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Putative anxiety-linked effects of the nitric oxide synthase inhibitor L-NAME in three murine exploratory behavior models

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Abstract

The aim of the current study was to extend investigation into possible linkage between nitric oxide (NO) and anxiety-linked behavior using a battery of tests. Effects of the NO synthase (NOS) inhibitor N^G-nitro-L-arginine methyl ester (L-NAME) were investigated in three murine models of anxiety—the light–dark, hole-board and elevated plus-maze—in between-groups designs. Treatment groups included L-NAME (0 [vehicle, or Veh], 10, 25, and 50 mg/kg) and 50 mg/kg of the inactive isomer N^G-nitro-D-arginine methyl ester (D-NAME) injected subcutaneously. Mice exhibited a robust anxiogenic-like response profile reflected by dose-related decreases in both light–dark (transitions and time in lighted area) and hole-board (head dips and time spent head dipping) test measures, reaching statistical significance at 25 and 50 mg/kg L-NAME when compared to Veh controls ($P < .05$ or .01; Dunnett's t test), while distance traveled and rearing showed no significant differential pattern in either model. In both models, there was a strong dissociation between nonspecific locomotion and putative exploratory behaviors. D-NAME was not significantly different from Veh condition in either model, indicating a stereospecific action and supporting NO involvement. A dose-related decrease was also observed for several traditional and ethological measures in the plus-maze; however, the effect was limited and relatively weak or absent; with the exception of open-arm and percent open-arm entries, putative anxiety-sensitive measures reached statistical significance only at the highest dose. Reductions in motor activity compromised ability to dissociate an anxiety linkage from a nonspecific motor effect in most measures. It is concluded that the hole-board and light–dark tests provide indication of anxiogenic-like action of NOS inhibition, suggesting that NO has an anxiolytic action. Data from the plus-maze are unclear, owing to a confounding motor influence in most measures.
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Keywords: Anxiety; Anxiogenic; Elevated plus-maze; Hole-board test; Light–dark test; N^G-Nitro-L-arginine methyl ester, L-NAME; N^G-Nitro-D-arginine methyl ester, D-NAME; Nitric oxide; Nitric oxide synthase inhibitor; Mouse

1. Introduction

The naturally occurring vasodilating gas, nitric oxide (NO), has been implicated in the recent literature as playing an important role in a broad spectrum of physiological and behavioral functions in animals. These include learning and memory, antinociceptive action/effects, sexual functioning, a number of ingestive-linked behaviors, regulation of autonomic functions, drug dependence, and anxiety-linked behaviors (for reviews, see Krukoff, 1999; Nelson et al., 1997; Riedel and Neeck, 2001; Szabo, 1996; Uzbay and Oglesby, 2001). There is evidence that NO functions as a neurotransmitter and intracellular messenger/signal in both central and peripheral nervous systems (Dawson and Snyder, 1994; Moncada et al., 1991). NO is synthesized from the amino acid L-arginine (L-arg) through action of the catalytic enzyme NO synthase (NOS) (Moncada et al., 1991).

Of particular interest to our laboratory have been a number of reports implicating NO signaling in mechanisms involved in anxiety-linked behaviors and which have also generated a number of conflicting findings. As is a common strategy in studies probing NO involvement, NO production was restricted through inhibiting NOS. Two of the earliest studies reported that acute systemic injection of the neuronal NOS inhibitor N^G-nitro-L-arginine (L-NOARG) antagonized the anxiolytic effect of the benzodiazepine drug chlordiazepoxide (CP) (Quock and Nguyen, 1992) and of the anesthetic gas nitrous oxide (N_2O) (Caton et al., 1994) in the elevated plus-maze in mice, thereby indicating an anxiogenic action of NOS inhibition. In both of these

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studies, L-NOARG's antagonist effect was reversed by intracerebroventricular administration of L-arg, the natural substrate for NOS, but not by the inactive isomer, D-arginine (D-arg), thus linking L-NOARG's influence to inhibition of NO production. More recently, an anxiogenic-like effect of NOS inhibition has also been reported in the elevated plus-maze in rat with systemic (De Oliveira et al., 1997; Vale et al., 1998; Pokk and Vali, 2002) and CNS (Monzón et al., 2001) administration of L-NOARG or N^G-nitro-L-arginine methyl ester (L-NAME) and in the light–dark test in mice following systemic administration of the selective neuronal NOS inhibitor 7-nitroindazole (7-NI) (Li and Quock, 2001). Most recently, it was reported that intracerebroventricular administration of an NO donor induced anxiolytic-like action in the light–dark test in mice (Li and Quock, 2002).

