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Learning in a 14-unit T-maze is impaired in rats following systemic treatment with N^{w}-nitro-L-arginine

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Abstract

We examined whether inhibition of nitric oxide synthase (NO synthase) impairs learning in male Fischer-344 rats (9 mo) in a shock-motivated 14-unit T-maze. Rats were pretrained in one-way active avoidance of foot shock to a criterion of 13/15 avoidances in a straight runway. The next day, rats received intraperitoneal (i.p.) injections of 0.9% NaCl as controls or N^{w}-nitro-L-arginine (*N*-Arg: 3.0, 4.5, or 6.0 mg/kg) to inhibit NO synthase 30 min before maze training. During 15 trials, rats were required to negotiate each of 5 segments within 10 s to avoid footshock. Performance variables included errors (deviations from the correct pathway), runtime from start to goal, shock frequency and duration. *N*-Arg treatment impaired performance on all variables in a dose-dependent manner. Specifically, only the 6 mg/kg *N*-Arg dose significantly increased errors compared to controls over the last 10 trials but not the first 5 trials. Controls and rats treated with 3 or 4.5 mg/kg *N*-Arg were retested in the maze 7–10 days following training, with half receiving *N*-Arg (6 mg/kg i.p.) 30 min in advance. In this retention test, maze performance was not significantly affected; thus, these results indicated that NO synthase inhibition primarily impaired acquisition without impacting upon noncognitive aspects of performance. This conclusion was further reinforced by the demonstration that 6 mg/kg *N*-Arg did not significantly affect sensorimotor performance in a rotarod task. When rats were treated with sodium nitroprusside, an NO donor, at 1 min, but not 30 min, prior to training, the *N*-Arg induced impairment (6 or 8 mg/kg i.p.) in maze learning was significantly attenuated. © 1998 Elsevier Science B.V.

Keywords: Nitric oxide (NO) synthase; Memory; Glutamate; Sodium nitroprusside; Blood pressure

1. Introduction

Nitric oxide (NO) is a soluble gas involved in a great variety of functions. It is synthesized from L-arginine by NO synthase which is regulated by three different genes. Production of NO catalyzed by NO synthase in endothelial cells is involved in smooth muscle contraction in the intestines and dilatation of blood vessels (Nanaev et al., 1995; Nemade et al., 1995; Morales-Ruiz et al., 1996). An inducible NO synthase also exists in various immune cells including microglia and catalyzes the production of NO for cytotoxic activity (Paakkari and Lindsberg, 1995; Szabo, 1996; Torres et al., 1996). A constitutive NO has also been identified in neurons (neuronal NO synthase; Bredt et al., 1990; Jaffrey and Snyder, 1995).

NO in brain has received increased attention as a putative neuromodulator involved in the formation of new memories (Jaffrey and Snyder, 1995; Hawkins, 1996). Specifically, it has been suggested that NO acts as a retrograde messenger to facilitate presynaptic release of glutamate. Following postsynaptic activation of the *N*-methyl-D-aspartate (NMDA) receptor, the influx of Ca^{2+} through the cationic channel of the receptor activates calmodulin, which in turn stimulates NO synthase to produce NO from its amino acid precursor (Garthwaite, 1991). The nascent NO is purported to diffuse into the presynaptic terminal to activate soluble guanylyl cyclase, thus producing increased levels of cyclic guanosine monophosphate (cGMP) and enhanced glutamate release. Experimental support for this pathway in vivo was provided in a study by Segovia et al. (1994), who infused the NO donor, 3-morpholino–sydnonimine (SIN-1), into the hippocampus

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and striatum of conscious rats and observed a significant increase of extracellular glutamate in both regions. In addition, Vallebuona and Maurizio (1994) reported increased extracellular cGMP in vivo following infusion of the NO donors, hydroxylamine and *S*-nitroso-*N*-penicillamine.

Evidence supporting a role for NO in long-term potentiation has also emerged (Collingridge and Bliss, 1995; Bailey et al., 1996). Although in vitro pharmacological inhibition of NO synthase in hippocampal-slice preparations blocks long-term potentiation (Bohme et al., 1991; Madison and Schuman, 1991; Haley et al., 1992), some laboratories have failed to confirm NO involvement in long-term potentiation (Bliss and Collingridge, 1993; Bannerman et al., 1994a).

