

## ***N-Methyl-D-aspartate unilaterally injected into the dorsal striatum of rats produces contralateral circling: antagonism by 2-amino-7-phosphonoheptanoic acid and cis-flupenthixol***

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(Accepted 24 March 1992)

**Key words:** Glutamate; *N*-Methyl-D-aspartate; 2-Amino-7-phosphonoheptanoic acid; Dopamine; Striatum; Circling

To evaluate the possible contribution of dorsal striatal glutamate receptors to motor behavior, circling responses were observed in rats following unilateral intrastratial microinjections of the agonist, *N*-methyl-D-aspartate (NMDA) or the antagonist, 2-amino-7-phosphonoheptanoic acid (APH). The role of dopamine (DA) in NMDA-produced circling also was evaluated. In experiment 1, an NMDA dose of 5.0 µg (in 0.5 µl), but not 0.5 or 0.05 µg produced significant contraversive circling. In experiment 2, an APH dose of 10.0 µg but not 1.0 or 0.1 µg produced significant ipsiversive circling. In experiment 3, microinjection of the ineffective 0.1 µg dose of APH or a dose (20 µg) of the DA antagonist, *cis*-flupenthixol, that did not produce circling when administered alone, significantly reduced the circling response produced by the 5.0 µg dose of NMDA. As NMDA produced circling in the same direction as that seen following similar unilateral injections of locomotion-stimulating DA agonists, the present results suggest that glutamate, acting via NMDA receptors in the dorsal striatum, may exert an excitatory influence on motor systems. The observation that a DA receptor blocker antagonized the NMDA response further suggests that the observed motor excitatory effect of glutamate at NMDA receptors requires concurrent stimulation of DA receptors in the same region of the striatum.

### **INTRODUCTION**

The striatum receives massive glutamatergic projections from all areas of the cortex<sup>36</sup>. In the striatum, glutamate has been found to stimulate the release of dopamine (DA)<sup>5,16,19,22,24,35</sup>. The *N*-methyl-D-aspartate (NMDA) glutamate receptor subtype may contribute to this effect. Thus, glutamate-stimulated DA release was blocked by NMDA receptor antagonists in *in vitro*<sup>22</sup> and *in vivo* microdialysis experiments<sup>24</sup> and high (but not low<sup>15</sup>) concentrations of NMDA itself stimulated striatal DA release<sup>14,17</sup>. Moghaddam et al.<sup>24</sup> suggested that the DA-releasing action of high *in vivo* concentrations of NMDA may be related to pathophysiological conditions created by NMDA. Although the mechanism by which NMDA increases striated DA release remains to be worked out, it is well established that increases in striatal dopaminergic neurotransmission lead to increases in locomotor activity<sup>2</sup>. Therefore, these results suggest that intrastratial NMDA might stimulate locomotor activity.

Several authors have reported that bilateral microinjection of glutamate agonists acting at the NMDA, (*R,S*)- $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropanoic acid (AMPA) or kainic acid (KA) receptor subtypes into the ventral striatum (nucleus accumbens) led to locomotor activation<sup>1,8-10,13,28</sup> and some argue that this effect depended on DA<sup>8</sup>. In one study, injections of NMDA into an anterior region of the striatum produced *decreased* activity<sup>34</sup>. We know of no other reports concerning the behavioural effects of glutamatergic agents injected bilaterally into the dorsal striatal region, viz. the caudate nucleus.