In contrast, several investigators have recently reported anxiolytic-like effects of L-NAME injected systemically (Faria et al., 1997) or centrally into the dorsal periaqueductal gray (PAG) (Guimarães et al., 1994) and of systemic injections of 7-NI (Dunn et al., 1998; Volke et al., 1997; Yildiz et al., 2000) in the elevated plus-maze in the rat. Anxiolytic-like action of 7-NI has also been observed for the rat social interaction test, and for the mouse plus-maze and light–dark exploratory tests (Volke et al., 1997). NOS inhibition has also been reported to reduce isolation-induced ultrasonic vocalizations in rat pups (Campbell et al., 1999; Podhorna and Brown, 1999). The basis(es) for these differing findings is unknown.

The present study further probed the effect of the nonselective NOS inhibitor L-NAME in the mouse light–dark exploratory test, a paradigm previously observed to reflect anxiolytic-like behavior only under quite high doses of 7-NI (Volke et al., 1997), an exploratory model in which L-NAME has not previously been evaluated—the mouse hole-board model—and the elevated plus-maze. In light of the widespread use of the plus-maze in the research herein cited, it was considered essential to include this paradigm in the battery of tests for comparison control purposes. We also included several ethological, as well as the more traditional/conventional, measures in the plus-maze—measures only recently reported in purported NO-linked studies.

2. Materials and methods

2.1. Animals

Adult male ICR mice from our breeding colony, weighing approximately 35–50 g at time of testing, were group housed in standard 15 × 26 × 12-cm-high opaque polypropylene tub-type cages (three to five animals per cage) and maintained on a 12:12 light–dark cycle (lights on from 0700 to 1900 h) in a temperature- and humidity-controlled colony room with ad lib access to pelleted food (Teklad rodent diet 8604) and tap water. Animals were used only once, and all testing procedures were carried out during the light period between 0900 and 1530 h.

2.2. Drugs

L-NAME and N^G-nitro-D-arginine methyl ester (D-NAME) were purchased from Sigma (St. Louis, MO) and were freshly prepared in sterile 0.9% NaCl vehicle (Veh) on the morning of testing. Drugs (or Veh) were injected subcutaneous in a volume of 0.1 ml/10 g of body weight.

2.3. Apparatus

2.3.1. Light–dark unit

The light–dark unit developed by Crawley and Goodwin (1980) was a polycarbonate cage (44 × 21 × 21 cm high) divided into two compartments: one twice as large as the other and separated by a partition containing a 5-cm-high by 13-cm-wide opening. The smaller compartment was covered, and all its surfaces were coated with flat black paint to provide a dark chamber. The larger compartment was illuminated from above with a fluorescent lamp providing approximately 10 ft-candles at floor level, which provided the only source of lighting in the test area. Small objects were placed in several locations on the floor of the lighted compartment. This was done, based on pilot data, to promote animals engaging in a baseline number of transitions under Veh condition and provide opportunity for drug-induced shifts in either direction—thereby reducing likelihood of ceiling or floor effects.

2.3.2. Hole-board unit

The hole-board, as modified by File and Wardill (1975), was a circular, enclosed polypropylene (opaque) arena 18 cm high and 31 cm in diameter with four holes (3 cm diameter) equally spaced in the floor. Infrared photodetector/emitter pairs were positioned below each hole to electronically monitor number and duration of head dips. The room was dimly lit (lighting at test area floor was approximately 6 ft-candles).

2.3.3. Plus-maze

The elevated plus-maze was a modification of the apparatus validated for NIH Swiss mice by Lister (1987) and consisted of two open (30×5.5×.25 cm) and two closed (both 30 × 5.5 × 15 cm) arms radiating from a common central platform (5.5 × 5.5 cm) at 90° shifts to form a plus shape. The maze floor was constructed of Plexiglas painted flat black, and the walls of the enclosed arms were clear Plexiglas. As previously reported (e.g., Rodgers and Johnson, 1995), a slightly raised edge (0.25 cm) along the perimeter of the open arms provided some protection against the animal falling off the maze. Data from animals that did fall off were discarded. The entire apparatus was elevated on a rigid stalk to a height of 60 cm above floor level. Lighting at floor level was approximately 6 ft-candles.