Several behavioral studies have demonstrated that treatment with NO synthase inhibitors results in impaired learning. For example, systemic administration of N^{w}-nitro-L-arginine methyl ester (NAME) or N^{w}-nitro-L-arginine (*N*-Arg) has been shown to disrupt the spatial learning ability of rats in a water maze (Chapman et al., 1992; Estall et al., 1993; Mogensen et al., 1995a,b). In addition, NAME treatment in rabbits impairs conditioning of an eyeblink response (Chapman et al., 1992), and *N*-Arg treatment prevents octopi from learning a simple target touching task (Robertson et al., 1994) and impairs passive avoidance performance in chicks (Holscher and Rose, 1992). Papa et al. (1994) linked changes in central NO synthase activity in rats to performance in a task involving habituation to novelty. When the brains from this study were stained histochemically for NADPH-d, an indirect marker of NO synthase activity, the rats exposed to the task showed significantly more staining of NADPH-d in several brain regions compared to rats that were handled but not exposed to the task. Furthermore, the administration of *N*-Arg disrupted habituation to the task and also reduced NADPH-d staining.

Despite the evidence emerging from these studies, other investigators have questioned whether the impaired performance associated with NO synthase inhibition reflected noncognitive factors primarily (Bannerman et al., 1994b; Sandi et al., 1995). Some studies have found only minimal or no behavioral effects related to NO synthase inhibition. For example, administration of *N*-Arg failed to block olfactory-based memory in mice, as measured by the ability of a pregnant female to recognize the pheromones unique to a mate (Okere et al., 1995). Similarly, Tobin et al. (1995) reported that rats treated with NAME were unimpaired in an operant task using both visual and spatial cues, and Holscher et al. (1995) found that rats were only mildly impaired in water maze performance following administration of *N*-Arg. Bannerman et al. (1994b) observed impaired spatial learning in a water maze only under certain training paradigms and thus questioned whether NO synthase inhibition directly affected mechanisms of spatial learning.

In the present report we demonstrate in rats that NO synthase inhibition by *N*-Arg impairs learning in an aversively-motivated 14-unit T-maze. Previous results involving rats in this maze have provided evidence that antagonism of NMDA receptor activation by the noncompetitive channel blocker, dizocilpine, impaired acquisition but not retention performance (Spangler et al., 1991). Therefore, if *N*-Arg was inhibiting NO production thereby also inhibiting cGMP-stimulated presynaptic glutamate release, we would expect to observe impaired learning at doses that did not adversely affect retention nor sensorimotor abilities involved in maze performance. As a further test of NO involvement in learning, we conducted additional experiments using the NO donor, sodium nitroprusside, to attenuate a *N*-Arg-induced impairment.

2. Materials and methods

2.1. Subjects

Young (3–5 mo), virgin male Fischer-344 rats were obtained from Harlan Sprague–Dawley (Indianapolis, IN). They were housed in conventional plastic cages (two per cage, cleaned weekly) with wood shavings in a vivarium at the Gerontology Research Center and were allowed to acclimate for at least 2 weeks before behavioral testing. A conventional diet (NIH-07) was provided ad libitum as was access to water through an automated and filtered system. In the vivarium constant temperature (22°C), humidity (70%), and a 12-h light: 12-h dark photocycle (lights on at 06.00) were maintained. All testing occurred during the light portion of the photocycle.

2.2. Apparatus

2.2.1. Straight runway

Pretraining for shock avoidance was conducted in a straight runway (approximately 2 m in length) constructed of clear Plexiglas© as described in detail previously (Spangler et al., 1986). The floor consisted of diagonally placed stainless steel grids wired in series to receive a constant-current scrambled shock (Model E13-08, Coulbourn Instruments, Lehigh Valley, PA). A hand-held switch initiated foot shock and started a clock that recorded the time to traverse the runway. Interchangeable start and goal boxes were made of black Plexiglas and fitted with a movable rear wall attached to a stainless steel arm. The boxes could be placed over the grid floors at either end of the runway.

