The role of NMDA receptors of the medial septum and dorsal hippocampus on memory acquisition

Fatemeh Khakpai ^a, Mohammad Nasehi ^b, Mohammad-Reza Zarrindast ^{a,b,c,d,e *}

^aInstitute for Cognitive Science Studies (ICSS), Tehran, Iran

^bMedical Genomics Research Center and School of Advanced Sciences in Medicine, Islamic Azad University, Tehran Medical Sciences Branch, Tehran, Iran

^cDepartment of Pharmacology School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

^dSchool of Cognitive Sciences, Institute for Research in Fundamental Sciences (IPM), Tehran, Iran

^eIranian National Center for Addiction Studies, Tehran University of Medical Sciences, Tehran, Iran

* Corresponding author at: Department of Pharmacology School of Medicine, Tehran University of Medical Sciences, P.O. Box 13145-784, Tehran, Iran.

Tel.: +98 21 66402569

fax: +98 21 66402569

E-mail address: zarinmr@ams.ac.ir (M.R. Zarrindast).

Abstract

The glutamatergic neurons in the medial septal/diagonal band of Broca (MS/DB) affect the hippocampal functions by modulating the septo-hippocampal neurons. Our study investigated the possible role of NMDA receptors of the medial septum nucleus (MS) and dorsal hippocampus (CA1) on memory acquisition in male Wistar rats. Animals were bilaterally implanted with chronic cannulae in the MS and CA1. Rats were trained in a step-through type inhibitory avoidance task, and tested 24 h after training to measure step-through latency as memory retrieval. Our results indicated that pre-training intra-MS or intra-CA1 infusions of NMDA (0.125 μ g/rat) and D-AP7 (0.012 μ g/rat) increased and decreased memory acquisition, respectively when compared to saline control group. Also, pre-training intra-CA1 and intra-MS injection of an effect dose of D-AP7 (0.012 μ g/rat) along with an effect dose of NMDA (0.125 μ g/rat) impaired memory acquisition. Interestingly, pre-training intra-CA1/MS infusion of D-AP7 (0.012 μ g/rat) diminished memory response produced by pre-training injection of NMDA (0.125 μ g/rat) in the MS/CA1, respectively (cross injection or bilateral injection). Also, all above doses of drugs did not alter locomotor activity. These results suggest that the glutamatergic pathway between the MS and CA1 regions is involved in memory acquisition process.

Key words: NMDA, D-AP7, Memory, Passive avoidance, Rat

1. Introduction

The septum and hippocampus are two regions of the limbic system (Cheeta *et al.* , 2000). The septum is anatomically and functionally connected to the hippocampus (Khakpai F, 2012, Roland and Savage, 2009). Studies using lesions show that the MS ablations are similar in many respects to the hippocampus lesions (Niewiadomska *et al.* , 2009). For example, Rawlins and Olton (1982) performed electrolytic coagulation experiments of the MS and observed disruption of spatial working memory in rats (Rawlins and Olton, 1982). The generation of theta rhythmicity is produced by a spread neuronal network. The MS/DB is an ultimate generator of theta waves. The current theta model proposes that the septal pacemaker system has a vital role, with cholinergic and GABAergic fibers projecting to the hippocampus (Cervera-Ferri *et al.* , 2012). The MS/DB contain cholinergic and GABAergic neurons. The glutamatergic neurons have also been recently defined in this area but their exact role still remains uncertain. The glutamatergic neurons within the MS/DB can stimulate the cholinergic and GABAergic neurons which upon proper stimulation may contribute to rhythm generation (Manseau *et al.* , 2005). Moreover, it is possible that a hippocampus-lateral septal (GABAergic CB-containing neurons)-supramammillary area (aspartate/glutamatergic cells)-medial septal (cholinergic neurons)-hippocampus signal loop involved in the generation and regulation of hippocampal theta rhythm

activity (Leranth *et al.* , 1999, Leranth and Kiss, 1996). In addition, anatomical studies indicated that there are frontocortico-septal glutamatergic pathway (Jaskiw *et al.* , 1991), and aspartatergic/glutamatergic projection from the nucleus reuniens thalami (RE) to the hippocampus and to the MS. The neurons of RE contribute to the excitatory innervation of the septo-hippocampal system (Bokor *et al.* , 2002). The glutamatergic septo-hippocampal connection has been assumed to regulate the activity of septal neurons projecting to the hippocampus (Ransome and Hannan, 2012). Several evidences demonstrated that a reciprocal pathway exists between septum and hippocampus (Okada and Okaichi, 2010). The hippocampus sends a GABAergic projection to the MS (Toth *et al.* , 1993) and a glutamatergic projection to the lateral septum (LS) (Khakpai *et al.* , 2013b). The glutamatergic projection originates from the pyramidal neurons and terminates on the GABAergic neurons of the LS (Khakpai *et al.*, 2013b). Both the GABAergic and glutamatergic projections could excite via exciting of the cholinergic receptors in the hippocampus. Stimulating of the GABAergic projection would probably induce a weakening of septal activity. Similarly, exciting of the glutamatergic projection would stimulate the GABAergic neurons of the LS and also result in a reduction of septal activity (Khakpai *et al.* , 2013a).

