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7-Nitroindazole, a neuronal nitric oxide synthase inhibitor, impairs passive-avoidance and elevated plus-maze memory performance in rats

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Abstract

The role of nitric oxide (NO) on cognitive performance in a modified elevated plus-maze (mEPM) and passive-avoidance (PA) task was investigated by using the NO synthase (NOS) inhibitor 7-nitroindazole (7-NI) and an NO precursor L-arginine. The interaction between the activation of *N*-methyl-D-aspartate (NMDA) receptors and NO synthesis on memory retention was also studied. 7-NI, L-arginine or MK-801, a non-competitive NMDA receptor antagonist were injected intraperitoneally (i.p) to male Wistar rats 30 min before the first training session of the PA test or 30 min before on the first day testing (acquisition session) of mEPM task. Transfer latency, the time rat took to move from the open arm to the enclosed arm, was used as an index of learning and memory in a mEPM test. The retention session was performed 24 h after the acquisition one. In the PA task, the retention test was carried out 24 h after training and reduction of retention latency was used to evaluate the acquisition of learning and memory. Blood glucose level and locomotor activity of the rats was also evaluated.

7-NI (10, 20, 25, 50 mg/kg) and MK-801 (0.15 mg/kg) significantly prolonged the transfer latency on retention session in a mEPM test and shortened step-through latency in PA test. 7-NI-induced impairment in memory and learning was partly reversed by L-arginine (200 mg/kg), a competitive substrate for NOS. However subeffective doses of 7-NI (5 mg/kg) and MK-801 (0.075 mg/kg) given in combination significantly impaired plus-maze and PA performances in rats. Thus NMDA receptor mediated NO pathways may be implicated in the PA and mEPM behaviours in rats. Since 7-NI does not affect blood pressure and did not alter blood glucose level and locomotor activity in conscious rats, 7-NI-induced impairment of memory is not due to either hypertension, changes in blood glucose level or effects on locomotor activity.

Keywords: 7-Nitroindazole; MK-801; Learning-memory; Elevated plus-maze; Passive-avoidance; Rat

1. Introduction

Nitric oxide (NO) is an intercellular messenger that has been suggested to play a critical role in learning and memory processes (Schumann and Madison, 1991). It is synthesized from L-arginine by the enzyme NO synthase (NOS) in response to Ca^{+2} influx induced by the activation of *N*-methyl-D-aspartate (NMDA) receptors by glutamate (Prast and Philippu, 2001). NO can diffuse out of the neuron and activate guanylyl cyclase in neighboring cells and serve as a messenger molecule mediating the neurophysiological events on behaviours in mammalian brain, such as long-term potentiation (LTP), modulation of neurotransmitter and neuropeptide release having a putative role in the regulation of behaviour and cognition (Bugnon et al., 1994; Lonart et al., 1992; Miki et al., 1991; Prast and Philippu, 1992;). Endogenous NO or NO secreted after the stimulation of NMDA receptors regulates secretion of neurotransmitters via acting presynaptically and changing synaptic activity (Hanbauer et al., 1992). It may be the fact that many of the effects of NO are mediated by cyclic guanosine monophosphate (cGMP). Treatment of mice with NMDA or non-NMDA receptor agonists enhances cGMP levels in the brain and this process is inhibited by NOS inhibitors (Wood et al., 1990). Activation of NMDA receptors greatly increases the outflow of

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cGMP whereby the enhanced cGMP outflow is inhibited by NOS inhibitors and soluble guanylate cyclase (sGS) (Consolo et al., 1999; Fedele et al., 1996; Luo et al., 1994). So stimulation of excitatory amino acid transmission promotes synthesis of NO which in turn increases cGMP synthesis via activation of sGS (Prast and Philippu, 2001).

NO may improve learning and memory (Plech et al., 2003) which is supported by the fact that NOS inhibitors impair learning and memory (Baratti and Kopf, 1996; Holscher and Rose, 1992; Holscher et al., 1996; Meyer, 1998). Although contradictory results exist about effects of NOS inhibitors on LTP and memory formation (Bannerman et al., 1994; Gribkoff and Lum-Ragan, 1992); however it is clear that NOS is involved in a form of hippocampal LTP (Son et al., 1996). This discrepancy has been attributed to their task-dependent effects on different forms of learning and memory.

Since NMDA receptors play an important role in the induction of LTP and memory (Morris et al., 1986) a highly specific non-competitive NMDA receptor antagonist MK-801 induces dose-dependent impairments of learning and memory in an elevated plus-maze test (Benvenga and Spaulding, 1988; Butelman, 1989; Hlinak and Krejci, 1998; 2000; 2002; 2003; Itoh et al., 1991). NO production in the brain is stimulated by the activation of glutamate receptor (Yamada and Nabeshima, 1997a,b). Some of the memory impairment caused by NMDA receptor antagonist MK-801 is due to the impairment of NO and subsequent cGMP production in brain (Yamada et al., 1996).

The aim of this study is to establish the role of NO on spatial and emotional learning by using the NOS inhibitor 7-NI and an NO precursor L-arginine in the modified elevated plus-maze (mEPM) and step-through passive-avoidance (PA) tasks in rats, which are both hippocampal dependent (Cahill and McGaugh, 1998; Holland and Bouton, 1999). Moreover a possible interaction between NO synthesis and NMDA receptors on cognitive performance is also investigated. EPM test is primarily used for measurement of anxiety in rodents and is modified to evaluate spatial learning and memory (Itoh et al., 1990; Itoh et al., 1991). The time for the rat to move from the open arm to the enclosed arm (transfer latency) is used as the measurement of learning and memory. Shortened transfer latency on the retention session is used as a parameter for retention or consolidation of memory, while treatment of drugs prior to first day may also be utilized for acquisition related action of drugs (Sharma and Kulkarni, 1992). The prolongation of the transfer latency on retention session shows that the drug has an amnesic effect and/or the animal does not remember the configuration of the open and enclosed arms. PA test is commonly used in studying the cognitive alterations following drug administration, lesions or behavioural manipulations (Sahgal, 1993). It is based on contextual fear conditioning and instrumental learning (Ogren, 1985) and it is dependent on hippocampus and amygdala (Cahill and McGaugh, 1998; Holland and Bouton, 1999). In this test the animal learns that a specific place should be avoided because of an aversive effect. Thus, longer step-through latency (STL) at retention compared with training indicates that the animal had acquired the task.