2.4. Behavioral testing procedures

On the day of testing, the animal was weighed to the nearest 0.5 g and injected subcutaneously with 10, 25, or 50 mg/kg of L-NAME, or 50 mg/kg of D-NAME, or 0.9% NaCl Veh. Then the animal was placed in an individual cage and transferred to a holding area adjacent and illuminated similar to the test area. Forty-five minutes later, the animal was moved to the test area and placed into the apparatus. Apparatus floor (subfloor as well in hole-board unit) and wall surfaces were cleaned between animals; they were first cleaned with water, then wiped with a 50% ethyl alcohol solution. A camcorder was positioned 160 or 175 cm above the apparatus floor and connected to a VCR and video monitor located in an adjoining room, where the investigator was also located during all testing. Order of treatment conditions was counterbalanced with different dose sequences being run on different days. Videotapes were submitted to blind review by one or, in the majority of cases, two trained observers per measure. In the latter instances, interobserver agreement was assessed with Pearson Product–Moment Correlation procedures yielding Pearson r's ranging from .78 to .97 (all $P < .01$). All research protocols were reviewed and approved by Marquette University's Institutional Animal Care and Use Committee (IACUC) and are in compliance with the USDA Animal Welfare Act.

2.4.1. Light–dark test

The mouse was placed in the center of the lighted compartment and observed over a 10-min test period, which began with the first transition into the dark compartment. Behavioral measures included time in light and dark compartments, number of between-compartment transitions, as well as distance traveled and number of rears in lighted compartment. Distance was measured by tracking horizontal movement of the mouse from the videotaped image displayed on a horizontally positioned video monitor with a hand-held electronic planimeter (Scalex 'PlanWheel XL') with a resolution of 1 mm.

2.4.2. Hole-board

The mouse was placed in the center of the arena and observed over an 8-min test period. Behavioral measures included number of head dips, total time spent head dipping, distance traveled, and number of rears.

2.4.3. Plus-maze

The mouse was placed on the central platform of the maze facing one of the open arms to begin a 10-min test period. Traditional spatiotemporal measures included frequency of open- and closed-arm entries and length of time (seconds) spent in the different sections of the maze. In addition, entries into, and time spent in, open arms as a percent of total arm activity were calculated (i.e., open-arm entries/total arm entries and open-arm time/total time in arms). Arm entry and exit were operationally defined as all four paws into or out of an arm. Distance traveled and rears were included as measures of general locomotor activity. Measures designated as ethological and described in Rodgers and Johnson (1995) and Rodgers et al. (1999) included frequency of rears, of head dips (exploratory movement of head/shoulders over side of maze) from different maze regions, of stretched-attend postures (exploratory posture whereby body is stretched forward and then retracted to original position without any forward locomotion) into arms, and of flat-back approach behavior (exploratory motion where animal stretches to its full length and cautiously moves forward) into arms. Head dips, stretch-attends, and flat-back approaches have been characterized as possibly reflecting risk assessment behavior.

2.5. Statistical analyses

Data for behavioral measures were evaluated separately with independent measures one-way analyses of variance (ANOVAs). Pairwise comparisons were made with Dunnett's t tests. Minimally acceptable alpha level was set at $P \leq .05$. Owing to differences in observation time window for general activity measures, distance traveled was initially adjusted for length of test (hole-board and plus-maze) or time in lighted compartment (light–dark test) and reported as mean centimeters per minute; rears were similarly adjusted and reported as mean rears per minute.

3. Results

3.1. Light–dark test

Light–dark measures are shown in Fig. 1. Both number of between-compartment transitions and time spent in lighted compartment were attenuated in a dose-related manner. The ANOVAs yielded $F(4,95) = 7.14$ and 3.52 for transitions and time in lighted compartment, respectively (P=.0001 and .010, respectively). When compared to Veh condition, mice administered doses of either 25 or 50 mg/kg of L-NAME showed statistically significantly fewer transitions and less time in lighted area ($P < .05$ or .01, Dunnett's t test). The group receiving 50 mg/kg D-NAME was not significantly different from the Veh condition on either measure ($P > .05$, Dunnett's t test). The ANOVAs for distance traveled and rears in light side/compartment were not statistically significant (P=.241 and .215, respectively).

