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Effect of Intrahippocampal CA3 Injection of Spexin on Passive Avoidance Learning and Memory in Normal and Castrated Rats

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Abstract

Background and Objectives: Spexin is a neuropeptide involved in learning and memory. The aim of the present study was to investigate the effect of intrahippocampal CA3 injection of spexin on passive avoidance learning and memory in normal and castrated rats.

Methods: A total of 42 adult male rats were divided into 6 groups. Sham 1 (received 0.5 μL ACSF); sham 2 (sham castrated rats that received 0.5 μL ACSF); experimental 1 and 2 (healthy rats that received 0.5 μL spexin 10 or 30 nmol/rat); experimental 3 and 4 (castrated rats that received 0.5 μL spexin 10 or 30 nmol/rat). In all groups, injection was done in the CA3 region of hippocampus. CA3 region of hippocampus was cannulated unilaterally by the stereotaxic procedure.

Results: Present data showed that castrated significantly ($P < 0.05$) increased lighting time in comparison to sham 2 group in memory sessions. In normal rats intrahippocampal CA3 injection of spexin in 2 doses significantly ($P < 0.05$) increased lighting time in comparison to the sham group in learning and memory sessions. In castrated rats, intrahippocampal CA3 injection of spexin 30 nmole/rat significantly ($P < 0.05$) induced even further increase in lighting time as compared to the sham group in memory sessions. In memory consolidation and memory retention sessions lighting time was significantly ($P < 0.05$) higher in castrated rats than normal animals.

Conclusions: According to the present results, castration and spexin at 2 doses in normal rats improve learning and memory; while in castrated rats spexin, in 30 nM/rat, produced more improvement in memory. Memory improvement in castrated rats was higher than that in normal rats.

Keywords: Spexin, Hippocampal CA3, Passive Avoidance Learning, Shuttle Box, Male Rats

1. Background

CA3 region of the hippocampus plays an important role in spatial learning and memory. Inhibition of excitatory afferents to CA3 impairs this phenomenon (1). Existence of androgen and estrogen receptors in the hippocampus, suggest the role of sex steroids in balancing and adjustment of spatial learning and memory (2). Efficiency of passive avoidance learning is dependent on healthy hippocampus and could be affected by environmental androgens (3). Androgens can raise synaptic plasticity and density of dendritic spines in CA1 and CA3 of the hippocampus of rats (4).

Recent studies have shown that neurons in the hippocampus contain a set of enzymes for synthesis of testosterone and estradiol (5). The environmental testosterone through conversion to estradiol may be able to affect memory and learning (6).

Spexin is a peptide that was discovered through bioinformatics methods (7). Porzionato et al., in 2010, suggested that spexin is expressed in different tissues such as the nervous system. In most centers of the brain, it is synthesized in the neurons that can also synthesize neurosteroids and are involved in learning and memory (8).

In a study conducted in 2014 by Yu et al., unilateral and bilateral injection of neurokinin1 receptor agonist in the hippocampus has similar effects on Y maze learning (9). The aim of the present study was to investigate the effect of unilateral intrahippocampal CA3 injection of spexin on memory and passive avoidance learning in normal and castrated rats.

2. Methods

2.1. Animals and Study Design

In this study, 42 male Sprague Dawley rats weighing 280 $\pm$ 30 g were used. Rats were keep in plexiglass cages

under standard conditions at a temperature of 22 ± 2°C, 12 hours light 12 hours dark (from 6 am to 6 pm), and maintained with free access to food and water. Keeping and working conditions with animals were based on the ethical and moral code of Shiraz University School of Veterinary Medicine.

In the present study, rats were randomly divided into the 6 groups as following: 1- sham 1, normal rats that received 0.5 μL ACSF by intrahippocampal CA3 injection; 2- sham 2, castrated rats that received 0.5 μL ACSF by intrahippocampal CA3 injection; 3 and 4- experimental 1 and 2- normal rats that received 0.5 μL spexin 10 or 30 nmol/rat by intrahippocampal CA3 injection; 5 and 6- experimental 3 and 4- castrated rats that received 0.5 μL spexin 10 or 30 nmol/rat by intrahippocampal CA3 injection. Intrahippocampal CA3 cannulation was done by stereotactic surgery and injection of ACSF and spexin at 2 doses was performed a week after stereotactic surgery and 30 minutes after injection. Passive avoidance learning test was done.

