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Differential Effects of Sildenafil (Viagra) on Processing Steps of Spatial Learning and Memory in Rat

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Background: Sildenafil (Viagra) has been introduced to treat erectile dysfunction by acting as phosphodiesterase 5 inhibitor and hence accumulation of guanosine cyclic monophosphate (cGMP). On the other hands, the nitric oxide/cGMP signaling pathway has crucial role in synaptic plasticity processes like long-term potentiation (LTP) in the central nervous system considered as a model of learning and memory.
Objectives: The aim of the present study was to determine the effects of sildenafil on different stages of spatial learning and memory of rat using radial maze.
Materials and Methods: The effects of pre-training, pre-retrieval oral administration or post-training i.p. injection of sildenafil (10 mg/kg) in radial maze task were investigated.
Results: Pre-training and post-training administration of sildenafil impaired radial maze task. Pre-retrieval injection of sildenafil decreased reference memory and working memory errors. Therefore, sildenafil impaired acquisition and consolidation of spatial learning and memory but improved retrieval of spatial memory in radial maze.
Conclusions: Sildenafil has differential effects on the spatial learning and memory processing. It seems this result is due to accumulation of cGMP in the neural structures related to learning and memory processing. As different neural structures are involved in different learning and memory tasks, these results might be due to the different actions of cGMP in different structures of brain.

*Keywords:*Sildenafil; Spatial Learning and Memory; Rat

1. Background

It has been confirmed that glutamate as a neurotransmitter which bind to its specific receptors has a critical role in learning and memory processing (1-4) and long-term potentiation (LTP) (4-6). Activated glutamate NMDA receptor stimulates an influx of calcium, which in turn activates multiple cascades. One of these pathways involves the production of nitric oxide (NO) (7-10) which stimulates guanylyl cyclase (sGC) and formation of the second messenger, 3́5́ guanosine cyclic monophosphate (cGMP) in the presynaptic terminal (8, 9, 11). Increased cGMP levels stimulate further release of glutamate (11, 12) and hence may constitute a presynaptic mechanism contributing to early phase of long-term potentiation of excitatory neurotransmission and perhaps some forms of learning and memory (13-15). Then, cGMP was degraded by phosphodiesterase (PDE). Sildenafil is an inhibitor of PDE type 5 (PDE5) which is the first oral medicine approved by the United States Food and Drug Administration for the therapeutic treatment of erectile dysfunction (16). The mRNA of PDE5 has been demonstrated in several brain areas of adult rat including brain cortex and hippocampus (17). Several lines of evidence indicate that sildenafil may offer novel strategy in therapeutic treatment of age related memory impairment, pain, pulmonary hypertension and multiple sclerosis (18).

2. Objectives

Based on mentioned reports, sildenafil which increases cGMP half-life may contribute to learning and memory processing. Few recent studies have shown that sildenafil could affect some kind of cognitive performance. It improves performance of object recognition task, inhibitory avoidance task and attenuates learning impairment induced by blockade of cholinergic muscarinic receptors (2, 19-24). On the other hand, there is another report which indicates that sildenafil had no significant effect on passive avoidance learning (13, 14). It has been report-

Implication for health policy/practice/research/medical education:
Sildenafil is an inhibitor of phosphodiesterase (PDE) which is the first oral drug approved by the United States Food and Drug Administration for the therapeutic treatment of erectile dysfunction. Inhibition of PDE increases cGMP levels. cGMP involves in learning and memory processing. The result of present study indicated that sildenafil improves retention of memory but impairs acquisition and consolidation stages of learning and memory processing.

ed that sildenafil consumption causes temporary amnesia (25-27). As different neural circuits involve in different types of learning and memory, the aim of this study was to evaluate the effects of sildenafil as a PDE5 inhibitor on acquisition, consolidation and retrieval of Y-maze spatial learning and memory in normal young rats.