3.2. Hole-board test

Hole-board measures are shown in Fig. 2. Both number of head dips and time spent head dipping were attenuated in a clear dose-related manner. The ANOVAs yielded $F(4,106) = 6.10$ and 9.52 for head dips (P=.0002) and total head dipping time (P=.0001), respectively. When compared to Veh con-

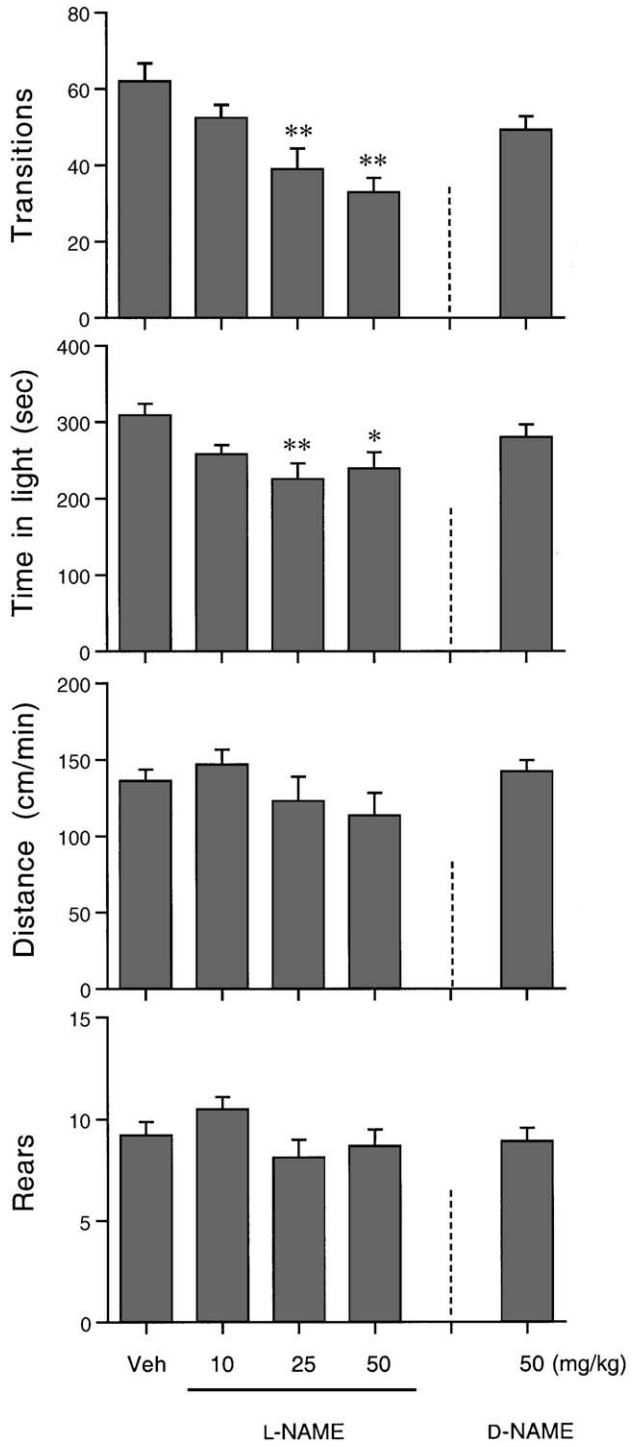

Fig. 1. Mean (±S.E.M.) number of between-compartment transitions (top panel), time spent in lighted compartment (second panel), distance (cm/min) traveled in lighted compartment (third panel), and number of rears per minute of time in lighted compartment (bottom panel) in light–dark unit under doses of L- or D-NAME. $*P<.05$, $**P<.01$ compared to Veh condition; Dunnett's t test, one tail. Group n's were 20 each.