Doses of spexin were based on a previous work is 10 and 30 nmol/rat in a volume of 0.5 μL (10). The spexin was used before the 1st session of shuttle box.

2.2. Stereotactic Surgery

1 week before behavioral testing, rats were anesthetized with an intraperitoneal injection of combination of ketamine hydrochloride (100 mg/kg) and xylazine (10 mg/kg). The rats were placed in a stereotaxic apparatus and the guide cannula (23 gauge needle), according to Paksinos, atlas (11) was unilaterally implanted in the intrahippocampal CA3. The coordinates for CA3 region were 2.92 mm posterior to Bregma and -2.1 mm lateral to midline.

2.3. Castration

Rats were castrated under anesthetic mixture of ketamine hydrochloride (100 mg/kg) and xylazine (10 mg/kg) intraperitoneally. The skin of the scrotum was cut and open, then epididymis was cut and testicles were cut off, then the wound was sutured. In sham 2, single incision in the scrotum area was established and the area was sutured.

2.4. Behavioral Testing

A two-way shuttle-box (made by Aryoazma Co) with acrylic walls and steel floor bars was used for the learning procedure. The box, 44 × 20 × 19 cm is bisected by a vertical partition with an opening in the middle that allows the animal to move freely from 1 compartment to another, including light and dark compartments. In the light compartment, the animal was safe while in the dark compartment it received a foot shock of 0.6 mA for 1 second with a latent period of 1 second.

In the 1st session, all animals were individually subjected to 2 minutes of adaptation to the shuttle box, in which the rat could explore the light compartment and move about freely. In the 2nd session, as initial latency, the rats were placed in the light compartment of the shuttle box and one second after entering the dark compartment they received a 0.6 mA foot shock for one second. In the 3rd session as learning, the procedure was similar to the initial latency session. In the 4th session, as memory consolidation, and 5th session, as memory retention, the procedure was like the learning sessions without foot shock. The rats were considered as completely learned, if they did not move to the dark compartment after 120 seconds during the 3rd, 4th, and 5th session of experiments; therefore, in these sessions, more stay in the light chamber represents reinforce learning and memory.

2.5. Statistical Analysis

Data were analyzed by the SPSS (Version 21) software. Results were expressed as mean ± SEM. For statistical analysis, nonparametric test kruskal-wallis and mann -whitney test as well as 2 way ANOVA and Tukey as post-hoc were used to compare groups and sessions. The significance level of $P < 0.05$ was considered.

3. Results

3.1. The Effect of Castration on Passive Avoidance Learning and Memory in Passive Avoidance Procedure

In the present study, in both memory sessions, castration without treatment significantly ($P < 0.05$) increased the time spent in light compartment of shuttle box in comparison to sham 1 and sham 2 groups; while in learning session castration without treatment significantly ($P < 0.05$) increased the time spent in light compartment of shuttle box in comparison to sham 1 group, however, not sham 2. In normal conditions, the time spent in the light compartment of the shuttle box had an increasing trend during learning and memory consolidation as well as memory retention sessions in all groups (Figure 2).

3.2. The Effect of Intrahippocampal CA3 Injection of Spexin on Passive Avoidance Learning and Memory in Normal Rats

In learning, memory consolidation and retention sessions of passive avoidance learning, spexin at both doses significantly ($P < 0.05$) increased the time spent in light compartment of shuttle box in comparison to the sham 1 group (Figure 3).

In normal rats, after intrahippocampal CA3 injection of spexin 10 and 30 nmol/rat, the time spent in light compartment of the shuttle box, in memory retention session

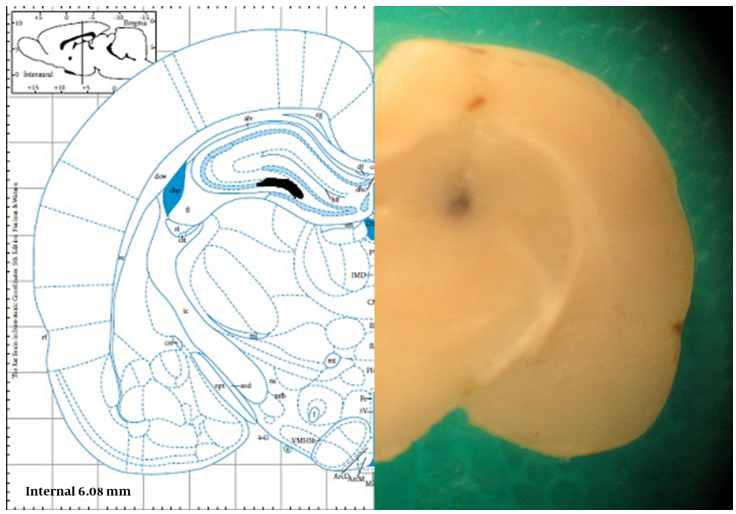

Figure 1. Unilateral ink injection site of CA3 region of hippocampus that represented the injection site of spexin in comparison to atlas of Paxinos and Watson.