The glutamate as an excitatory neurotransmitter is mainly abundant in the mammalian brain (Watson and Stanton, 2009). These receptors are found in high

density in the septum and CA1 (Jafari-Sabet, 2011, Khakpai F, 2012). The NMDA receptors of the MS and hippocampus are implicated in cognitive performance, specifically in learning and memory processes (Lu *et al.* , 2011, Rezayof *et al.* , 2010). Memory is often considered to be a process that has numerous stages including acquisition, consolidation and retrieval (Nasehi *et al.* , 2013). Our previous study revealed that intra-CA1 or intra-MS injection of NMDA and D-AP7 improved and impaired memory acquisition, respectively (Khakpai *et al.* , 2012). In this investigation, based on the role of NMDA receptors of the MS and hippocampus in memory processes (Khakpai *et al.*, 2013b), on one hand and the regulatory role of the glutamatergic septo-hippocampal connection in the activity of septal neurons projecting to the hippocampus (Niewiadomska *et al.*, 2009), on the other hand, we attempt to determine the role of glutamatergic pathway between the MS and hippocampus in memory acquisition and locomotor activity in the step-through type inhibitory avoidance task and locomotion apparatus.

2. Materials and methods

2.1. Animals

Naïve adult male Wistar rats (Institute of Cognitive Science, Tehran, Iran) weighing 230–270 g and aging 2-3 months at the time of surgery, were used. The animals were housed four per cage and had free access to standard food pellets

(22% protein, 53% carbohydrate, and 4.5% lipid; Behparvar Co., Tehran, Iran) and water in a room with a 12:12 h light/dark cycle (lights on at 08:00 h) and controlled temperature ($22 \pm 2^{\circ}\text{C}$). Animals were allowed to adapt to the laboratory conditions for at least 1 week prior to surgery. Rats were handled about 3 min each day previous to behavioral testing. Rats were handled from surgery day to testing day. All experiments were performed between 9:00 and 13:00 h and each rat was tested only once. All procedures in this investigation are in agreement with the guide for the Care and Use of Laboratory Animals as adopted by the Ethics Committee of Faculty of Science, Tehran University (357: November 2000).

2.2. Drugs

Drugs used in this study were ketamine, xylazine (For surgical procedure; Alfasan Chemical Co, Woerden, and Holland), N-methyl-d-aspartate (NMDA, selective NMDA receptor agonist) and dl-2-amino-7-phosphonoheptanoate (D-AP7, selective NMDA receptor antagonist; Tocris, Bristol, UK). Based on the pharmacological properties, glutamate ionotropic receptors have been classified into three major subtypes, that is, the α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), kainate and NMDA receptor (Petrie *et al.* , 2000). With respect to learning and memory, scientists are able to recognize more specifically the role played through the three main glutamate receptor classes in learning and

memory: center stage is evidently the NMDA receptor, with overwhelming evidence proving its participation in the actual learning process (encoding), throughout the animal kingdom. Evidence for the involvement of the AMPA receptors is less clear-cut due to the general problem of specificity: block of AMPA receptors will shut down neuronal communication, and this will affect many components necessary for learning. Hence, the role of AMPA receptors cannot be recognized in isolation (Riedel *et al.* , 2003). The dosages of drugs were chosen according to our previous investigation. In the following of our previous research, significant dose of NMDA and sub-threshold doses of D-AP7 were chosen (Khakpai *et al.*, 2012). The drugs were tested at doses: NMDA 0.125 $\mu\text{g}/\text{rat}$ and D-AP7 0.006 and 0.012 $\mu\text{g}/\text{rat}$. NMDA and D-AP7 were dissolved in sterile 0.9% saline and were injected into the intra-MS and intra-CA1 in a volume of 0.5 $\mu\text{l}/\text{site}$.