2.2.2. Maze

A clear Plexiglas 14-unit T-maze used for testing has been described previously (Spangler et al., 1986). The maze was separated into five distinct sections by guillotine doors that prevented reentry into previous sections of the

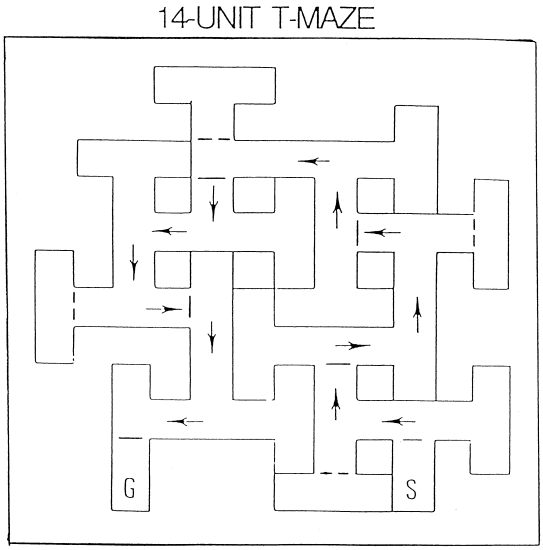

Fig. 1. Configuration of the 14-unit T-maze. Arrows denote correct pathway. Errors are scored as any deviations from this pathway. Solid lines in pathway denote guillotine doors; broken lines denote false guillotine doors; S, start box; G, goal box.

maze. Fig. 1 shows the schematic layout of the maze and the correct pathway from startbox to goalbox. To eliminate the guillotine doors from being used as cues to the correct pathway, identical, but nonfunctional, doors were located at the entry to each cul-de-sac of the maze. A manually operated switchbox triggered a clock which, when timed out, initiated scrambled shock to the grid floor of the maze. At the same time another clock was initiated to record the duration of shock, and a counter recorded the number of shocks (maximum = 5 shocks per trial). Infrared photocells were located throughout the maze and were wired in series to a microprocessor, which recorded movement through the maze, time elapsed from start to goal, and time between photocell interruptions. The particular sequence of photocell interruptions and time between interruptions were analyzed by the microprocessor to provide the number of errors (defined as a deviation from the correct pathway) and runtime. Data from the microprocessor were transferred to a personal computer for more detailed analysis as well as for storage of raw data.

The maze was surrounded by gray-painted wooden walls to reduce extramaze visual cues, and speakers were located under each of the four corners and provided music to mask auditory cues. The maze was hoisted by a motor-driven series of pulleys so that the grid floor could be mopped between trials to reduce odor cues.

2.2.3. Rotarod

A rotarod test was used to assess possible motor effects of *N*-Arg treatment. Described in detail previously (Spangler et al., 1994), the apparatus used was a scored, plastic cylinder 47 cm in diameter with metal dividers 48 cm high to prevent the rat from escaping. The cylinder was mounted onto a plastic frame housing a motor that rotated the cylinder at 4 rpm by means of a pulley system. The base of the frame was 47 cm below the cylinder and 13 cm of foam padding was placed onto the base to cushion the fall of the rat from the rotarod.

2.3. Procedure

2.3.1. Maze pretraining

Prior to one-way active avoidance training in the straight runway, rats were taken from the vivarium and transported in their home cages to the maze room, where they were allowed to acclimate for at least 30 min. On the first trial each rat was taken from the home cage and placed into one of the black boxes. The box was inserted into the start portion of the runway, and the rat was pushed from the box into the runway by pressing the steel arm of the box forward. The rat then had 10 s to avoid a footshock (0.8 mA) by locomoting to the goal box at the opposite end of the runway. Once the rat entered the goal box, a guillotine door was lowered, and the goal box was removed to a holding area for a 90 s intertrial interval. Following the intertrial interval, the box was returned to the start area of the runway for the next trial. Each rat continued to receive massed training trials until a criterion of 13/15 shock avoidances had been made (maximum = 30 trials). All rats successfully achieved this criterion.

2.3.2. Maze acquisition

Maze training began 24 h following pretraining in the straight runway. Consistent with pretraining, all rats were brought into the maze room and allowed to acclimate for at least 30 min prior to maze training.