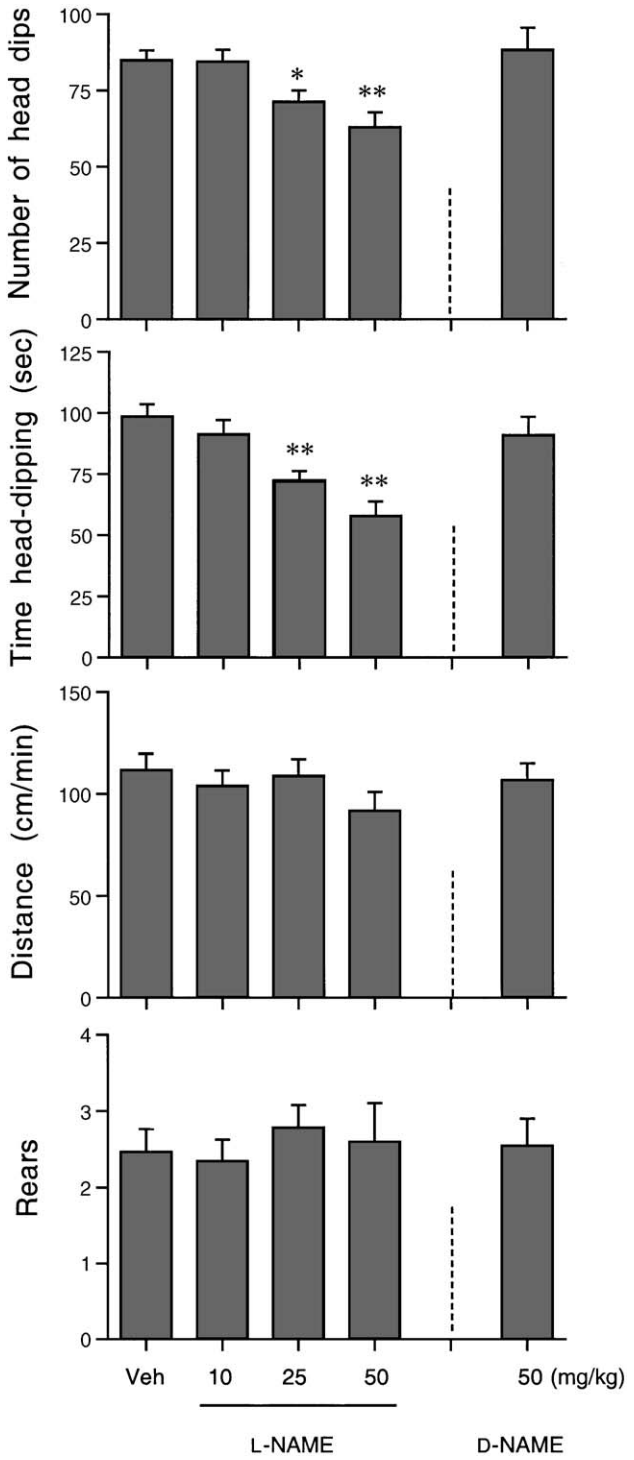

Fig. 2. Mean (±S.E.M.) number of head dips (top panel), total time spent head dipping (second panel), distance (cm/min) traveled (third panel), and number of rears (bottom panel) in hole-board unit under doses of L- or D-NAME. $*P<.05$, $**P<.01$ compared to Veh condition; Dunnett's t test, one tail. Group n's were 25, 25, 25, 23, and 13, respectively, for 0 (Veh), 10, 25, and 50 mg/kg L-NAME and 50 mg/kg D-NAME.

dition, mice administered either 25 or 50 mg/kg of L-NAME showed significant reductions in both behaviors ($P<.05$, or .01, Dunnett's t test). Again, the 50 mg/kg D-NAME group was not significantly different from Veh condition on either measure ($P>.05$, Dunnett's t test). Again, the ANOVA for distance traveled ($P=.461$) and rears ($P=.920$) failed to reach statistical significance.

3.3. Plus-maze

All plus-maze measures are shown in Table 1. Of the more traditionally used measures, number of arm entries was significantly affected in a dose-related manner. The ANOVAs yielded $F(4,98) = 4.06$, 3.80, and 6.59 for open (P=.004), closed (P=.007), and total (P=.001) arm entries, respectively. When compared to Veh condition, all three doses of L-NAME reduced open-arm entries ($P < .05$ or .01), closed-arm entries were reduced only at 50 mg/kg ($P < .05$), and total arm entries were reduced at both 25 and 50 mg/kg ($P < .01$), all Dunnett's t tests. The ANOVA for percentage of open-arm entries yielded $F(4,98) = 2.53$ (P=.045), with both 25 and 50 mg/kg of L-NAME reaching statistical significance ($P < .05$) when compared with Veh condition. While a dose-related attenuating pattern was also exhibited for time spent in open arms, no time-related ANOVA reached statistical significance (all P>.05). The ANOVAs yielded $F(4,98) = 3.97$ and 3.88 for distance traveled (P=.005) and rears (P=.006), respectively; when compared to Veh condition, both measures reached statistical significance only at the 50 mg/kg dose ($P < .05$ or .01). Findings for ethological measures were as follows. ANOVAs yielded $F(4,98) = 4.52$ and 4.34 for number of head dips from center (P=.002) and from closed arms (P=.003), respectively. When compared to Veh condition, number of head dips was significantly lower from center area ($P < .05$) and closed arms ($P < .01$) under 50 mg/kg of L-NAME. Head dipping from open arms was not significant (P=.147). ANOVAs yielded $F(4,98) = 4.08$ and 3.44 for number of stretch-attend postures into open (P=.004) and closed (P=.011) arms, respectively. When compared to Veh condition, posturing from both arms was significantly attenuated at 50 mg/kg of L-NAME ($P < .05$ or .01). An ANOVA yielding $F(4,98) = 2.62$ (P=.040) for flat-back approach behavior into open arms was a consequence of relatively higher flat-back activity under 10 mg/kg of L-NAME; Dunnett's tests revealed no significant difference between Veh condition and any dose of L-NAME. No significant differences were found between Veh and D-NAME conditions for any traditional or ethological measure.