Many studies concerned with the role of dorsal striatal DA in the control of locomotor activity have taken advantage of the rotational model originally described by Ungerstedt<sup>40</sup>. (For a recent review see ref. 23.) Rats with unilateral dorsal striatal DA depletions were observed to circle in a direction ipsilateral to the side of the lesion following systemic amphetamine showing that rats turn away from (contralateral to) the side of higher DA activity. Subsequent studies showed

that otherwise intact rats could be made to circle contraversively following direct unilateral dorsal striatal microinjections of DA agonists<sup>30,31</sup>. From this it follows that unilateral intrastriatal microinjections of glutamatergic agents, known to stimulate DA release<sup>5,14,16,17,19,21,22,24,35</sup>, may produce contraversive turning. One preliminary report recently described results supporting this hypothesis following injections of the excitatory amino acid agonists, L-glutamate, L-asparatate, NMDA, quisqualate or KA but no quantitative data were presented<sup>39</sup>. Others have reported contralateral rotation following unilateral intrastriatal microinjections of KA<sup>20,38</sup>.

The purpose of experiment 1 of the present study was to evaluate the effects of several intrastriatal doses of NMDA on circling behavior. Dose-dependent contraversive circling was observed. Experiment 2 evaluated the effects of intrastriatal injections of the NMDA antagonist, 2-amino-7-phosphonoheptanoic acid (APH) on circling behaviour in a second group of rats. Dose-dependent ipsiversive circling was seen. Experiment 3 evaluated the ability of doses of APH or the DA antagonist, *cis*-flupentixol, that were ineffective in producing circling when injected alone, to antagonize the circling response seen following NMDA.

## MATERIALS AND METHODS

Treatment of the rats in the present study was in accordance with the Animals For Research Act, the Guidelines of the Canadian Council on Animal Care and relevant University policy and was approved by the Queen's University Animal Care Committee.

### Animals

Forty-three male albino Wistar rats weighing 250–300 g, obtained from Charles River Canada, were individually housed in a climatically controlled ( $21 \pm 1^\circ\text{C}$ ) colony room on a 12 h light (06.00–18.00 h)/dark cycle. Food and water were continuously available in the home cages.

### Surgeries

Rats were anaesthetized with sodium pentobarbital (Somnotol, 60 mg/kg, i.p.) and prepared with chronically indwelling stainless steel guide cannulae (0.64 mm diam) stereotactically implanted at the following coordinates: 0.26 mm posterior to bregma, 3.0 mm lateral to the midline and 3.5 mm ventral to the skull with the incisor bar at 3.2 mm below the horizontal plane passing through the interaural line<sup>29</sup>. The ventral coordinate was 1.0 mm above the target site as the injection cannulae extended 1.0 mm beyond the tip of the guide. Cannulae were anchored to the skull with stainless steel screws and acrylic cement. Half of the rats were implanted on the right side and the other half on the left.

### Apparatus

Testing was carried out in three polyurethane-sealed circular wooden bases, with wire mesh sides 30 cm in diameter and 30 cm in height, fitted with plexiglass covers.

### Drugs

NMDA (Sigma) was dissolved in warm 0.9% saline at concentrations of 0.05, 0.5, and 5.0  $\mu\text{g}/0.5 \mu\text{l}$  and neutralized with 0.5 N

NaOH (pH 7.0–7.5). Racemic APH (Research Biochemicals) was dissolved in 0.9% saline at concentrations of 0.1, 1.0 and 10.0  $\mu\text{g}/0.5 \mu\text{l}$ . *cis*-Flupentixol (Lundbeck) was dissolved in 0.9% saline at a concentration of 20  $\mu\text{g}/0.5 \mu\text{l}$ . All drugs were dissolved daily prior to behavioral testing.

### Central injections

Microinjections were delivered in a volume of 0.5  $\mu\text{l}$  with a 10  $\mu\text{l}$  Hamilton microsyringe. Injection cannulae were constructed with stainless steel tubing (0.31 mm diam), cut to extend 1.0 mm beyond the tip of the guide cannulae and were attached to the microsyringe by a length of polyethylene tubing. The injections were delivered by hand over 30 sec and the injection cannula was left in place for an additional 30 s to allow for diffusion.