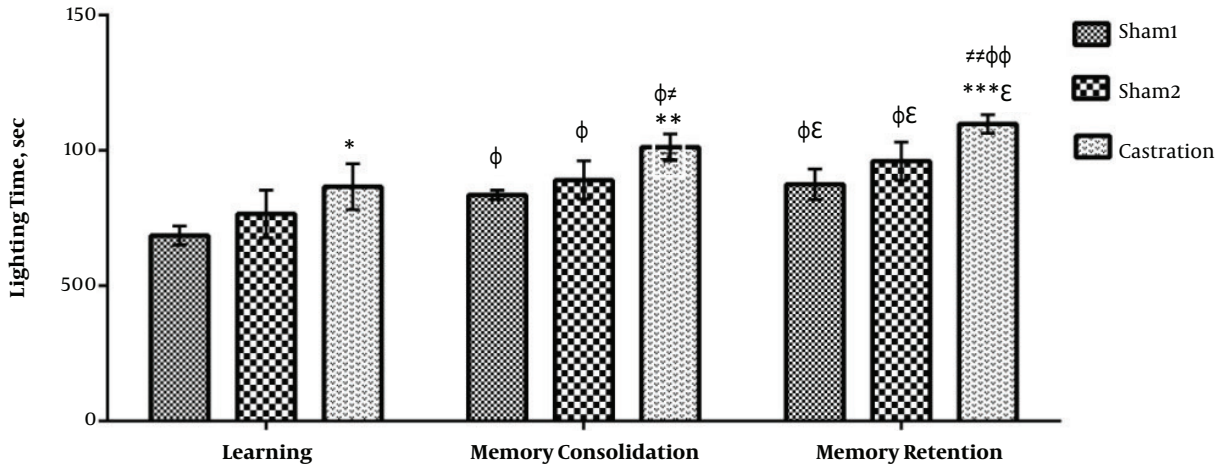

Figure 2. Effect of castrated on time spent in light compartment of shuttle box. * significant difference relative to sham 1; ≠ significant difference relative to sham 2; Φ significant difference relative to learning session; ϵ significant difference relative to memory consolidation. *: P < 0.05; **: P < 0.01; ***: P < 0.001; †:≠ P < 0.05; ≠≠: P < 0.01; Φ: P < 0.05; ΦΦ: P < 0.01; ϵ: P< 0.05.

of passive avoidance learning, was significantly (P< 0.05) higher than that in learning and memory consolidation sessions. In addition, the time spent in the light compartment of the shuttle box in memory consolidation session of passive avoidance learning was significantly (P < 0.05) higher than that in the learning session of rats with intrahippocampal CA3 injection of the spexin 10 and 30 nmol/rat (Figure 3).

3.3. The Effect of Intrahippocampal CA3 Injection of Spexin on Passive Avoidance Learning and Memory in Castrated Rats

In learning, memory consolidation and memory retetion sessions of passive avoidance learning, spexin 10 and 30 nmol/rat significantly (P < 0.05) increased the time spent in light compartment of shuttle box in comparison to sham 2 and castrated groups (Figure 4).

In all groups with castrated rats, the time spent in light compartment of shuttle box was significantly (P < 0.05) higher in memory retention session than that of in learning and memory consolidation sessions of passive avoidance learning in the rats with intrahippocampal CA3 in-