4. Discussion

The aim of the current study was to further investigate the possible effect(s) of NOS inhibition on putative anxiety-linked behaviors, herein using a battery of three of the more widely used and validated animal models employed in probing drug effects/influence on anxiety-linked mechanisms and behavior and using a series of systemically administered moderate doses of the nonspecific NOS inhibitor L-NAME-doses often reported in the NO literature. We report findings for the hole-board test, which has, to our knowledge, not previously been reported in the NO-anxiety literature, and also incorporate a number of putative risk assessment measures in the plus-maze model. We operated under the premise that testing under multiple models in the same laboratory under similar conditions of handling and other procedural influences would provide opportunity for more direct comparison and serve as a distinct control advantage with respect to extraneous factors that might influence behavioral outcomes.

Table 1
Effect of L- or D-NAME on various behavioral measures in the elevated plus-maze

Behaviors	Dose (mg/kg)					
	L-NAME				D-NAME	
	Veh (0)	10	25	50	50	
ENT-open	8.8 ± 1.4	5.1 ± 1.1 *	3.7 ± 0.8 **	3.6 ± 0.8 **	7.1 ± 1.3	F=4.06, P=.004
ENT-closed	15.0 ± 0.9	16.3 ± 1.0	12.3 ± 1.0	11.0 ± 1.3 *	15.3 ± 1.4	F=3.80, P=.007
ENT-total	23.8 ± 1.7	21.4 ± 1.2	16.0 ± 1.5 **	14.6 ± 1.8 **	22.4 ± 1.6	F= 6.59, P=.001
% ENT-open	34.3 ± 4.0	22.3 ± 4.1	21.3 ± 3.4 *	19.6 ± 3.6 *	30.5 ± 5.1	F=2.53, P=.045
Rears	7.7 ± 1.3	5.5 ± 0.9	6.2 ± 1.3	3.2 ± 0.9 *	9.9 ± 1.7	F=3.88, P=.006
Distance	59.5 ± 4.1	49.9 ± 3.9	49.7 ± 5.6	39.1 ± 4.0 **	62.0 ± 4.9	F=3.97, P=.005
TI-open	115.7 ± 16.2	81.2 ± 17.5	64.5 ± 11.2	66.8 ± 13.7	80.4 ± 13.8	F=2.03, P=.096, ns
TI-closed	322.6 ± 19.7	361.2 ± 20.3	330.3 ± 13.8	385.9 ± 24.8	312.6 ± 20.3	F=2.26, P=.069, ns
% TI-open	26.7 ± 3.7	18.2 ± 3.8	15.8 ± 2.6	15.6 ± 3.2	20.8 ± 3.4	F=1.94, P=.110, ns
HD-center	21.8 ± 1.8	16.7 ± 1.9	25.1 ± 2.0	15.5 ± 1.9 *	22.2 ± 1.7	F=4.52, P=.002
HD-open	32.5 ± 5.3	19.1 ± 3.9	23.7 ± 4.8	16.7 ± 3.7	23.5 ± 5.1	F=1.74, P=.147, ns
HD-closed	24.1 ± 2.1	17.8 ± 2.5	17.2 ± 2.7	11.8 ± 2.2 **	23.4 ± 2.6	F=4.34, P=.003
SAP-open	14.6 ± 2.4	10.1 ± 2.5	8.9 ± 1.7	4.2 ± 1.1 **	15.7 ± 3.2	F=4.08, P=.004
SAP-closed	24.0 ± 2.3	25.8 ± 3.1	19.3 ± 2.6	13.8 ± 2.3 *	25.5 ± 3.3	F=3.44, P=.011
FBA-open	2.4 ± 0.5	2.8 ± 0.8	0.8 ± 0.3	1.0 ± 0.4	2.0 ± 0.6	F=2.62, P=.040
FBA-closed	1.6 ± 0.9	0.7 ± 0.3	2.7 ± 0.7	1.3 ± 0.6	3.1 ± 1.0	F=1.78, P=.139, ns

