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Decreased spontaneous motor activity and startle response in nitric oxide synthase inhibitor-treated rats

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Abstract

In the central nervous system, nitric oxide has been proposed to be a retrograde messenger mediating learning and synaptic plasticity. Since only pretraining injections of nitric oxide synthesis inhibitors were shown to impair learning, we examined the possibility that systemic administration of these inhibitors might influence some non-specific aspects related to the organism's general psychophysiological status. Intraperitoneal administration of N^G-nitro-L-arginine methyl ester (30 or 100 mg/kg) 60 min pre-test to adult rats resulted in: (i) altered exploratory pattern and reduced locomotion in a novel environment; (ii) reduced startle response to either acoustic or electric stimuli; and (iii) cardiovascular alterations. In addition, intracerebroventricular administration of *N*-nitro-L-arginine (10 μl of a 10 mM solution) diminished the acoustic startle response. Specificity of these effects through nitric oxide was supported by the ability of the nitric oxide precursor, L-arginine, to prevent the inhibitors actions. These findings indicate that nitric oxide inhibitors interfere with the general psychophysiological status of the organism.

Keywords: Nitric oxide (NO); Locomotor activity; Startle response; Psychophysiological status; Learning

1. Introduction

Nitric oxide, a free radical gas initially identified as the 'endothelium-derived relaxing factor', is now known to play a wide array of roles in different physiological systems (Moncada et al., 1991). Nitric oxide is generated from L-arginine by the enzyme, nitric oxide synthase, which exists in different isoforms, one or more being inducible, identified in hepatocytes, neutrophils, macrophages and glial cells, and at least two constitutive forms, identified in endothelial cells and neurones (Schuman and Madison, 1994). In the brain, nitric oxide synthase has been localised in discrete neuronal populations, in particular in areas such as the cerebellum, hippocampus, striatum, cortex, hypothalamus, midbrain, olfactory bulb, and medulla of the rat (Bredt et al., 1990; Bredt and Snyder, 1992; Garthwaite, 1991).

Recently, evidence has accumulated, showing that nitric oxide acts as an intercellular messenger in the central nervous system (Garthwaite et al., 1988; Vincent, 1994) and plays an important role in certain types of synaptic plasticity (Schuman and Madison, 1994). Given its easy diffusibility, nitric oxide was regarded as the possible retrograde messenger hypothesised by learning and memory neurobiologists to increase the synaptic strength underlying memory formation (O'Dell et al., 1991). Several studies have shown that nitric oxide synthase inhibitors prevent the induction of long-term potentiation in vitro (Böhme et al., 1993; Haley et al., 1992; O'Dell et al., 1991; Schuman and Madison, 1994) and in vivo (Mizutani et al., 1993), although some forms of long-term potentiation appear insensitive to treatment with nitric oxide synthase inhibitors (Haley et al., 1993). Long-term depression, another type of synaptic plasticity consisting of persistent decreases in synaptic strength, is also blocked by extracellular application of nitric oxide synthase inhibitors (Crepel and Jaillard, 1990). Psychopharmacological studies have also implicated nitric oxide in the neural mechanisms involved in learning and memory processes. Thus, systemic injection of nitric oxide synthase inhibitors has been proved to interfere with formation of memory for spatial learning (Böhme et al., 1993; Chapman et al., 1992) and olfactory learning in rats (Böhme et al., 1993), conditioned eyeblink learning in rabbits (Chapman et al., 1992), and passive avoid-

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ance learning in chicks (Hölscher and Rose, 1992) but not in rats (Böhme et al., 1993). It should also be noted that intracerebral injection of nitric oxide synthase inhibitors into learning-related brain structures was also found to interfere with certain types of learning tasks in rats (Ohno et al., 1993) and chicks (Hölscher and Rose, 1993). Interestingly, these studies showed that nitric oxide synthase inhibitors were effective to prevent learning when administered prior to the training trials but, although some experiments included post-training injections, there are no reports of memory impairment by post-training injections. Certainly, this is consistent with data from long-term potentiation studies suggesting that nitric oxide production is only required for long-term potentiation induction, but not necessary once long-term potentiation has been established (Haley et al., 1992; O'Dell et al., 1991). However, the possibility remains that in vivo pre-training treatments, particularly systemic administration, could be inducing non-specific effects which could influence acquisition of the task. This possibility is particularly conceivable given the well known vasoconstrictor effects of nitric oxide synthase inhibitors (Moncada et al., 1991).