### Procedure

#### Experiment 1: NMDA-induced circling behavior

Behavioral testing began approximately 7 days after surgery and involved 7 sessions per animal, as follows: (1) no central injection; (2) central injection of the vehicle (saline); (3,4,5) each of the 3 NMDA doses (0.05, 0.5 and 5.0  $\mu\text{g}$  in 0.5  $\mu\text{l}$ ) administered in a counterbalanced order across rats over three sessions; (6) replication of the vehicle injection; (7) replication of no central injection condition. Test sessions were separated by 48 h.

All complete turns (360°) were recorded and their direction with respect to the side of the cannula (ipsilateral or contralateral) was noted across four 5-min observation periods (0–5, 15–20, 30–35, 45–50 min) composing a 50-min session. The use of this time sampling procedure made it possible to test 3 animals at a time, each starting at staggered intervals of 5 min with the clock being stopped during the time required to make the central injection. Circling behavior was expressed as the ratio of ipsilateral turns over the total number of turns (ipsilateral + contralateral). Values greater than 0.5 indicated ipsilateral circling. Thus, the dependent measures were the circling ratio and the total number of turns.

#### Experiment 2: APH-induced circling behavior

Behavioral testing was similar to that described for experiment 1 with the following changes: (1) the drug injected in sessions 3, 4 and 5 was APH at doses of 0.1, 1.0, and 10  $\mu\text{g}$  in 0.5  $\mu\text{l}$ ; (2) the duration of testing now consisted of two 5-min observation periods (0–5 and 15–20 min); this change was made following the finding in experiment 1 that minimal circling behavior was seen during the last two observation periods.

#### Experiment 3: antagonism of NMDA-induced circling behavior

Behavioral testing began 7 days after surgery and involved 7 sessions per animal, as follows: (1,7) no central injection; (2,4,6) NMDA in a dose of 5.0  $\mu\text{g}$  in 0.5  $\mu\text{l}$ ; (3,5) NMDA (5.0  $\mu\text{g}$  in 0.5  $\mu\text{l}$ ) preceded 15 min earlier by APH (0.1  $\mu\text{g}$  in 0.5  $\mu\text{l}$ ) or *cis*-flupentixol (20  $\mu\text{g}$  in 0.5  $\mu\text{l}$ ). Half the animals received APH in session 3 and *cis*-flupentixol in session 5 and the other half in the reverse order. As in experiment 2, observations were made for two 5-min periods (0–5 and 15–20 min).

### Histology

Upon conclusion of behavioral testing, all animals were injected with a lethal dose of sodium pentobarbital and perfused intracardially with saline followed by 10% formalin. Frozen coronal sections (50  $\mu\text{m}$ ) were mounted and stained with thionine.

### Statistical analyses

For the turning ratio and total turns data, *t*-tests for correlated measures were conducted on the first and second no-injection scores and the first and second sessions of vehicle for experiments 1 and 2; for experiment 3, NMDA sessions 2, 4 and 6 were compared using one-way repeated measures analysis of variance (ANOVA). Where no differences were found, scores were averaged across those condi-

