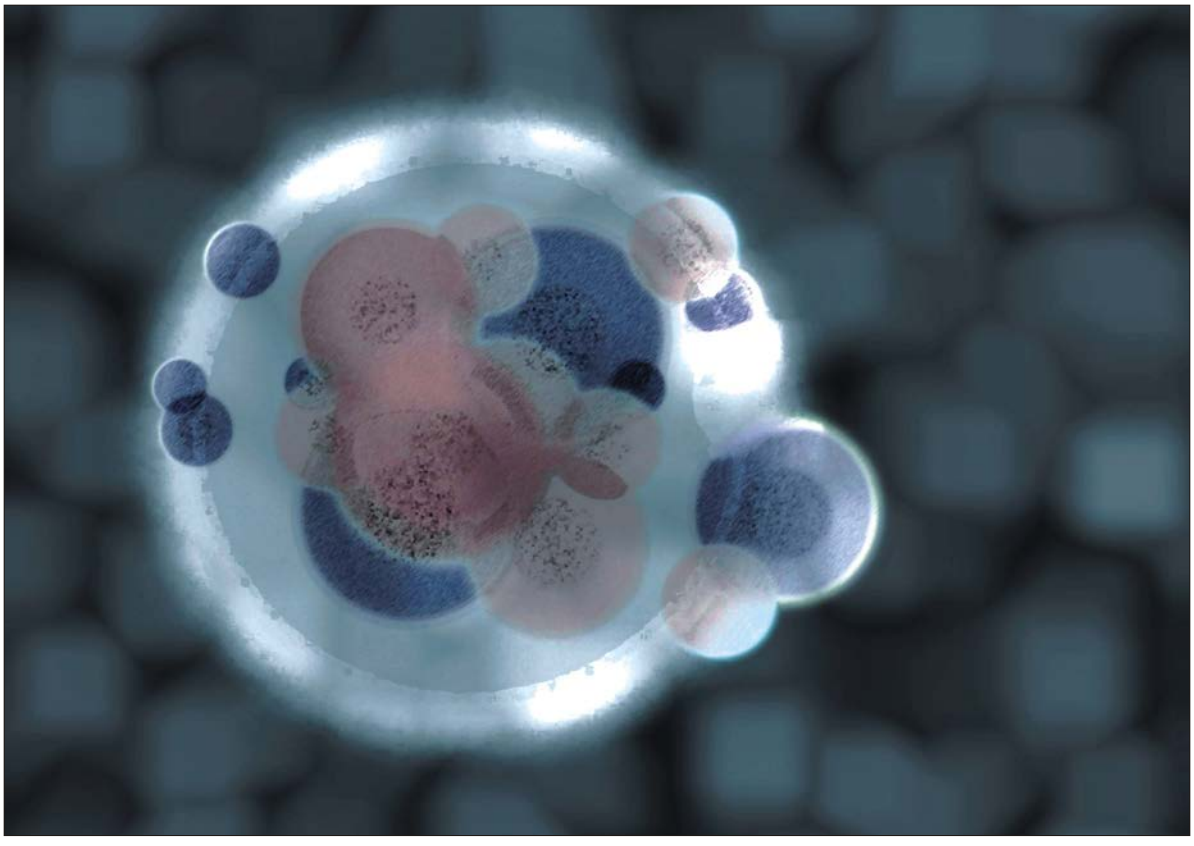

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The Effects of Pre and Post Training Administration of MK-801 in Dorsal Hippocampus on Learning and Memory in Adult Male Rats

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Abstract: The aim of the present study was to investigate the effects intra-dorsal hippocampus (intraCA_1) injection of NMDA receptor noncompetitive antagonists (MK-801) on memory retention using passive avoidance learning. The antagonist was administered pre and post-behavioral training. In the first experiment adult Wistar rats were given saline or MK-801 (1 μg rat^{-1}) 10 min prior to training for 4 days, while in the second experiment saline or MK-801 (1 μg rat^{-1}) were given immediately after training for 4 days. Post-training administration of MK-801 did not affects memory retention, in contrast to pre-training injection of MK-801 which decreased memory retention. The results suggest that pre-training injection of the non competitive antagonist NMDA receptor (MK-801) in hippocampus impaired learning and memory but post-training administration of MK-801 did not effect on learning and memory in passive avoidance learning task.