2.3. Stereotaxic surgery

Male Wistar rats were anaesthetized with intraperitoneal injection of a solution including ketamine hydrochloride (50 mg/kg) plus xylazine (5 mg/kg) and located in a stereotaxic frame (Stoelting Co, IL, USA) with flat-skull position. A midline cut was made in the skin of the skull, and then the underlying periosteum was pulled back. A stainless steel guide cannula (22-gauge) was inserted 2 mm

above the medial septum nucleus in agreement with stereotaxic coordinate. Two 22-gauge stainless steel guide cannulae were also placed (bilaterally) 2 mm above the CA1 regions of the dorsal hippocampus again in agreement with stereotaxic coordinates atlas (Paxinos, 2007). Stereotaxic coordinates for the medial septum nucleus were +0.3 to +1.1 mm (depending on body weight) anterior to bregma, 0 to ± 0.1 mm lateral to the midline and -4.5 to -6.5 mm ventral to the dorsal surface of the skull. Stereotaxic coordinates for the CA1 areas were -2.6 to -2.9 mm (depending on body weight) posterior to bregma, ± 1.6 to -1.8 mm lateral to the midline and -2.5 to -2.8 mm ventral to the dorsal surface of the skull. The cannulae were assured to the bone using dental acrylic cement. A stylet was inserted into the guide cannula to avoid possible impediment. All rats were permitted about 5–7 days to recuperate from surgery and the influence of anesthetic drugs. [The protocols and target coordination of cannula-implantation were according to our previous research \(Khakpai *et al.*, 2012\).](#)

2.4. Behavioral procedures

2.4.1. Passive avoidance (PA) apparatus

Passive avoidance is one of the most frequently used behavioral paradigms to study neurotransmitter functions involved in learning, long-term memory

(Robinson *et al.* , 2011, Zarrindast MR, 2002, Zarrindast *et al.* , 2011), memory consolidation (Izquierdo *et al.* , 2006), diverse forms of cognition (Jafari-Sabet, 2006), and rewarded alternation task (Nagahara and McGaugh, 1992). Passive avoidance task have been many successful inferences to other types of memory, particularly those of fear-motivated learning and spatial tasks. The apparatus composed of two compartments, one dark (20 cm $\times$ 20 cm $\times$ 15 cm) with a stainless steel grid floor and the other light (20 cm $\times$ 20 cm $\times$ 30 cm). The walls of the dark compartment were black, with no light there. The walls and floor of light compartment composed of white opaque resin and were lit with a 20 W electric bulb placed $\sim$ 50 cm above the floor of the apparatus. A guillotine door opening (7 cm $\times$ 9 cm) was made on the floor in the middle of the section intermediate to the two divisions. Stainless steel grids (2.5 mm in diameter) were located at 1-cm intermission (area between the centers of grids) on the floor of the dark compartment to produce foot shock. Intermittent electric shocks (50 Hz, 3 s, and 1 mA intensity) were given to the grid floor of the dark compartment by an isolated stimulator (BorjSanat Co, Tehran, Iran).

2.4.2. Memory testing

Passive avoidance (PA) training was based on the protocol used in our previous studies (Khakpai *et al.*, 2012, Yousefi *et al.* , 2012). The rats were

permitted to habituate to the experimental room 1 h previous each training or testing sessions. All training and testing sessions were performed between 09:00 and 13:00 h. Each animal was usually positioned in the light compartment for 5 s, following the guillotine door was opened. The time which animal waited prior crossing to the dark (shock) compartment was registered as the latency. The latency to cross the dark compartment was recorded for each animal. If an animal waited greater than 100 s to cross to the other compartment, it was eliminated from the experiment (in the current experiments, all of the animals crossed the dark compartment before 100 s). Once the animal crossed with all four paws to the following side, the guillotine door was closed and was immediately pulled back from the compartment into its home cage. After 25 min which is equal to 5 min before training animals were given pre-training administration of drugs, afterwards the session was repeated for each animal as in the acquisition session except the rat that immediately crossed to the dark compartment, the door was closed and a foot shock (50 Hz, 3 s, and 1 mA intensity) was at once delivered to the grid floor. 20 s later, the rat was ejected from the apparatus and placed into its home cage. Two minutes later, the animal was retested in a similar way which described for previous session. A successful acquisition of inhibitory avoidance was for animals with 120 s latency to enter into the dark compartment. Otherwise, when the animal entered the dark compartment (before 120 s) a second time, the door was closed

and the rat received the shock again. It is possible that the drug potency last as long as the acquisition. So the acquisition session was performed for each animal once or twice. The goal of this study with pre-training drug injection was to examine effects of drugs on the acquisition process.