As the peripheral effects of systemic administration of NOS inhibitors, especially those related to arterial blood pressure (Ribeiro et al., 1992) and blood glucose level (Salehi et al., 1996) are able to change the animal's learning and memory processes, effects of drugs on these parameters are also investigated. Since NO is an endothelial-derived relaxing factor, NOS inhibitors also produce blood pressure changes. But it is known that 7-NI, is a NOS inhibitor which inhibits the neuronal isoform without affecting vascular system (Babbedge et al., 1993; Moore et al., 1993). In addition, NO may also play an important role in insulin and glucagon secretion resulting in blood glucose level regulation (Salehi et al., 1996). The locomotor activity of the animals was also tested before a change in performance can be attributed to learning and memory process.

2. Materials and methods

2.1. Animals

Adult male Wistar rats (Istanbul University Medical Sciences Research Center, Turkey) weighing 200–250 g, were housed five to six per cage in an animal colony facility for 2 weeks before the start of the experiment. The animals were maintained in constant room temperature (22±2 °C) under a 12-h light/dark cycle (lights on at 07:00 h). Tap water and food pellets were provided ad libitum. All animals used for the experiments were naive to the EPM and PA apparatus. Each rat was tested only once.

All procedures for the treatment of animals were in compliance with the European Community Council Directive of 24 November 1986 and ethical approval was granted by the Kocaeli University of Ethics Committee (Number: AEK 90/6, Kocaeli, Turkey).

2.2. Drugs and treatments

7-Nitroindazole (7-NI), MK-801, and L-arginine were purchased from Sigma Chemicals (St. Louis, MO). MK-801 and L-arginine were dissolved in 0.9% saline. 7-Nitroindazole was dissolved in saline using a few drops of Tween-80 (Volke et al., 2003). All drugs were prepared immediately prior to use and given intraperitoneally (i.p) in a volume of 0.2 ml per 100 g body weight of rats.

2.3. Modified elevated plus-maze test

Cognitive behaviour was evaluated by using the mEPM learning task, which measures spatial long-term memory (Reddy and Kulkarni, 1998). Transfer latency (the time in which the animal moves from the open arm to the enclosed arm) was utilized as an index of learning and memory processes.

The plus-maze was made of wood and consisted of two open arms (50×10 cm) surrounded by a short (1 cm) plexiglas edge to avoid falls, and two enclosed arms (50×10×40 cm) arranged such that two open arms were opposite to each other. The arms were connected by a central platform (10×10 cm). The maze

was elevated to a height of 50 cm above the floor. The open arms and central platform were painted white and enclosed arms were painted black. To remove any confounding olfactory cues the maze was cleaned with alcohol–water solution after each rat. The principle in this experiment is based upon the aversive behaviour of rodents to open and high spaces. The animals dislike open and high spaces and move from them to an enclosed arm, the protected areas of the maze.

The procedure was performed as described by Hlinak and Krejci (Hlinak and Krejci, 1998; 2000; 2002). The animals were randomly assigned to the different experimental and control groups. In the acquisition session (on day 1), each rat was gently placed at the distal end of an open arm of the apparatus facing away from the central platform and the time it took for the rat to move from the open arm to either of the enclosed arms (transfer latency) was recorded. Training (repeated exposure of animal to open arms) shortens this parameter, possibly as a consequence of learning acquisition and retention. If the rat did not enter the enclosed arm within 90 s, it was excluded from further experimentation. The criterion of an animal's entry into the enclosed arm was crossing with all four legs of an imaginary line separating the enclosed arm from the central space. After entering the enclosed arm, the rat was allowed to move freely in the maze regardless of open and enclosed arms for 10 s. Then, the rat was returned to its home cage. The retention session followed 24 h after the acquisition session (on day 2). The rats were put into the open arm and the transfer latency was recorded again. The experiments were conducted between 10:00 and 14:00 h in a semi-soundproof room under a natural illumination. The maze was cleaned after each rat. Each rat was tested only once.

2.4. Effect of 7-NI on the transfer latency of rats in the modified elevated plus-maze

In order to evaluate the effect of systemic administration of different doses of 7-NI on the transfer latency in the mEPM, 0.9% saline ($n=16$) or 7-NI (5, 10, 20, 50 mg/kg, $n=11$, 14, 16, 14 respectively) was administered i.p 30 min prior to acquisition session (on day 1), since it has been reported that maximal inhibition of NOS by 7-NI occurs at 30 min after i.p injection (MacKenzie et al., 1994).

2.5. Effect of L-arginine on the transfer latency of rats in the modified elevated plus-maze

The aim of this experiment is to evaluate the effect of systemic administration of L-arginine on the transfer latency in the mEPM. 0.9% saline (SAL+SAL, $n=12$) or 200 mg/kg L-arginine (SAL+L-ARG$_{200}$, $n=10$) was administered intraperitoneally 30 min before prior to acquisition session (on day 1).

To investigate whether if the impairment of transfer latency induced by 7-NI could be counteracted by co-administration of L-arginine, saline plus 20 mg/kg 7-NI (SAL+7-NI$_{20}$, $n=14$), 200 mg/kg L-arginine plus 20 mg/kg 7-NI (L-ARG$_{200}$+7-NI$_{20}$, $n=10$) was given to different groups of animals prior to acquisition session (on day 1). L-arginine was administered i.p 10 min prior to 7-NI.

2.6. Effect of MK-801 on the transfer latency of rats in the modified elevated plus-maze

In order to determine the subeffective and effective doses of MK-801 inducing impairment on the transfer latency upon the mEPM, saline (SAL, $n=12$) or MK-801 (0.075, 0.15 mg/kg, $n=10$, 12 respectively) was given to different groups of rats prior to acquisition session (on day 1). Based on the previous studies (Hlinak and Krejci, 1998; 2000; 2002; 2003) the ineffective dose (0.075 mg/kg) and the lowest dose of MK-801 (0.15 mg/kg) inducing short-term amnesia was used in our experiments. 30 min after drug administration, each group was submitted to the transfer latency procedure as described above.

