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Interactions between *N*-methyl-D-aspartate and nitric oxide in the modulation of ultrasonic vocalizations of infant rats

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Abstract

The effects of interactions between *N*-methyl-D-aspartate (NMDA) and nitric oxide on ultrasonic vocalizations, motor activity and body temperature was investigated in 9–10-day-old rat pups. The competitive NMDA receptor antagonist, 3-((±)-2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP), and the nitric oxide synthase inhibitor, nitro-L-arginine-methyl ester (L-NAME), decreased the emission of ultrasonic vocalizations while NMDA and the nitric oxide precursor, L-arginine, produced a trend toward increased emission of ultrasonic vocalizations. CCP also attenuated the geotaxic response. Co-administration of CPP with L-NAME virtually abolished the emission of ultrasonic vocalizations and the ability to show the geotaxic response while co-administration of NMDA with L-arginine increased the emission of ultrasonic vocalizations and decreased body temperature with no effect on the geotaxic response. NMDA and L-arginine reversed the effects of L-NAME, but not of CPP, on ultrasonic vocalizations. L-arginine but not NMDA antagonized the effect of CPP on the geotaxic response. Our results confirmed the functional coupling between NMDA receptor activation and nitric oxide in modulating anxiety-like behavior and motor coordination in infant rats.

Keywords: Nitric oxide (NO); NMDA (*N*-methyl-D-aspartate); Anxiety; Motor behavior; Ultrasonic vocalization; (Rat)

1. Introduction

The neurobiology of fear and anxiety involves numerous neurotransmitter systems including γ-aminobutyric acid (GABA) (Kalueff and Nutt, 1996), serotonin (Olivier et al., 1992), noradrenaline (Charney and Redmond, 1983), and cholecystokinin (Harro et al., 1993). The glutaminergic neurotransmission via the modulation of NMDA receptors has also been implicated in the pathophysiology of anxiety (Sanger et al., 1991), and both competitive and non-competitive NMDA receptor antagonists have been shown to produce anxiolytic-like effects in animals (Bennett and Amrick, 1986; Reddy and Kulkarni, 1997; Winslow et al., 1990).

Recent in vitro findings indicate the coupling of NMDA receptor activation with the synthesis of the gaseous messenger molecule, nitric oxide (Zhang and Snyder, 1995). Nitric oxide is synthesized from its biological precursor, arginine, by the catalytic enzyme, nitric oxide synthase (NOS), and acts as a retrograde messenger to enhance presynaptic glutamate release (for review see Garthwaite, 1991). The coupling of NMDA receptor activation with nitric oxide synthesis occurs in brain regions that contain nitric oxide synthesizing neurons (Garthwaite, 1991). Since the neural substrates of anxiety and defensive behavior are rich in nitric oxide synthesizing neurons (Vincent and Kimura, 1992), one might hypothesize a role for nitric oxide in the modulation of anxiety. Behavioral experiments confirmed this hypothesis as drugs that inhibit the synthesis of nitric oxide produce an anxiolytic-like effect in both adult (Faria et al., 1997; Volke et al., 1997) and infant rodents (Campbell et al., 1999; Podhorna and Brown, 1999).

Although in vitro studies showed coupling between NMDA receptor activation and nitric oxide synthesis, there are no studies that confirm such coupling on a behavioral level. It is known that studies done in vivo often give different results than those done in vitro as results from behavioral experiments are influenced by uncontrolled variables that are absent with in vitro preparations (Aronin et al., 1986). For example, nitric oxide synthase inhibitors decrease the synthesis of nitric oxide in vitro (Salter et al.,

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1996) and reduce anxiety-like behavior in vivo (Campbell et al., 1999; Podhorna and Brown, 1999; Volke et al., 1997) while the biological precursor of nitric oxide, arginine, increases nitric oxide production in vitro (Salter et al., 1996) but has no effect on anxiety-like behavior in vivo (Faria et al., 1997; Podhorna and Brown, 1999).