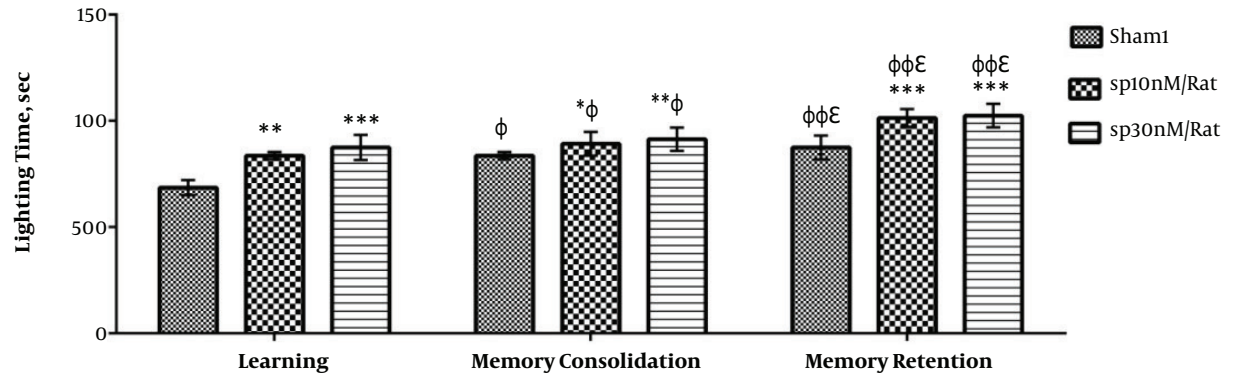

Figure 3. Effect of intrahippocampal CA3 injection of the spexin 10 and 30 nmol/rat on time spent in light compartment of shuttle box in normal rats. * Significant difference relative to sham 1; Φ: Significant difference relative to learning session; ϵ: Significant difference relative to memory consolidation. * P < 0.05; ** P < 0.01; *** P < 0.001; Φ: P < 0.05; ΦΦ: P < 0.01; ϵ: P < 0.05.

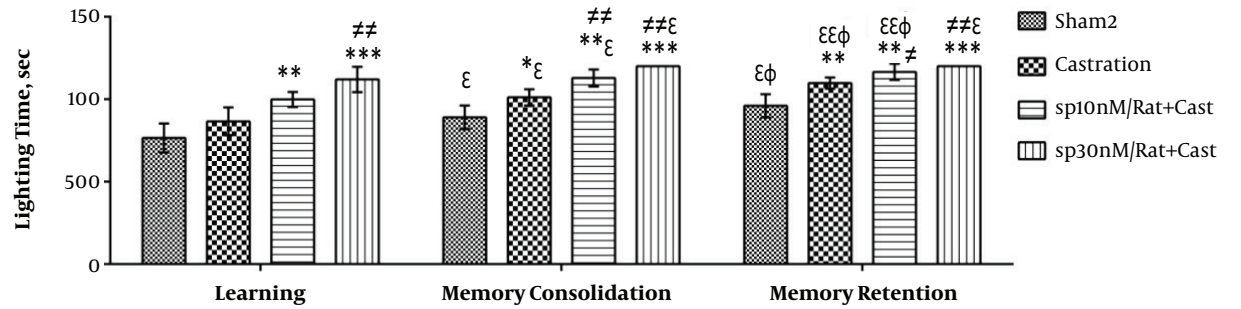

Figure 4. Effect of intrahippocampal CA3 injection of the spexin 10 and 30 nmol/rat on time spent in light compartment of shuttle box in castrated rats. * Significant difference compared to sham 2; ≠ Significant difference relative to castrated; ϵ: Significant difference relative to learning session; Φ Significant difference relative to memory consolidation. *: P < 0.05 <; **: P < 0.01 <; ***: P < 0.001; ≠: P < 0.05; ≠≠: P < 0.01; ϵ: P < 0.05; $\epsilon\epsilon$: P < 0.01; Φ: P < 0.05.

jection of the spexin 10 and 30 nmol/rat. In addition, the time spent in light compartment of shuttle box was significantly (P < 0.05) higher in the memory consolidation session than that in learning of rats with intrahippocampal CA3 injection of the spexin 10 and 30 nmol/rat of passive avoidance learning (Figure 4).

3.4. Comparison of Intrahippocampal CA3 Injection of Spexin on Passive Avoidance Learning and Memory in Normal and Castrated Rats

In learning, memory consolidation and retention sessions of passive avoidance learning intra hippocampal CA3 injection of spexin 10 and 30 nmol/rat in castrated rats, in comparison to normal rats at the same dose, significantly (P < 0.05) increased the time spent in light compartment of the shuttle box (Figure 5).