Group n's were 22, 20, 21, 20, and 20, respectively for 0, 10, 25, and 50 mg/kg L-NAME and 50 mg/kg D-NAME, df (4,98) for all F tests.
ENT = entries; TI = time; HD = head dips; SAP = stretch-attend postures; FBA = flat-back approaches.

* $P < .05$ compared to Veh control condition; Dunnett's t test, one tail.

** $P < .01$ compared to Veh control condition; Dunnett's t test, one tail.

In both the light–dark and hole-board environments, L-NAME induced a robust, dose-related decrease in exploratory behaviors generally considered to reflect anxiety-linked action of drugs in these models (Crawley, 1985; Crawley and Goodwin, 1980); although the specificity of such linkage for transitions in the light–dark test appears to be somewhat controversial (Kilfoil et al., 1989). The direction of these putative exploratory behavioral shifts indicates an anxiogenic-like action of L-NAME. Distance traveled by mice over the observation period, reflecting general locomotor activity, showed no significant differential pattern across doses of L-NAME in either model, thus revealing a clear dissociation between drug-induced reduction of exploration and nonspecific locomotion. Vertical motor activity (rears) also failed to show a significant pattern, indicating absence of compromised motor function, as e.g., ataxia. Finally, the inactive isomer D-NAME, which is not a substrate for NOS, was essentially without effect in either paradigm when compared to Veh control in the current study, thus supporting a stereospecific drug action and clearly indicating involvement of NO.

Our light–dark test findings are consistent with a recent study revealing antagonism of both N_2O- and CP-induced anxiolysis, where anxiety reduction by these drugs is shown by increases in time spent in lighted compartment, by the selective neuronal NOS inhibitor 7-NI; an N_2O-induced increase in transitions was not reversed (Li and Quock, 2001). The light–dark exploration test is based on natural tendencies of rodents to explore a novel environment, but to avoid a brightly lit open area (for reviews, see Crawley, 1985; Menard and Treit, 1999). Li and Quock's (2001) findings, however, are in partial conflict with findings earlier reported by Volke et al. (1997). Volke's group observed that in mice 7-NI produced a significant decrease in number of between-compartment transitions, but an increase in time spent in the lighted compartment, the latter suggesting an anxiolytic effect; both shifts, however, were seen only at quite high doses (80 and 120 mg/kg). Indeed, this might not be unexpected; while reduced horizontal locomotion in rats (Sandi et al., 1995) and mice (Moore et al., 1991; Starr and Starr, 1995) in unfamiliar or novel environments and altered exploratory patterns (Sandi et al., 1995; Moore et al., 1991) have been linked to systemic injection of NOS inhibitors, these were also observed only at relatively high doses (e.g., $\geq$100 mg/kg). Volke's group argued that time in lighted compartment is a more sensitive/specific indicator of anxiolytic action of drugs (Kilfoil et al., 1989), and that an observed reduction in open-field behavior at the higher doses suggests that the drop in transitions might reflect a sedative rather than anxiogenic effect of the drug. Our animals, however, did not exhibit signs of sedation, and both distance traveled and rears showed no differential pattern across doses, as did transitions. Transitions might be a function of the novelty of connecting environments having different characteristics, and could reflect/promote exploratory activity (Crawley and Goodwin, 1980). Good agreement has been observed between relative potency of drugs clinically used in the treatment of anxiety in humans and their ability to facilitate exploratory activity in the light–dark paradigm in mice (Crawley, 1981). It has been reported as well that agents having anxiogenic properties can reduce number of transitions in the light–dark test in mice (Shimada et al., 1995). Most recently, Li and Quock (2002) provided further support for an anxiolytic effect of NO, reporting that administration of the NO donor, 3-morpholinosyndnonime (SIN-1) significantly increased time spent in lighted compartment in control mice in the light–dark test; SIN-1 also significantly increased/facilitated the anxiolytic effect (increase) of N_2O on both time in lighted compartment and number of between-compartment transitions. These findings are consistent with a putative anxiogenic effect of NOS inhibition.