Retention session was performed 24 h after the training session. During the retention session, each animal was located in the light compartment for 5 s, the door was opened, and the latency for entering into the dark compartment (as mentioned in the training session) was measured. The testing process was finished when the animal entered the dark compartment or remained in the light compartment for 300 s. During testing sessions no electric shock was given and the entrance latency into the dark chamber of the inhibitory avoidance apparatus was recorded as an index of the inhibitory avoidance memory. After step-through latency test, general activity of each animal is allowed to be estimative still after the fear learning.

2.4.3. Measurement of locomotor activity

The locomotion apparatus (Borj Sanat Co, Tehran, Iran) consist of clear perspex receptacle box (30 cm × 30 cm × 40 cm high). The apparatus has a gray perspex band (30 cm × 30 cm × 2.2 cm thick) with 16 photocells which separated

the box to 16 equal-sized squares. Locomotion was registered as the number of crossings from one square to another during 5 min.

2.5. Intra-MS/CA1 injections

For drug micro-injection, the rats were quietly handled; the stylets were removed from the guide cannulae and substituted with dental 27-gauge injection needles (2 mm below the tip of the guide cannula). The needle was linked to a 2.5 µl Hamilton micro syringes by means of polyethylene tubing and the injection were carrying out by hand. The forward motion of a small air bubble within the polyethylene tubing interposed among the superior end of the needle and the micro syringe was taken as a proof for the drug flow. The medial septum nucleus, left and right CA1 regions were infused with 0.5 µl solution on each site over a 60-s period. Drug injections were done 5 min previous testing.

2.6. Statistical analysis

Since data revealed normality of distribution and homogeneity of variance, the results were statistically assessed through one-way ANOVA and two-way ANOVA, in which mean $\pm$ S.E.M. of step-through latencies of experimental groups on the test day were estimate. If the F -value was significant, post hoc

analysis (Tukey test) was employed to evaluate specific group comparisons. In all comparisons, $P < 0.05$ was regarded to show statistical significance.

2.7. Experimental design

Eight animals were used in each experimental group. In experiments where rats received one or two injections, the control groups also received one or two saline injections. The comparisons conducted under the between condition for the six groups that contains 8 rats respectively. In all experimental groups, comparisons within groups were between saline/D-AP7 group and comparisons between groups were between NMDA/D-AP7 group which indicated treatment effect, dose effect and treatment–dose interaction of drugs. Thus, the order of several types of drug-administrations was counterbalanced. Because the glutamatergic projection between the medial septum and hippocampus (septo-hippocampal pathway) is a direct pathway (Colom *et al.* , 2005), but the glutamatergic pathway between the hippocampus and medial septum (hippocampo-septal pathway) is an indirect pathway (Alonso and Kohler, 1982), the first injection of drugs was done 10 min prior training into the medial septum and the second one was administered 5 min after first injection into the CA1. The experimental design and experimental results have been summarized in Table 1.

[Table 1 near here]

2.7.1. Experiment 1

In this experiment, the effect of pre-training intra-CA1 co-injection of D-AP7 and NMDA on acquisition of memory formation was evaluated. Six groups of animals were used. The animals received intra-CA1, saline (0.5 μ l/site) or different doses of D-AP7 (0.006 and 0.012 μ g/rat) or a dose of NMDA (0.125 μ g/rat), 5 min before training. The locomotor activity of animals was estimated 5 min after memory testing.

2.7.2. Experiment 2

In this experiment, the effect of pre-training intra-MS co-administration of different doses of D-AP7 and a dose of NMDA on acquisition of memory formation was examined. Six groups of animals were used. The animals received intra-MS saline (0.5 μ l/site), diverse doses of D-AP7 (0.006 and 0.012 μ g/rat) or a dose of NMDA (0.125 μ g/rat) 5 min prior to second infusion. The locomotor activity of animals was estimated 5 min after memory testing.

2.7.3. Experiment 3

In this experiment, we attempt to assess if NMDA receptors block in the CA1 and these receptors stimulate in the MS, how memory response change. For

this purpose, the effect of pre-training intra-CA1 administration of various doses of D-AP7 on improvement of memory induced by NMDA in the MS was examined. Six groups of animals were employed. The animals received intra-MS saline (0.5 μ l/site) or a dose of NMDA (0.125 μ g/rat) 10 min before training. Moreover, these animals also received intra-CA1, saline (0.5 μ l/rat) or different doses of D-AP7 (0.006 and 0.012 μ g/rat) 5 min before training. The locomotor activity of animals was estimated 5 min after memory testing.

