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# Do NMDA receptor-mediated changes in motor behaviour involve nitric oxide?

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**Abstract**

Nitric oxide (NO) synthase inhibitors were investigated for their effects on motor behaviour. In normal mice, $N^G$-nitro-L-arginine (5–125 mg/kg i.p.) and 7-nitroindazole (10–50 mg/kg i.p.), but not aminoguanidine (60–150 mg/kg i.p.) suppressed species-typical behaviours. In 24 h reserpine-treated mice, akinesia was reversed with the dopamine $D_1$ receptor agonist 2,3,4,5-tetrahydro-7,8-dihydroxy-1-phenyl-1*H*-3-benzazepine hydrochloride (SKF 38393, 3–30 mg/kg i.p.) and by the dopamine $D_2$ receptor agonist *N*-*n*-propyl-*N*-phenylethyl-*p*-(3-hydroxyethyl) ethylamine hydrochloride (RU 24213, 0.5–5 mg/kg s.c.), but not by any of the NO synthase inhibitors. $N^G$-Nitro-L-arginine and 7-nitroindazole (not aminoguanidine) suppressed $D_1$ and $D_2$ receptor agonist-induced locomotion, but L-arginine (500 mg/kg i.p.) was not always able to prevent this effect. These results suggest that continued activity of constitutive NO synthase is necessary for normal body movements to occur. The difference in the interaction profiles of constitutive NO synthase inhibitors and NMDA antagonists with dopaminergic drugs, indicates that inhibition of NO generation is not a factor in the well-known $D_1$-facilitatory effect of glutamate receptor blockade.

*Keywords:* Nitric oxide (NO); Dopamine; Motor behavior; Reserpine; (Mouse)

## 1. Introduction

One of glutamate's many functions in the brain is to regulate motor behaviour, which it does in concert with dopamine (Graybiel and Ragsdale, 1983; Iversen, 1984; Kornhuber and Kornhuber, 1986). According to one school of thought, a principal site at which glutamate and dopamine systems interact to control motor output from the basal ganglia is the striatum (Schmidt et al., 1990), where cortical glutamatergic and mesencephalic dopaminergic fibres converge to terminate in close apposition to each other on striatal efferent neurones (Bouyer et al., 1984; Somogyi et al., 1981). This anatomical arrangement allows ample opportunity for one transmitter to modulate the release and/or actions of the other (Brown and Arbuthnott, 1983; Fujimoto et al., 1981; Garcia-Munoz et al., 1981; Scatton et al., 1982).

Our interest in the motor role of glutamate stems from the observation that compounds which attenuate the action of glutamate at *N*-methyl-D-aspartate (NMDA)-type receptors, dramatically potentiate the motor stimulant property of L-dihydroxyphenylalanine (L-DOPA) in monoamine-depleted animals (Klockgether et al., 1991; Klockgether and Turski, 1990; Löschmann et al., 1991; Morelli et al., 1992; Morelli and Di Chiara, 1990; Wüllner et al., 1992). It has been proposed that this synergism could indicate a potential use for glutamate antagonists as adjuvants to L-DOPA treatment in the therapeutic management of Parkinson's disease in man (Greenamayre and O'Brien, 1991; Klockgether and Turski, 1989; Schmidt et al., 1990).

In attempting to clarify the nature of this glutamate-dopamine interaction, we have investigated how NMDA receptor antagonists modify the motor responses to selective dopamine $D_1$ and $D_2$ receptor agonists in the mouse reserpine model of parkinsonism (Goodwin et al., 1992; Starr and Starr, 1993a,b; 1994),

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since the remedial actions of L-DOPA are believed to reflect the combined stimulation of these two receptors by dopamine (Clark and White, 1987; Robertson, 1992; Waddington and O'Boyle, 1989). We have found that whilst $D_2$ receptor-dependent locomotion is little affected, or even suppressed by NMDA receptor antagonism, depending on the particular NMDA receptor antagonist and $D_2$ receptor agonist used, $D_1$ receptor-dependent movements on the other hand are invariably facilitated (Goodwin et al., 1992; Starr and Starr, 1993a,b; 1994). These data suggest that an interplay between NMDA receptors and dopamine $D_1$ receptors is fundamental to the antiparkinson potential of NMDA antagonists.