3. Materials and Methods

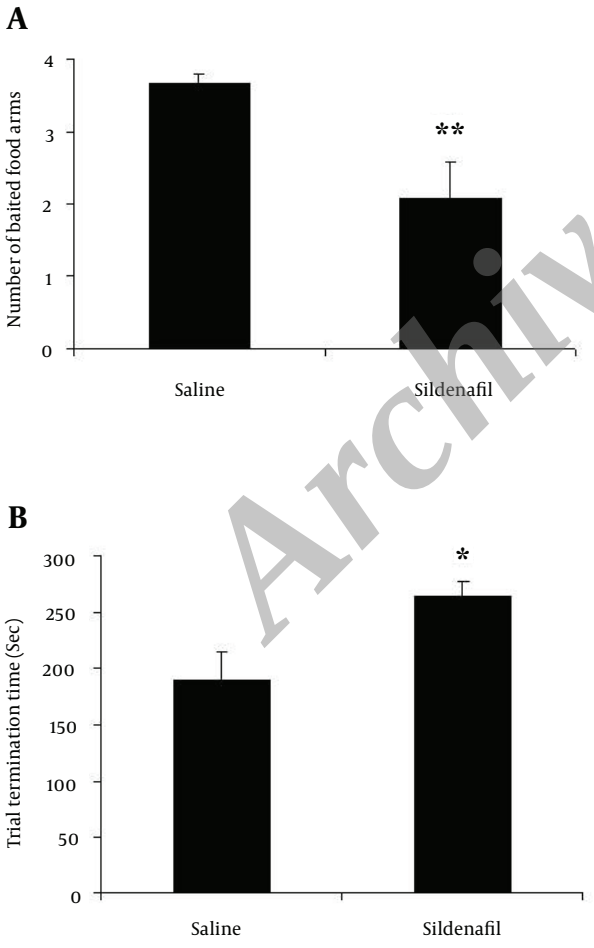
3.1. Animals

Sixty-two three-month-old male Wistar rats weighting 250 - 300 g were used in this study. Rats were housed three per cage. They were maintained at 20 ± 2 °C on a 12:12-hour light–dark photocycle (lights on 07:00 a.m.). Water and food were available ad libitum. All rats were acclimatized to the environment for at least ten days prior to initiation of behavioral testing. Body weights were reduced to about 85-90% of the primary weights by reduction of daily food.

3.2. Radial Maze

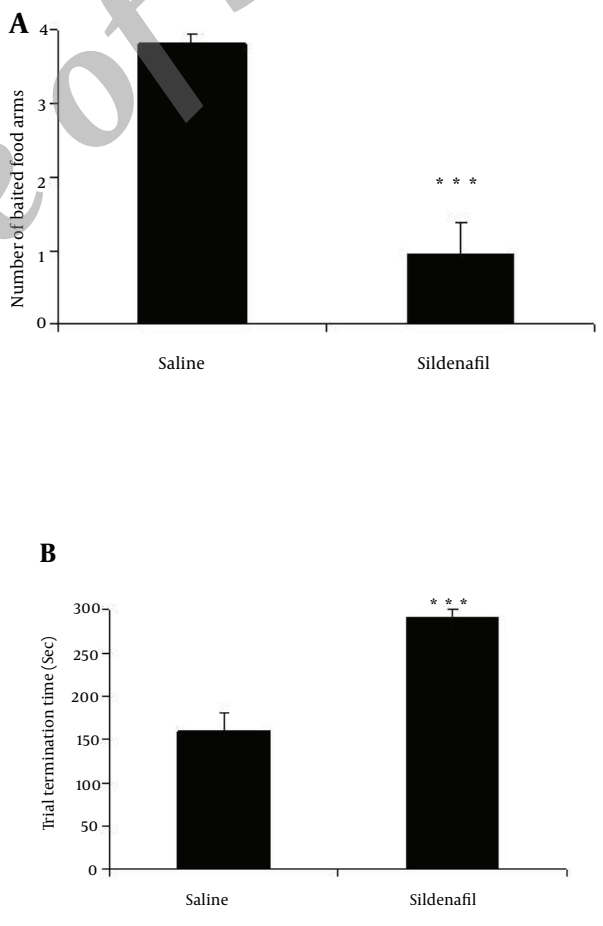
The rats were tested in an 8-arm radial maze. The apparatus consisted of one central compartment and eight arms radiated from it in equal intervals. The central part of the radial-maze was 20 cm in diameter. Its arms (50 cm long, 15 cm high, 10 cm wide) were closed and made of transparent Plexiglas and their entrance was blockade by transparent Plexiglas guillotine doors. There was a cup at the end of each arm filled with food pellets. Four arms were baited randomly (arms number: 1, 4, 5, 7). The sequence never changed through the experiments. The maze was always oriented in space in the same way. Several extra-maze cues were provided close to the arms. All of experiments were performed between 12:00 to 15:00 p.m. In each trial, the rat was placed into central compartment (start position) closed off by door. After 15 seconds to navigate central compartment, guillotine doors were raised and were allowed to find the food pellets in arms until all 4 pellets arms had been eaten or 5 minutes was elapsed.