This study, therefore, examined the effects of in vivo interactions between NMDA receptor modulation and nitric oxide synthesis on anxiety-like behavior, using the rat pup ultrasonic vocalization model (Gardner, 1985; Nastiti et al., 1991). Drugs that increase or decrease NMDA receptor activation and drugs that increase or decrease nitric oxide synthesis were given alone or in combination to amplify or counteract each other's effects. In order to use a split-litter design, only one dose of each drug was used. Selection of drugs and drug doses was based on previous studies from our laboratory (Podhorna and Brown, 1999) and from other investigators, using the same experimental model (Campbell et al., 1999; Winslow et al., 1990). Justification of doses is given in detail in Section 2.5. The following drugs were used: the prototypic NMDA receptor agonist, *N*-methyl-D-aspartate (NMDA); the competitive NMDA receptor antagonist, 3-((±)-2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP); the NO precursor, L-arginine; and the NOS inhibitor, nitro-L-arginine-methyl ester (L-NAME).

2. Method

2.1. Subjects

The offspring of mated pairs of Long–Evans hooded rats originally purchased from Charles River, Canada (St. Constant, Quebec) and bred in the Psychology Department, Dalhousie University were used as subjects. The adults were housed in standard Plexiglas cages ($23 \times 45 \times 25$ cm) in a vivarium with constant room temperature (22 ± 1°C) and a reversed 12:12 light:dark cycle with lights on from 9 p.m. to 9 a.m. Agribrand laboratory rodent chow #5001(Abribrand Purina, Strathoy, Ontario) and tap water were available at libitum.

Pairs of rats were mated for 10 days and then the male was removed. Following birth, each litter was housed with its mother in a Plexiglas cage with wood shavings for bedding and shredded paper for nesting material. Litters were culled to 12 pups on Day 4 (parturition as Day 0) and their bedding material was changed. All pups were tested once when 9–10 days of age. The mean (±S.E.M.) weight, when tested, was 20.47 ± 0.22 g. Fifteen litters were used in this study.

2.2. Apparatus

Testing for ultrasonic vocalizations and locomotor behavior took place in a Plexiglas chamber ($26 \times 15 \times 12$ cm) which had the floor divided into eight rectangles (6.5×7.5 cm). To facilitate emission of ultrasonic vocalizations by rat pups, the room temperature was lowered to 20 ± 1°C (Okon, 1970). Ultrasonic vocalizations were recorded via an ultrasonic microphone connected to a SM2 bat detector (Ultrasound Advice, UK). The broadband output of the bat detector was fed into a custom-built four-channel digitizer based on that designed by Harrison and Holman (1978). This digitizer contained four variable band pass filters that were set to 28, 36, 44 and 52 kHz. When input was detected at one of these frequencies, the digitizer produced a pulse for the duration of the signal. The output of the digitizer was connected via a terminal panel and interface card (Strawberry Tree, Sunnyvale, CA, USA) to a Macintosh 2cx computer on which a custom program written under the Strawberry Tree Workbench Mac software (McGregor, 1996) recorded the occurrence of each ultrasonic vocalization on a minute-by-minute basis.

2.3. Procedure

On the experimental day, pups were transported together with their mother in their home cages to the room adjacent to the experimental room and left undisturbed for at least 10 min. Litters were tested in two phases with six pups in each phase. Thus, half of the pups were removed from the nest and placed into a cage with clean wood shavings that was placed in a water bath with temperature between 34–37°C, and left undisturbed for 5–10 min. Each pup was then weighed, marked with a nontoxic permanent marker and individually placed into the test chamber where its baseline ultrasonic vocalizations were recorded for a 1-min screening period. The number of ultrasonic vocalizations emitted during the 1-min screening period determined the assignment of pups into experimental groups (see Section 2.4). After all pups from one litter were screened, they were assigned to a treatment based on a split-litter design (see below). Pups were then taken in order and their basal temperature (pre-treatment temperature) was measured. This was done by placing a thermocouple, connected to a Physitemp Model B Thermometer, in the left armpit and kept there until the temperature on the digital display stabilized. Each pup then received the first injection and was returned to the water bath cage for 10 min. Each pup then got the second injection and was returned to the water bath cage for another 25 min.

Pups were individually removed from the water-bath cage and their temperature was measured the second time (post-treatment temperature). Pups were then individually placed in the center of the test chamber for 3 min and the number of ultrasonic vocalizations emitted was automatically recorded. Locomotor behavior was recorded by an observer sitting quietly in the testing room. The behaviors scored were the number of lines crossed (defined as any

traversing of a line by a half of the pup's body), rearing (climbing walls of the chamber with front paws on the wall), head raising (raising the head from the horizontal position to an angle of 30°), and rolling (rolling onto their back with all four paws up). The frequency of rolling was used as a measure of ataxia.