Therefore, we considered the possibility that systemic administration of nitric oxide synthase inhibitors could influence some aspects of the organism's general psychophysiological status, as evaluated here on spontaneous locomotion, startle responses, and cardiovascular responses. In addition, knowledge about behavioural modulation of nitric oxide is scarce. Given the relevance of exploratory/locomotor components in most animal learning test designs, as well as the number of tasks based on responsiveness to aversive stimulation, a novel environment 'activity cage' and different versions of startle response tasks were selected to test for possible behavioural effects of nitric oxide synthase inhibitors. Thus, the present work was designed to investigate whether systemic administration of the nitric oxide synthase inhibitor, N^G-nitro-L-arginine methyl ester, might interfere with performance of these behavioural tasks. To check for possible physiological alterations, blood pressure was also assessed at the same time after N^G-nitro-L-arginine methyl ester administration as the behavioural tests were done. Finally, we also assessed whether central inhibition of nitric oxide synthase through intracerebroventricular administration of a nitric oxide synthase inhibitor could mimic the systemic effects observed on behaviour.

2. Materials and methods

2.1. Animals

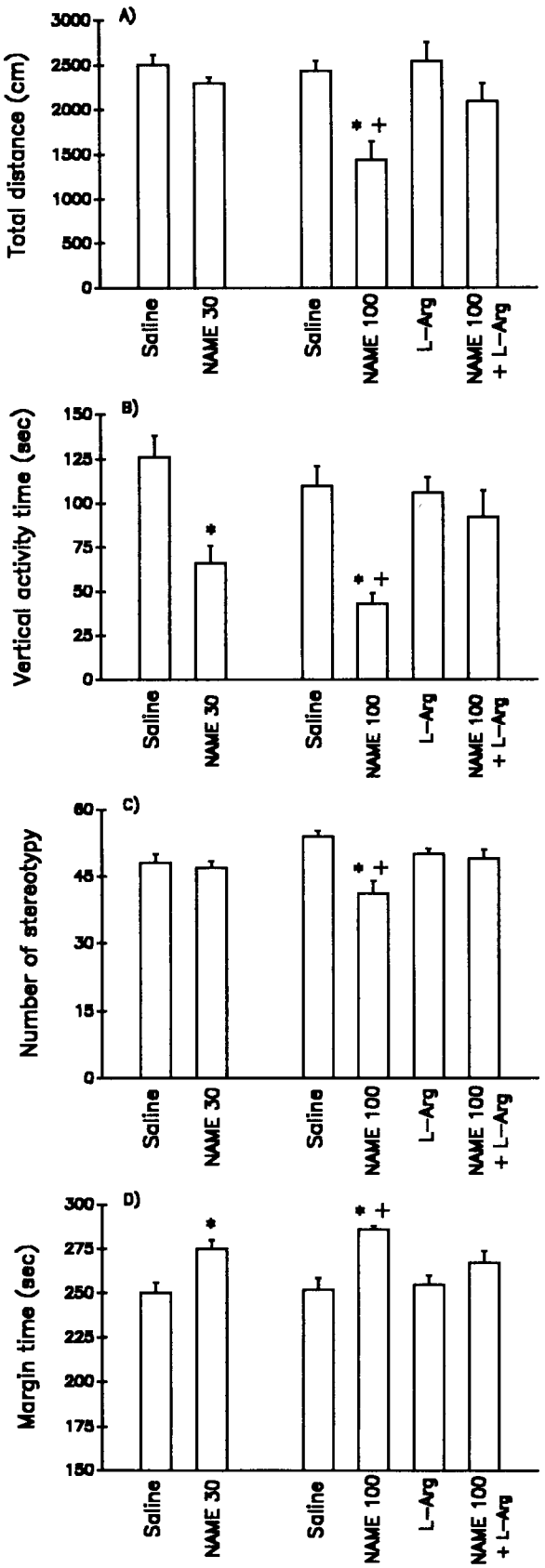
Adult male Wistar rats (250–300 g) from our in-house colony were used. They were housed 4–5 per cage under temperature ($22 \pm 2°$ C) and light (12:12 light-dark cycle; lights on at 7.00 a.m.) controlled conditions and had free access to food and water. Testing always occurred between 10:00 and 14:00 h. Animal care procedures were conducted in accordance with the guidelines set by the European Community Council Directives (86/609/EEC).