Figure 1. Effect of Sildenafil on Acquisition of Spatial Learning



The effects of daily pre-training oral administration of sildenafil or saline on the number of baited food arms (A), and the trial termination time (B) on sixth day of training. *: P < 0.05, **: P < 0.01, Ordinate: Mean ± SEM.

Figure 2. Effect of Sildenafil on Consolidation of Spatial Memory



The effects of daily post-training i. p. injection of sildenafil or saline on the mean (±SEM) number of baited food arms (A) and trial termination time (B) on sixth day of training. ***: P < 0.001.

All rats were trained 3 trials per day for 6 consecutive days. The interval between trials was 5 minutes. In the last day, the trial termination time and number of baited food arms were recorded. If rat learns to find baited food arms, numbers of memory errors were calculated. The number of entries into never baited arms was regarded as reference memory errors (RME), while the number of re-entries into the arms where the pellet had already been eaten was regarded as working memory errors (WME) (28).

3.3. Effects of Pre-Training Sildenafil Administration on Acquisition in Radial Maze

The rats were randomly divided into two experimental groups: saline (n = 11) and sildenafil (n = 11) treated groups which were given saline or sildenafil (10 mg/kg) orally by gavage 1 hour prior to the daily training trials.

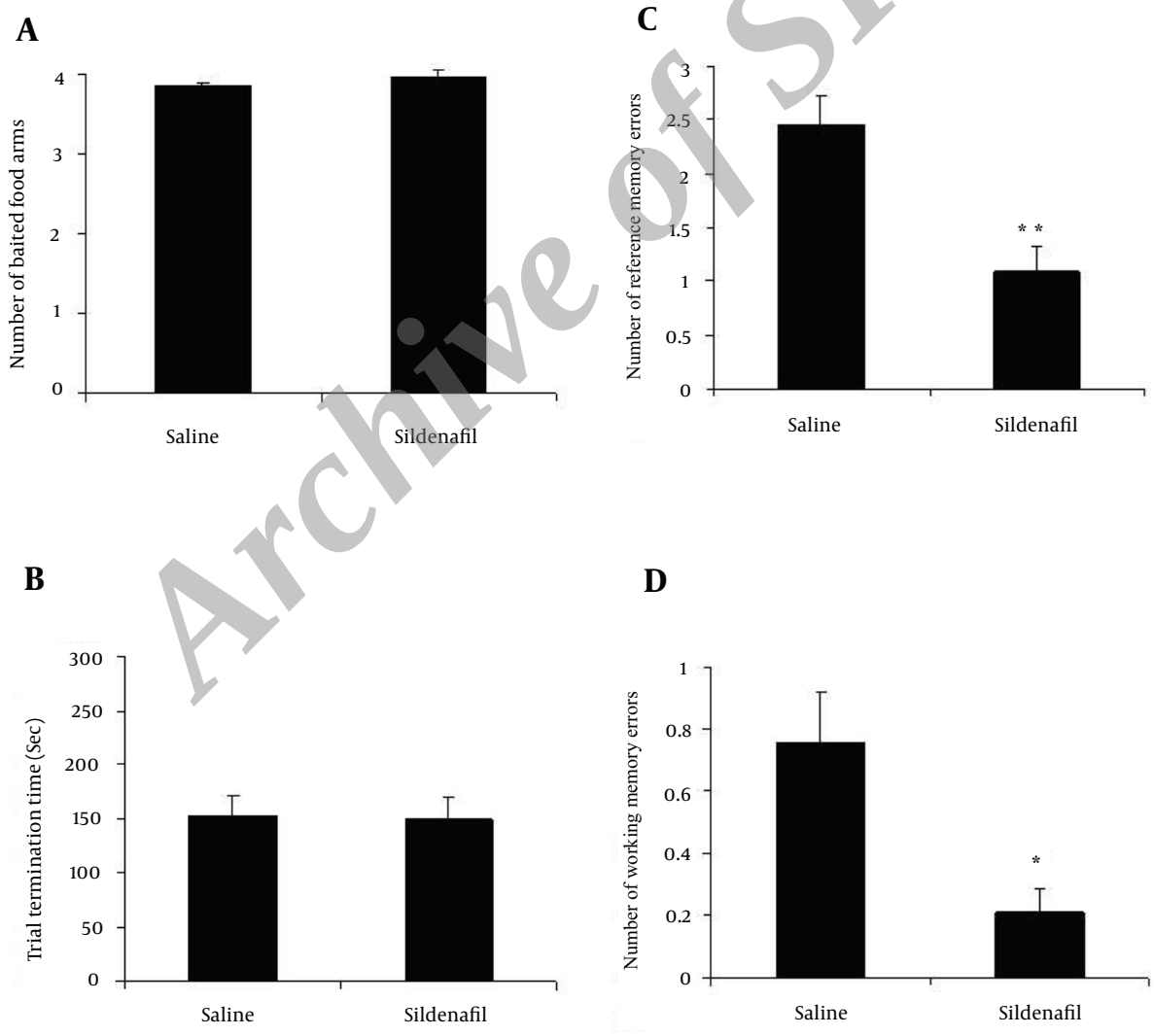
3.4. Effects of Post-Training Sildenafil Administration on Consolidation of Memory in Radial Maze