How this interplay is manifested at the cellular level is still not clearly understood, but one important transduction mechanism for NMDA receptors is nitric oxide (NO; Garthwaite et al., 1989). The striatum is enriched in both NMDA receptors (Albin et al., 1992) and NO synthase (Snyder and Bredt, 1991), and recent evidence has established a link between NO and dopamine function within the striatonigral axis (Hanbauer et al., 1992; Tsou et al., 1993). The present study therefore considers whether NO participates in motor control in general, and in the influence that NMDA receptor antagonists have on dopamine receptor-mediated motor activity in particular, by examining the behavioural properties of several NO synthase inhibitors. These include $N^G$-nitro-L-arginine (Rees et al., 1990) and 7-nitroindazole (Moore et al., 1993), both of which inhibit the constitutive isoform of NO synthase, and aminoguanidine (Misko et al., 1993), which preferentially inactivates the inducible form of the enzyme. We reasoned that if NO mediates motor responses to NMDA receptor stimulation at excitatory synapses in the brain, then inhibiting NO synthesis with $N^G$-nitro-L-arginine or 7-nitroindazole, but not aminoguanidine, should induce behavioural changes similar to those of NMDA receptor blockade. This paper details the effects of the three NO synthase inhibitors on exploratory motor activity in non-habituated mice, and their interactions with selective $D_1$ and $D_2$ dopamine receptor agonists in monoamine-depleted mice.

## 2. Materials and methods

### 2.1. Animals

Male albino mice (TO strain, A.R. Tuck), weighing 28–35 g, were housed in groups of 20 at 22 ± 1°C, under fluorescent lighting from 07.00–17.00 h, and allowed free access to food and water. Experiments were conducted between 10:00–15:00 h, and each animal was used once only.

### 2.2. Behavioural measurements

Working dose ranges for the effects of the three NO synthase inhibitors on unconditioned motor behaviour were first determined in normal, dopamine-intact mice. Animals were injected with $N^G$-nitro-L-arginine (5–125 mg/kg i.p.) or the inactive enantiomer $N^G$-nitro-D-arginine (25–125 mg/kg i.p.), 7-nitroindazole (10–50 mg/kg i.p.) or aminoguanidine (60–150 mg/kg i.p.) and returned to their home cage. Thirty min later, mice were placed singly and without prior acclimatisation onto the floor of a clear Perspex container (30 × 25 × 20 cm high). Horizontal movements were recorded for 10 min by underfloor sensors, using a Panlab model 0603 detector unit placed at setting 5 (Starr and Starr, 1986). The number of sensor activations gave a measure of the animals' locomotion. The presence or absence of other behaviours was noted by a trained observer, with the aid of a checklist, but these were not quantified.

Other experiments were conducted with monoamine-depleted mice. Animals were injected with reserpine (5 mg/kg i.p.) and returned to their home cage. The ambient temperature was raised to 28 ± 1°C to prevent the animals becoming hypothermic. Twenty four h later, the mice were given $N^G$-nitro-L-arginine (5–125 mg/kg i.p.), 7-nitroindazole (10–50 mg/kg i.p.), aminoguanidine (60–150 mg/kg i.p.) or L-arginine (500 mg/kg i.p.), and/or the selective dopamine $D_1$ receptor agonist 2,3,4,5-tetrahydro-7,8-dihydoxy-1-phenyl-1*H*-3-benzazepine hydrochloride (SKF 38393, 3–30 mg/kg i.p.; Setler et al., 1978) or the selective dopamine $D_2$ receptor agonist *N*-*n*-propyl-*N*-phenylethyl-*p*-(3-hydroxyphenyl)ethylamine (RU 24213, 0.5–5 mg/kg s.c.; Euvrard et al., 1980). Thirty min later locomotor scores (10 min) and other behaviours were determined as described above.

### 2.3. Statistics

One-factor analysis of variance (ANOVA) was used to determine $F$ ratios and levels of significance of the effects of individual drug treatments on locomotion, with post hoc analysis of individual dose points by Dunnett's $t$-test. Two-factor ANOVA was used to reveal any interaction between $N^G$-nitro-L-arginine or 7-nitroindazole with L-arginine and either of the dopamine receptor agonists in the drug combination studies. In all cases significance was taken as $P < 0.05$.