2.2. Spontaneous locomotor activity

A Digiscan Animal Activity Monitor System (activity cage), model RXYZCM TAO (Omnitech Electronics, Colombus, OH, USA) previously described (Sandi et al., 1992), was used to assess the activity of the animals in a novel environment. Briefly, the apparatus consists of a square area in which a plastic animal cage ($40 \times 40 \times 35$ cm) is placed. It contains two perpendicular arrays of 15 horizontal infrared beams and two vertical light screens (infrared). Each interruption of the beam generates an electric impulse counted by an internal electronic counter. Rats were tested in the activity cage for a 5-min session and different types of movement were precisely recorded. Data for the following variables of locomotor activity detected by the activity monitor were collected by an IBM compatible computer system: (a) the distance travelled by the animal in cm; (b) vertical activity, the total number of beam interruptions in the vertical sensor; (c) number of stereotypic movements, this parameter increased when the same beams were broken repeatedly within a 1-s interval; (d) margin time, the time in seconds that the animal spent in the margins of the cage; (e) average speed, the average speed of the animal's movement in cm/s.

2.3. Startle response

Startle responses were measured in a stabilimeter cage (Responder Economy, Colombus, OH, USA). It was constructed of Plexiglas and wire mesh and suspended within a steel frame between compression springs and an accelerometer. Cage movement resulting from a startle response was transduced by the accelerometer into a voltage that was proportional to the displacement velocity. The stabilimeter was housed in a sound-attenuating chamber with a background noise. For acoustic startle measurements, the cage was located 10 cm from a high-frequency speaker that delivered the acoustic stimuli. After a 5-min acclimation period, during which time there was no stimulation, each rat received 10 startle stimuli (each had a duration of 20 ms, including 0.4-ms rise-fall times) separated by a variable interval averaging 15 s. In different experiments, two stimulation conditions were tested: (i) 400 Hz, 105 dB; and (ii) 4000 Hz, 105 dB. These acoustic stimulation conditions were previously



reported to be around the startle threshold of the rat (Pilz et al., 1987). They were, therefore, selected to evaluate the ability of nitric oxide synthesis inhibitors to modulate startle responses close to the threshold, for its potential implication in training situations demanding an intermedial responsiveness. For electric shock-induced startle measurements, electric stimulation was delivered with a current shock generator through the floor of the box, which consisted of a grid of stainless steel rods. After a 5-min pre-period, rats received 10 shocks of 0.3-mA intensity with a variable intertrial interval averaging 15 s.

2.4. Surgery

In one experiment, the rats were intracerebroventricularly (i.c.v.) cannulated. The animals were anaesthetised with an i.p. injection of pentobarbital (Euta-Lender, Normon, Spain) dissolved in saline, in a dose of 40 mg/kg. When anaesthesia became apparent, the rats were placed in a stereotaxic apparatus where their temperature was maintained by an electric heating pad. A unilateral craniotomy was performed following the stereotaxic coordinates of Paxinos and Watson (1982) at 1.4 mm lateral to the midline and 0.8 mm anterior to the bregma. A sterile stainless 21-gauge guide cannula of 4 mm was inserted below the dura into the right lateral cerebral ventricle. Seven days were allowed for recovery after cannulation. After completion of the experiment, methylene blue dye was injected (10 μl) and the placement of the cannula was confirmed by observing the site and extent of staining.

2.5. Blood pressure recording procedure

Measurements of blood pressure were made using the tail-cuff procedure (Pressure Computer LE 5007, Letica, Spain). The rat was placed in a restrainer and vascular dilation was induced by warming the animal for 20 min on a metallic heat source. A photoelectric devise, placed around the rat's tail, measured blood pressure. Five subsequent recordings separated by a 60-s interval were taken for each rat. In addition to blood pressure, heart rate was also recorded. The data for each animal were expressed as means of five measurements. Blood pressure was calculated as the mean arterial pressure in mm Hg, using the formula [systolic

Fig. 1. Effects of intraperitoneal injection of saline, N^G-nitro-L-arginine methyl ester (L-NAME; 30 or 100 mg/kg), L-Arg (350 mg/kg) or N^G-nitro-L-arginine methyl ester (L-NAME; 100 mg/kg) plus L-Arg (350 mg/kg) on the different parameters of spontaneous locomotor activity in a novel environment evaluated 60 min post-injection. Results are the means ± S.E.M. for 8–11 animals per group. * $P < 0.01$ vs. corresponding saline, + $P < 0.05$ vs. L-NAME + L-Arg group.

blood pressure − diastolic blood pressure)/3] + diastolic blood pressure, and heart rate was recorded as beats/min.