Immediately after the end of the first day of training trials, the rats were randomly divided into two groups which were received intra-peritoneal (i.p.) injection of saline (n = 12) or sildenafil (10 mg/kg, n = 9), respectively. This procedure was continued after the daily training.

3.5. Effects of Pre-Retrieval Sildenafil Administration on Retention of Memory in Radial Maze

The rats were trained for 6 consecutive days. Then after 2 days, retention test was performed. The rats were randomly divided into saline (n = 11) or sildenafil (n = 8), (10 mg/kg) which were given orally by gavages 1 hour prior to the retention test.

Figure 3. Effect of Sildenafil on Retrieval of Spatial Memory



The effects of pre-retrieval oral administration of sildenafil or saline on the number of baited food arms (A), trial termination time (B), number of reference memory errors (C) and number of working memory errors (D) in the retention test. Ordinate: Mean ± SEM. *: $P < 0.05$, **: $P < 0.01$.

3.6. Statistical Analysis

The data of each experiment was analyzed using student t-test. $P < 0.05$ was considered statistically significant. All data was represented as mean ± SEM.

4. Results

4.1. Effects of Pre-Training Sildenafil Administration on Acquisition in Radial Maze

As shown in Figure 1, in last day of training the number of baited food arms in sildenafil group was significantly fewer than the saline group [$t(20) = -3.06$; $P = 0.006$]. In addition, sildenafil group of rat took more time to get foods in the arms [$t(20) = -2.57$; $P = 0.01$].

4.2. Effects of Post-Training Sildenafil Administration on Consolidation of Memory

The results of second experiment showed that sildenafil treated group significantly found and ate less arms food [$t(19) = -6.98$; $p < 0.0001$] and took more time than control group [$t(19) = 5.17$; $p < 0.0001$] (Figure 2).

4.3. Effects of Pre-Training Sildenafil Administration on Retention of Memory

As indicated in Figure 3. A & B, there were no significant differences in the number of baited food arms [$t(17) = 0.94$; $P = 0.35$] and trial termination times [$t(17) = 0.13$; $P = 0.89$] between the two groups of rats on sixth day of training. On the other hands, the number of reference [$t(17) = 3.87$; $P = 0.0015$] and working memory errors [$t(17) = 2.67$; $P = 0.016$] in the treated group of rat, which received sildenafil 1 hour before retention, were significantly fewer than the control group (Figure 3 C and D).