2.7. Effect of systemic co-administration of subeffective doses of MK-801 and 7-NI on the transfer latency of rats in the modified elevated plus-maze

The possible interaction between the NMDA receptors and NO synthesis in the transfer latency learning was investigated by co-administering ineffective doses of MK-801 and 7-NI to different groups of rats as saline plus saline (SAL+SAL, $n=11$), saline plus 5 mg/kg 7-NI (SAL+7-NI$_5$, $n=11$), saline plus 0.075 mg/kg MK-801 (SAL+MK-801$_{0.075}$, $n=11$), and 5 mg/kg 7-NI plus 0.075 mg/kg MK-801 (7-NI$_5$+MK-801$_{0.075}$, $n=14$) prior to acquisition session (on day 1).

2.8. Passive-avoidance test

Animals were trained in a one-trial, step-through, passive-avoidance apparatus (Ugo Basile model 7551, Italy) for evaluating memory based on contextual fear conditioning and instrumental learning (Ogren, 1985). In this task the animal learns that a specific place should be avoided since it is associated with an aversive event. Decrease in step-through latency (STL) indicates an impairment in memory in the PA task.

The training apparatus consisted of two compartments, each measuring 22×21×22 cm. The illuminated white chamber was connected to the dark chamber (i.e. conditioning chamber) which was equipped to with an electric cable grid floor and the shock was delivered to the animal's feet via a shock generator. The two chambers were separated by a flat-box partition, including an automatically operated sliding door at floor level.

Training trial was carried out as described by Hiramatsu et al. (1998), and Monleon et al. (2002). The animals received drugs prior to PA training. On the first day of training, rats were placed individually into the light compartment and allowed to explore the boxes. After 30 s, the door between these two boxes was opened and the animal moved into the dark compartment freely (preacquisition trial).

The acquisition (training) trial was carried out 15 min after the preacquisition trial. Rats were again placed in the light compartment of the PA apparatus. After a 30 s adaptation period in the safe chamber, the door between the compartments was opened. Having completely entered the dark compartment, the

sliding door was closed automatically and an electric foot-shock (0.5 mA) of a 3 s duration was delivered to the animal via grid floor immediately. The time taken to reenter the dark compartment was recorded (training latency). Any animal failing to cross from the illuminated to the dark compartment within 300 s was discarded from the experiment. Animals were then removed from the dark chamber and returned to their home cages. Between each training session, both compartments of the chamber were cleaned to remove any confounding olfactory cues.

Retention trial: Recall of this inhibitory stimulus was evaluated 24 h post-training by returning the animals into the light compartment and recording their latency to enter the dark compartment (four paws in). No foot-shock was applied in this trial. If the animal had not entered to the dark compartment within 300 s, it was returned to its cage and a maximum latency of 300 s was recorded. This latency served as a measure of retention performance of the step-through avoidance response (retention latency).

2.9. Effect of systemic administration of 7-NI on passive-avoidance performance of rats

In order to evaluate the effect of systemic administration of 7-NI on STL in the PA task, 0.9% saline ($n=15$) or 7-NI (5, 25, 50 mg/kg, $n=14$, 12, 12 respectively) was given intraperitoneally 30 min prior to training trial.

2.10. Effect of systemic administration of L-arginine on the reversal of 7-NI-induced passive-avoidance performance of rats

To evaluate the effect of systemic administration of L-arginine on STL in the PA test, 0.9% saline ($n=11$) or 200 mg/kg L-arginine ($n=10$) was administered intraperitoneally 30 min before the training trial. In order to investigate whether if the shortened STL induced by 7-NI could be counteracted by co-administration of L-arginine, L-arginine (200 mg/kg) was administered 10 min prior to 7-NI (25 mg/kg,) or 40 min before training ($n=11$).

2.11. Effect of MK-801 on passive-avoidance performance of rats

The ineffective dose and the lowest dose of MK-801 inducing decrease in STL in PA test were evaluated by giving 0.075 and 0.15 mg/kg MK-801 ($n=12$ and 11 respectively) intraperitoneally to different groups of rats prior to training trial.

2.12. Effect of systemic co-administration of subeffective doses of 7-NI and MK-801 on passive-avoidance performance of rats

In an another separate experiment the possible interaction between the NMDA receptors and NO synthesis on the STL in PA test was investigated by co-administering ineffective doses of MK-801 (0.075 mg/kg) and 7-NI (5 mg/kg) to different groups of rats ($n=12$) prior to training trial.

2.13. Effects of systemically given 7-NI, L-arginine or MK-801 on locomotor activity

The aim of this experiment is that the modified plus-maze or PA test being affected by the changes in locomotor activity, may lead to false negative or positive results. The spontaneous locomotor activity of the animals was therefore assessed by monitoring the activity of the animals in a locomotor activity cage (May 9803 Activity Monitoring System, Commat Iletisim Ltd. May Pentium Computer, Ankara, Turkey). Rats ($n=10$ for each group) were individually placed in a plexiglas cage ($42\times42\times30$ cm) and the total distance travelled and number of movements were evaluated for a 5 min period.

2.14. Effects of systemically given 7-NI, L-arginine or MK-801 on blood glucose level

Effects of systemically given 7-NI, L-arginine or MK-801 on blood glucose level were determined in a separate group of rats. Animals were treated i.p with saline, 5, 10, 20, 50 mg/kg 7-NI, 200 mg/kg L-arginine or 0.075, 0.15 mg/kg MK-801 ($n=10$ for each group). 30 min later, a few drops of blood were collected directly through the aorta under light ether anesthesia and blood glucose level was evaluated using blood glucose sensor electrode (Abbott, Lot: 53093).

2.15. Statistics

To compare the differences between the first and the second transfer latencies in a group in the mEPM test Wilcoxon *t*-test was used. To evaluate the differences among drug treated groups during the first as well as during the second transfer latencies in the mEPM test the Kruskal–Wallis non-parametric one-way analysis of variance (ANOVA) followed by Dunn's test was used. The behavioural scores of PA and locomotor activity was evaluated by one-way analysis of variance (ANOVA) followed by Tukey test. Data for blood glucose level was analyzed by Kruskal–Wallis and Mann–Whitney *U* test. Results are expressed as mean±SEM. The criterion for statistical significance was $p<0.05$.

3. Results

3.1. Effect of 7-NI on the transfer latency of rats in the modified elevated plus-maze

Mean transfer latencies of 7-NI (5, 10, 20 or 50 mg/kg, i.p) or vehicle (on first day and second day), given 30 min before acquisition session in the mEPM in rats are presented in Fig. 1.

