) and, as a trend, 2.4 times less than in the control amnesia. The percentage of animals that have reached the learning criterion for Semax® was 42.9% more than in NP9 and the amnesia control group ($p \leq 0.01$). The antiamnestic activity in the study of phase II of memory for Semax® is almost absent (only 12.7%). For NP9, the negative antiamnestic activity index was even observed (–59.2%), which signalizes impairment of the memory consolidation processes and retention under the influence of the studied nonapeptide.

In studying the effects on phase III of memory, peptide NP9 increased the retention latency against the training latency by 4.1 times ($p \leq 0.05$). In contrast, Semax® increased it only as a trend by 47%. The percentage of animals that have reached the learning criterion under the influence of peptide NP9 and Semax® was insignificant (1 animal in each group – 14.3%) and did not differ significantly from the amnesia control group. According to the effect in the study of phase III of memory, the antiamnestic activity index of Semax® had a negative value (–49.2%); thus, the reference drug impaired the reproduction of

Table 2. The effect of nonapeptide NP9 on passive avoidance test in mice with scopolamine-induced amnesia (M ± SEM, n = 56)

Group, n	Transfer latency, sec		The number of incomplete attempts to enter	% of animals which have reached the learning criterion	AA, %	ρ
	training (1st day)	retention test (in 24 hours)				
Vehicle control, n = 7	17.9 ± 2.4	145.6 ± 23.8§	3.6 ± 0.9	57.1$^{\#\#}$	–	0.31
Amnesia control group (scopolamine) n = 7	16.4 ± 4.9	69.1 ± 21.1*§	3.4 ± 1.0	0.0**	–	0.83$^{\&}$
I phase of memory						
Scopolamine + Semax® n = 7	49.3 ± 20.1	164.3 ± 8.6$^{\#\#\S}$	5.3 ± 1.0	57.1$^{\#\#}$	83.1	–0.33
Scopolamine + NP9 n = 7	26.1 ± 7.5	138.0 ± 27.4§	6.7 ± 1.1$^{\#}$	71.4$^{\#\#}$	78.9	0.82$^{\&}$
II phase of memory						
Scopolamine + Semax® n = 7	47.4 ± 22.4	109.6 ± 31.5	3.7 ± 1.0	42.9$^{\#\#}$	12.7	0.99$^{\&}$
Scopolamine + NP9 n = 7	20.6 ± 3.5	28.9 ± 12.7**	0.9 ± 0.3**	0.0**	–59.2	0.53
III phase of memory						
Scopolamine + Semax® n = 7	33.3 ± 18.1	49.1 ± 23.0*	1.9 ± 0.6	14.3*	–49.2	0.69
Scopolamine + NP9 n = 7	14.0 ± 2.4	57.9 ± 23.7*§	2.6 ± 0.8	14.3*	–11.7	0.94$^{\&}$

Significant relative to the vehicle control group: *$p \leq 0.05$, **$p \leq 0.01$
Significant relative to the amnesia control group: $^{\#}p \leq 0.05$; $^{\#\#}p \leq 0.01$
Significant relative to the training latency with Wilcoxon signed-rank test: $^{\S}p \leq 0.05$
Statistically significant correlation: $^{\&}p < 0.05$
n – number of animals in the experiment, AA – antiamnestic activity index, ρ – Spearman's rank correlation coefficient between the number of incomplete attempts to enter and the transfer latency after 24 hours

memory, and the nonapeptide NP9 had practically no effect on these processes (Table 2).

Additionally, we recorded the NIAT during retention testing. The NIAT can be used to evaluate both anxiolytic and mnemotropic action of the compound, and it correlates well with the retention latency (Table 2). In the vehicle control group, there was no significant relationship between the retention latency and the NIAT ($\rho = 0.31$), in the amnesia control group, a positive relationship is formed ($\rho = 0.83$, $p < 0.05$), which may indicate an increase in the retention latency due to the doubts of animals when entering the dark compartment. As shown in Table 2, in the study of phase I of memory, peptide NP9 increased the NIAT by 97.1% ($p = 0.053$) relative to the amnesia control group attempts, and Semax® as a trend by 55.8%. Also, NP9, as a strong trend, increased the NIAT by 86.1% ($p = 0.072$) relative to vehicle control. However, the correlation under the influence of the studied substances was multidirectional: $\rho = -0.33$ for the Semax® group and $\rho = 0.82$ ($p < 0.05$) for the NP9 group. It may mean that under the influence of Semax®, animal doubts about entering the dark compartment were less important for making a final decision than under the influence of NP9. The NIAT in the study phase II of memory in the Semax® group did not differ significantly from the group of vehicle control and the amnesia control group; and for NP9, it was four times less than in the vehicle control ($p = 0.017$) and 3.8 times less than in the amnesia control group ($p = 0.097$). In contrast to phase I of memory, under the influence of Semax®, there is a high positive correlation between the retention latency and the NIAT ($\rho = 0.99$, $p < 0.05$), and under the influence of peptide NP9 it is lower ($\rho = 0.53$). In phase III of the memory study, Semax® reduced the NIAT relative to the vehicle control by 47.2%, relative to the amnesia control by 44.1%, and NP9 reduced by 27.8% and 23.5%, respectively. The correlation between the retention latency and the NIAT in both groups is positive, but, in contrast to phases II of memory, under the influence of Semax®, it is of medium strength ($\rho = 0.69$), and for NP9 – high ($\rho = 0.94$, $p < 0.05$).