At the end of the 3-min testing period, each pup was removed from the test chamber and tested for the negative geotaxic response. This was done by placing the pup head-down on a wire-mesh covered Plexiglas inclined plane (30° angle). The median latency to turn around in a head-up (±10°) position obtained from three consecutive trials was taken as a measure of the geotaxic response. The cut-off time was 60 s. A score of 60 s was given to any pup that fell off the inclined plane.

2.4. Drugs and experimental design

N-methyl-D-aspartate (NMDA, 2.0 mg/kg), 3-((±)-2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP, 1.5 mg/kg), nitro-L-arginine-methyl ester (L-NAME, 20 mg/kg) and L-arginine (L-ARG, 100 mg/kg) were purchased from Sigma and all were dissolved in saline. All drugs were injected subcutaneously in the neck in a volume of 2 ml/kg. Each pup received two injections. To avoid any possible precipitation of drugs caused by simultaneous administration, the two drugs were given 10 min apart. The first injection was 35 min prior to testing, and the second injection was 25 min prior to testing. As all drugs were soluble in saline, only one vehicle (VEH) was required.

There were 11 treatment groups: VEH + VEH (control), VEH + NMDA, VEH + CPP, L-NAME + VEH, L-ARG + VEH, NMDA + CPP, L-NAME + CPP, L-NAME + NMDA, L-ARG + NMDA, L-ARG + L-NAME, and L-ARG + CPP. Using a split-litter design, one pup from each litter was tested in each treatment condition (one pup/group/litter), the spare pup got one of the treatment conditions at random.

It is well known that there is considerable variability in the frequency of ultrasonic vocalizations emitted by isolated pups (Podhorna and Brown, 1999, 2000; Vivian et al., 1997, Winslow et al., 1990), with some pups emitting a high number and others emitting a low number of ultrasonic vocalizations. In order to ensure that all experimental groups had a similar average basal vocalization rate, we kept a record of the basal vocalization rate in the 1-min screening period and assigned pups to each treatment group so that they were matched on the results of the 1-min screening period.

2.5. Justification of drug doses

Drug doses for L-NAME (20 mg/kg) and L-arginine (100 mg/kg) were based on the previous findings from our laboratory (Podhorna and Brown, 1999) as well as those of other investigators (Campbell et al., 1999), using the rat pup ultrasonic vocalization model. In those studies, L-NAME produced an anxiolytic effect at doses ranging from 5 to 100 mg/kg but high doses (50 and 100 mg/kg) also produced motor impairment. Thus, we selected 20 mg/kg of L-NAME as an effective anxiolytic dose with no side effects. L-arginine previously failed to increase anxiety at a wide range of doses in both infant (Campbell et al., 1999; Podhorna and Brown, 1999) and adult rodents (Faria et al., 1997; Volke et al., 1997). We, therefore, decided to use 100 mg/kg of L-arginine as this dose produced the biggest trend toward increased anxiety in our previous study (Podhorna and Brown, 1999).

Drug doses for NMDA and for the 3-((±)-2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP) were based on the study of Winslow et al. (1990). In this study, 1 mg/kg of NMDA had no effect on the number of ultrasonic vocalizations, 2.5 mg/kg was anxiogenic with no side effects, 5.0 mg/kg was anxiogenic but also produced tail twitching and turning while higher doses caused seizures in rat pups. The only dose of CPP used in this study was 2.5 mg/kg, which Winslow et al. (1990) found to decrease ultrasonic vocalizations by 63.6% from the control level without causing motor impairment. Thus, we decided to use 2.5 mg/kg of NMDA and 2.5 mg/kg of CPP. However, our pilot study showed that those doses produced severe locomotor side effects (data not shown). Pups treated with 2.5 mg/kg of NMDA showed tail twitching, turning and circling while pups treated with 2.5 mg/kg of CPP had signs of severe muscle relaxation. Therefore, we lowered the dose of NMDA to 2.0 mg/kg and reduced the dose of CPP to 1.5 mg/kg.