### 2.4. Drugs

These included reserpine, $N^G$-nitro-L-arginine methyl ester, $N^G$-nitro-D-arginine methyl ester, aminoguanidine hemisulphate and L-arginine (Sigma), 7-nitroindazole (Lancaster Synthesis), SKF 38393 (Re-

search Biochemicals) and RU 24213 (Roussel). All drugs were dissolved in demineralised water and administered in a dose volume of 5 ml/kg. Reserpine and 7-nitroindazole were dissolved with the aid of a minimum quantity of glacial acetic acid and 5 M NaOH solution, respectively.

## 3. Results

### 3.1. Effects of NO synthase inhibitors on normal motor behaviour

Low doses of $N^G$-nitro-L-arginine (5–25 mg/kg i.p.) did not appear to affect exploratory motor activity in mice, whilst a higher dose (125 mg/kg i.p.) produced a light sedation and a 46% reduction in locomotion (Table 1). ANOVA disclosed a significant main effect with $F(3,29) = 3.53$, $P = 0.029$. The mice looked alert but less active than normal, with a corresponding decrease in the amount of time spent rearing and grooming, and an increase in the time spent sitting still. However, we saw no signs of the abnormal posture or loss of muscle tone that are characteristic of NMDA receptor antagonists (Starr and Starr, 1994). Equivalent doses of $N^G$-nitro-D-arginine, had no detectable effect on mouse exploratory behaviour (Table 1).

A similar quiescence and bradykinesia were also observed with high doses of 7-nitroindazole (ANOVA main effect $F(3,26) = 14.28$, $P < 0.001$), but not with aminoguanidine (Table 1).

### 3.2. Effects of NO synthase inhibitors on motor activity of reserpine-treated mice

Twenty four h after receiving a single dose of reserpine (5 mg/kg i.p.), mice were almost completely akinetic (Table 1) and other species-typical behaviours such as rearing and grooming were absent. The low baseline locomotor scores of vehicle-treated control animals reflect an early attempt by the animals to explore their surroundings on first entering the test arena, otherwise the mice sat still for the whole of the observation period. Pretreatment with $N^G$-nitro-L-arginine (5–125 mg/kg i.p.), 7-nitroindazole (10–50 mg/kg i.p.) or aminoguanidine (60–150 mg/kg i.p.) did not significantly alter the locomotor activity of reserpine-treated mice (Table 1).

### 3.3. Effects of NO synthase inhibitors on locomotor activity induced by SKF 38393 in reserpine-treated mice

As noticed in previous work (Starr and Starr, 1993a,b; 1994), SKF 38393 (3–30 mg/kg i.p.) induced robust and fluent locomotion in reserpine-treated mice (Table 2), which was accompanied by rearing and bouts of whole body grooming. The animals moved about the test box in a rapid and well-coordinated fashion which appeared little different from that of dopamine-intact animals. ANOVA revealed a significant main effect of SKF 38393 on locomotion ($F(3,98) = 32.87$, $P < 0.001$).

$N^G$-Nitro-L-arginine (5–125 mg/kg i.p.) dose dependently inhibited the reinstatement of locomotion by

Table 1
Effects of NO synthase inhibitors on locomotor activity of normal and reserpine-treated mice

| Treatment | Dose (mg/kg) | Locomotor score | |
|---|---|---|---|
| | | Normal | Reserpine |
| Saline | – | 394.2±27.5 | 11.0±2.1 |
| $N^G$-Nitro-L-arginine | 5 | 455.5±26.2 | 20.3±3.6 |
| $N^G$-Nitro-L-arginine | 25 | 348.3±48.6 | 10.3±3.5 |
| $N^G$-Nitro-L-arginine | 125 | 213.0±29.0 * | 5.0±2.0 |
| $N^G$-Nitro-D-arginine | 25 | 417.6±29.0 | 21.9±4.2 |
| $N^G$-Nitro-D-arginine | 125 | 379.8±18.5 | 16.4±3.1 |
| 7-Nitroindazole | 10 | 291.1±47.0 | 11.6±3.3 |
| 7-Nitroindazole | 25 | 153.4±22.2 * | 12.6±3.2 |
| 7-Nitroindazole | 50 | 56.4± 6.1 ** | 4.0±3.6 |
| Aminoguanidine | 60 | 400.6±29.4 | 17.1±5.5 |
| Aminoguanidine | 150 | 414.5±15.1 | 9.1±2.0 |
| L-Arginine | 500 | 400.7±31.4 | 9.8±2.1 |

The enzyme inhibitors were administered i.p. to normal mice, or 24 h after treatment with reserpine (5 mg/kg i.p.). Thirty min later, locomotor scores were determined over 10 min by means of a Panlab model 0603 detector unit. Each result is the mean±S.E.M. of at least eight determinations. * $P < 0.05$, ** $P < 0.001$ versus saline.