2.7.4. Experiment 4

In this experiment, we attempt to evaluate if NMDA receptors stimulate in the CA1 and these receptors block in the MS, how memory response alter. For this purpose, the effect of pre-training intra-CA1 administration of a dose of NMDA upon memory response induced by D-AP7 in the MS was evaluated. Six groups of animals were employed. The animals received intra-CA1 saline (0.5 μ l/site) or a dose of NMDA (0.125 μ g/rat) 5 min before training. Moreover, these animals also received intra-MS, saline (0.5 μ l/rat) or diverse doses of D-AP7 (0.006 and 0.012 μ g/rat) 10 min before training. The locomotor activity of animals was estimated 5 min after memory testing.

3. Results

3.1. Effects of D-AP7 on memory acquisition improvement by NMDA in the CA1

Fig. 1 indicated the influence of pre-training intra-CA1 co-administration of D-AP7 and NMDA on step-through latency and locomotor activity. Our results indicated that pre-training intra-CA1 injection of D-AP7 reduced, while NMDA improved memory acquisition. Two-way ANOVA was performed with two levels of NMDA (0 and 0.125 μ g/rat) as factor A, and three levels (0, 0.006 and 0.012 μ g/rat) of D-AP7 (as factor B). Results of two-way ANOVA indicated that there is an interaction between Saline/D-AP7 group and NMDA/D-AP7 group on memory acquisition in the CA1 area: significant main effects for NMDA [F (1, 42) = 4.006, P < 0.05], for D-AP7 [F (2, 42) = 25.395, P < 0.001], and a significant interaction for both factors [F (2, 42) = 14.733, P < 0.001]. Post-hoc analyses revealed that in groups with administrations of sub-threshold dose- not to be appropriate dose- of D-AP7 (0.012 μ g/rat) along with meaningful dose- appropriate dose- of NMDA (0.125 μ g/rat) compared with a group that received saline plus D-AP7, memory acquisition was impaired in the CA1 which is possible due to plateau effect of D-AP7 (Fig. 1A, left and right panels). Furthermore, one-way ANOVA indicated that there is no interaction between D-AP7 and NMDA on locomotor activity in the CA1 [F (5, 42) = 0.309, P > 0.05, Fig. 1B; left and right panels].

[Fig.1 near here]

3.2. Effects of D-AP7 on memory acquisition improvement by NMDA in the MS

The effects of pre-training intra-MS co-infusion of D-AP7 and NMDA on step-through latency and locomotor activity are illustrated in Fig. 2. The current study showed that pre-training intra-MS injection of D-AP7 impaired, while NMDA increased memory acquisition. In this fig. two levels of NMDA (0 and 0.125 $\mu\text{g}/\text{rat}$) as factor A, and three levels (0, 0.006 and 0.012 $\mu\text{g}/\text{rat}$) of D-AP7 (as factor B) were used to assess two-way ANOVA. Results of Two-way ANOVA showed that there is an interaction between Saline/D-AP7 group and NMDA/D-AP7 group on memory acquisition [treatment effect: $F(1, 42) = 3.657$, $P < 0.05$, dose effect: $F(2, 42) = 28.358$, $P < 0.001$, treatment–dose interaction: $F(2, 42) = 14.276$, $P < 0.001$, Fig. 2A; left and right panels] in the MS. Post hoc analysis exhibited that sub-threshold dose of D-AP7 (0.012 $\mu\text{g}/\text{rat}$) diminished improvement of memory induced by significant dose of NMDA (0.125 $\mu\text{g}/\text{rat}$) in the MS. Moreover, one-way ANOVA exhibited that there is no interaction between D-AP7 and NMDA on locomotor activity in the MS [$F(5, 42) = 0.261$, $P > 0.05$, Fig. 2B; left and right panels].

[Fig.2 near here]

3.3. Effects of pre-training infusion of intra-MS NMDA and intra-CA1 D-AP7 on memory acquisition in the septo-hippocampal pathway