Key words: MK-801, NMDA receptor, passive avoidance learning, rat

INTRODUCTION

The amino acid glutamate is principal excitatory neurotransmitter which acts on several receptor subtypes in the mammalian nervous system. This includes the kainite, quisqualate and N-methyl-D-aspartate (NMDA) receptors. The NMDA glutamate receptors are widely distributed in the brain, whit high densities in the amygdala basolateral nuclei. The highest concentrations of NMDA binding sites are found in area CA1, of the hippocampus, with substantial concentration also localized within the dentate gyrus (Jafari-Sabet, 2006). Studies using either pharmacological or genetic approaches have shown that NMDA receptors are intimately involved in developing memory for aversion conditioning, spatial memory training (Ahlander *et al*., 1999) and nonspatial, nonaversive tasks (Baker and Kim, 2002). Systemic or interacerbral administration of the NMDA receptor blocker MK-801 [(+) 5-methyl-10, 11-dihyro-5H-dibenzo-[a, b] cyclo-hepten-5-10-imine-maleae: dizocilpine)] has been shown to effect formation of memory for a number of tasks, including different types of aversive conditioning (Gould *et al*., 2002), water maze (Ahlander *et al*., 1999), radial arm maze (Noemia *et al*., 2005). Whereas some of the studies using MK-801 to memory acquisition (Takatsuki *et al*., 2001). Other reports have shown that MK-801 affects memory consolidation when given after training (Ciamei *et al*., 2001). Experimental evidence suggests that the NMDA receptor play a crucial role in acquisition of spatial information. NMDA antagonists such as noncompetitive channel blocker MK-801, block the induction of hippocampal Long-Term Potentiation (LTP) *in vivo* (Abraham and Mason, 1988) and *in vitro* (Aaron *et al*., 1998). In addition the NMDA antagonists also impair the learning of spatial working and reference memory tasks when learning is assessed by measuring changes in performance across several days. However NMDA receptor activation does not appear to be necessary for spatial learning when learning is measured within a single day (Aaron *et al*., 1998). In the present study the effects of pre and post training administration of NMDA channel blocker, MK-801 in CA1 of the hippocampus on passive avoidance task was investigated.

MATERIALS AND METHODS

Animals: Male Wistar rats weighting 180±20 at the time of the experiment were used, the animals were individually housed in stainless cages at a temperature of 23±2°C and 12/12 h day-night cycle (7:00 am light on, 7:00 pm light off). All animals were provided from animal facility of

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Ahvaz University of Medical Science. Animal's food was provided from standard Laboratory feed as flowing; 5% soybean protein isolate 0.3% DL-metioninm, 32.7% corn starch, 25% sucrose, 2% cellulose powder, 5% mineral mixture, 1% vitamin mixture.

Animals had access to food and water *ad libitum* and were allowed to adapt to the laboratory conditions for at least 1 week before the experiment. Rats were divided in to 5 groups (n = 8/group). The control group, with free access to food and water, a second group which received MK-801 after training; a third group which received MK-801 before training; a fourth sham group which received saline under the same conditions as second group. A fifth sham group which received saline under the same condition a third group. Rats were handling about 3 min each day prior to behavioral testing. All experiments were performed between 8:00 am and 12:00 pm from Jun to Sep. All experiments were performed in the Laboratory of Learning and Memory in the Biology Department of Shahid Chamran in Iran.

Passive avoidance apparatuses: The experimental device is a 30×30×30 cm avoidance-response-chamber, made of Plexiglas on its four sides. The chamber has a bottom of parallel 0.5 cm stainless steel bars spaced 1 cm apart. A rubber platform (5 cm high, 8 cm in diameter at its top surface) was fixed in place in the center at the bottom of the chamber as described by Chen (2005).

Stereotaxic surgery and microinjection: The animals were anesthetized with intraperitonerl injection (100 mg kg^{-1}) of ketamine hydrochloride (Flecknell, 1996) plus (10 mg kg^{-1}) xylazine (Flecknell, 1996) and positioned in a Stoelting sterotaxic instrument. A 22 gauge stainless steel guide cannula as was placed 2 mm above the intended site of injection, according to the method described by Jafari-Sabet (2006). Stereotaxic coordinates for the CA1 regions of the dorsal hippocampus were -3.8 mm posterior to bergma, -2.2 nim lateral to the midline and -3 mm ventral of the dorsal surface of the skull. Cannula as was secured to anchor jeweler's screws with dental acrylic. To prevent clogging, stainless steel stylets (27 gauge) was placed in the guide cannula until the animals were given the CA1 injection. All animals were allowed 1 week to recover from surgery and anesthesia. The animals were gently restrained by hand and the styles were removed from the guide cannula and replaced by 27 gauge injection needles (2 mm below the tip of the guide annual). Each injection unit was connected by polyethylene tubing to a 10 μL Hamilton syringe. The right CA1 infused with 0.5 μL solution (0.5 μL rat^{-1}) over a 60 sec period. The injection needles were left in place for an additional 60 sec to allow diffusion and the stylets was reinserted into the guide cannula.