5. Discussion

The present findings demonstrated that systemic administration of sildenafil (Viagra) could influence different phases of spatial learning and memory of rats in radial maze task. Briefly, our results showed that pre-training administration of sildenafil attenuated learning of radial maze task. In addition, post-training administration of sildenafil impaired consolidation of spatial memory. On the other hand, pre-retrieval administration of sildenafil enhanced retrieval of spatial memory by decreasing the number of working memory and reference memory errors. Pre-retrieval administration of sildenafil could improve both spatial reference and working memory. Therefore, sildenafil has differential effects on different steps of spatial learning and memory. Several lines of evidence indicated that sildenafil improves learning and memory (2, 19, 20, 22-24, 29-32). Some of these were resulted in memory deficit conditions. Devan et al. reported that pre-training i.p. administration of L-NAME, scopolamine or intracerebroventricular infusion of nitric oxide synthase inhibitor impaired 14-unit T-maze performance and that pre-training i.p. administration of sildenafil significantly attenuated this impairment (23, 33, 34). Erceg et al. reported that in rats with chronic liver failure or hyperammonemia, the brain glutamate-NO-cGMP was impaired. chronic treatment with sildenafil restored the ability of rats to learn a conditional discrimination task (32). There are some reports indicating beneficial effects of sildenafil on learning and memory in some types of learning and memory tests in normal animals. For instance, administration of sildenafil directly into the hippocampus after the first trail in object recognition task, improved memory in mice (19, 35) and enhanced the processes of consolidation of object information (20). Prickaerts et al. reported that post trial oral administration of sildenafil (0.3, 1, 3 mg/kg) improved the object discrimination performance of Swiss mice at a dose of 1 mg/kg (30). Prickaerts et al. reported that sildenafil given before or immediately after the first trial improved the memory performance of object recognition task after 24 hours (31). Singh et al. reported that, sildenafil (2, 4, 8 mg/kg, i.p.) administered immediately after training on first day led to a dose-dependent improvement of memory in mice in elevated plus maze test (2).

PDE5 inhibitors, such as sildenafil, provide a means to enhance the amplitude and duration of cGMP signal transduction. Therefore, it is believed that the effects of sildenafil on learning and memory are due to accumulation of cGMP in neural structures like hippocampus. In vitro experiments demonstrated that sildenafil increases NO-mediated cGMP accumulation in the dorsal hippocampus as assessed with radioimmunoassay and immunocytochemistry (19, 20, 36). Therefore, memory enhancing effect of sildenafil which improved reference and working memories might be due to increasing the level of cGMP in neural structures like hippocampus. Although, our results are in contrast with most previous reports concerning the benefit effects of sildenafil on learning and memory, our results are in agree with few reports which indicated that sildenafil had no significant facilitatory effects on learning and memory. Few cases of transient global amnesia have been reported after sildenafil medication (25-27). one of the hypothesizes suggests that the pathophysiology of transient global amnesia is related to intracranial vasomotor changes, especially due to venous congestion and venous ischemia of bilateral hippocampal structures. Therefore, it is suggested that a single dose of sildenafil may stimulate transient global amnesia (25). Shahidi et al. reported that immediate post-training i.p. administration of sildenafil (1, 3, 10, 20 mg/kg) did not facilitate retention performance of a passive avoidance response in both young and middle aged rats. Comparison of retention time between young and middle aged rats showed that the memory of the latter had been significantly reduced. Sildenafil had no significant effect on consolidation of passive avoidance response in young rats and decreased consolidation in middle aged

rats (13, 14). Devan et al. reported that different doses of sildenafil (1, 1.5,3, 4.5 mg/kg) alone did not significantly alter complex maze performance (23). The decreasing effects of sildenafil on consolidation and acquisition of spatial learning and memory in our study might be due to the inhibition of PDE5 within other brain structures and/or may inhibit other PDE ribozymes (e.g. the recently cloned PDE10A enzyme). Sildenafil has a beneficial role in preventing the decline of learning and memory in different tasks. It had no beneficial effects on acquisition and consolidation of learning and memory of healthy subjects in more complex tasks such as radial maze task, but it could be considered as an enhancer of memory retrieval of such a complex task in normal young rats. In addition, effects of sildenafil in normal subjects are varied which might be due to the type of memory test or the genius of the subjects. Furthermore, as different neural pathways involve in different types of learning and memory task, sildenafil may acts and accumulates cGMP in other neural structures in addition to hippocampus. Further researches with local cerebral infusions of sildenafil are suggested to determine the site(s) of drug action within different brain regions contributing learning and memory processing using behavioral and electrophysiological methods.

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Authors' Contributions

Study concept and design: Shahidi, Arjipour, Komaki, Mahmoodi; Acquisition of data: Shahidi, Arjipour; Analysis and interpretation of data: Shahidi, Komaki, Mahmoodi; Drafting of the manuscript: Shahidi, Mahmoodi; Critical revision of the manuscript for important intellectual content: Shahidi, Arjipour, Komaki, Mahmoodi; Statistical analysis: Shahidi, Mahmoodi; Administrative, technical, and material support: Shahidi, Komaki; Study supervision: Shahidi.

Financial Disclosure

There was no conflict of interests.