In fig.3 two-way ANOVA was done with two levels of NMDA (0 and 0.125 $\mu\text{g}/\text{rat}$) as factor A, and three levels (0, 0.006 and 0.012 $\mu\text{g}/\text{rat}$) of D-AP7 (as factor B). The data of Fig. 3 displayed that there is an interaction between intra-MS injection of NMDA and intra-CA1 infusion of D-AP7 (Saline/D-AP7 group and NMDA/D-AP7 group) on memory acquisition [Two-way ANOVA analysis, treatment effect: $F(1, 42) = 4.045$, $P < 0.05$, dose effect: $F(2, 42) = 26.909$, $P < 0.001$, treatment–dose interaction: $F(2, 42) = 12.316$, $P < 0.001$, Fig. 3A; left and right panels]. Moreover, post hoc analysis exhibited that intra-CA1 injection of D-AP7 at a dose of 0.012 $\mu\text{g}/\text{rat}$ weakened memory response induced by NMDA (0.125 $\mu\text{g}/\text{rat}$) in the MS. In addition, one-way ANOVA revealed that pre-training intra-CA1 injection of various doses of D-AP7 (0.006 and 0.012 $\mu\text{g}/\text{rat}$) plus also intra-MS administration of a dose of NMDA (0.125 $\mu\text{g}/\text{rat}$) [$F(5, 42) = 0.29$, $P > 0.05$, Fig. 3B; left and right panels] did not change locomotor activity.

[Fig.3 near here]

3.4. Effects of pre-training injection of intra-MS D-AP7 and intra-CA1 NMDA on memory acquisition in the septo-hippocampal pathway

In fig.4 two-way ANOVA was performed with two levels of NMDA (0 and 0.125 µg/rat) as factor A, and three levels (0, 0.006 and 0.012 µg/rat) of D-AP7 (as factor B). Two-way ANOVA shown that there is an interaction between intra-MS injection of D-AP7 and intra-CA1 administration of NMDA (Saline/D-AP7 group and NMDA/D-AP7 group) on memory acquisition [treatment effect: $F(1, 42) = 4.14$, $P < 0.05$, dose effect: $F(2, 42) = 25.094$, $P < 0.001$, treatment–dose interaction: $F(2, 42) = 11.636$, $P < 0.001$, Fig. 4A; left and right panels]. Furthermore, post hoc analysis revealed that intra-MS infusion of D-AP7 at a dose of 0.012 µg/rat declined memory response produced by NMDA (0.125 µg/rat) in the CA1. Also, one-way ANOVA shown that pre-training intra-MS infusion of different doses of D-AP7 (0.006 and 0.012 µg/rat) plus also intra-CA1 injection of a dose of NMDA (0.125 µg/rat) [$F(5, 42) = 1.625$, $P > 0.05$, Fig. 4B; left and right panels] had no effect on locomotor activity.

[Fig.4 near here]

3.5. Histology

After the testing sessions each rat was deeply anesthetized (according to the surgery section) and 0.5 µl of a 4% methylene-blue solution was infused into the CA1 and MS (0.5 µl/side), as described in the drug section. After decapitation, the brains removed and located in formaldehyde (10%) for several days. Then, the

brains were sliced (10- μ m thick coronal sections) and the sites of infusions were verified according to the atlas of Paxinos and Watson (2007) (fig. 5). Cannulae were implanted into the medial septum and CA1 regions of a total of 205 animals, but only the data from 192 animals with correct cannulae implants were included in the statistical analyses. The histological protocol was conducted in accordance with the Principles of Laboratory Animal Care (NIH publication No. 85-23, revised 1985) and with the Animal Care and Use guidelines of Tehran University of Medical Science.

[Fig.5 near here]

4. Discussion

4.1. The effect of glutamatergic receptor system of the CA1 and MS on memory acquisition

According to our previous investigation, this study also indicated that pre-training intra-CA1 or intra-MS injection of NMDA improved, while D-AP7 reduced memory acquisition (Khakpai *et al.*, 2012). The septum and hippocampus operate corresponding with memory processes in the inhibitory avoidance task (Jafari-Sabet, 2011). Some evidences revealed that NMDA receptors in the medial septum and hippocampus are very important in the learning and memory processes (Huerta *et al.* , 2000, Larkin *et al.* , 2008, Mei *et al.* , 2011, Stephens *et al.* , 2011,

Zarrindast *et al.*, 2011). The glutamatergic system in the medial septum, probably via NMDA receptors is involved in the hippocampal-dependent learning and memory (Gartner *et al.* , 2001). It has been shown that intra-MS injections of NMDA agonists or antagonists improve or impair retention of avoidance tasks, respectively (Puma *et al.* , 1998). Furthermore, infusion of NMDA receptor antagonists impaired memory performance of rodents in spatial and non-spatial learning tasks that depended on the hippocampal function (Larkin *et al.*, 2008, Ohno and Watanabe, 1998, Pourmotabbed *et al.* , 1998). Other investigators reported that NMDA receptor antagonists, D-AP7 or MK-801 facilitate retention in a step-down inhibitory avoidance task, but impair retention in place navigation and step-through dark avoidance tasks (Mondadori and Weiskrantz, 1993, Zoladz *et al.* , 2006). Therefore, it has been suggested that the effects of NMDA receptor antagonists on learning and memory are dependent on the type of the task (Mondadori and Weiskrantz, 1993). In addition, it has been reported that NMDA receptor activation is involved directly in the primary neural plasticity mechanisms underlying memory formation (Jafari-Sabet *et al.* , 2005). In spite of such positive findings, some investigators failed to detect a positive effect of NMDA agonists on memory (Holmes *et al.* , 1993), and in some studies, even impairment was observed (Cohn and Cory-Slechta, 1994).