In the EET, neither NP9 nor Semax® provided a significant difference in the escape time. But the peptide significantly increased the main criterion of the test – the percentage of animals that escaped the cylinder by diving ($p < 0.05$) relative to vehicle control, which is by 12.5% better than in animals of the Semax® group (Table 3).

Discussion

The results of the first stage of research show that peptide NP9 in the dose range of 0.04–0.4 mg/kg dose-independent increases the percentage of animals that have reached the learning criterion compared with both vehicle control and the effect of the peptidic nootropic drug Semax®. It indicates the strong nootropic (positive mnemotropic) properties of NP9.

Also, the results indicate the absence of nonapeptide NP9 anxiogenic properties in contrast to Semax®, which significantly reduced the latency of entry into the dark compartment. A threefold decrease in this indicator after a single IN administration of Semax® may indicate the anxiogenic properties of the reference drug in intact random-bred mice. It contradicts the data[25–28], according to which Semax has anxiolytic properties. However, in the cited studies, results were obtained in a different species of animals (rats)[25–28], by a different route of administration (intraperitoneally)[26–28], using other doses (from 0.02 to 0.4 mg/kg), mainly after a long course of administration (up to 2 weeks)[25, 28]. Features of the phenotype of an emotional stress reaction in mice and rats may also be important: it is known that this factor is essential for the action of anxiolytics[29]. Finally, unexpected effects in the form of increased anxiety can also be observed in classical benzodiazepine anxiolytics[30–32]. So, the exact reason for the revealed anxiogenic effect of Semax® on mice requires clarification, which is beyond the scope of our study. These data can help expand knowledge about the pharmacological properties of Semax. But it is noteworthy that in the same PAT on the model of scopolamine amnesia, we did not find such properties of Semax®. In contrast, in all series of these experiments, the training latency in the Semax® group was tendentiously higher than in the vehicle control and amnesia control groups, which may be due to the peculiarities of the drug under the influence of blockade of brain cholinergic receptors.

The lowest effective antiamnesic dose of nonapeptide NP9 0.04 mg/kg for mice was used in further experiments on the model of scopolamine

Table 3. The effect of nonapeptide NP9 on the behavior of naive rats in the extrapolation escape task (M ± SEM, n = 24)

Group, n	Escape time, sec	% of animals fulfilling the test criterion
Vehicle control, n = 8	37.3 ± 4.7	75.0
Semax®, 0.1 mg/kg, n = 8	40.1 ± 2.8	87.5
Peptide NP9, 0.02 mg/kg, n = 8	45.7 ± 4.7	100.0*

Significant relative to the vehicle control group: *$p < 0.05$
n – number of animals in the experiment

amnesia. Results of the study on this model indicate a strong positive effect of peptide NP9 at a dose of 0.04 mg/kg on phase I of memory. For this phase of memory, the studied peptide is equal to the reference nootropic drug Semax® by the antiamnestic activity index. The percentage of animals that have reached the learning criterion even trends to exceed it by 14.3%. In the assessment of phase II of memory, NP9 did not increase the retention latency. The percentage of animals that have reached the learning criterion did not exceed that in the group of amnesia control compared to Semax®, which showed a slight antiamnestic effect. In the assessment of phase III of memory, NP9 did not show a significant positive impact. Still, it was slightly better than the reference drug, which impaired the retrieval of memory.