Each rat was removed from the home cage, placed into the black start box, and the box was inserted into the start area of the maze. The rat was then pushed gently from the start box into the first section of the maze, and the first guillotine door was closed behind the rat. A switch was manually triggered which initiated the clock controlling the shock contingency. The rat then had 10 s to navigate the first section of the maze and pass through the door into the second maze section. If the rat failed to pass through the door in 10 s, scrambled footshock (0.8 mA) was delivered to the grid floor until the rat escaped through the door. When the rat passed through the door into the second section, the guillotine door was lowered, and the shock contingency was reset. The contingency was reset each time the rat moved through the remaining three sections of the maze. Once the rat entered the goal box, the box was removed to a holding area for an intertrial interval of 90 s. During this time the maze was hoisted and the grid floor was cleaned with a 95% solution of ethanol. Each rat received a total of 15 trials.

2.3.3. Drug treatment

Rats were assigned randomly to a control group or to experimental groups receiving *N*-Arg (Sigma). In an initial

experiment, rats received intraperitoneal (i.p.) injections of saline (0.9% NaCl) as controls ($n = 7$) or 3.0 ($n = 6$), 4.5 ($n = 7$) or 6.0 ($n = 7$) mg/kg *N*-Arg 30 min prior to maze training. Dwyer et al. (1991) had shown that a dose of 5 mg/kg i.p. could inhibit cerebellar NO synthase activity about 50 percent. In experiments designed to determine if an NO donor could overcome NO synthase inhibition, rats were given i.p. injections of either saline ($n = 13$) or 6 mg/kg ($n = 7$) or 8 mg/kg ($n = 8$) *N*-Arg 35 min prior to maze training, and then treated with sodium nitroprusside (1 mg/kg) either 1 min ($n = 10$ following 8 mg/kg *N*-Arg) or 30 min ($n = 7$ following 6 mg/kg *N*-Arg) before the first trial in the maze. Prior to these experiments, a pilot study was conducted to determine if the 1 mg/kg dose of sodium nitroprusside would produce hypotension as evidence of its activity as an NO donor.

2.3.4. Blood pressure measurement

To measure effects of sodium nitroprusside on blood pressure, rats were placed into a plastic rat restrainer, which permitted the tail to protrude. A tail cuff attached to a blood pressure monitor (Narco Biosystems, Galveston, TX) was placed around the tail about 1 inch from its base. After 15 min a baseline measure of systolic blood pressure was recorded. The rat was then given either saline ($n = 4$) or sodium nitroprusside ($n = 4$), and blood pressure was recorded at 1 and 5 min thereafter.

2.3.5. Maze retention

A group of 12 rats that had been trained in acquisition as described above were retested in the 14-unit T-maze 7–10 days later. About half this group had previously served as saline controls and the others had received either 3 or 4.5 mg/kg *N*-Arg. Thirty minutes prior to the retention test, one group ($n = 6$) received 6 mg/kg *N*-Arg i.p. and the other ($n = 6$) received saline as control. Prior treatment condition during acquisition was balanced across these conditions. According to the same procedures used during acquisition training, these rats received an additional 10 trials in the maze.

2.3.6. Rotarod

A new group of 14 rats was tested in a rotarod task. Thirty min prior to this session, half the group received 6 mg/kg *N*-Arg i.p. and half received saline as controls. As described previously (Spangler et al., 1994), each rat was placed onto the rotarod with its body oriented parallel to the axis of the cylinder, and a timer was initiated. Each time the rat fell, the timer was stopped, a fall was recorded, the rat was returned to the rotarod, and the timer was reset. The dependent measure was the number of falls during a 3-min exposure to the rotarod.

2.3.7. Statistical analysis

Maze performance variables analyzed included errors, runtime from start to goal, and shock frequency and duration. Data on maze acquisition were submitted to a 4 (drug group) by 3 (blocks of 5 trials) repeated measures analysis of variance (ANOVA). Data on maze retention were analyzed in a 2 (drug group) by 2 (blocks of 5 trials) ANOVA. To analyze the effects of sodium nitroprusside, we utilized a 5 (drug group) by 3 (blocks of 5 trials) ANOVA with repeated measures. Dose effects within blocks were examined using one-way ANOVA followed by Dunnett's test comparing different treatment groups to controls. The variable assessed in the rotarod test was the number of falls during the 3-min exposure to the apparatus. These data were analyzed with a two-tailed *t*-test comparing the 6 mg/kg group to controls. For all comparisons, statistical significance was accepted as $P < 0.05$.