7-NI, at doses studied, had no effect on the transfer latencies of the first day compared to that of the vehicle-treated group (Kruskal–Wallis analysis: $H=0.69$, $p=0.95$). On the second day 7-NI at 10, 20, 50 mg/kg doses significantly prolonged the transfer latency, indicating that the rats only poorly remembered the presence of the enclosed arms, while 5 mg/kg 7-NI had no effect. Post hoc comparisons evidenced that the animals given 7-NI (10, 20, 50 mg/kg) had significantly prolonged the transfer

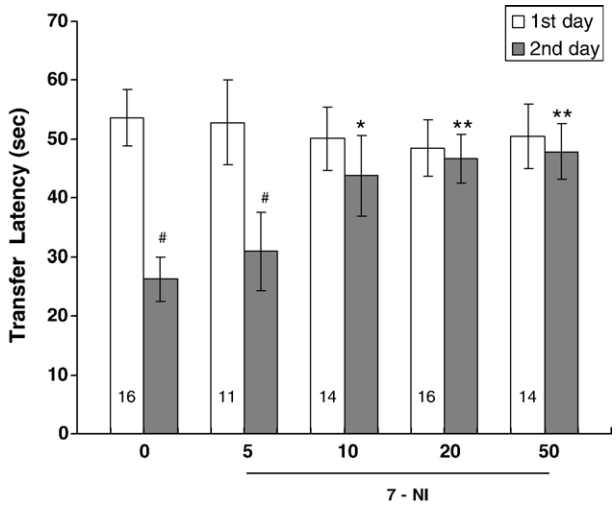

Fig. 1. Effect of 7-NI administration on the transfer latencies (of first day and second day) to the enclosed arm of the elevated plus-maze in rats. 7-NI (5, 10, 20, 50 mg/kg) or saline (0.9%) was administered intraperitoneally 30 min before first day's trial. The number of rat is shown in the columns. Transfer latency data (s) are expressed as mean±SEM values. $^{\#}p<0.01$ Wilcoxon *t*-test, first day vs. second day; $^{*}p<0.05$, $^{**}p<0.01$ Kruskal–Wallis ANOVA followed by Dunn's test, vs. control group on the second day.

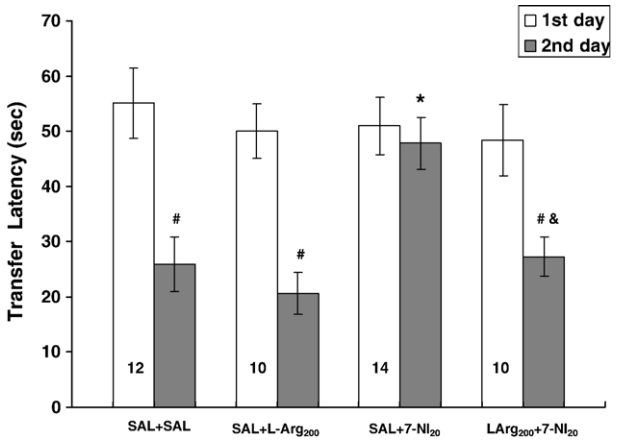

Fig. 2. Effect of systemic L-arg (200 mg/kg) administration on the transfer latency(s) to the enclosed arm of the elevated plus-maze in rats and reversal of the 7-NI-induced impairment in the transfer latency of rats by L-arg. The animals received saline plus saline (0.9%), saline plus L-arg (200 mg/kg), saline plus 7-NI (20 mg/kg), or L-arg (200 mg/kg) plus 7-NI (20 mg/kg) i.p 30 min before the first day's trial. L-arg was administered 10 min prior to 7-NI. The number of rat is shown in the columns. Transfer latency data (s) are expressed as mean± SEM values. $^{\#}p<0.05$ Wilcoxon *t*-test, first day vs. second day; $^{*}p<0.05$ Kruskal–Wallis ANOVA followed by Dunn's test, vs. control group on the second day; $^{\&}p<0.05$ Kruskal–Wallis ANOVA followed by Dunn's test, 7-NI treated group vs. L-arg+7-NI treated group.

latency on the second day as compared to that of vehicle-treated group indicating an impairment of learning and memory of the mEPM test in rats (Kruskal–Wallis analysis: $H=21.97$, $p=0.0002$).

3.2. Effect of L-arginine on the transfer latency of rats in the modified elevated plus-maze

As shown in Fig. 2, L-arginine (200 mg/kg) given 30 min before the acquisition session had no effect on the transfer latency of the first day and the second day. The groups treated with L-arginine exhibited the same transfer latency of the groups treated with saline on the first and second day.

20 mg/kg 7-NI given 30 min before the acquisition session significantly prolonged the transfer latency on the second day compared to that of vehicle-treated group ($p<0.05$). However, L-arginine given 10 min before 7-NI, partly reversed 20 mg/kg 7-NI-induced prolongation in the transfer latency of rats on the second day (Kruskal–Wallis analysis: $H=16.52$, $p=0.0015$). Since the effects of 7-NI were inhibited as a result of increasement in NO synthesis by the induction of L-arginine, this finding suggests that NO production is important for emotional learning underlying the transfer latency procedure in rats.

3.3. Effect of MK-801 on the transfer latency of rats in the modified elevated plus-maze

The effects of MK-801 given before acquisition session on the transfer latencies of the first and second days are shown in Fig. 3. MK-801 failed to change the transfer latency on the first day (Kruskal–Wallis analysis, $H=1.17$, $p=0.55$), but significantly prolonged the transfer latency on the second day at 0.15 mg/kg doses ($H=14.64$, $p=0.0007$). The transfer latency on the second day in the 0.15 mg/kg MK-801 treated group was significantly prolonged compared to that of the vehicle-treated and 0.075 mg/kg MK-801 treated group (Dunn's test).