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References

1. Perez-Otano I, Ehlers MD. Learning from NMDA receptor trafficking: clues to the development and maturation of glutamatergic synapses. *Neurosignals.* 2004;**13**(4):175–89.
2. Singh N, Parle M. Sildenafil improves acquisition and retention of memory in mice. *Indian J Physiol Pharmacol.* 2003;**47**(3):318–24.
3. Traverso LM, Ruiz G, Camino G, De la Casa LG. Ketamine blocks the formation of a gustatory memory trace in rats. *Pharmacol Biochem Behav.* 2008;**90**(3):305–11.
4. Zorumski CF, Izumi Y. Modulation of LTP induction by NMDA receptor activation and nitric oxide release. *Prog Brain Res.* 1998;**118**:173–82.
5. Bortolotto ZA, Fitzjohn SM, Collingridge GL. Roles of metabotropic glutamate receptors in LTP and LTD in the hippocampus. *Curr Opin Neurobiol.* 1999;**9**(3):299–304.
6. Cain DP. LTP, NMDA, genes and learning. *Curr Opin Neurobiol.* 1997;**7**(2):235–42.
7. Schuman EM, Madison DV. A requirement for the intercellular messenger nitric oxide in long-term potentiation. *Science.* 1991;**254**(5037):1503–6.
8. Hawkins RD, Son H, Arancio O. Nitric oxide as a retrograde messenger during long-term potentiation in hippocampus. *Prog Brain Res.* 1998;**118**:155–72.
9. Garthwaite J. Glutamate, nitric oxide and cell-cell signalling in the nervous system. *Trends Neurosci.* 1991;**14**(2):60–7.
10. Bohme GA, Bon C, Stutzmann JM, Doble A, Blanchard JC. Possible involvement of nitric oxide in long-term potentiation. *Eur J Pharmacol.* 1991;**199**(3):379–81.
11. Garthwaite J, Charles SL, Chess-Williams R. Endothelium-derived relaxing factor release on activation of NMDA receptors suggests role as intercellular messenger in the brain. *Nature.* 1988;**336**(6197):385–8.
12. Haley JE, Dickenson AH, Schachter M. Electrophysiological evidence for a role of nitric oxide in prolonged chemical nociception in the rat. *Neuropharmacology.* 1992;**31**(3):251–8.
13. Shahidi S, Komaki A, Mahmoodi M, Lashgari R. The role of GABAergic transmission in the dentate gyrus on acquisition, consolidation and retrieval of an inhibitory avoidance learning and memory task in the rat. *Brain Res.* 2008;**1204**:87–93.
14. Mahmoodi M, Shahidi S, Hasanein P. Involvement of the ventral tegmental area in the inhibitory avoidance memory in rats. *Physiol Behav.* 2011;**102**(5):542–7.
15. Bon CL, Garthwaite J. On the role of nitric oxide in hippocampal long-term potentiation. *J Neurosci.* 2003;**23**(5):1941–8.
16. McCullough AR. Four-year review of sildenafil citrate. *Rev Urol.* 2002;**4 Suppl 3**:S26–38.
17. Van Staveren WC, Steinbusch HW, Markerink-Van Ittersum M, Repaske DR, Goy MF, Kotera J, et al. mRNA expression patterns of the cGMP-hydrolyzing phosphodiesterases types 2, 5, and 9 during development of the rat brain. *J Comp Neurol.* 2003;**467**(4):566–80.
18. Uthayathas S, Karuppagounder SS, Thrash BM, Parameshwaran K, Suppiramaniam V, Dhanasekaran M. Versatile effects of sildenafil: recent pharmacological applications. *Pharmacol Rep.* 2007;**59**(2):150–63.
19. Rutten K, Vente JD, Sik A, Ittersum MM, Prickaerts J, Blokland A. The selective PDE5 inhibitor, sildenafil, improves object memory in Swiss mice and increases cGMP levels in hippocampal slices. *Behav Brain Res.* 2005;**164**(1):11–6.
20. Prickaerts J, de Vente J, Honig W, Steinbusch HW, Blokland A. cGMP, but not cAMP, in rat hippocampus is involved in early stages of object memory consolidation. *Eur J Pharmacol.* 2002;**436**(1-2):83–7.
21. Patil CS, Singh VP, Kulkarni SK. Modulatory effect of sildenafil in diabetes and electroconvulsive shock-induced cognitive dysfunction in rats. *Pharmacol Rep.* 2006;**58**(3):373–80.
22. Devan BD, Duffy KB, Bowker JL, Bharati IS, Nelson CM, Daffin LW Jr, et al. Phosphodiesterase type 5 (PDE5) inhibition and cognitive enhancement. *Drugs Future.* 2005;**30**(7):725.
23. Devan BD, Bowker JL, Duffy KB, Bharati IS, Jimenez M, Sierra-Mercado D Jr, et al. Phosphodiesterase inhibition by sildenafil citrate attenuates a maze learning impairment in rats induced by nitric oxide synthase inhibition. *Psychopharmacology (Berl).* 2006;**183**(4):439–45.
24. Baratti CM, Boccia MM. Effects of sildenafil on long-term retention of an inhibitory avoidance response in mice. *Behav Pharmacol.* 1999;**10**(8):731–7.
25. Shihman B, Auriel E, Bornstien NM. [Two cases of transient global amnesia (TGA) following sildenafil use]. *Harefuah.* 2006;**145**(9):656–7. 703.
26. Savitz SA, Caplan LR. Transient global amnesia after sildenafil (Viagra) use. *Neurology.* 2002;**59**(5):778.