Drug administration: The drug in the present study was (M107-5MG) (+)-MK-801 hydrogen maleate (Jafari-Sabet, 2006). The drug dissolved in sterile 0.9% saline and was injected into the intra-CA1 of hippocampus it a volume of 0.5 μL rat^{-1}. In the first experiment rats received intra-CA1 injection of MK-801 (1 μg rat^{-1}) immediately after shock for 4 days in training trial (Jafari-Sabet, 2006) and in the second experiment, animals received intra-CA1 injection of MK-801 (1 μg rat^{-1}), 10 min before training trial for 4 days. Li-Sha *et al.* (2005) and the sham groups received saline solution under the same conditions.

Procedure: Rats were continually trained in one-trial step down inhibitory avoidance task for 4 times (one time/day, conducted between 8:00 am and 12:00 pm) and tested for their memory retention at the same time 24 h after training. Rats were placed on the platform and their latency to step down placing their four paws on the grids, were measured. At the meantime, their times of placing on the platform were recorded. In training sessions, immediately upon stepping down, the rats received a 0.5 mA, 2 S, scramble foot shock. No foot shock was given in test sessions. Test session step down latencies and errors (during 3 min) were taken as a measure of memory retention (Chen, 2005).

Histology: After completion of the experimental sessions, each animal was killed with an overdose of chloroform. Animals received a 0.5 μL/side injection of ink (1% aqueous methylene blue solution). The brains were then removed and immersion fixed in a 10% formalin solution for 5 days before sectioning the fixed brains. Brains were sectioned directly across the injection sites and the cannula placement verified using the atlas of Paxino and Watson (1986). The result of histological examination CA_1 region of dorsal hippocampus injection cannula placement is shown in Fig. 1. Data from rats with injection site located outside the CA_1 area of the dorsal hippocampus were not used in the analysis.

Statistic analysis: Statistical analysis of data using one way analysis of variance (ANOVA) and post hoc analysis (Tukey test) using the Least Significant Difference (LSD). The decision for statistic test of significance was $p<0.05$. Calculation were performed using the SPSS statistical package.

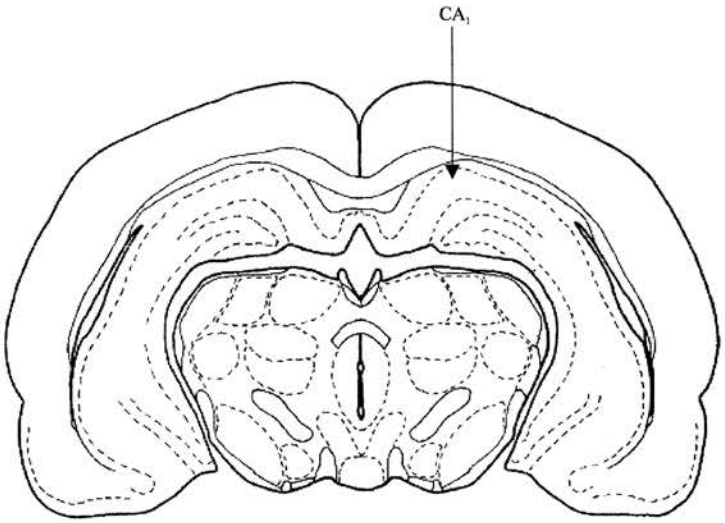

Fig. 1: The approximate placement of injection cannula within the CA1 is indicated in figure

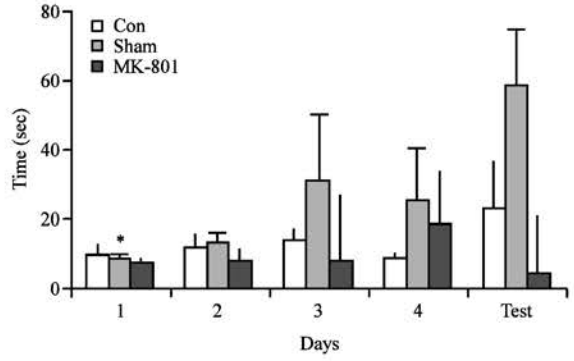

Fig. 2: The effect of post training injection of 1 μg rat^{-1} MK-801 on step down latency. MK-801 was injected immediately after shock in training session. * indicate statistical differences at $p<0.05$ n = 8/group

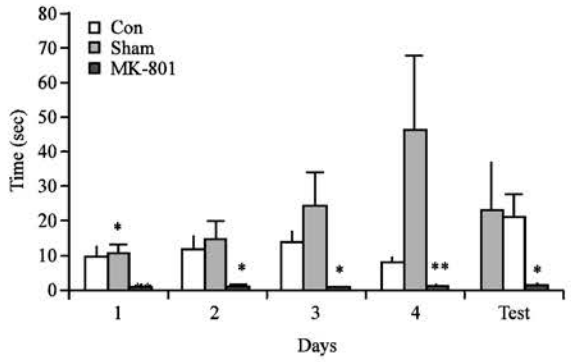

Fig. 3: The effect of pre training injection of 1 μg rat^{-1} MK-801 on step down latency. MK-801 was injected 10 min before training session. *, ** indicates statistical differences at $p<0.05$ or $p<0.001$, respectively, n = 8/group