2.6. Drug administration

N^G-Nitro-L-arginine methyl ester (30 or 100 mg/kg; Sigma) and L-arginine (L-Arg, 350 mg/kg; Sigma) were dissolved in saline and injected intraperitoneally in a volume of 0.5 ml (pH 7.2). The effect of central administration of a nitric oxide synthase inhibitor was studied with *N*-nitro-L-arginine (10 mM; Sigma) dissolved in acidified normal saline with sonication immediately before use and injected intracerebroventricularly in a volume of 10 μl/rat (pH 6.8). Rats were injected with the drugs (cf. specific protocols) 60 min prior to exposure to the different tests. Control rats received a vehicle injection (same pH as their correspondingly treated group).

2.7. Statistics

The results were expressed as means ± S.E.M. Data from the startle response tasks were previously submitted to logarithmic transformation. In experiments involving two experimental groups, the data were analysed using Student's *t*-test. When experiments involved three or more groups of animals, the means were compared using one- or two-way analysis of variance (ANOVA) followed, when appropriate, by a Newman-Keuls post-hoc test.

3. Results

3.1. Effects of systemic administration of N^G-nitro-L-arginine methyl ester on the exploratory behaviour displayed in a novel environment

The effects of i.p. administration (60 min prior to testing) of the inhibitor of nitric oxide synthase, N^G-nitro-L-arginine methyl ester, were evaluated on the exploratory behaviour shown by rats exposed to a novel environment (activity cage). At the lower dose tested (30 mg/kg), N^G-nitro-L-arginine methyl ester reduced 'vertical activity' ($df = 18$, $t = 3.87$, $P < 0.001$) and increased the time rats spent in the margins of the cage ($df = 18$, $t = 3.12$, $P < 0.005$), but not any other parameter involved in exploratory behaviour in the activity cage (Fig. 1). However, the dose of 100 mg/kg profoundly influenced the general pattern of behaviour. As shown in Fig. 1, in addition to influencing 'vertical activity' (ANOVA for the four treatment groups: $F(3,33) = 8.88$, $P < 0.0002$; Newman-Keuls test N^G-nitro-L-arginine methyl ester vs. saline: $P < 0.01$) and 'margin time' (ANOVA for the four treatment groups: $F(3,33) = 9.06$, $P < 0.0002$; Newman-Keuls test N^G-nitro-L-arginine methyl ester vs. saline: $P < 0.01$) parameters in the same way as the 30 mg/kg dose, this higher dose also decreased the horizontal activity parameter 'total distance' (ANOVA for the four treatment groups: $F(3,33) = 6.93$, $P < 0.001$; Newman-Keuls test N^G-nitro-L-arginine methyl ester vs. saline: $P < 0.01$) and the 'stereotypy number' (ANOVA for the four treatment groups: $F(3,33) = 7.36$, $P < 0.0007$;