Table 2
Effects of NO synthase inhibitors on the locomotor response to SKF 38393 in reserpine-treated mice

| Treatment | Dose | Locomotor score (mg/kg) |
|---|---|---|
| SKF 38393 | 3 | 34.9± 4.2 |
| SKF 38393 | 10 | 120.8±15.9 |
| SKF 38393 | 30 | 194.6± 6.6 |
| + $N^G$-nitro-L-arginine | 5 | 178.0±12.4 |
| + $N^G$-nitro-L-arginine | 25 | 71.4± 6.0 ** |
| + $N^G$-nitro-L-arginine | 125 | 5.8± 2.4 ** |
| + L-arginine | 500 | 222.1±25.4 |
| + L-arginine and | 500 | |
| $N^G$-nitro-L-arginine | 25 | 83.0±22.8 |
| + L-Arginine and | 500 | |
| $N^G$-nitro-L-arginine | 125 | 54.9±24.5 |
| + 7-nitroindazole | 10 | 197.1±23.6 |
| + 7-nitroindazole | 25 | 192.3±18.2 |
| + 7-nitroindazole | 50 | 40.6± 6.4 ** |
| + L-arginine and | 500 | |
| 7-nitroindazole | 50 | 209.5±35.0 * |
| + aminoguanidine | 60 | 212.4±17.8 |
| + aminoguanidine | 150 | 180.7±18.4 |

Mice were injected with reserpine (5 mg/kg i.p.), and 24 h later with the dopamine $D_1$ receptor agonist SKF 38393 (30 mg/kg i.p.) plus other treatments as shown (all i.p.). Locomotor activity was recorded after 30 min for a 10 min period. All results are means±S.E.M. of at least eight determinations. * $P < 0.005$ versus SKF 38393 (30 mg/kg) + 7-nitroindazole (50 mg/kg), ** $P < 0.001$ versus SKF 38393 (30 mg/kg).

Table 3
Effects of NO synthase inhibitors on the locomotor response to RU 24213 in reserpine-treated mice

| Treatment | Dose (mg/kg) | Locomotor score |
|---|---|---|
| RU 24213 | 0.5 | 18.4 ± 2.1 |
| RU 24213 | 1.5 | 47.6 ± 5.3 |
| RU 24213 | 5 | 222.9 ± 23.8 |
| + $N^G$-nitro-L-arginine | 5 | 156.0 ± 46.2 |
| + $N^G$-nitro-L-arginine | 25 | 101.7 ± 32.0 * |
| + $N^G$-nitro-L-arginine | 125 | 45.7 ± 11.7 ** |
| + L-arginine | 500 | 198.9 ± 33.2 |
| + L-arginine and $N^G$-nitro-L-arginine | 500<br>25 | 81.6 ± 15.1 |
| + L-arginine and $N^G$-nitro-L-arginine | 500<br>125 | 67.9 ± 20.1 |
| + 7-nitroindazole | 10 | 183.8 ± 34.1 |
| + 7-nitroindazole | 25 | 174.3 ± 15.0 |
| + 7-nitroindazole | 50 | 107.9 ± 12.3 * |
| + L-arginine and 7-nitroindazole | 500<br>50 | 164.8 ± 28.4 |
| + aminoguanidine | 60 | 201.0 ± 25.7 |
| + aminoguanidine | 150 | 217.7 ± 11.8 |

Animals were injected with reserpine (5 mg/kg i.p.), and 24 h later with the dopamine $D_2$ receptor agonist RU 24213 (5 mg/kg s.c.) plus other treatments as shown. Thirty min later, their locomotor scores were determined for a period of 10 min. Each result is the mean ± S.E.M. of at least eight determinations. * $P < 0.05$, ** $P < 0.001$ versus RU 24213 5 mg/kg.