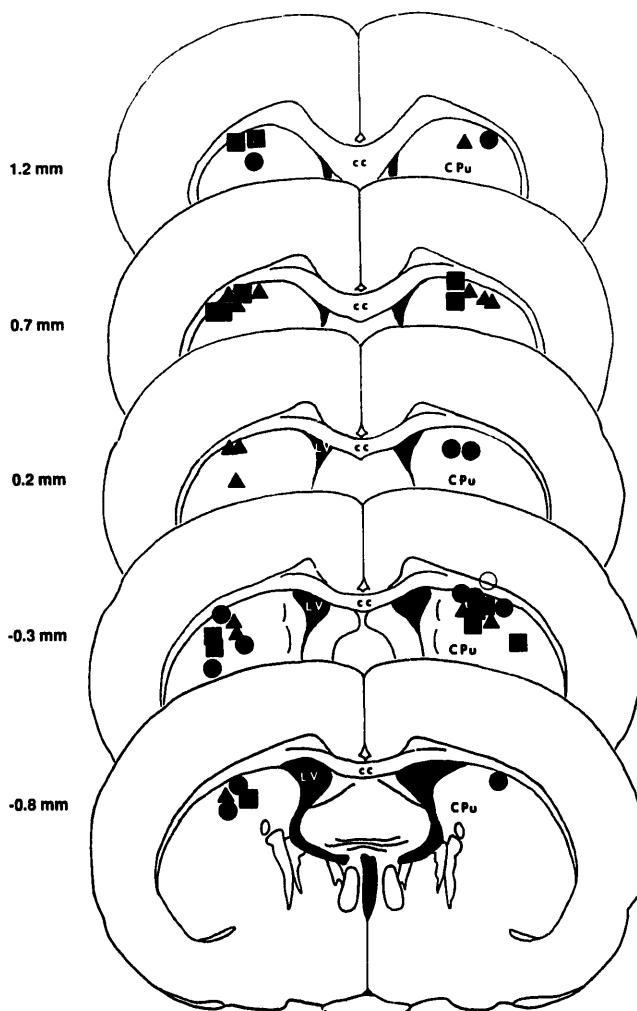


Fig. 1. Cannulae placements for rats from experiments 1 (▲), 2 (■) and 3 (●). The one open symbol indicates a miss. Coronal sections were adapted from Paxinos and Watson<sup>29</sup> and numbers beside each section indicate the distance in mm anterior to bregma.

tions. ANOVAs were then conducted to analyze treatment effects. The Geisser-Greenhouse correction for degrees of freedom in repeated measures designs was used. Individual comparisons employed Dunnett's tests using the saline session as control in experiments 1 and 2 and the three NMDA sessions combined in experiment 3.

## RESULTS

Histological results confirmed that of the 15, 14 and 14 rats originally operated for each of experiments 1, 2 and 3, the cannulae were in the appropriate site for 15, 14 and 13 rats, respectively. These placements and the rejected placement are shown in Fig. 1.

### Experiment 1: NMDA-induced circling behaviour

The four observation periods were combined for each treatment and circling ratios (ipsilateral turns/total turns in both directions) were calculated; a ratio below 0.5 would indicate a contralateral turning bias. Separate correlated *t*-tests were performed comparing

the first and second no-injection treatments (sessions 1 and 7) and the first and second saline treatments (sessions 2 and 6). Neither of these *t*-tests resulted in significant differences and the scores were averaged for each pair of sessions (Fig. 2A).

The no injection and saline treatments produced circling ratios of approximately 0.5, indicating the absence of a directional bias. In the doses range tested, NMDA produced progressively lower circling ratios,