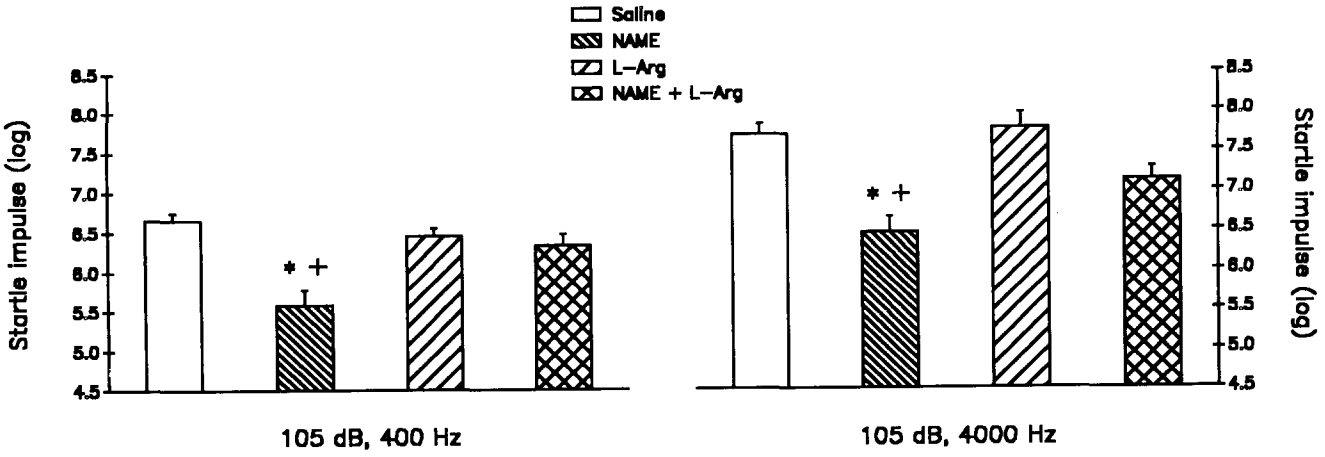


Fig. 2. Effects of intraperitoneal injection of saline, N^G-nitro-L-arginine methyl ester (L-NAME; 30 mg/kg), L-Arg (350 mg/kg) or N^G-nitro-L-arginine methyl ester plus L-Arg on the magnitude of the acoustic startle response to two different acoustic stimuli conditions. Results are means ± S.E.M. for 7–10 animals per group. * $P < 0.01$ vs. corresponding saline, + $P < 0.01$ vs. L-NAME + L-Arg group.

Newman-Keuls test N^G-nitro-L-arginine methyl ester vs. saline: $P < 0.01$). However, another relevant parameter evaluated, the 'average speed', remained unchanged by any of the doses of the inhibitor tested (data not shown). If the effects of the competitive inhibitor, N^G-nitro-L-arginine methyl ester, on the exploratory behaviour pattern were due to specific inhibition of nitric oxide synthesis, the concurrent administration of the nitric oxide precursor, L-Arg, should prevent such effects. Indeed, as Fig. 1 shows, L-Arg (350 mg/kg) reversed the behavioural changes induced by 100 mg/kg N^G-nitro-L-arginine methyl ester, since the group injected with N^G-nitro-L-arginine methyl ester plus L-Arg always differed from the N^G-nitro-L-arginine methyl ester group with at least $P < 0.05$, but did not differ from saline (n.s. at the four parameters showed in Fig. 1). However, L-Arg did not affect any of these parameters when administered alone.

3.2. Systemic administration of N^G-nitro-L-arginine methyl ester and startle response to acoustic and electric shock stimulation

We assessed the effect of i.p. administration of N^G-nitro-L-arginine methyl ester (30 mg/kg) 60 min before testing on the startle response to two different types of stimuli: acoustic and electric shock stimulation (Fig. 2). Firstly, the inhibitor induced a reduction of the startle response to either of the two frequency parameters of acoustic stimulation used, 400 Hz (ANOVA of data from Fig. 2, left panel: $F(3,31) = 11.73$, $P < 0.0001$; Newman-Keuls test N^G-nitro-L-arginine methyl ester vs. saline: $P < 0.01$) or 4000 Hz (ANOVA of data from Fig. 2, right panel: $F(3,31) = 12.39$, $P < 0.0001$; Newman-Keuls test N^G-nitro-L-arginine methyl ester vs. saline: $P < 0.01$). Two-way

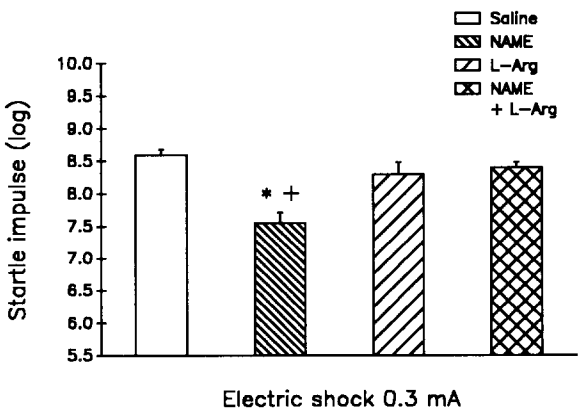


Fig. 3. Effects of intraperitoneal injection of saline, N^G-nitro-L-arginine methyl ester (L-NAME; 30 mg/kg), L-Arg (350 mg/kg) or N^G-nitro-L-arginine methyl ester (L-NAME) plus L-Arg on the magnitude of the startle response to electric shock stimulation. Results are means ± S.E.M. for 8–13 animals per group. * $P < 0.01$ vs. corresponding saline, + $P < 0.01$ vs. L-NAME + L-Arg group.