2.6. Data analysis

Data that showed homogeneity of variance were analyzed using one-way analysis of variance (ANOVA). Non-homogenous data were transformed using a square root transformation before analysis. Data that did not meet the criteria for parametric statistics were analyzed using a non-parametric ANOVA on ranks (Kruskall–Wallis). Parametric or non-parametric versions of post-hoc multiple comparisons (Student–Newman–Keuls) were used to analyse individual differences between experimental groups (Glantz, 1992).

3. Results

3.1. Ultrasonic vocalizations

There was a significant difference between experimental groups in the number of ultrasonic vocalizations ($H_{(10)} = 78.36$, $P < 0.0001$; Fig. 1A). Compared to controls (VEH + VEH), the competitive NMDA receptor antago-

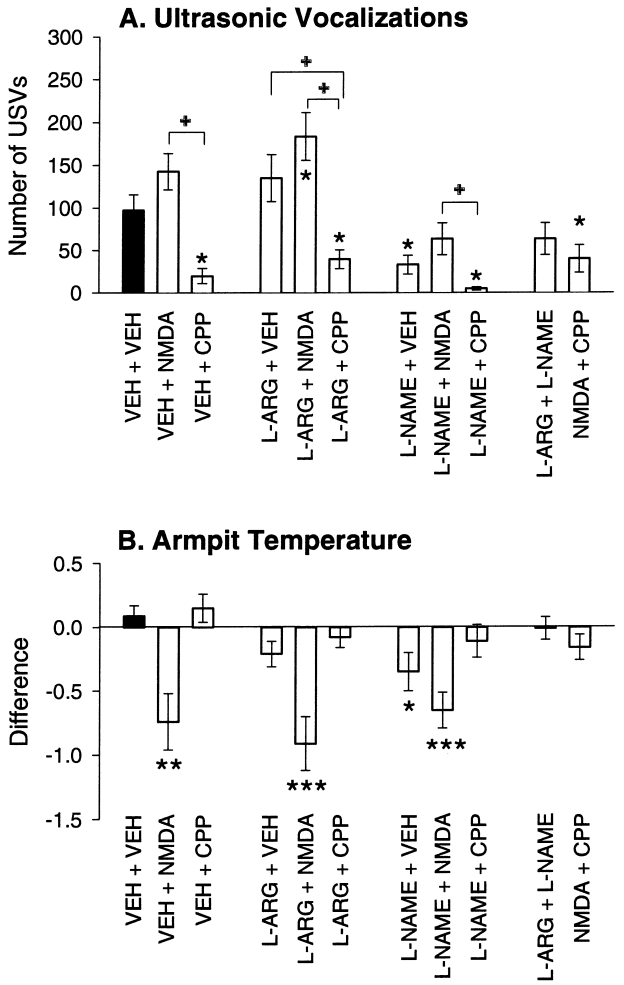

Fig. 1. Effects of the prototypic NMDA receptors agonist, NMDA, the competitive NMDA receptor antagonist, CPP, the biological precursor of nitric oxide, L-arginine (L-ARG), and the nitric oxide synthase inhibitor, L-NAME on (A) the number of ultrasonic vocalizations emitted by 9–10-day-old Long–Evans hooded rat pups and (B) on the change in body temperature (the difference between pre- and post-treatment body temperature in °C is shown). Saline was used as a vehicle (VEH) for all drugs tested. $^{*}P < 0.05$ vs. control (VEH + VEH; black bar), $^{+}P < 0.05$ between the depicted groups.

nist, 3-((±)-2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (VEH + CPP), and nitric oxide synthesis inhibitor, nitro-L-arginine-methyl ester (L-NAME + VEH), decreased the number of ultrasonic vocalizations emitted by rat pups. A combination of CPP + L-NAME virtually abolished emission of ultrasonic vocalizations (to about 5% of the control value), although this group of pups was not significantly different from either the VEH + CPP or L-NAME + VEH groups, presumably due to the floor effect (Student–Newman–Keuls post-hoc multiple comparisons). The other two groups of pups that received CPP (L-ARG + CPP; NMDA + CPP) emitted significantly fewer ultrasonic vocalizations than controls ($P < 0.05$; Student post hoc). Groups of pups treated with VEH + CPP and L-ARG + CPP also emitted significantly fewer vocalizations than pups treated with VEH + NMDA and L-ARG + VEH or the combination of L-ARG + NMDA. Pups treated with L-NAME + CPP emitted fewer vocalizations than pups treated with L-NAME + NMDA and VEH + NMDA.