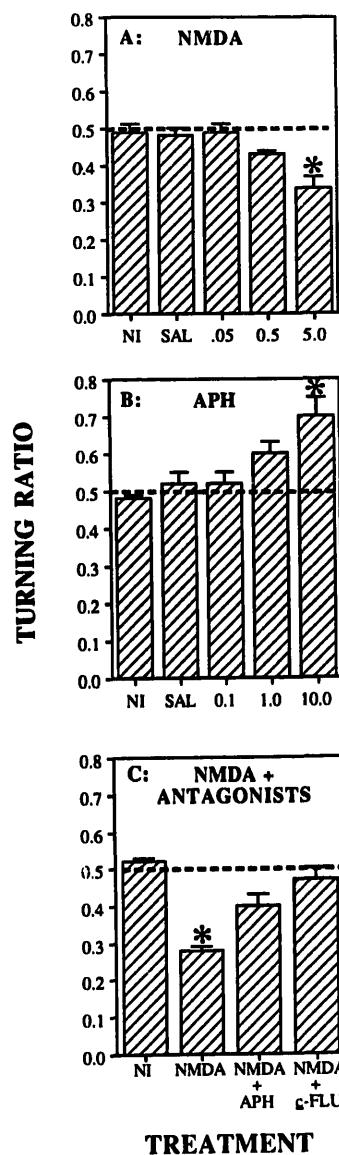


Fig. 2. Mean ( $\pm$  S.E.M.) turning ratios (total ipsilateral turns/total ipsilateral + contralateral turns) for the two no-injection (NI) sessions combined, the two saline (SAL) sessions combined (experiments 1 and 2), or the three doses ( $\mu$ g in 0.5  $\mu$ l) of NMDA in experiment 1 (A) or APH in experiment 2 (B). Treatments in experiment 3 (C) include the three NMDA (5.0  $\mu$ g in 0.5  $\mu$ l) sessions combined, NMDA (5.0  $\mu$ g) preceded 15 min earlier by APH (0.1  $\mu$ g) or NMDA preceded 15 min earlier by *cis*-flupentixol (*c*-FLU, 20  $\mu$ g). Asterisks indicate treatments that were significantly different from SAL in experiments 1 and 2; in experiment 3, the contralateral turning bias produced by NMDA was significantly attenuated by APH or *c*-FLU.

demonstrating a dose-dependent contralateral bias. It is noteworthy that the circling observed was not the tight nose-to-tail type reported following unilateral DA denervation of the striatum and injection of apomorphine<sup>30</sup>; rather, animals tended to move around the perimeter of the circular testing arena but with a quantifiable directional bias. A one-way repeated measures ANOVA for 5 treatments (no injection, saline, 0.05, 0.5 and 5.0 µg NMDA) revealed a significant main effect of treatment,  $F_{3,87,54,2} = 6.25$ ,  $P < 0.001$ . Post-hoc Dunnett's tests comparing the treatment groups to saline showed that the 5.0 µg dose of NMDA was significantly lower than saline (Fig. 2A).

Besides affecting directional bias, NMDA might influence activity as indicated by total turns. For total turns, the first and second sessions with no-injection (sessions 1 and 7) and saline (sessions 2 and 6) were combined as the differences between them were not significant (Table I). There appeared to be a dose-dependent increase in the number of total turns. This was confirmed by an ANOVA revealing a significant main effect of treatment,  $F_{3,13,43,82} = 5.63$ ,  $P < 0.01$ . Dunnett's test revealed that only the 5.0 µg dose of NMDA produced significantly more turns than the saline treatment.

#### Experiment 2: APH-induced circling behaviour

For circling ratios, no significant differences were found in the *t*-tests comparing the first and second no injection and the first and second saline treatments. Thus, these scores were averaged for each pair of sessions (Fig. 2B).

In the dose range tested, APH produced progressively higher circling ratios, demonstrating a dose-de-

pendent ipsilateral bias. A one-way repeated measures ANOVA, performed on the five treatments (no injection, saline, 0.1, 1.0 and 10.0 µg APH) revealed a significant treatment effect,  $F_{2,79,36,27} = 9.99$ ,  $P < 0.0001$ . Dunnett's tests comparing the treatment groups to saline indicated that the 10.0 µg dose of APH produced significantly higher circling ratios.

For total turns, the first and second sessions of no injection and saline were not combined because the differences between them were significant,  $t_{13} = 2.36$  and 3.21,  $P < 0.05$ , respectively. The ANOVA revealed a significant treatment effect,  $F_{6,78} = 3.93$ ,  $P < 0.002$ , and post-hoc comparison revealed significant differences between the first saline treatment and the three APH treatments (Table I).

#### Experiment 3: antagonism of NMDA-induced circling behaviour

For circling ratios, a correlated *t*-test comparing the first and second no injection treatment showed no significant difference and these scores were averaged. Circling ratios ( $\pm$ S.E.M.) for the first, second and third NMDA treatments (sessions 2, 4 and 6) were 0.26 ( $\pm$ 0.04), 0.26 ( $\pm$ 0.04) and 0.32 ( $\pm$ 0.02), respectively, replicating the contralateral circling effect seen in experiment 1. A one-way ANOVA comparing the 3 NMDA treatments revealed no significant difference,  $F_{1,67,20,04} = 0.58$ ,  $P > 0.05$ , and these scores were averaged (Fig. 2C).