RESULTS AND DISCUSSION

There were no significant differences between control and sham groups in step-down latencies 24 h after training. Similarity, there were no differences between post-training injection of MK-801 and sham groups. These data indicates that the post-training administration of MK-801 did not effect learning and memory (Fig. 2). Statistical analysis of data showed that on first day of training there were significant difference between control and sham groups and between rats received MK-801 10 min before training in second, third, forth day of training and 24 h after training and sham groups (Fig. 3). These data indicated that MK-801 when injection before training impaired learning and memory.

The effect of NMDA receptor antagonists on leaning task and memory task is controversial. The present study investigated the effects of pre and post-training intra-dorsal hippocampal (intra CA_1) administration of MK-801 a non-competitive antagonist of the NMDA receptor on memory retention of passive avoidance learning in rats. Present data indicates that post-training intra-CA1 administration of 1 μg rat^{-1} of MK-801, does not affect on learning and memory retention in any days, while pre-training administration of MK-801 significantly decreases learning and memory retention in 2, 3 and 4 days of learning and test day. These results suggest that pre-training administration of MK-801 intra CA1 impairment both learning and memory. These results are in contrast whit other published reports. There are

different reports about the effect of NMDA receptor antagonists on cognitive behavior and memory that pointed to some of them. Noemia *et al.* (2005) reported that intra-peritoneal injection of MK-801 impaired short-term retention of object recognition memory when given before and immediately after training. With results of the present study showed that MK-801, when injected into CA1 immediately after shock does not effects on memory retention. This opposing result might be due to the choice of infusion site. Administration of MK-801 to rats has been shown to cause a deficit in recall of a reversal task (Wozniak *et al.*, 1990), while Mc Lamb *et al.* (1990) showed that systematic treatment with MK-801 interferes with acquisition of a water maze task, but does not influence its recall. Ward *et al.* (1990) showed that MK-801 can produce a dose and time dependent disruption of radial-arm maze memory. Some studies using MK-801 have suggested that NMDA receptors contribute specifically to memory acquisition (Robinson *et al.*, 1989). Levin *et al.* (2003) reported that none of three dose of MK-801 used in their studies (2, 6 and 18 μg/side) caused significant working memory impairments when administered 20 min before training in ventral side of the hippocampus in adult female rats. This suggests that the activity of dorsal hippocampal NMDA glutamate receptors may have different effects than these in the ventral hippocampus. Other reports have shown that MK-801 affects memory consolidation when given after training. The effects of NMDA receptor blocker by MK-801 on memory may depend on factors such as familiarity with the training environment and training duration (Noemia *et al.*, 2005). MK-801 significantly impairs working memory function in radial arm maze when testing was conducted in an un familiar environment but not in a familiar environment (Caramanos and Shapior, 1994). Studies describing a crucial role for NMDA receptors in synaptic activity and memory and the effects of NMDA receptor antagonists in different rodent models of learning and memory have been extensively reviewed by Riedel *et al.* (2003). Jafari-Sabet (2006) indicated that Intra-CA1 administration of 2 μg rat^{-1} MK-801 a noncompetitive NMDA receptor antagonist significantly decreased the retention latency. It is opposite with our result in this experiment. This contrasting result might be due to using different doses. Buck *et al.* (2006) have shown, enhancement of LTP at CA1-subiculum synapses in MK-801 treated rats. Systemic application of MK-801 causes an initial suppression of LTP at CA1-subiculum synapses 4 h after treatment that was not significance, followed by a facilitation of LTP 24 h after treatment as compared with control LTP. The plasma half-life of MK-801 is in the range of 1-4 h (Buck *et al.*, 2006). Several studies have shown that the density of NMDA receptor in the hippocampus in increases as early as 2 h after MK-801 treatment and remains elevated 24 after treatment (Gao and Tamming, 1995). The memory deficits associated with NMDA antagonist MK-801 are similar to hippocampectomy (Levin *et al.*, 2003). In addition, al thought most studies show memory-impairing effects of NMDA receptor antagonists, there is evidence that at least under some experiment condition, MK-801 can display memory enhancing properties (Noemia *et al.*, 2005). In conclusion, present result show that NMDA receptor blocker MK-801 impair memory acquisition and suggesting that NMDA receptor are require for formation of short-term memory and don't effect on memory consolidation. Thus, memory effects of MK-801 are rather complex and should be further investigate using different training procedures.

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