30 mg/kg SKF 38393 (Table 2). What little activity occurred in the presence of 25–125 mg/kg $N^G$-nitro-L-arginine was very slow and the animals looked lethargic. Similar results were obtained with 125 mg/kg i.p. $N^G$-nitro-L-arginine in combination with lower doses of SKF 38393 (3–10 mg/kg i.p.; significant drug × drug interaction by two-factor ANOVA, $F(3,128) = 3.82$, $P = 0.012$; data not shown).

Cotreatment with 7-nitroindazole at 50 mg/kg i.p. (but not 10–25 mg/kg i.p.) also markedly reduced the locomotor effects of SKF 38393 (ANOVA $F(3,52) = 9.47$, $P < 0.001$), but administration of the inducible NO synthase inhibitor aminoguanidine (60–150 mg/kg i.p.) failed to affect $D_1$ motor responding (Table 2).

### 3.4. Effect of NO synthase inhibitors on locomotion induced by RU 24213 in reserpine-treated mice

In line with earlier observations (Starr and Starr, 1993a,b; 1994), the $D_2$-selective dopamine receptor agonist, RU 24213 (0.5–5 mg/kg s.c.), dose dependently ameliorated reserpine-induced akinesia, but qualitatively less effectively than SKF 38393 (ANOVA main effect $F(3,80) = 17.48$, $P < 0.001$; Table 3). RU 24213 promoted a more unnatural-looking locomotion, characterised by head-down posture and sniffing directed at the floor, whilst the animals moved slowly forward in a perseverative fashion. The animals occasionally groomed themselves and reared against the sides of the container.

$N^G$-Nitro-L-arginine (5–125 mg/kg i.p.) significantly attenuated $D_2$ receptor-dependent locomotion, as illustrated in Table 3. With 25–125 mg/kg i.p. $N^G$-nitro-L-arginine, all movements induced by 5 mg/kg RU 24213 were slowed and the animals spent longer periods sitting still (ANOVA $F(3,23) = 3.62$, $P = 0.026$). $N^G$-Nitro-L-arginine (125 mg/kg i.p.) similarly suppressed locomotor responses to lower doses of RU 24213 (0.5–1.5 mg/kg s.c.; significant drug × drug interaction by two-factor ANOVA, $F(3,104) = 5.19$, $P = 0.019$; data not shown).

7-Nitroindazole, at the highest dose of 50 mg/kg i.p., only marginally suppressed RU 24213-induced locomotion (ANOVA $F(3,34) = 2.82$, $P = 0.049$), whilst aminoguanidine was without effect (Table 3).

### 3.5. Effect of L-arginine on $D_1$- and $D_2$ receptor-dependent locomotion in reserpine-treated mice

On the basis of an earlier study (Starr and Starr, 1993c), we sought to prevent inhibition of NO synthase with $N^G$-nitro-L-arginine and 7-nitroindazole by coadministering L-arginine, the biological precursor of NO. By itself, the amino acid (500 mg/kg i.p.) had no effect on the spontaneous activity of normal mice or the akinesia of reserpine-treated mice (Table 1).

Treatment with L-arginine, 500 mg/kg i.p., did not alter the motor responses of reserpine-treated mice to SKF 38393 (30 mg/kg i.p.) or RU 24213 (5 mg/kg s.c.), as shown in Tables 2 and 3. There was a tendency for this same dose of the amino acid to reverse the inhibitory effects of $N^G$-nitro-L-arginine (125 mg/kg i.p.) on $D_1$ receptor-dependent (but not $D_2$ receptor-dependent) locomotion in monoamine-depleted mice, but this trend failed to reach statistical significance (Tables 2 and 3). By contrast, L-arginine (500 mg/kg i.p.) counteracted the inhibitory effect of 7-nitroindazole on SKF 38393- but not RU 24213-induced locomotion (Tables 2 and 3).