Compared to controls (VEH + VEH), a combination of L-ARG + NMDA significantly increased emission of ultrasonic vocalizations while each drug alone (VEH + NMDA, L-ARG + VEH) produced only a trend toward increased vocalizations. NMDA and L-arginine antagonized the vocalization-suppressant effect of L-NAME as pups treated with the combination of L-NAME + NMDA and L-ARG + L-NAME were not significantly different from controls. In contrast, neither NMDA nor L-arginine antagonized the vocalization-suppressant effect of CPP.

3.2. Body temperature

Fig. 1B shows the differences between pre-treatment and post-treatment measures of body temperature. No changes in body temperature were found after treatment with vehicle or CPP while NMDA significantly decreased body temperature (paired *t*-test: $t_{(14)} = 3.39$, $P < 0.01$). L-arginine had no effect on body temperature if given alone or with CPP but the combination of L-arginine with NMDA decreased body temperature ($t_{(14)} = 4.35$, $P < 0.001$). The NOS inhibitor, L-NAME, decreased armpit temperature if given alone ($t_{(14)} = 2.37$, $P < 0.05$) or in combination with NMDA ($t_{(14)} = 4.64$, $P < 0.001$) but not when co-administered with CPP. Co-administration of L-arginine with L-NAME and of NMDA with CPP had no effect on armpit temperature of 9–10-day-old rat pups.

3.3. Geotaxic response

There was a significant difference between experimental groups in the latency to show the geotaxic response ($F_{(10, 157)} = 7.29$, $P < 0.0001$; Fig. 2A). The competitive NMDA receptor antagonist, CPP, significantly increased the latency to the geotaxic response when given alone (VEH + CPP) or with NMDA ($P < 0.05$, Student post hoc). Co-administration of CPP and L-NAME virtually abolished the ability of pups to turn on the geotaxic plane, but the combination of CPP with L-arginine did not significantly increase the latency to show the geotaxic response. No other drug treatments altered the latency to show the geotaxic response (Fig. 2A).

3.4. Locomotion

There was no significant difference between groups in the frequency of line crossing ($F_{(10, 157)} = 0.92$, NS), rearing ($H_{(10)} = 6.54$, NS) or head raising ($H_{(10)} = 13.13$, NS). The frequency of rolling as a measure of ataxia was significantly different between experimental groups ($H_{(10)} = 42.12$, $P < 0.0001$; Fig. 2B). Post-hoc comparisons showed that rolling was significantly increased in groups treated with the competitive NMDA receptor antagonist,