3.4. Effect of systemic co-administration of subeffective doses of MK-801 and 7-NI on the transfer latency of rats in the modified elevated plus-maze

Effect of systemic co-administration of subeffective doses of 7-NI and MK-801 given before acquisition session on the

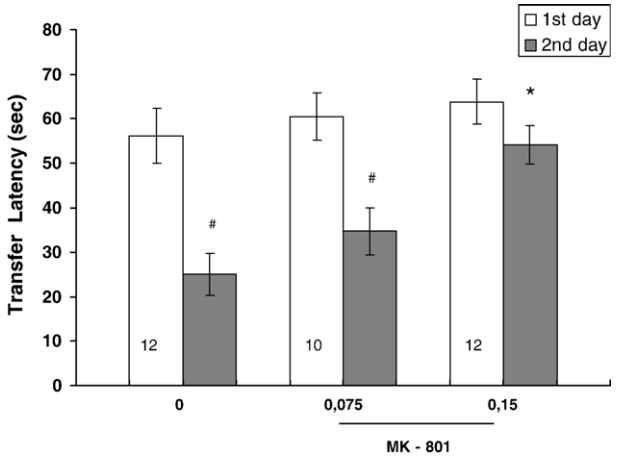

Fig. 3. Effect of systemic MK-801 administration on the transfer latency(s) to the enclosed arm of the elevated plus-maze in rats. The animals received saline (0.9%) or MK-801 (0.075, 0.15 mg/kg) i.p 30 min before first day's trial. The number of rat is shown in the columns. Transfer latency data (s) are expressed as mean±SEM values. $^{\#}p<0.05$ Wilcoxon *t*-test, first day vs. second day; $^{*}p<0.001$ Kruskal–Wallis ANOVA followed by Dunn's test, vs. control group on the second day.

transfer latencies to the enclosed arm of the mEPM on the first and second day in rats is shown in Fig. 4. As also shown in Figs. 1 and 3, 5 mg/kg 7-NI or 0.075 mg/kg MK-801 had no effect on the transfer latency on the first day and the second day. 7-NI or MK-801 at these doses exhibited the same transfer latency of the groups treated with saline on the first day and second day (Dunn's test).

However animals given subeffective doses of 7-NI and MK-801 together before acquisition session, while having no effect on the transfer latencies of the first day ($H=2.55$, $p=0.46$) compared to that of the vehicle-treated group, on the second day significantly prolonged the transfer latency ($H=14.62$, $p=0.0022$) (Kruskal–Wallis analysis of variance followed by Dunn's test). Post hoc comparisons evidenced that the animals given subeffective doses of MK-801 and 7-NI together had significantly prolonged the transfer latency on the second day as compared to that of vehicle-treated group.

3.5. Effect of systemic administration of 7-NI on passive-avoidance performance of rats

During the training session (on day 1) of step-through type PA task, vehicle-treated and 7-NI (5, 25, 50 mg/kg) treated rats showed a similar STL (data not shown). However, 7-NI (25, 50 mg/kg) treated rats showed a significant lower STL as compared to that of vehicle-treated rats ($F_{3,\ 49}=46.78$ $p<0.001$) during the retention test, performed 24 h after the training test (Fig. 5). Decrease in STL indicates an impairment in memory retention of the PA task.

3.6. Effect of systemic administration of L-arginine on the reversal of 7-NI-induced passive-avoidance performance of rats

200 mg/kg L-arginine alone or in combination with 25 mg/kg 7-NI had no effect on the latencies of step-through PA on day 1

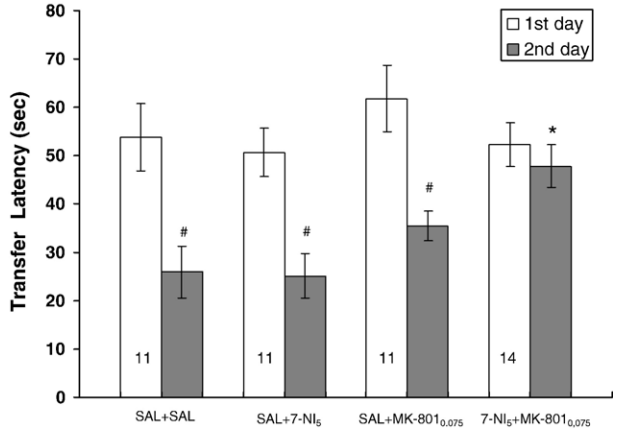

Fig. 4. Effect of systemic co-administration of subeffective doses of MK-801 and 7-NI on the transfer latency(s) to the enclosed arm of the elevated plus-maze in rats. The animals received intraperitoneal saline plus saline (0.9%), saline plus MK-801 (0.075 mg/kg), saline plus 7-NI (5 mg/kg), or 7-NI (5 mg/kg) plus MK-801 (0.075 mg/kg) 30 min before first day's trial. The number of rat is shown in the columns. Transfer latency data (s) are expressed as mean±SEM values. $^{\#}p<0.01$ Wilcoxon t-test, first day vs. second day; $^{*}p<0.01$ Kruskal–Wallis ANOVA followed by Dunn's test, vs. control group on the second day.

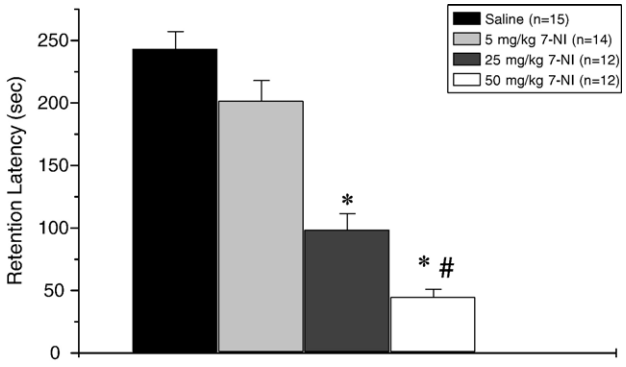

Fig. 5. Effect of 7-NI (5, 25, 50 mg/kg), on the step-through latency (STL) of rats during retention test of passive-avoidance task. Vertical bars represent mean± SEM of retention latencies. $^{*}p<0.001$ compared to control group, $^{\#}p<0.05$ compared to 25 mg/kg 7-NI group (ANOVA post hoc Tukey test). The number of rat is shown in parenthesis.

(data not shown). As shown in Fig. 6, 200 mg/kg L-arginine alone while having no effect on STL in the test phase, in combination with 25 mg/kg 7-NI it significantly ranversed the effect of 7-NI on STL of PA task ($F_{3,\ 40}=17.24$ $p<0.001$).