## 4. Discussion

The present data indicate that $N^G$-nitro-L-arginine and 7-nitroindazole, but not $N^G$-nitro-D-arginine or aminoguanidine, suppress exploratory motor activity in normal mice, and reverse the motor stimulation elicited by dopamine $D_1$ and $D_2$ receptor agonists in monoamine-depleted mice. $N^G$-Nitro-L-arginine and 7-nitroindazole inhibit the constitutive form of NO synthase (Moore et al., 1993; Rees et al., 1990), which participates in synaptic transmission in the brain (Garthwaite et al., 1988; Bredt and Snyder, 1989). Increasing the availability of L-arginine, the biological

precursor of NO, is normally sufficient to offset the actions of these enzyme inhibitors (Garthwaite et al., 1989; Moore et al., 1993; Starr and Starr, 1993c), but this strategy was only partially effective in the present experiments. Thus coadministration of L-arginine with other drug treatments in reserpine-treated mice, allowed a greater restoration of movement in response to dopamine $D_1$ and $D_2$ receptor stimulation, but this only reached statistical significance with the combination of SKF 38393 plus 7-nitroindazole (Table 2). It is possible that a different dosage regimen of L-arginine would sustain NO synthesis more efficiently, and this is something we shall consider more carefully in future studies. Nonetheless, these data coupled with the inefficacy of the inactive enantiomer $N^G$-nitro-D-arginine, or aminoguanidine, which preferentially inhibits the inducible form of NO synthase (Misko et al., 1993), support the notion that ongoing NO production in the brain is essential for motor activity to occur.

Given the widespread distribution of NO synthase throughout the brain, there is a strong possibility that NO participates in cell signalling in many pathways that constitute, or impinge upon, motor circuits of the basal ganglia (Snyder and Bredt, 1991; Vincent and Hope, 1992). However, there are a number of reasons for believing that NO synthesis in the striatum is particularly influential in this respect. This structure is a key component of the basal ganglia (Mehler, 1981) and receives a massive, topographically ordered and putatively glutamatergic input from all major motor and sensory regions of the overlying cerebral cortex (Graybiel and Ragsdale, 1983). It is richly endowed with both NMDA receptors (Albin et al., 1992) and NO synthase (Vincent and Hope, 1992), and there is evidence that NO modulates the glutamate-induced release of dopamine (Hanbauer et al., 1992). Guevara-Guzman et al. (1994) recently extended the latter observation, by demonstrating that NO gas exerted profound effects on the in vivo output of a wide range of striatal amino acid and amine neurotransmitters, in urethane-anaesthetised rats. It is entirely possible, therefore, that glutamate released from corticostriatal axon terminals acts upon NMDA receptors, that are coupled via $Ca^{2+}$/calmodulin to NO synthesis (Garthwaite et al., 1989; McCall and Vallance, 1992), and that NO participates in sensorimotor integration by the striatum via its influences on these local transmitter systems.

If this hypothesis is correct, we would expect to see similarities between the behavioural profiles of NMDA receptor blockers and NO synthase inhibitors. We have previously found that all types of NMDA antagonists, regardless of their site of action within the NMDA receptor-ion channel complex (Wong and Kemp, 1991), have a sedative effect at higher dose levels (Starr and Starr, 1994). The mechanism of this sedation, and the deterioration in posture and gait that often accompany it, is not known, but it is noteworthy that quiescence was a constant feature of the behavioural actions of the NO synthase inhibitors $N^G$-nitro-L-arginine and 7-nitroindazole. Some NMDA receptor antagonists, especially those which occlude the ion channel non-competitively (e.g. MK 801), cause a pronounced hyperexcitability at lower doses through the release of dopamine and other neurotransmitters (e.g. Löscher and Hönack, 1992). We never saw any signs of behavioural stimulation with $N^G$-nitro-L-arginine or 7-nitroindazole, only depression, even with extended periods of observation (up to 3 h). Dopaminergic hypoactivity could be a contributory factor in the behavioural depression accompanying NO synthase inhibition, since Hanbauer et al. (1992) reported that exogenously applied NO stimulated dopamine release in striatal slices. If this is the case, then it will be interesting to determine if NO donors injected discretely into the striatum, are able to elicit dopamine-like increases in motor activity.