3. Results

3.1. Effects of N-Arg on maze acquisition

When *N*-Arg was injected 30 min prior to maze training, the rats demonstrated a dose-dependent learning impairment, evidenced by an increased number of errors (Fig. 2). At the final block of trials, rats treated with 6 mg/kg *N*-Arg were making over twice as many errors compared to controls.

Results of the ANOVA of errors showed significant main effects of drug treatment, $F(3,23) = 4.28$, $P = 0.015$, and blocks, $F(2,46) = 184.2$, $P < 0.001$. To determine the *N*-Arg dose effects within each block of trials, a one-way ANOVA was conducted for each block followed by Dunnett's comparisons between each *N*-Arg group and controls. For the first block of trials, the group effect was not significant, $F(3,23) < 1$. The group effect was significant for blocks 2 and 3 ($F(3,23) = 4.3, P < 0.02$; $F(3,23) = 8.6, P = 0.0005$, respectively). However, the 6 mg/kg dose was the only group to show significantly increased errors compared to controls over the last two blocks.

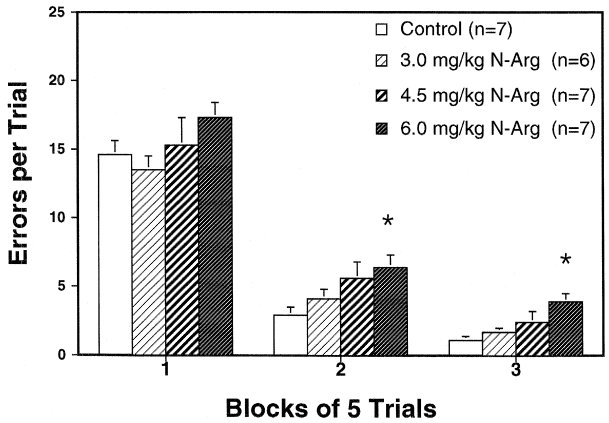

Fig. 2. Mean (SEM) errors per trial in a 14-unit T-maze during acquisition training according to dose of N^{w}-nitro-L-arginine (*N*-Arg). * Significantly different from control group, $P < 0.05$.

Table 1
Effects of N^{w}-nitro-L-arginine (*N*-Arg) on noncognitive performance variables during acquisition training (mean values (S.E.M.) per trial)

Variable	Dose of *N*-Arg (mg/kg)			
	control ($n = 7$)	3.0 ($n = 6$)	4.5 ($n = 7$)	6.0 ($n = 7$)
Runtime (s)	56.6 (5.3)	58.1 (2.3)	89.7[a] (10.6)	111.8[a] (38.5)
Shock frequency	1.6 (0.2)	2.2 (0.1)	2.7 (0.1)	3.5[a] (0.1)
Shock duration (s)	32.0 (5.5)	25.9 (2.4)	42.1 (8.8)	67.8[a] (13.6)

[a] Significantly different from control group, Dunnett's test, $P < 0.05$.

The results of the ANOVAs of the other performance variables, runtime, shock frequency and duration, also yielded a similar pattern, i.e. significant main effects of drug group (all F's$(3,23) > 4.0$, P's < 0.02) and block (all F's$(2,46) > 64$, P's < 0.001). These data have been collapsed across blocks and summarized in Table 1. *N*-Arg treatment produced negative effects on these performance variables in a dose-dependent manner. Consistent with the analysis of errors, *N*-Arg produced a significant increase compared to controls in shock duration and frequency only at the dose of 6 mg/kg. A similar effect was observed in runtime at this dose, but the 4.5 mg/kg dose also significantly increased runtime.

3.2. Retention test

As summarized in Table 2, treatment with 6 mg/kg *N*-Arg had no significant effect on performance in the retention test. Although runtime, shock frequency and duration appeared modestly elevated in the *N*-Arg treated group, very few errors were made, and the control and *N*-Arg groups were virtually identical in performance. Results of the ANOVAs for errors, runtime, shock duration and frequency yielded significant main effects only for blocks (All F's$(1,11) > 9.11$, P's < 0.02), but no significant main effect of group, $P > 0.05$. These findings in the retention test indicated that the *N*-Arg induced increase in errors in the acquisition test was not likely due to motoric effects produced by NO synthase inhibition.

3.3. Rotarod test

The lack of motoric effects induced by *N*-Arg was further confirmed in the rotarod test. The mean (SEM) number of falls for controls was 7.5 (0.8) compared to 8.7 (1.2) for the group treated with 6 mg/kg *N*-Arg, which did not differ significantly, $t(12) < 1.0$.