A large NIAT may indicate the presence of a residual memory engram, the animal anxiety, which is directly related to the conflict of desire to enter the dark compartment (natural instinct) and the experience (memory) of the danger of this compartment. Then a low NIAT may indicate a lack of anxiety (negative memory trace) and, therefore, a lack of memory of the danger. The NIAT was significantly higher in animals treated with NP9 before training (testing phase I of memory) than in animals' group of amnesia control, and, as a trend, higher than in animals of the vehicle control group. It further indicates the preservation of memory engram by enhancing the primary processing of information. But with the administration of NP9 after training, it significantly reduced the NIAT versus vehicle control and as a trend versus amnesia control, which may indicate inhibition of the consolidation and retention of negative experience.

Nonapeptide NP9, as a derivative of neuropeptide Y, can potentially exhibit the activity of the original peptide. Therefore, the available information about NPY allows us to avoid a blind and non-directional search for biological activities of NP9 and helps to understand the results of experiments with NP9 better. Studies of the effect of native NPY on memory indicate that it can reduce the expression of fear due to the extinction of memory of negative experiences[6, 33]. NPY is important for controlling emotional responses because the short-term increase in NPY levels after stress can make a person more resistant to this irritant in the future[34], that is, to be potentially helpful for treating PTSD. It may be typical of NP9 which has been confirmed to have anxiolytic properties[13] and are consistent with the results of the impact on memory when administrated after the training (strong irritant). Such pharmacological effects indicate its potential as a treatment for PTSD by selectively suppressing negative memories. A study by Verma D. et al.[35] has found a significant role of Y1 and Y2 receptors in the basolateral amygdala in the processes of acquisition, consolidation, and extinction of memory[35]. There is a lot of information about the impact of the NPY system on memory processes, but there is still no clear understanding of all processes. Therefore, it is difficult to say which specific receptors are affected by NP9 and whether the effects obtained are due to the effect on the NPY receptor system.

The opposite effects of NP9 on the models of phases I and II of memory do not have an exhaustive explanation. As mentioned above, NPY, to some extent, has a similar effect, which may help explain the effects of NP9. It is clear from the data of Gøtzsche C. et al.[6] that the effects depend on the receptors it affects and on the brain areas in which they are located, on the balance of binding between different receptors that can exhibit multidirectional effects, as well as on the dose and time administration of the peptide. Despite the typically short period of action of peptide compounds[12], NP9 probably induces a certain cascade of reactions responsible for the obtained effect, and the type and manifestation of reactions depend on the context of events upon administration of the studied nonapeptide.

In the EET, the animals showed approximately the same time for escape from the aversive environment. However, in contrast to the vehicle control and Semax®, 100% of animals that received NP9 before testing successfully fulfilled a test that confirms the positive effect of the studied nonapeptide on cognitive function.

One of the significant differences between the studies of nonapeptide NP9 and native peptide NPY is the route of administration. Fundamental knowledge of the role and effects of NPY on memory has been obtained in experiments with intracerebroventricular injection (ICV) of the peptide into specific areas of the brain (e.g, Flood et al., 1989)[7]. It is evident that IN administration differs from ICV by pharmacokinetic characteristics and distribution in different brain structures; and extrapolation of results of influence IN administration of NP9 from animals on people is relative. However, in the context of the study of NP9 for potential use in medicine, the preclinical study should be conducted with a less invasive and clinically acceptable IN route of administration, which is used in our studies.

The results obtained cannot be simply explained by the extrapolation of known information about NPY. The NP9 peptide is another chemical compound with its own characteristics, effects, and likely mechanisms of action. Further study is required to understand the impact of NP9 specifically on the NPY system. Therefore, the logical next step in the study of NP9 will be to elucidate its biochemical mechanisms of influence on the central nervous system, influence on neuroplasticity, and other cognitive processes, particularly spatial memory.

The results of the study show that nonapeptide NP9 improved the process of memorization and, like NPY, probably causes the attenuation of traumatic memory when administered immediately after severe stress (foot shock) and increases the cognitive potential

of animals. Thus, nonapeptide NP9 is a promising peptide for further study as a drug for treating cognitive impairment and therapy of PTSD.

Conclusions

The results obtained on the ability of nonapeptide NP9 to restore scopolamine-induced memory impairment are consistent with the data of the experiments of Flood et al.[8]. The administration of NP9 before training improves memorization, acting precisely on phase I of memory – the acquisition and primary processing of information. The administration of NP9 after training, which characterizes the effect on phase II of memory, accelerates the extinction of the negative learning experience (foot electric irritation). The administration of NP9 before the retention test indicates no effect on memory retrieval processes (phase III of memory). The EET results demonstrate the ability of NP9 to improve the cognitive function of animals.

Conflict of interest: none.

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