27. Gandolfo C, Sugo A, Del Sette M. Sildenafil and transient global amnesia. *Neurol Sci.* 2003;**24**(3):145–6.
28. Huang YW, Hu WW, Chen Z, Zhang LS, Shen HQ, Timmerman H, et al. Effect of the histamine H3-antagonist clobenpropit on spatial memory deficits induced by MK-801 as evaluated by radial maze in Sprague-Dawley rats. *Behav Brain Res.* 2004;**151**(1-2):287–93.
29. Prickaerts J, van Staveren WC, Sik A, Markerink-van Ittersum M, Niewohner U, van der Staay FJ, et al. Effects of two selective phosphodiesterase type 5 inhibitors, sildenafil and vardenafil, on object recognition memory and hippocampal cyclic GMP levels in the rat. *Neuroscience.* 2002;**113**(2):351–61.
30. Prickaerts J, Sik A, van Staveren WC, Koopmans G, Steinbusch HW, van der Staay FJ, et al. Phosphodiesterase type 5 inhibition improves early memory consolidation of object information. *Neurochem Int.* 2004;**45**(6):915–28.
31. Prickaerts J, Sik A, van der Staay FJ, de Vente J, Blokland A. Dissociable effects of acetylcholinesterase inhibitors and phosphodiesterase type 5 inhibitors on object recognition memory: acquisition versus consolidation. *Psychopharmacology (Berl).* 2005;**177**(4):381–90.
32. Erceg S, Monfort P, Hernandez-Viadel M, Rodrigo R, Montoliu C, Felipo V. Oral administration of sildenafil restores learning ability in rats with hyperammonemia and with portacaval shunts. *Hepatology.* 2005;**41**(2):299–306.
33. Devan BD, Sierra-Mercado D, Jr., Jimenez M, Bowker JL, Duffy KB, Spangler EL, et al. Phosphodiesterase inhibition by sildenafil citrate attenuates the learning impairment induced by blockade of cholinergic muscarinic receptors in rats. *Pharmacol Biochem Behav.* 2004;**79**(4):691–9.
34. Devan BD, Pistell PJ, Daffin LW, Jr., Nelson CM, Duffy KB, Bowker JL, et al. Sildenafil citrate attenuates a complex maze impairment induced by intracerebroventricular infusion of the NOS inhibitor Nomega-nitro-L-arginine methyl ester. *Eur J Pharmacol.* 2007;**563**(1-3):134–40.
35. Monfort P, Munoz MD, Kosenko E, Llansola M, Sanchez-Perez A, Cauli O, et al. Sequential activation of soluble guanylate cyclase, protein kinase G and cGMP-degrading phosphodiesterase is necessary for proper induction of long-term potentiation in CA1 of hippocampus. Alterations in hyperammonemia. *Neurochem Int.* 2004;**45**(6):895–901.
36. de Vente J, Hopkins DA, Markerink van Ittersum M, Steinbusch HWM. Effects of the 3′,5′-phosphodiesterase inhibitors isobutylmethylxanthine and zaprinast on NO-mediated cGMP accumulation in the hippocampus slice preparation: an immunocytochemical study. *Journal of Chemical Neuroanatomy.* 1996;**10**(3-4):241–8.