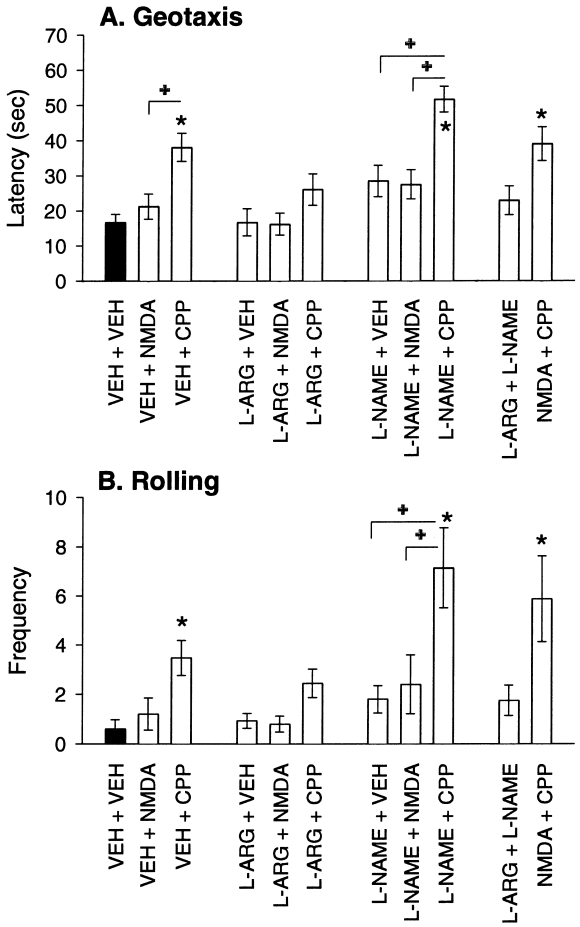

Fig. 2. Effects of the prototypic NMDA receptors agonist, NMDA, the competitive NMDA receptor antagonist, CPP, the biological precursor of nitric oxide, L-arginine (L-ARG), and the nitric oxide synthase inhibitor, L-NAME on (A) the latency to show negative geotaxic response and (B) the frequency of rolling in 9–10-day-old Long–Evans hooded rat pups. Saline was used as a vehicle (VEH) for all drugs tested. $^{*}P < 0.05$ vs. control (VEH + VEH; black bar), $^{+}P < 0.05$ between the depicted groups.

CPP alone (VEH + CPP), or in combination with NMDA (NMDA + CPP) and with L-NAME (L-NAME + CPP). However, animals given CPP with L-arginine showed no increase in rolling. The NOS inhibitor, L-NAME, the prototypic NMDA receptor agonist, NMDA, and the nitric oxide precursor, L-arginine, did not increase rolling.

4. Discussion

Rodent pups, if separated from their mother and littermates, emit ultrasonic vocalizations (Winslow and Insel, 1991). A decreased number of ultrasonic calls following pharmacological manipulation reflects an anxiolytic effect while an increased number of ultrasounds represents an anxiogenic effect (Gardner, 1985). The competitive NMDA receptor antagonist, CPP, and the nitric oxide synthase inhibitor, L-NAME, were both anxiolytic as each drug alone reduced ultrasonic vocalizations, and when given together, they virtually abolished emission of ultrasounds, indicating an additive anxiolytic effect. The prototypic NMDA receptor agonist, NMDA (given at a low dose), and the biological precursor of NO, L-arginine, produced a trend toward increased number of ultrasonic vocalizations but co-administration of NMDA with L-arginine significantly increased ultrasonic vocalizations, producing a clear-cut anxiogenic effect. NMDA and L-arginine antagonized the anxiolytic effect of L-NAME but neither NMDA nor L-arginine antagonized the anxiolytic effect of CPP on the emission of ultrasonic vocalizations.

Effects of the prototypic NMDA receptor agonist, NMDA, on anxiety-like behaviors are rather inconsistent. In adult rodents, NMDA has been shown to be anxiogenic in rats and mice in the elevated plus maze test (Dunn et al., 1989; Vasar et al., 1993) and in the social interaction test in rats (Dunn et al., 1989), but ineffective in the Geller–Seifter punished conflict paradigm in rats (Wiley et al., 1998). In Sprague–Dawley rat pups, Winslow et al. (1990) found that 2.5 mg/kg of NMDA was anxiogenic in the infant rodent ultrasonic vocalization test without producing side effects, but the same dose of NMDA resulted in severe side effects, such as tail twitching, in Long–Evans rat pups in our pilot study. Genetic background is known to influence the differential effects of drugs on behavior of rats (Sauter and Rudin, 1995) and mice (Wang and Fowler, 1999). Strain-dependent reactivity to drugs might explain the different side effects of 2.5 mg/kg of NMDA in our pilot study with Long–Evans rat pups and the study of Winslow et al. (1990) with Sprague–Dawley rat pups. Since we reduced the dose of NMDA to 2.0 mg/kg in our study, we found only a trend toward increased ultrasonic vocalization in rat pups. However, co-administration of the nitric oxide precursor, L-arginine, with NMDA had an additive effect, resulting in anxiogenesis.

NMDA decreased body temperature of rat pups and the competitive NMDA receptor antagonist, CPP, antagonized this effect. It is known that isolated rat pups respond to cold exposure by increasing metabolic heat production and by emitting ultrasonic vocalizations (Blumberg and Alberts, 1990). However, these two responses are not tightly linked as metabolic heat production is neither necessary nor sufficient to stimulate the emission of ultrasonic vocalizations (Hofer and Shair, 1991). For example, the nitric oxide synthase inhibitor, L-NAME, which reduces body temperature of rat pups, also reduced the emission of ultrasonic vocalizations (Campbell et al., 1999; Podhorna and Brown, 1999). It is, therefore, impossible to hypothesize that the decrease in body temperature caused by the administration of NMDA must automatically cause the increased emission of ultrasonic vocalizations.