4.2. Effect of NMDA receptor of the CA1 and MS on impairment of memory induced by D-AP7 in the CA1 and MS

The result of this study revealed that intra-MS and intra-CA1 administration of D-AP7 weakened improvement of memory produced by NMDA in the medial septum and CA1, indicating involvement of the septal and hippocampal NMDA receptors in memory acquisition. Physiological investigations revealed that NMDA receptors play an important role in the regulation of synaptic plasticity and the process of learning and memory (Larkin *et al.*, 2008, Riedel *et al.*, 2003). It has been reported that NMDA receptors of the septum and hippocampus participates in the acquisition of object recognition memory (Puma *et al.*, 1998, Puma and Bizot, 1998), and spatial memory (Huerta *et al.*, 2000, Martin *et al.* , 2000), respectively. According with our results, some investigations displayed that blockade of NMDA receptors with AP5 impaired acquisition of hippocampal-dependent tasks (Morris *et al.* , 1986), and septal memory formation (Puma *et al.*, 1998). Nonetheless, other studies investigating the influences of NMDA receptor blockade during the acquisition period of memory have produced various findings. It has been reported that intra-septal (Puma and Bizot, 1998) and intra-hippocampal infusions of AP5 enhanced memory in rats (Villarreal *et al.* , 2002).

4.3. Influence of NMDA receptor of the MS and CA1 on impairment of memory induced by D-AP7 in the septo-hippocampal pathway

The present data indicated that pre-training intra-CA1 injection of D-AP7 weakened memory response induced by pre-training infusion of NMDA in the MS. Also, pre-training intra-MS administration of D-AP7 deteriorated memory response produced by pre-training injection of NMDA in the CA1. The MS/VB is mutually connected with the hippocampus which may show a main input for spatial learning and hippocampal theta rhythm (Givens and Olton, 1995, Givens and Sarter, 1997). Although cholinergic septo-hippocampal projections have been well described, but the functional significance of the glutamatergic projection has not been demonstrated (Huh *et al.* , 2010). It has been revealed that about 4–23% of the septo-hippocampal projection is glutamatergic (Colom *et al.*, 2005, Henderson *et al.* , 2010). The glutamatergic system in the MS/DB, possibly via the NMDA receptors is involved in the hippocampal-dependent learning and memory (Izquierdo *et al.* , 1992, Ogren *et al.* , 2008). For example, intra-MS administration of glutamate receptor antagonists decreased hippocampal learning and memory (Izquierdo *et al.*, 1992), hippocampal spatial and emotional learning (Cervera-Ferri *et al.*, 2012). Glutamate is in the position to regulate the activity of septal fibers projecting to the hippocampus, since the medial septum cholinergic and GABAergic neurons are synaptically innervated by vesicular glutamate transporter

2-immunoreactive glutamatergic boutons (Elvander-Tottie *et al.* , 2006). Our results indicated that intra-MS/CA1 administration of NMDA and D-AP7 change memory acquisition through modulating the glutamatergic septo-hippocampal pathway. These results implicated that glutamatergic system in the septo-hippocampal pathway play a role in acquisition of memory.

5. Conclusion

The obtained results indicated that pre-training intra-CA1 or intra-MS infusion of NMDA increased, while D-AP7 decreased memory acquisition. Moreover, pre-training administration of D-AP7 in the MS and CA1 decreased memory response induced by NMDA in the MS and CA1. [In addition, pre-training intra-CA1/MS injection of D-AP7 weakened memory response produced by pre-training infusion of NMDA in the MS/CA1, respectively. It can be concluded that the glutamatergic pathway between the MS and CA1 areas is participated in memory acquisition process.](#)

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Legends

Table1 summarizes all experiments, groups of animals, time of drugs injection and doses of drugs.