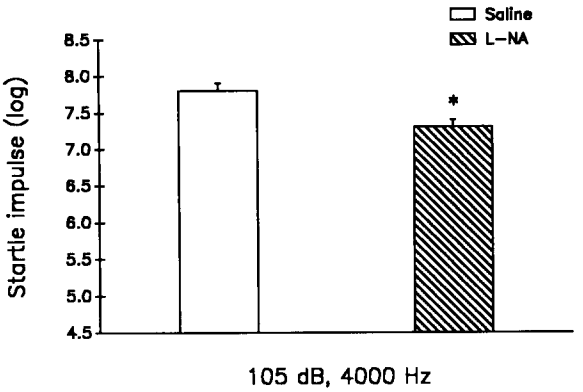


Fig. 4. Effects of intracerebroventricular injection of saline or N-nitro-L-arginine (L-NA; 10 μl of a 10 mM solution) on the magnitude of the acoustic startle response. Results are means ± S.E.M. for 6 animals per group. * $P < 0.01$ vs. corresponding saline.

ANOVA for repeated measures with drug and acoustic startle trials as the factors showed a drug effect as with the previous analyses, but not a drug × trial interaction (n.s.), indicating that the inhibitory effect of the inhibitor was maintained throughout the different trials. The simultaneous administration of L-Arg (350 mg/kg) with N^G-nitro-L-arginine methyl ester prevented the effects of the inhibitor on the acoustic startle response under both experimental conditions used (Newman-Keuls test N^G-nitro-L-arginine methyl ester + L-Arg: $P < 0.01$ vs. N^G-nitro-L-arginine methyl ester, n.s. vs. saline), whereas L-Arg did not have any effect when injected alone.

In addition, N^G-nitro-L-arginine methyl ester (30 mg/kg), injected i.p. 60 min before testing, was also able to decrease the startle response to electric shock stimulation (Fig. 3, ANOVA from the four treatment groups: $F(3,36) = 12.90$, $P < 0.0001$; Newman-Keuls test N^G-nitro-L-arginine methyl ester vs. saline: $P < 0.01$). The simultaneous administration of L-Arg (350 mg/kg) with N^G-nitro-L-arginine methyl ester prevented the effects of the inhibitor on the electric shock-induced startle response (Newman-Keuls test N^G-nitro-L-arginine methyl ester + L-Arg: $P < 0.01$ vs. N^G-nitro-L-arginine methyl ester, n.s. vs. saline), whereas L-Arg did not have any effect when injected alone.

3.3. Effect of intracerebroventricular administration of N-nitro-L-arginine on the startle response to acoustic stimulation

In order to assess whether the behavioural effects of inhibiting nitric oxide synthesis via a systemic route could be also observed when administering the inhibitor centrally, we evaluated the effect of i.c.v. administration of N-nitro-L-arginine (10 μl/rat of a 10 mM solution) 60 min pre-test on the startle response to

Table 1
Effects of intraperitoneal administration, 60 min prior to testing, of different doses of N^G-nitro-L-arginine methyl ester (L-NAME) on blood pressure and heart rate

	Blood pressure (mm Hg)	Heart rate (beats/min)
Saline	123.3±5.4	370.7±7.5
L-NAME 30 mg/kg	151.8±5.1 [a]	333.2±6.7 [a]
L-NAME 100 mg/kg	147.8±3.9 [a]	322.4±6.6 [a]

Results are the means±S.E.M. for 10–12 animals per group. [a] $P <$ 0.01 vs. saline.

acoustic stimulation (105 dB, 4000 Hz). Again, the nitric oxide synthase inhibitor was able to decrease the startle response ($df = 10$, $t = 3.28$, $P < 0.009$) (Fig. 4), even though the percentage reduction as compared to that in the saline group was less than for i.p. administration of N^G-nitro-L-arginine methyl ester.

3.4. Effects of N^G-nitro-L-arginine methyl ester on arterial blood pressure

The effect of N^G-nitro-L-arginine methyl ester (30 and 100 mg/kg i.p.) on blood pressure and heart rate 60 min post-injection is shown in Table 1. There was a treatment effect on both blood pressure ($F(2,31) = 9.80$, $P < 0.0005$) and heart rate ($F(2,31) = 12.60$, $P < 0.0001$). Neuman-Keuls post-hoc analyses indicated that N^G-nitro-L-arginine methyl ester, at both doses used, induced a rise in blood pressure ($P < 0.01$) and a decrease in heart rate ($P < 0.01$). No dose-dependent effects were observed.