3.7. Effect of MK-801 on passive-avoidance performance of rats

During the training session (on day 1) of step-through type PA task, vehicle-treated and MK-801 (0.075 and 0.15 mg/kg) treated rats showed a similar STL (data not shown) while 0.15 mg/kg MK-801 significantly shortened the STL of the rats on the test phase ($F_{2,\ 32}=16.61$ $p<0.001$) (Fig. 7).

3.8. Effect of systemic co-administration of subeffective doses of 7-NI and MK-801 on passive-avoidance performance of rats

Subeffective doses of MK-801 alone or in combination with 5 mg/kg 7-NI while having no effect on the latencies of step-

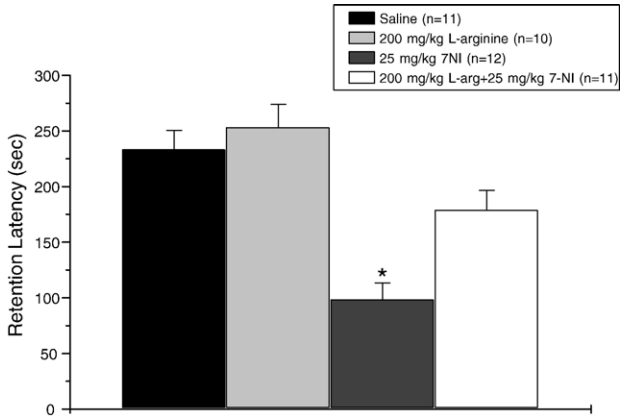

Fig. 6. Effects of systemic administration of L-arginine given alone or in combination with 7-NI on the step-through latency (STL) of rats during retention test of passive-avoidance task. Vertical bars represent mean±SEM of retention latencies. $^{*}p<0.001$ compared to control group (ANOVA post hoc Tukey test). The number of rat is shown in parenthesis.

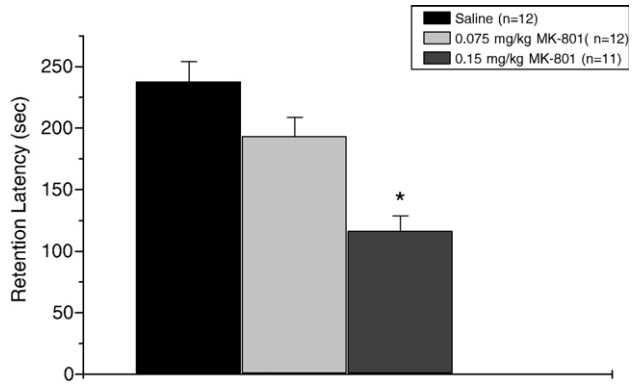

Fig. 7. Effects of systemic administration of MK-801 on the step-through latency (STL) of rats during retention test of passive-avoidance task. Vertical bars represent mean±SEM of retention latencies. *$p<0.001$ compared to control groups (ANOVA post hoc Tukey test). The number of rat is shown in parenthesis.

through PA on day 1 (data not shown), MK-801 alone also revealed no effect on STL in the test phase. However concurrent administration of subeffective doses of 7-NI (5 mg/kg) and MK-801 (0.075 mg/kg) significantly shortened STL as compared to that of vehicle-treated rats ($F_{3,\ 46}=21.60$, $p<0.001$) during the retention test, performed 24 h after the training test (Fig. 8).

3.9. Effects of systemically given 7-NI, L-arginine or MK-801 on locomotor activity

Increase in locomotor activity may produce a behavioural disinhibition. To clarify this possibly, the locomotor activity of the animals was also tested before a change in performance can be attributed to learning and memory process. Locomotor activity of the animals was assessed by measuring the distance travelled (Fig. 9A) and the number of movements (Fig. 9B) for a 5 min period. Statistical analysis of the present (ANOVA) and our previous studies (Yildiz et al., 2000a,b) showed that 7-NI at 5, 10, 20 mg/kg doses did not significantly modify locomotor activity of rats whereas it significantly decreased locomotor activity at 50 mg/kg doses ($p<0.05$). Statistical evaluation of the data revealed that MK-801 produced locomotor stimulation at 0.15 mg/kg doses ($p<0.001$) as it is in convenience with literature findings (Itoh et al., 1991; Murray et al., 1995). 0.075 mg/kg MK-801 and 200 mg/kg L-arginine did not produce any changes in locomotor activity of the animals. Data was expressed as means±SEM ($n=10$ for each group).

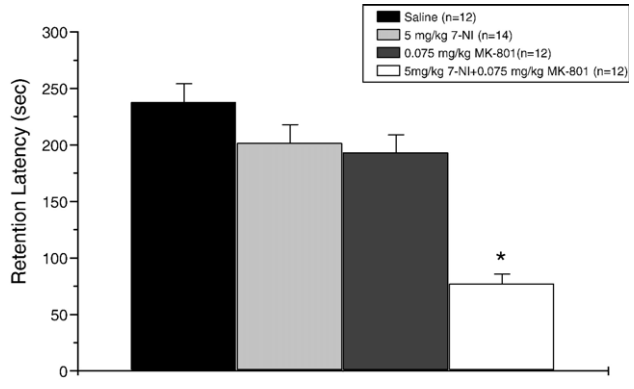

Fig. 8. Effect of systemic co-administration of subeffective doses of MK-801 and 7-NI on the step-through latency (STL) of rats during retention test of passive-avoidance task. Vertical bars represent mean±SEM of retention latencies. *$p<0.001$ compared to control groups (ANOVA post hoc Tukey test). The number of rat is shown in parenthesis.