4. Discussion

Data of the present study showed that castration improves memory; however, it had no significant effect on learning. The results are, however, inconsistent. In a study analyzing the effects of a single testosterone injection on elderly men, the treatment caused a worsening of verbal memory (12). Similarly, biweekly injections of testosterone during 90 days resulted in memory decline (13). Fedotova (1999) showed that higher testosterone levels in male rats led to derangement of active learning (14). Naghdi et al., (2004), showed that testosterone can impair long-term memory in passive avoidance conditioning both via intracellular receptors and through nongenomic pathway (2).

Data of the present study show that spexin at 2 doses in normal rats significantly improves both the learning and memory as compared with sham 1. How can spexin affect learning and memory? Research has shown that spexin exerts its physiological effects through galanin receptor type 2 and 3 (GAL2 and GAL3) (15). Hippocampus receives

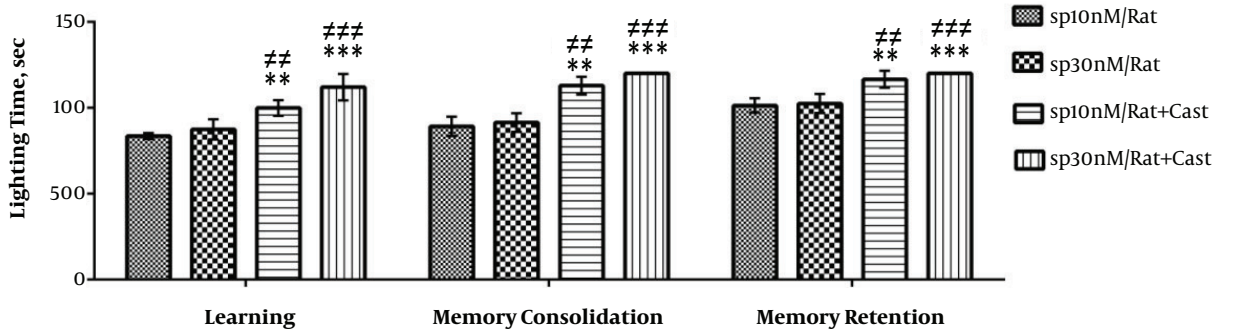

Figure 5. Comparison of intrahippocampal CA3 injection of the spexin on learning and memory in normal and castrated rat. * Significant difference relative to spexin 10 nmol/rat of normal rat; # significant difference relative to spexin 30 nmol/rat of normal rat ($P < 0.05$). **; $P < 0.01$; ***; $P < 0.001$; ≠≠: $P < 0.01$; ≠≠≠: $P < 0.001$.

a lot of galaninergic input from the medial septum, locus coeruleus, and hypothalamus (16). Li and colleagues, 2013, showed that galanin receptor GAL2 has protective effects on spatial memory deficits caused by the accumulation of Aβ in the hippocampus (17). It seems that galanin has both inhibitory and facilitatory effects on spatial learning and these opposite effects are according to the type of galanin receptor in the cell body and axon terminal of cholinergic cells (18). Spexin and galanin genes originate from a common ancestor gene, therefore, these 2 peptides have enough similarity to bind with the same receptor (15). In the hippocampus there is no GAL3 receptor (19), thus, in the present study it seems that an effect of spexin on learning and memory is through GAL2 receptor. In the present study, intrahippocampal CA3 injection of spexin potentiated learning and memory, while intrahippocampal CA3 injection of galanin attenuates learning and memory (20). Therefore, it seems that the effect of these 2 peptides in learning is contradictory.

In the present study, intrahippocampal CA3 injection of spexin 30 nmol/rat improved both memory consolidation and retention in castrated rats in comparison to the sham 2 groups. These results indicate that improvement of memory consolidation and retention by spexin in 2 doses in castrated rats was more powerful than that in normal rats. The most expression of spexin is in neuronal and reproductive system; spexin expression is related to the gonadal stage. On the other hand, spexin reduces LH release in gold fish. Therefore, there is a reciprocal relationship between spexin and gonadal hormone (21). In the present study, castration improves memory. According to our previous study, in castrated rats passive avoidance learning lead to increase in density of glutamate NR1 subunit of NMDA receptor in hippocampus relative to castration alone (22). It seems that changes in the learning and memory in the present study was due to changes in glutamate NR1 subunit of NMDA receptor. Ovariectomy leads to increases in spexin gene expression (21). Therefore, castration maybe increases the spexin gene expression. Therefore, castration, plus intrahippocampal injection of spexin, increases learning and memory more powerful than castration or intrahippocampal injection of spexin alone.