4. Discussion

This study showed that systemic injection of the nitric oxide synthase inhibitor, N^G-nitro-L-arginine methyl ester, alters the exploratory pattern displayed by rats in a novel environment, as well as the startle response elicited by either acoustic or electric stimulation. The specificity of these effects exerted through nitric oxide was supported by the ability of the nitric oxide precursor, L-Arg, to prevent the actions of the competitive inhibitor, N^G-nitro-L-arginine methyl ester. At the same time after injection as the behavioural effects were observed, N^G-nitro-L-arginine methyl ester was also found to induce cardiovascular alterations. Thus, these results indicate that N^G-nitro-L-arginine methyl ester, when administered systemically, modifies certain behavioural and physiological responses related to the general psychophysiological status of the organism. Central administration of *N*-nitro-L-arginine, an irreversible inhibitor of the endothelial and brain parenchymal enzyme, nitric oxide synthase (Lambert et al., 1991) was also able to influence the acoustic startle response.

The doses for N^G-nitro-L-arginine methyl ester systemic administration found to inhibit the psychophysiological response to either a novel environment or a new stimulus were within the range of nitric oxide synthase inhibitor doses reported to interfere with memory formation (Böhme et al., 1993; Chapman et al., 1992). From these studies, those carried out in rats included tasks such as a radial arm maze, a water maze, and a social memory test, performance of which requires exploration, locomotor behaviour, and reactivity to novel stimulation. Certainly, these studies tried to control for possible non-specific effects of the treatments. Thus, exploratory/locomotor effects of the nitric oxide synthase inhibitor were discarded since the total exploration time in the radial arm maze remained unchanged (Böhme et al., 1993). However, based on a more accurate activity measurement, our results clearly show that N^G-nitro-L-arginine methyl ester-treated rats reduce both horizontal and vertical scanning movements, which results in inhibited exploration. This might imply that inhibitor-treated rats would be less prone to explore new environments, which subsequently could result in a poorer performance of spatial/exploratory training tasks and acquisition impairments. Nevertheless, it should be taken into account that we evaluated spontaneous locomotor behaviour in a novel environment and, even though the situation involves certain learning components about the new place, it does not necessarily make the same behavioural demands as a proper learning-designed task. On the other hand, we also observed a reduced startle response to sudden stimuli, which suggests a reduced capability of rats to react to new stimulation. This deficit might also interfere with learning demands eliciting a behavioural response from sensory stimulation. However, our results for acoustic and electric stimulation might not account for olfactory reactivity, which was shown to remain unaltered when inhibitor-treated rats were exposed for the first time to an unfamiliar juvenile (Böhme et al., 1993). Therefore, nitric oxide synthase inhibitor-treated rats show a diminished ability to react to novel situations which could interfere with their performance in training tasks. Nevertheless, this does not mean that these non-specific effects could account entirely for the learning deficits described for this kind of treatment. The possibility exists that the inhibitor simultaneously affects plasticity at learning-related neural structures. In fact, a systemic injection of 100 mg/kg N^G-nitro-L-arginine methyl ester efficiently enters the brain to block hippocampal long-term potentiation (Böhme et al., 1993). Moreover, nitric oxide synthase inhibitor administration into discrete brain regions, such as the hippocampus in rats (Ohno et al., 1993) or into the intermediate and medial hyper-

striatum ventrale – a learning-related area – in chicks (Hölscher and Rose, 1993) has been shown to induce acquisition impairments.

The precise site of the behavioural effects now found for N^G-nitro-L-arginine methyl ester is difficult to establish. Since this treatment also increased blood pressure and decreased heart rate, in agreement with previous findings (Moncada et al., 1991; Kelly et al., 1994), the possibility exists that these peripheral alterations might account for the behavioural modifications. However, that i.c.v. administered N^G-nitro-L-arginine methyl ester, a treatment which does not affect peripheral blood pressure (Moore et al., 1991), also reduces the startle response strongly suggests an effect within the central nervous system.