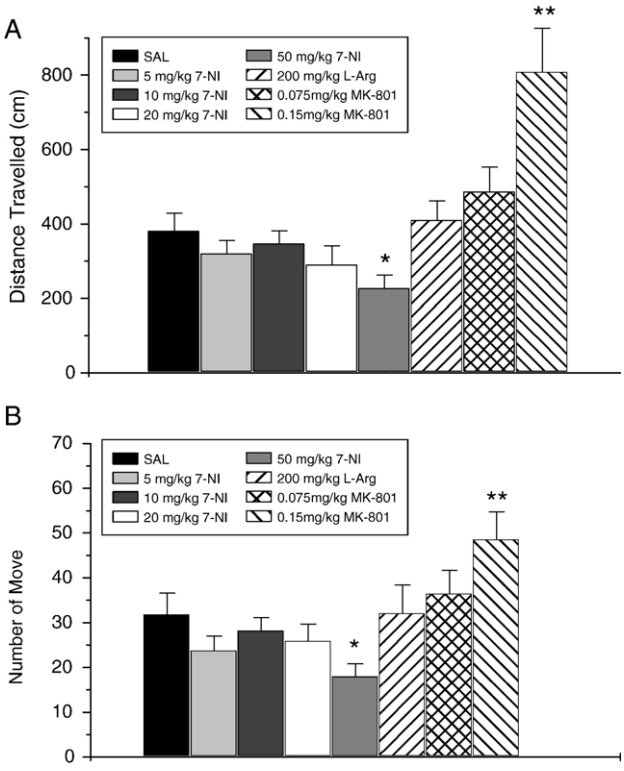

Fig. 9. Effects of 7-NI, L-arg and MK-801 on spontaneous locomotor activity of rats in the activity cage. The distance travelled is represented in (A) and the number of movements of rats in the activity cage is represented in (B). Results are calculated as mean±SEM of 5 min evaluation in the activity cage ($n=10$ for each group) *$p<0.05$ control vs. 50 mg/kg 7-NI, **$p<0.001$ control vs. 0.15 mg/kg MK-801 (ANOVA, post hoc Tukey test).

Table 1
Blood glucose level of rats treated with different doses of 7-NI, L-arginine or MK-801

Groups	Blood glucose levels (mg/dl)
Saline	126.8±3.6
5 mg/kg 7-Nitroindazole	123±3.5
10 mg/kg 7-Nitroindazole	128.4±3.5
20 mg/kg 7-Nitroindazole	120.5±3.8
50 mg/kg 7-Nitroindazole	111.4±3.6
200 mg/kg L-arginine	118.4±3.8
0.075 mg/kg MK-801	125.7±5
0.15 mg/kg MK-801	119.1±4.1

Animals were treated i.p with saline, 5, 10, 20, 50 mg/kg 7-NI, 200 mg/kg L-arginine or 0.075, 0.15 mg/kg MK-801 ($n=10$ for each group). 30 min later, blood samples were collected through the aorta and blood glucose level was evaluated. Each point represents the mean±SEM. The statistical analysis performed by Kruskal–Wallis and Mann–Whitney U test showed no noticeable difference between the drug-treated and control groups.

3.10. Effects of systemically given 7-NI, L-arginine or MK-801 on blood glucose level

Effects of systemically given 7-NI, L-arginine or MK-801 on blood glucose level were determined in a separate group of rats. Animals were treated i.p with saline, 7-NI (5, 10, 20, 50 mg/kg), L-arginine (200 mg/kg) or MK-801 (0.075, 0.15 mg/kg). 30 min later, a few drops of blood samples were collected through the aorta and blood glucose level was evaluated by blood glucose sensor electrode (Abott, Lot: 53093). The statistical analysis exhibited no significant difference between 7-NI (5, 10, 20, 50 mg/kg), MK-801 (0.075, 0.15 mg/kg) treated versus control groups (Kruskal–Wallis) and L-arginine (200 mg/kg) treated versus control groups (Mann–Whitney *U* test) (Table 1).

4. Discussion

The present study showed that 7-NI impaired spatial learning and memory in the mEPM task and emotional memory in the PA task. The mEPM test is suggested to be a simple method for the evaluation of learning and memory processes. Since the animals are able to remember the configuration of the open and enclosed arms, they escape from the unsafe open arm more rapidly on the second trial. It is possible to evaluate the fear-motivated learning, which underlies the transfer latency procedure in this test. Shortened transfer latency on second days trial in rats and mice is used as a parameter for retention or consolidation of memory, while treatment of drugs prior to first day may also be utilized for acquisition related action of drugs (Itoh et al., 1990; Sharma and Kulkarni, 1992).

Passive-avoidance task is also commonly used for examining long-term (24 h) emotional memory. It is based on contextual fear conditioning and instrumental learning (Ogren, 1985) and it is dependent on hippocampus and amygdala (Cahill and McGaugh, 1998; Holland and Bouton, 1999). In this test the animal learns that a specific place should be avoided because of an aversive effect. Thus, longer STL at retention compared with training indicates that the animal had acquired the task.

NO has been postulated to be a critical mediator in learning and memory. It is reported to play a role in excretion and reuptake of excitator and inhibitor amino acids, catecholamines and various neurotransmitters in CNS (Bugnon et al., 1994; Lonart et al., 1992; Miki et al., 1991; Prast and Philippu, 1992). Endogenous NO or NO secreted after the stimulation of NMDA receptors regulates secretion of neurotransmitters via acting presynaptically and changing synaptic activity (Hanbauer et al., 1992). NO is involved in formation of several types of memory and it preferentially affects the acquisition of memory, a process thought to be related to the induction phase of LTP (Prast and Philippu, 2001). It is postulated that NOS immunoreactivity greatly increases in hippocampus, caudate putamen and somatosensory cortex during avoidance learning or exposure to spatial learning (Wood et al., 1990). Inhibition of neuronal NO production by NOS inhibitors has been reported to disturb the experimental memory processes in animals (Estall et al., 1993; Prendergast et al., 1997) but other reports failed to confirm such effects (Bannerman et al., 1994; Bohme et al., 1993; Gribkoff and Lum-Ragan, 1992). In the present study effects of 7-NI on spatial and emotional learning were studied using mEPM and step-through PA tasks, which are both hippocampal dependent. 7-NI (10–50 mg/kg i.p) administered before the first trial (training) significantly prolonged the transfer latency to the enclosed arm of the mEPM on the second day (retention test). It has been reported that transfer latency may be one of the indicators of learning and memory, since the transfer latency in the retention test is significantly shorter than that on training (Itoh et al., 1990). In the step-through PA task 7-NI (25, 50 mg/kg i.p) administered before the training, again reduced the retention latency on the second (test) day and thus significantly impaired the retention of memory. Contradictory results exist about effects of NOS inhibitors on learning experiments and this discrepancy has been attributed to their task-dependent effects on different forms of learning and memory.