Table 2
Effects of N^{w}-nitro-L-arginine (*N*-Arg) on maze performance variables during retention testing (mean values (S.E.M.) per trial)

Variable	Dose of *N*-Arg (mg/kg)	
	control ($n = 6$)	6.0 ($n = 6$)
Errors	1.4 (0.2)	1.8 (0.6)
Runtime (s)	16.2 (1.6)	26.6 (5.9)
Shock frequency	0.3 (0.09)	0.8 (0.3)
Shock duration (s)	1.5 (0.7)	4.5 (1.5)

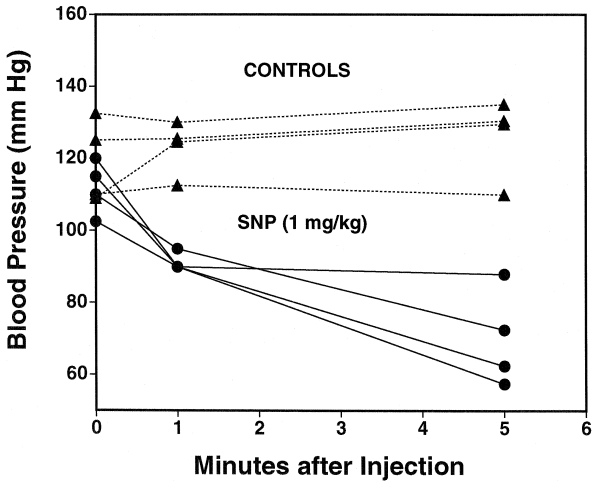

Fig. 3. Systolic blood pressure following i.p. injection of sodium nitroprusside (SNP).

3.4. Effects of sodium nitroprusside

Results of the pilot study indicated that the effects of the 1 mg/kg dose of sodium nitroprusside on blood pressure were immediate and substantial. As shown in Fig. 3, clear reductions in systolic pressure were evident as early as 1 min and were 30–40% below baseline by 5 min; thus, this dose of sodium nitroprusside appeared to produce a biologically active level of NO at least peripherally.

A replication of the *N*-Arg induced learning impairment is shown in Fig. 4 along with evidence that sodium nitroprusside treatment could attenuate the impairment. Results of the ANOVA for errors yielded a significant group effect, $F(4,40) = 3.1$, $P = 0.026$, and block effect,

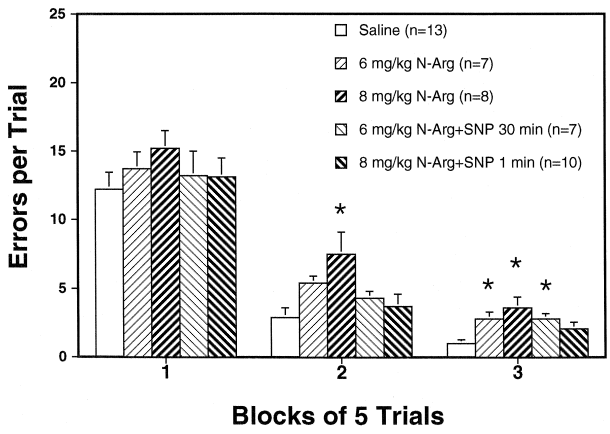

Fig. 4. Mean (SEM) errors per trial in a 14-unit T-maze during acquisition training according to dose of N^{w}-nitro-L-arginine (*N*-Arg) and dose of sodium nitroprusside (SNP). * Significantly different from control group, $P < 0.05$.

Table 3
Effects of N^{w}-nitro-L-arginine (*N*-Arg) or sodium nitroprusside (SNP) in various combinations on noncognitive performance variables during acquisition training (mean values (S.E.M.) per trial)

	Variable			
	n	runtime (s)	shock duration (s)	shock frequency
Saline + Saline	13	47.3 (5.7)	16.2 (3.7)	1.4 (0.1)
6 mg/kg *N*-Arg + Saline	7	66.0 (4.9)	29.1 (3.5)	2.6 (0.3)[a]
8 mg/kg *N*-Arg + Saline	8	85.3 (14.2)[a]	47.1 (11.4)[a]	2.5 (0.3)[a]
6 mg/kg *N*-Arg + SNP at 30 min	7	53.6 (3.9)	21.9 (4.6)	2.3 (0.3)[a]
8 mg/kg *N*-Arg + SNP at 1 min	10	65.9 (8.7)	35.5 (6.7)	2.4 (0.3)[a]

[a] Significantly different from control group, Dunnett's test, $P < 0.05$.