One of the important functions of glutamate is the regulation of motor behavior via NMDA receptors (Kretschmer, 1998; Starr and Starr, 1995). The competitive NMDA receptor antagonist, CPP (1.5 mg/kg), produced not only an anxiolytic effect but also severe motor impair-

ment (inhibiting geotaxis and increasing rolling) in 9–10-day-old rat pups. CPP also produces muscle relaxation in adult rodents (Lehmann et al., 1987; Turski et al., 1987). Since proper functioning of laryngeal muscles is essential for the production of ultrasounds (Hofer and Shair, 1993), the ultrasound-suppressant effect of CPP may be due to its muscle relaxant properties rather than its anxiolytic properties. Surprisingly, 9–11-day-old rat pups treated with 2.5 mg/kg of CPP showed no motor impairment in the study of Winslow et al. (1990). As mentioned above, strain-dependent sensitivity to drug effects (Sauter and Rudin, 1995; Wang and Fowler, 1999) may explain the different side effect profiles found by Winslow et al. (1990) and our results. None of the behavioral effects of CPP were antagonized by NMDA; thus, confirming that CPP is a very potent competitive NMDA receptor antagonist (Lehmann et al., 1987; Turski et al., 1987). However, the motor impairment effects of CPP on measures of geotaxis and rolling were antagonized by the nitric oxide precursor, L-arginine. Theoretically, increased nitric oxide production by L-arginine should have no effect on glutamate transmission via NMDA receptors if these receptors are blocked with CPP. Thus, reversal of CPP-induced muscle relaxation by L-arginine in our study implies the involvement of another neurotransmitter system. There is evidence that nitric oxide modulates the release of dopamine (Hanbauer et al., 1992). It is, therefore, possible that increased synthesis of nitric oxide reversed the CPP-induced motor impairment via modulation of the dopaminergic system (Starr and Starr, 1995).

The lack of an anxiogenic effect on ultrasonic vocalizations following administration of L-arginine is consistent with previous studies (Campbell et al., 1999; Podhorna and Brown, 1999; Volke et al., 1997), although systemic administration of L-arginine increases nitric oxide production in vitro (Salter et al., 1996). This confirms again that in vitro and in vivo results are not always identical and stresses the importance of confirming in vitro findings with in vivo behavioral tests.

The anxiolytic effect of L-NAME and of other NOS inhibitors is well documented in both adult (Volke et al., 1997) and infant rodents (Campbell et al., 1999; Podhorna and Brown, 1999). The 20 mg/kg dose of L-NAME was 'anxioselective' as it reduced the emission of ultrasonic vocalizations, but did not produce motor impairment in 9–10-day-old pups. As previously reported, doses higher than 20 mg/kg of L-NAME are necessary to produce motor impairment in rodents (Podhorna and Brown, 1999; Starr and Starr, 1995).

Although L-NAME is anxiolytic, it also decreases body temperature of adult (Branco et al., 1997; Steiner et al., 1998) and infant rats (present results; Campbell et al., 1999; Podhorna and Brown, 1999). The anxiolytic and hypothermic effects of L-NAME were both antagonized by the nitric oxide biological precursor, L-arginine. On the other hand, the prototypic NMDA receptor agonist, NMDA, antagonized the anxiolytic, but not the hypothermic, effects of L-NAME. This is not surprising as NMDA decreased the body temperature of pups on its own. This suggests no relationship between modulation of NMDA receptors and nitric oxide synthesis for the regulation of body temperature and confirms that changes in body temperature and in the emission of ultrasonic vocalizations by infant rats are not tightly linked (Hofer and Shair, 1991).

The results showed that functional interactions between NMDA receptors and nitric oxide plays an important role in anxiety-like behavior, although the primary neurotransmitters involved in the regulation of fear and anxiety are GABA (Kalueff and Nutt, 1996) and serotonin (Olivier et al., 1992). Our data also showed that NMDA receptor activation and nitric oxide synthesis are essential in the control of motor coordination and that their effect on motor behavior is linked to another neurotransmitter system, presumably dopaminergic (Hanbauer et al., 1992; Starr and Starr, 1995). This study demonstrated that coupling between NMDA receptor activation and nitric oxide synthesis, previously found in vitro (Garthwaite, 1991; Zhang and Snyder, 1995), can also be observed on an in vivo behavioral level.

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