The hole-board model, as noted above, also yielded a consistent anxiogenic-like action of L-NAME. Both frequency of head dips and total time spent head dipping were attenuated in a dose-related manner, reaching statistical significance at 25 mg/kg of L-NAME and suggesting an anxiogenic action. In this model, it has been established that head dipping behavior in mice and rats reflects exploration distinct from general motor activity, studies having shown that it reflects novel aspects of the environment and that it results in information storage, the latter shown by response habituation upon reexposure to the hole-board environment (File, 2001). Comparison studies using NOS inhibitors are not currently available for the hole-board model.

L-NAME also induced a significant dose-related reduction in several traditional and ethological, putative risk assessment, exploratory behavioral measures in the elevated plus-maze, again suggestive of an anxiogenic-like action. A reduction in a number of measures considered to reflect a nonspecific locomotor influence were observed as well, principally at the highest dose of L-NAME. Consequently, dissociation between putative exploratory behaviors and a nonspecific locomotor effect was relatively weak or absent, respectively, for traditional and ethological measures. In all cases of L-NAME effects achieving statistical significance, D-NAME was without significant effect when compared to Veh group, again indicating a stereospecific action.

Of the more traditional spatiotemporal measures purportedly indicating an anxiety-linked effect, the number and percentage of open-arm entries were significantly lower under 10 and 25 mg/kg, respectively, of L-NAME than under Veh condition, suggesting an anxiogenic effect. At the same time, several measures seemingly reflecting a nonspecific motor influence, including closed and total arm entries and distance traveled and rears, were also statistically significantly lower than controls, although at the highest dose of L-NAME only, for all except total arm entries (significant at 25 mg/kg dose). Total arm entries, however, arguably do not reflect exclusively a locomotor influence, given that open-arm entries contribute as well. Factor analytic studies reveal

that while closed-arm entries load only on an 'activity' factor, total arm entries load on both 'anxiety' and 'activity' (Rodgers and Dalvi, 1997). These data, while not dissociatively robust, do however reveal trends in agreement with a number of previous reports of anxiogenic action of acute systemic (Caton et al., 1994; Quock and Nguyen, 1992) or CNS (Monzón et al., 2001) administration of NOS inhibitors in the plus-maze. Monzón et al. (2001) observed that acute L-NAME produced anxiogenic-like decreases in a broad spectrum of traditional measures when injected into amygdala and hippocampus, regions known for their role in anxiety. In sharp contrast, however, it has also been reported that acute systemic (Faria et al., 1997; Dunn et al., 1998; Volke et al., 1997; Yildiz et al., 2000) or CNS (Guimarães et al., 1994) injection of NOS inhibitors can have an anxiolytic action. Interestingly, Volke et al. (1997) noted a biphasic effect of 7-NI in both percent of open-arm entries and time spent in open arms, with a significant increase being observed only at a high dose of 90 mg/kg of 7-NI. Data from several ethological measures, while significant and in the predicted direction, were observed only at the highest dose of L-NAME and could be accounted for by a nonspecific locomotor effect as well. As already noted, motor-linked measures were also significantly lower under 50 mg/kg of L-NAME in the plus-maze.

In summary, results or aspects of the current study are (1) L-NAME can produce an anxiogenic-like effect, although not consistently observed, in three prominent murine exploratory models used to screen drugs for anxiety-linked behaviors including a model not previously evaluated in the NO-anxiety literature; (2) the effect appears to be stereospecific, not being exhibited under the inactive isomer D-NAME, thus lending support for NO involvement; and (3) while robust effects were found in the light–dark and hole-board models, the effect was relatively weak and inconclusive in the elevated plus-maze.

Based on current findings, we conclude that NO is involved in mechanisms mediating anxiety and that inhibiting NO production can, under certain conditions, produce an anxiogenic effect. This was expressed quite robustly in the light–dark and hole-board tests. While dose-related trends in the plus-maze were observed and might point to an anxiogenic effect as well, these data were clearly inconclusive. Current protocols and findings do not address inconsistencies noted as existing in the literature. It is suggested that further studies will need to include employing protocols involving administration of NOS inhibitors directly into CNS sites.

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