$F(2,80) = 226.63$, $P < 0.001$. Results of one-way ANOVAs indicated that significant group differences occurred only at block 2, $F(4,40) = 3.45$, $P = 0.016$, and block 3, $F(4,40) = 4.53$, $P = 0.004$. For these blocks, Dunnett's tests were used to compare each dose group to controls. At block 2, the only group significantly different from controls was the group that received the 8 mg/kg dose. At block 3, both the 6 and 8 mg/kg *N*-Arg groups were significantly different from controls as was the 6 mg/kg *N*-Arg group that had received sodium nitroprusside 30 min prior to training. The 8 mg/kg *N*-Arg + sodium nitroprusside group did not differ significantly from controls at either block 2 or 3; thus, the only consistent evidence that sodium nitroprusside could attenuate the *N*-Arg induced learning impairment was when it was delivered 1 min prior to training.

The results of ANOVAs for runtime, shock duration and frequency yielded slightly different results than those observed for error performance (Table 3). The main effects of group and block were significant for all analyses (P's < 0.05). Based on Dunnett's comparisons of data collapsed across trials, *N*-Arg treatment at 8 mg/kg significantly increased runtime, shock duration and frequency. Sodium nitroprusside delivered at 1 min prior to training attenuated these performance deficits with the exception of the increase in shock frequency. The 6 mg/kg dose of *N*-Arg significantly increased shock frequency but not shock duration or runtime. Sodium nitroprusside delivered at 30 min prior to training significantly decreased runtime and shock duration relative to the 8 mg/kg *N*-Arg group.

4. Discussion

The 14-unit T-maze has been utilized primarily to assess neurobiological mechanisms involved in age-related memory dysfunction (Ingram et al., 1996b). A major hypothesis is that age-related loss of various components of the NMDA receptor might underlie the age-related declines in learning observed in this maze (Ingram et al., 1996b). To this end, results of previous studies in young rats have demonstrated involvement of the NMDA receptor/glutamate system. Specifically, learning in young rats can be impaired by blocking the NMDA recognition site with 3-(2-carboxy-piperazin-4-)propyl-1-phosphonic acid (Meyer et al., 1997a) or blocking the NMDA receptor-gated calcium channel with dizocilpine (Spangler et al., 1991). However, consistent evidence supporting an age-related loss of hippocampal binding sites of the NMDA receptor complex has not been forthcoming (Ingram et al., 1996a). Therefore, examination of signal transduction events, such as NO production, mediated via the NMDA receptor, has become the focus of further studies applying this paradigm.

The current results demonstrate that learning in the 14-unit T-maze is impaired in young rats treated with the NO synthase inhibitor, *N*-Arg. Error performance is the most salient variable supporting this conclusion. However, there was negative impact on other performance variables as well. In the dose–response study, *N*-Arg treated rats (6 mg/kg) took longer to complete the maze and received more shocks and of longer duration. Since these variables are also highly correlated with error performance, other lines of evidence must be presented to argue that the *N*-Arg induced impairment was not attributed to noncognitive factors. First, no significant effect of *N*-Arg on error performance was observed during the first block of training. Second, a 6 mg/kg dose of *N*-Arg, the highest dose used during acquisition training, did not significantly affect any performance variable including errors in the retention test. This observation of a negative effect of *N*-Arg on acquisition but not retention performance is generally consistent with the literature (Hawkins, 1996). Third, the 6 mg/kg dose of *N*-Arg had no significant effect on rotarod performance. Thus, it appeared that inhibiting NO synthase with this compound negatively affected memory acquisition processes involved in the maze task rather than influencing performance through noncognitive mechanisms. Further evidence of NO involvement in the *N*-Arg induced impairment was demonstrated when we observed that sodium nitroprusside could attenuate the learning deficit but only when delivered 1 min prior to maze training. When delivered 30 min before training, this rapidly acting NO donor may have lost sufficient activity.