Investigations represented that the efficiency of passive avoidance learning is dependent to the hippocampus; it is affected by peripheral androgens (3). Harooni et al., in 2008, showed that hippocampal injection of testosterone reduces passive avoidance learning; however, removal of the testis has no effect on this kind of learning (3). Daniel et al., 2003, showed that removal of the testis in male rats does not affect passive avoidance learning (23). Sandstorm et al., 2006, have shown that removal of the testis has no effect on spatial memory in Morris water maze (4). Although this study reported no negative effect of androgens on spatial learning and memory in water maze, it should be noted that effects of administration of testosterone at pharmacologic dose on learning and memory might be very different to physiological concentrations of testosterone in male rats (4). In this regard, Daniel et al., (2003) found that normal rats had better performance in selected types of spatial memory than castrated rats. They found that physiologic concentrations of testosterone had positive effects on learning and memory (23).

4.1. Conclusion

The results of present study showed that:

1- Castrated leads to passive avoidance learning and memory impairment.

2- Intrahippocampal CA3 injection of spexin at 2 doses 10 and 30 nmol/rat in normal rats ameliorates passive avoidance learning and memory, which might be through the GAL2 receptor.

3- Intrahippocampal CA3 injection of spexin at 2 doses 10 and 30 nmol/rat in castrated rats ameliorates passive avoidance memory.

4- Intrahippocampal CA3 injection of spexin at 2 doses 10 and 30 nmol/rat in castrated rats has a more powerful effect on passive avoidance memory than that in normal rats.

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Footnote

Declaration of Interest: All the authors confirm that there is no financial or other relationship, which could cause a conflict of interest.