As NO is an endothelial-derived relaxing factor 7-NI may induce blood pressure changes. 7-NI, as a result of its neuronal NOS inhibition, do not produce changes in blood pressure (Babbedge et al., 1993; Moore et al., 1993). So it is unlikely that impairment of memory caused by 7-NI is associated with its cardiovascular effects. It is also unlikely that alteration in blood glucose level is involved in the impairment of transfer latency or STL of rats in the mEPM and PA tasks since 7-NI had no effect on the blood glucose levels of rats. Moreover it is conceivable that impairment of learning and memory in these tasks is due to the inhibition of NO production in the brain. One might consider that the inhibitory effect of 7-NI on transfer learning of rats in the mEPM task or retention latency of rats in PA test is due to a general impairment of locomotor activity. However this is unlikely since 7-NI did not cause a significant difference in the locomotor activity of the animals except at high doses (Fig. 9A,B). Furthermore, as other investigators (Yamada et al., 1996) and we (Yildiz et al., 2000a,b) have previously demonstrated that 7-NI had no effect on locomotor activity of the animals at the low doses.

Systemic administration of L-arginine, an NO precursor, increases NO production in the rat brain (Salter et al., 1996; Yamada and Nabeshima, 1997b). Thus L-arginine improves memory consolidation and learning (Plech et al., 2003). It was interesting to observe that L-arginine alone had no effect on mEPM or PA learning. This was surprising to our expectation because L-arginine is a precursor of NO and NO is known to play an important role in learning and memory formation (Plech et al., 2003). One plausible explanation for the observed effect of L-arginine might be due to the conversion of exogenously given L-arginine to agmatine via arginine decarboxylase activity (Raasch et al., 2001; Reis and Regunathan, 2000). Agmatine is known to cause inhibition of nNOS and other NOS isoforms (Demady et al., 2001). Therefore we believe that agmatine may balance the effect caused by L-arginine. A second explanation that L-arginine alone did not enhance performance may be explained quite simply as a ceiling effect that is the system is working at near maximum efficiency and cannot be improved upon.

If prevention of NO production produces amnesic activity, then administration of a competitive NO precursor should reverse

this effect. In our study, 7-NI-induced impairment of mEPM and PA memory was partly reversed by co-administration of L-arginine (200 mg/kg) that was ineffective in altering the transfer latency or retention latency; indicating that NO is important in these paradigm. In support of this suggestion, 7-NI (30 mg/kg, i.p) impaired spatial learning in the rat (Holscher et al., 1996) and 7-NI-induced learning and memory was reversed by L-arginine (Zou et al., 1998).

Glutamergic NMDA receptor mechanism plays an important role in a spatial orientation of mice placed on the mEPM and on the memory based on the negative reinforcement. MK-801, a non-competitive NMDA antagonist, produces amnesic effect in several behavioural tests of learning and memory with rodents, which is considered to be elicited via the inhibition of LTP in the hippocampus (Butelman, 1989). In our study MK-801 (0.15 mg/kg) significantly prolonged the transfer latencies in the retention test as compared with those of the corresponding control in the mEPM test, similarly to previous studies of the literature (Butelman, 1989; Da Cunha et al., 2005; Hlinak and Krejci, 1998; 2002) and shortened the STL of the rats on the retention test in the PA task. However doses of 7-NI (5 mg/kg) and MK-801 (0.075 mg/kg) that had no effect per se on the PA and mEPM behaviours, given in combination regimen significantly impaired spatial and emotional learning and memory in rats. Thus NO-dependent mechanism may be involved in MK-801 induced impairment of learning and memory processes.

It is suggested that NMDA receptors play an important role in hippocampal-dependent spatial learning and that LTP is involved in certain forms of learning (Morris et al., 1986). The importance of hippocampal glutamate, acting via the NMDA receptor, in the encoding of new information is supported with subsequent studies (Elvander-Tottie et al., 2006; Kawabe et al., 1998; Liang et al., 1994) observing competitive NMDA receptor antagonist D-AP5 infused 15 min prior to training, impaired spatial learning and memory in water maze and step-through passive-avoidance tasks and postulating changes in septal glutamate transmission and NMDA receptor activity can influence activity dependent synaptic plasticity in the hippocampus and thereby learning and memory.

Multiple modulatory systems may be subject to the pharmacology of transfer latency and retention latency such as nitrergic and glutamatergic. We examined that, as in agreement with previous studies, systemic administration of NOS inhibitor 7-NI impaired the retention of memory in spatial and emotional learning in rats and also MK-801 (0.15 mg/kg) exhibited memory deterioration in these tasks. In support of this suggestion scopolamine, MK-801, and co-administration of subeffective doses of MK-801 and scopolamine impaired the transfer latency learning in mice (Da Cunha et al., 2005; Hlinak and Krejci, 1998; 2000; 2002; Itoh et al., 1991; Sharma and Kulkarni, 1992). Thus NMDA receptor mediated NO pathways may be implicated in this effect. In investigation the relationship between the activation of the NMDA receptor and NO synthesis in the transfer latency learning of rats, while co-administration of subeffective doses of L-NAME and MK-801 failed to impair the transfer latency learning of rats (Da Cunha et al., 2005), in our study co-administration of subeffective doses of 7-NI and MK-801 impaired spatial and emotional learning in mEPM and step-through PA tasks in rats. It has been suggested that some of the memory impairment caused by the NMDA receptor antagonist MK-801 is due to the impairment of NO and subsequent cyclic GMP production in brain (Yamada et al., 1996); and the same mechanism is suggested to play a role in the LTP in the hippocampus (Zhuo et al., 1994). Thus, it can be postulated that NO synthesis may represent a system of signal amplification for the NMDA receptors in the spatial orientation of mice placed on the elevated plus-maze; since it is known that NO production in the brain is stimulated by the activation of glutamate receptors (Yamada and Nabeshima, 1997a,b). This finding clearly indicates that there is a relationship between NO and glutamatergic system and moreover that both systems play an important role in a spatial orientation of rat on the mEPM.

In conclusion, the present study suggests that pretraining administration of 7-NI impaired the PA and plus-maze memory performance in rats. Subeffective doses of 7-NI and MK-801 given in combination significantly impaired mEPM and PA performances in rats. So NMDA receptor mediated NO pathways may be implicated in the PA and mEPM behaviours in rats.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/pbb.2007.05.019.

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