Fig. 1. The effects of pre-training intra-CA1 co-administration of NMDA and D-AP7 on memory acquisition and locomotor activity. A. Left and right panels show the effects of pre-training intra-CA1 injection of saline ($0.5 \mu\text{l}/\text{site}$) or different doses of D-AP7 (0.006 and $0.012 \mu\text{g}/\text{rat}$) or a dose of NMDA ($0.125 \mu\text{g}/\text{rat}$) on memory acquisition, respectively. The memory was measured 24 h after injection of drugs. B. Left and right panels show the effect of drugs on locomotor activity in the test's day. Data are expressed as mean $\pm$ S.E.M of eight animals per group. ****P<0.01** different from saline/saline control group. **+P<0.05 as compared to NMDA/saline group.**

Fig. 2. The effects of pre-training intra-MS co-administration of NMDA and D-AP7 on memory acquisition and locomotor activity. The animals received pre-training intra-MS injection of saline ($0.5 \mu\text{l}/\text{site}$) or diverse doses of D-AP7 (0.006 and $0.012 \mu\text{g}/\text{rat}$, A; left panel) or NMDA ($0.125 \mu\text{g}/\text{rat}$, A; right panel). The memory was measured 24 h after injection of drugs. B. Left and right panels

indicate the effect of drugs on locomotor activity in the test's day. Data are expressed as mean $\pm$ S.E.M of eight animals per group. * $P < 0.05$ and ** $P < 0.01$ different from saline/saline control group. + $P < 0.05$ as compared to NMDA/saline group.

Fig. 3. The effects of pre-training administration of intra-MS NMDA and intra-CA1 D-AP7 on memory acquisition and locomotor activity. A. Left and right panels display the effects of pre-training intra-CA1 saline (0.5 μ l/rat) or D-AP7 (0.006 and 0.012 μ g/rat) 5 min after intra-MS injection of NMDA (0.125 μ g/rat). The memory was measured 24 h after injection of drugs. B. Left and right panels exhibit the effect of drugs on locomotor activity in the test's day. Data are expressed as mean $\pm$ S.E.M of eight animals per group. ** $P < 0.01$ different from saline/saline control group. + $P < 0.05$ as compared to NMDA/saline group.

Fig. 4. The effects of pre-training infusion of intra-MS D-AP7 and intra-CA1 NMDA on memory acquisition or locomotor activity. The animals received pre-training intra-MS saline (0.5 μ l/rat) or D-AP7 (0.006 and 0.012 μ g/rat, A; left panel) 5 min before intra-CA1 injection of NMDA (0.125 μ g/rat, A; right panel). The memory was measured 24 h after injection of drugs. B. Left and right panels indicate the effect of drugs on locomotor activity in the test's day. Data are

expressed as mean $\pm$ S.E.M of eight animals per group. *P < 0.05 and **P<0.01 different from saline/saline control group. + P<0.05 as compared to NMDA/saline group.

Fig. 5. Approximate location of the infusion cannulae tips in the CA1 area and MS for all animals included in the data analyses was taken from the atlas of Paxinos and Watson (Paxinos, 2007).

Table 1

Table1 summarizes all experiments, groups of animals, time of drugs injection and doses of drugs.

Experiment	Figure	Pre-training treatment		Step-through latency (panel A)	locomotor activity (panel B)
		First injection in septum*(0.5 µl/rat)	Second injection in CA1 (1 µl/rat)		
1	Left	-	D-AP7 (0.006 and 0.012 µg/rat)	Decreased	Not effective
	Right	-	D-AP7 (0.006 and 0.012 µg/rat) + NMDA (0.125µg/rat)	Decreased	Not effective
2	Left	D-AP7 (0.006 and 0.012 µg/rat)	-	Decreased	Not effective
	Right	D-AP7 (0.006 and 0.012 µg/rat) + NMDA (0.125µg/rat)	-	Decreased	Not effective
3	Left	Saline (1 µl/rat)	D-AP7 (0.006 and 0.012 µg/rat)	Decreased	Not effective
	Right	NMDA (0.125µg/rat)	D-AP7 (0.006 and 0.012 µg/rat)	Decreased	Not effective
4	Left	D-AP7 (0.006 and 0.012 µg/rat)	Saline (1 µl/rat)	Decreased	Not effective
	Right	D-AP7 (0.006 and 0.012 µg/rat)	NMDA (0.125µg/rat)	Decreased	Not effective

*In all experiments, the first injection of drugs was done 5 min previous training into the medial septum and the second one was infused 5 min after first injection into the CA1.

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Bregma: -2.7 mm

Microinjection site MS

Bregma: +0.60 mm

Fig. 5