The learning impairment observed in the present study was obtained with *N*-Arg doses that were much lower than doses of *N*-Arg (Mogensen et al., 1995a,b) or NAME (Estall et al., 1993; Holscher and Rose, 1993; Barnes et al., 1994; Yamada et al., 1995; Jewett et al., 1996) that had been used in previous studies involving learning and memory paradigms. Because we did not measure NO synthase activity, the degree of NO synthase inhibition achieved with the *N*-Arg doses applied in the current study is uncertain. Following 4 days of *N*-Arg treatment at 5 mg/kg, Dwyer et al. (1991) reported that cerebellar NO

synthase was inhibited about 50% compared to about 95% NO synthase inhibition observed at 50 mg/kg. Thus, it is likely that the acute doses of 6–8 mg/kg which we used were providing only partial NO synthase inhibition.

Perhaps the current task is more sensitive to NO synthase inhibition compared to other tasks, or perhaps strain differences in response to NO synthase inhibitors can account for this discrepancy in behavioral responses observed across studies. In pilot studies using *N*-Arg doses above 30 mg/kg, we observed marked motoric impairments that handicapped performance in the 14-unit T-maze. With the exception of those by Barnes et al. (1994) and Tobin et al. (1995), no other study of *N*-Arg or NAME blockade of learning and memory has used Fischer-344 rats. To clarify the possibility of strain differences in response to NO synthase inhibition, direct comparisons between different rat strains or different behavioral paradigms would have to be conducted in the same laboratory.

The duration of treatment with NO synthase inhibitors is also important to consider. For example, in their study of Fischer-344 rats, Barnes et al. (1994) delivered *N*-Arg at 50 mg/kg i.p. over a period of 4 days prior to training in a Morris water maze over 2 days. NO synthase activity was reduced dramatically, but this loss was not correlated with impairment of performance in the water maze nor with excitatory postsynaptic potentials measured in the dentate gyrus. Two explanations can be offered for this observation. First, because Barnes et al. used a chronic regimen of NO synthase inhibition (>4 days of daily injections), perhaps compensatory mechanisms, not seen with acute treatments, were activated. This explanation would be consistent with the general lack of phenotypic abnormalities initially reported for neuronal NO synthase knock-out mice (O'Dell et al., 1994). Moreover, it should be noted that NO synthase inhibitors used in long-term potentiation studies appear to be more effective with relatively weak tetanic stimulation (Haley et al., 1993; O'Dell et al., 1994). Second, the learning paradigm may be critical to observing effects of NO synthase inhibition. We are currently exploring both hypotheses.

Regarding the aforementioned possibility of compensatory mechanisms, results of a recent study using knock-out mice have demonstrated that reduction of both neuronal and endothelial NO synthase is necessary to observe impaired long-term potentiation in the CA1 hippocampal region (Son et al., 1996). Since endothelial NO synthase has been identified in CA1 pyramidal cells, it appears that neuronal NO synthase is not exclusively involved in long-term potentiation and that other retrograde messengers, such as carbon monoxide (Stevens and Wang, 1993) or arachidonic acid (Williams et al., 1993), can function in the absence of NO synthase. Our results with *N*-Arg were obtained with only acute treatments at relatively low doses, which might not have invoked these NO synthase-independent components of long-term potentiation.

Perhaps the most important issue regarding the present results is whether the learning impairment following *N*-Arg treatment was specific to events involving interneuronal communication. *N*-Arg and NAME are not specific for neuronal NO synthase and indeed cause marked hypertension when delivered systemically via their effects on endothelial NO synthase (Boger et al., 1994; Hajj-Ali et al., 1994). Thus, the learning impairment that we observed in the current study could have been due to the effects of these inhibitors on endothelial NO synthase to produce an acute hypertensive state. It is well known that rats with a genetic predisposition for spontaneous hypertension show impairment in several learning paradigms (Fujishima et al., 1995; Meneses et al., 1996). It follows that the attenuation of the *N*-Arg induced impairment by sodium nitroprusside could have also been mediated through effects on blood pressure. The issue of endothelial NO synthase involvement could be better addressed in studies in which NO synthase inhibition is produced through central injection or with a compound that is more specific to neuronal NO synthase to avoid the hypertension associated with *N*-Arg treatment. These questions have been addressed in two other reports (Ingram et al., 1997; Meyer et al., 1997b).

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