References

1. Meilandt WJ, Barea-Rodriguez E, Harvey SA, Martinez JJ. Role of hippocampal CA3 mu-opioid receptors in spatial learning and memory. *J Neurosci*. 2004;**24**(12):2953–62. doi: 10.1523/JNEUROSCI.5569-03.2004. [PubMed: 15044534].
2. Naghdi N, Asadollahi A. Genomic and nongenomic effects of intrahippocampal microinjection of testosterone on long-term memory in male adult rats. *Behav Brain Res*. 2004;**153**(1):1–6. doi: 10.1016/j.bbr.2003.10.027. [PubMed: 15219700].
3. Harooni HE, Naghdi N, Sepehri H, Rohani AH. Intra hippocampal injection of testosterone impaired acquisition, consolidation and retrieval of inhibitory avoidance learning and memory in adult male rats. *Behav Brain Res*. 2008;**188**(1):71–7. doi: 10.1016/j.bbr.2007.10.017. [PubMed: 18054400].
4. Sandstrom NJ, Kim JH, Wasserman MA. Testosterone modulates performance on a spatial working memory task in male rats. *Horm Behav*. 2006;**50**(1):18–26. doi: 10.1016/j.yhbeh.2005.09.008. [PubMed: 16263125].
5. Mukai H, Kimoto T, Hojo Y, Kawato S, Murakami G, Higo S, et al. Modulation of synaptic plasticity by brain estrogen in the hippocampus. *Biochim Biophys Acta*. 2010;**1800**(10):1030–44. doi: 10.1016/j.bbagen.2009.11.002. [PubMed: 19909788].
6. Naftolin F. Brain aromatization of androgens. *J Reprod Med*. 1994;**39**(4):257–61. [PubMed: 8040841].
7. Mirabeau O, Perlas E, Severini C, Audero E, Gascuel O, Possenti R, et al. Identification of novel peptide hormones in the human proteome by hidden Markov model screening. *Genome Res*. 2007;**17**(3):320–7. doi: 10.1101/gr.5755407. [PubMed: 17284679].
8. Porzionato A, Rucinski M, Macchi V, Stecco C, Malendowicz LK, De Caro R. Spexin expression in normal rat tissues. *J Histochem Cytochem*. 2010;**58**(9):825–37. doi: 10.1369/jhc.2010.956300. [PubMed: 20530460].
9. Yu Y, Zeng C, Shu S, Liu X, Li C. Similar effects of substance P on learning and memory function between hippocampus and striatal marginal division. *Neural Regen Res*. 2014;**9**(8):857–63. doi: 10.4103/1673-5374.131603. [PubMed: 25206901].
10. Toll L, Khroyan TV, Sonmez K, Ozawa A, Lindberg I, McLaughlin JP, et al. Peptides derived from the prohormone proNPQ/spexin are potent central modulators of cardiovascular and renal function and nociception. *FASEB J*. 2012;**26**(2):947–54. doi: 10.1096/fj.11-192831. [PubMed: 22038051].
11. Paxinos G, Watson C. *The Rat Brain in Stereotaxic Coordinates*. 6th ed. Berlin: Elsevier Academic Press; 2004.
12. Wolf OT, Preut R, Hellhammer DH, Kudielka BM, Schurmeyer TH, Kirschbaum C. Testosterone and cognition in elderly men: a single testosterone injection blocks the practice effect in verbal fluency, but has no effect on spatial or verbal memory. *Biol Psychiatry*. 2000;**47**(7):650–4. doi: 10.1016/S0006-3223(99)00145-6. [PubMed: 10745058].
13. Maki PM, Ernst M, London ED, Mordecai KL, Perschler P, Durso SC, et al. Intramuscular testosterone treatment in elderly men: evidence of memory decline and altered brain function. *J Clin Endocrinol Metab*. 2007;**92**(11):4107–14. doi: 10.1210/jc.2006-1805. [PubMed: 17726086].
14. Fedotova YO. Comparative characteristics of learning and behavior processes in conditions of elevated sex hormone levels. *Neurosci Behav Physiol*. 1999;**29**(5):605–7. doi: 10.1007/BF02461154. [PubMed: 10596798].
15. Kim DK, Yun S, Son GH, Hwang JI, Park CR, Kim JI, et al. Coevolution of the spexin/galanin/kisspeptin family: Spexin activates galanin receptor type II and III. *Endocrinology*. 2014;**155**(5):1864–73. doi: 10.1210/en.2013-2106. [PubMed: 24517231].
16. Merchenthaler I, Lopez FJ, Negro-Vilar A. Anatomy and physiology of central galanin-containing pathways. *Prog Neurobiol*. 1993;**40**(6):711–69. doi: 10.1016/0301-0082(93)90012-H. [PubMed: 7683433].
17. Li L, Yu L, Kong Q. Exogenous galanin attenuates spatial memory impairment and decreases hippocampal beta-amyloid levels in rat model of Alzheimer's disease. *Int J Neurosci*. 2013;**123**(11):759–65. doi: 10.3109/00207454.2013.800976. [PubMed: 23687905].
18. Ogren SO, Kuteeva E, Elvander-Tottie E, Hokfelt T. Neuropeptides in learning and memory processes with focus on galanin. *Eur J Pharmacol*. 2010;**626**(1):9–17. doi: 10.1016/j.ejphar.2009.09.070. [PubMed: 19837050].
19. Mennicken F, Hoffert C, Pelletier M, Ahmad S, O'Donnell D. Restricted distribution of galanin receptor 3 (GalR3) mRNA in the adult rat central nervous system. *J Chem Neuroanat*. 2002;**24**(4):257–68. doi: 10.1016/S0891-0618(02)00068-6. [PubMed: 12406501].
20. Schott PA, Bjelke B, Ogren SO. Time-dependent effects of intrahippocampal galanin on spatial learning. Relationship to distribution and kinetics. *Ann N Y Acad Sci*. 1998;**863**:454–6. [PubMed: 9928198].
21. Liu Y, Li S, Qi X, Zhou W, Liu X, Lin H, et al. A novel neuropeptide in suppressing luteinizing hormone release in goldfish, Carassius auratus. *Mol Cell Endocrinol*. 2013;**374**(1-2):65–72. doi: 10.1016/j.mce.2013.04.008. [PubMed: 23623870].
22. Mirzaei SH, Taherianfard M, Tadjali M, Kohkaloyeh M. Castration and passive avoidance learning alter the distribution of N-Methyl D-Aspartate receptor. *Am J Neurosci*. 2013;**4**(1):39–45. doi: 10.3844/amjnsp.2013.39.45.
23. Daniel JM, Winsauer PJ, Moerschbaecher JM. Castration in rats impairs performance during acquisition of a working memory task and exacerbates deficits in working memory produced by scopolamine and mecamylamine. *Psychopharmacology (Berl)*. 2003;**170**(3):294–300. doi: 10.1007/s00213-003-1537-4. [PubMed: 12898124].