A prominent feature of NMDA receptor blockade in dopamine-depleted animals, is a potentiation of the motor activation induced by stimulating $D_1$ receptors, which could form the basis of a beneficial antiparkinsonian effect (Goodwin et al., 1992; Klockgether and Turski, 1990; Löschmann et al., 1991; Morelli et al., 1992; Starr and Starr, 1993a,b; 1994). Once again the cellular mechanism of this glutamate-dopamine interaction remains poorly understood, although a plausible explanation has been offered by Girault et al. (1990). These authors proposed that NMDA and $D_1$ receptors located within the cell membrane of striatal neurones, are reciprocally coupled to the phosphorylation of dopamine and cyclic AMP-regulated phosphoprotein (DARPP-32). This phosphatase inhibitor is a marker for striatonigral output neurones (Berretta et al., 1992), which are currently considered to preferentially mediate the expression of $D_1$ receptor-dependent motor behaviour (Gerfen, 1992). If NO acted as an intermediary for glutamate-induced deactivation of DARPP-32 in these neurones, then inhibiting NO synthase should mimic NMDA receptor antagonism and potentiate SKF 38393-induced locomotion in reserpine-treated mice (Starr and Starr, 1993a, 1994). That $N^G$-nitro-L-arginine and 7-nitroindazole clearly had the opposite effect in this study, and suppressed the motor stimulant action of SKF 38393, suggests that glutamate and NO control $D_1$ receptor-mediated behaviours in an opposite fashion, rather than as components of a common mechanism (Goodwin et al., 1992; Morelli and Di Chiara, 1990; Morelli et al., 1992; Starr and Starr, 1993a, 1994; Svensson et al., 1992).

Whereas NMDA receptor antagonists generally accentuate dopamine $D_1$ receptor-mediated locomotion in dopamine-depleted animals, their interactions with

$D_2$ receptor agonists are much more variable, depending on the particular NMDA receptor antagonist and $D_2$ agonist that are used in combination (Goodwin et al., 1992; Starr and Starr, 1994). Nevertheless, the most common finding is that blocking glutamate transmission also disables $D_2$ motor responding (Goodwin et al., 1992; Morelli and Di Chiara, 1990; Morelli et al., 1992; Starr and Starr, 1993a, 1994; Svensson et al., 1992). The abolition of RU 24123-induced locomotion by $N^G$-nitro-L-arginine and 7-nitroindazole could therefore involve a similar mechanism, but as yet no satisfactory explanation has been advanced to suggest what this mechanism might be. Girault et al. (1990) pointed out that any interference with $D_1$/DARPP-32 activity would automatically limit $D_2$ motor responding as well, since $D_1$ and $D_2$ receptors are held to function interdependently as far as the expression of normal motor activity in dopamine-intact animals is concerned (Clark and White, 1987; Waddington and O'Boyle, 1989). However, $D_1/D_2$ receptor cooperativity tends to be lessened under conditions of dopamine depletion and dopamine receptor supersensitivity, such as prevail following 24 h reserpine treatment (Neisewander et al., 1991; Starr et al., 1987). It is therefore unlikely that $N^G$-nitro-L-arginine and 7-nitroindazole lowered the efficacy of the $D_2$ agonist RU 24213, by attenuating the enabling function of $D_1$ receptors in the dopamine-depleted striatum.

An alternative explanation could involve a negative interaction between glutamate and dopamine within the executive pathways of the basal ganglia (Gerfen, 1992). In contrast to the close association of $D_1$ receptor-mediated behaviours with the striatonigral projection, $D_2$ receptor-mediated influences on motor behaviour are thought to be conducted by the striatopallidal output pathway (Anderson et al., 1992; Gerfen, 1992). This is a polysynaptic circuit involving excitatory glutamatergic neurones in the subthalamus, and so it is conceivable that NO synthase inhibitors will inhibit motor responding to RU 24213 by interrupting NMDA receptor-mediated transmission in subthalamic efferent pathways (Gerfen, 1992). A direct test of this hypothesis, would be to note whether NO synthase inhibitors are similarly able to abolish $D_2$ receptor-mediated motor recovery when they are deposited directly into the subthalamic nucleus, or its target structures (i.e. entopeduncular nucleus, substantia nigra) by stereotaxic injection.

In conclusion, the motor inhibitory properties of the NO synthase inhibitors $N^G$-nitro-L-arginine and 7-nitroindazole, suggest that NO functions as an intercellular messenger in motor circuits in the brain, but not necessarily as a consequence of NMDA receptor stimulation. There are probably many ways in which NO can participate in the regulation of motor output from the basal ganglia, for instance by altering the availability of striatal neurotransmitters (Guevara-Guzman et al., 1992) or the activity of their signal transduction mechanisms (Tsou et al., 1993), or the electrical properties of thalamocortical feedback circuits (Pape and Mager, 1992). No doubt others will be discovered as the relationship between this enigmatic molecule and motor behaviour is unravelled further.

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