The mechanisms involved in the locomotor and startle response effects now described can be speculated upon. Striatal dopaminergic transmission has largely been implicated in the control of locomotion (Angulo and McEwen, 1994; Randrup and Munkvad, 1979). Interestingly, nitric oxide synthase is widely distributed in neurons of the striatum and the nucleus accumbens (Snyder and Bredt, 1991). Moreover, although there is some controversy in the literature (Guevara-Guzman et al., 1994), both in vivo (Zhu and Lou, 1992) and in vitro studies (Hanbauer et al., 1992; Lonart et al., 1993) indicate that nitric oxide might induce dopamine release in the striatum. Therefore, a possible explanation for our results could be that nitric oxide synthesis inhibition could result in decreased locomotor activity by interfering with dopaminergic transmission. In fact, N^G-nitro-L-arginine methyl ester has also been proven to reduce dopamine-mediated morphine effects on locomotion (Calignano et al., 1993). Along with dopamine, other neurotransmitters and neuropeptides now appear to participate in the regulation of locomotor activity by the striatum and nucleus accumbens (Angulo and McEwen, 1994). Evidence is accumulating for a role of nitric oxide in the release of a number of neurotransmitters and neuropeptides (Guevara-Guzman et al., 1994; Karanth et al., 1993; Lonart et al., 1992; Montague et al., 1994; Prast and Philippu, 1992; Sandi and Guaza, 1995). In particular, there is evidence supporting the involvement of a glutamatergic input in the control of locomotor behaviour involving mesolimbic and mesostriatal dopamine (Witkin, 1993). Moreover, the striatal dopamine release stimulated by *N*-methyl-D-aspartate (NMDA), an excitatory glutamate receptor agonist, has been reported to be blocked by a nitric oxide synthase inhibitor or by the nitric oxide scavenger, haemoglobin (Hanbauer et al., 1992). Therefore, it could be cautiously hypothesised that inhibiting nitric oxide synthesis could result in reduced exploratory/locomotor behaviour by interfering with striatal dopaminergic transmission either directly or through the modulation of other dopamine modulating agents. Nevertheless, an effect through other brain areas with a putative role in the control of locomotion, such as the mesencephalic locomotor region (Coles et al., 1989), and other neurotransmitter systems, cannot be discarded.

As for the startle response, the neural mechanisms best understood are those for the acoustic version. The acoustic startle response, a short-latency behaviour in response to sudden and intense acoustic stimulation, is mediated by a relatively simple neural circuit including neurons in the pontine reticular formation and spinal motoneurons (Davis et al., 1982). There is evidence supporting that, among the neurotransmitters involved in its regulation, glutamatergic transmission, mainly of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA)/kainate type, on reticulospinal pontine brain stem neurons might mediate this response (Ebert and Koch, 1992; Krase et al., 1993; Miserendino and Davis, 1993). In addition, this response can be modulated by the neural pathway described from the central amygdaloid nucleus to the pontine reticular brainstem. Again, glutamatergic transmission within this pathway, through both NMDA and non-NMDA receptors (Koch, 1993; Koch and Ebert, 1993; Krase et al., 1993; Miserendino and Davis, 1993), has also been shown to mediate/facilitate the acoustic startle response. Since nitric oxide can both release glutamate (Montague et al., 1994) and mediate glutamatergic actions (Garthwaite, 1991; Garthwaite et al., 1988; Montague et al., 1994), it could be reasoned that the reduction in the acoustic startle response induced by N^G-nitro-L-arginine methyl ester might be due to interference with glutamate transmission underlying either the expression and/or the modulation of the startle response. Since nitric oxide appears to stimulate central corticotropin-releasing factor release – as shown for the hypothalamus – (Karanth et al., 1993), and amygdaloid corticotropin-releasing factor also facilitates this behavioural response (Liang et al., 1992), interference with corticotropin-releasing factor transmission could be another potential target for N^G-nitro-L-arginine methyl ester startle action. Thus, i.c.v. N^G-nitro-L-arginine methyl ester effects on acoustic startle response could have resulted from the diffusion of the inhibitor to modulatory areas, such as the amygdala.

Given the well known interactions of nitric oxide and glutamate, and the proposed involvement of glutamatergic systems in auditory transmission (Ebert and Koch, 1992), we evaluated the effect of N^G-nitro-L-arginine methyl ester in electric shock-induced startle response to check for the possibility that the observed effects were not on the startle response, but on auditory processing. However, startle responses were also reduced by the systemic treatment, suggesting that inhibition of the startle response by the nitric oxide synthase inhibitor could be a generalised phenomenon

independent of the eliciting stimulus. Nevertheless, since N^G-nitro-L-arginine methyl ester has also been proven to elicit antinociception (Moore et al., 1991), the possibility of non-specific effects attenuating the impact of the electric shock cannot be discarded.

Therefore, it is suggested that endogenous nitric oxide might play a role in the general psychophysiological status of the organism, as indicated by reduced exploration/locomotor behaviour and startle responses, as well as altered cardiovascular parameters after N^G-nitro-L-arginine methyl ester administration. This study also emphasises the need to cautiously interpret data from behavioural studies, particularly of studies focused on learning and memory processes, using systemic administration of nitric oxide synthase inhibitors.

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