Both APH and *cis*-flupenthixol appeared to block the contralateral circling effect of NMDA. Using a one-way repeated measures ANOVA, the 4 treatments (no injection, NMDA, APH + NMDA and *cis*-flupenthixol + NMDA) were analyzed and a significant treatment effect was found,  $F_{1,87,22,43} = 11.63$ ,  $P <$

TABLE I

Mean ( $\pm$ S.E.M.) total number of turns for each experiment

Abbreviations: Exp., experiment; NI, no-injection; Sal, saline; Low, Medium and High drug doses were 0.05, 0.5 and 5.0 µg in Exp. 1 and 0.1, 1.0 and 10.0 µg in Exp. 2.

Exp. (n)	NI	Sal	Drug dose		
			Low	Medium	High
1 (15)	9.4 $\pm$ 0.7	11.2 $\pm$ 1.2	12.1 $\pm$ 1.0	14.0 $\pm$ 1.2	15.5 $\pm$ 1.4 *
2 (14)	9.5 $\pm$ 1.0 **	10.1 $\pm$ 0.9 **	6.1 $\pm$ 0.6	7.2 $\pm$ 0.8	7.6 $\pm$ 0.9
	6.8 $\pm$ 0.8	6.6 $\pm$ 0.7			
	NI	NMDA (5.0 µg)	NMDA (5.0 µg) + APH (0.1 µg)		NMDA (5.0 µg) + <i>cis</i> -flupenthixol (20.0 µg)
3 (13)	5.3 $\pm$ 0.3	5.4 $\pm$ 0.4	4.8 $\pm$ 0.4		5.4 $\pm$ 0.4

\* There was a significant ( $P < 0.01$ ) treatment effect in experiment 1 and post-hoc tests showed the high (5.0 µg) dose to differ significantly from Sal.

\*\* The first and second NI and the first and second SAL scores differed significantly from one another ( $P < 0.05$ ). There was a significant ( $P < 0.002$ ) overall treatment effect and the first SAL score differed from each APH dose.

0.001. Dunnett's tests comparing NMDA to the other two treatments revealed that both APH and *cis*-flupenthixol significantly decreased the contralateral directional bias produced by NMDA alone.

A *t*-test comparing total turns in the first and second no-injection sessions showed no significant difference and the scores were averaged. An ANOVA of the total turns data among the three NMDA sessions revealed no significant effect,  $F_{2,26} = 1.42$ ,  $P > 0.05$ , and the data were averaged (Table I). An ANOVA comparing total turns in the 4 treatments (No injection, NMDA, APH + NMDA and *cis*-flupenthixol + NMDA) revealed no significant treatment effect,  $F_{3,39} = 0.61$ ,  $P > 0.05$ .

## DISCUSSION

The present results revealed that unilateral dorsal striatal microinjections of NMDA produced a dose-dependent increase in contralateral circling. Similar treatments with the NMDA receptor antagonist, APH produced dose-dependent ipsilateral circling. The effects of NMDA on circling were blocked by co-injections of doses of APH or *cis*-flupenthixol that failed to affect circling when given alone. These results suggest that the motor effects of glutamate at NMDA receptors in the dorsal striatum require concurrent stimulation of DA receptors in the same region.

The series of 5 central injection sessions in each experiment was preceded and followed by no-injection sessions. Mean turning ratios were seen to be near 0.5 in each case, indicating no directional bias, and in no case did the first no-injection score differ from the last. These results showed that neither chronic cannulation nor the series of five central injection sessions had significant enduring effects on directional bias, as reported in previous studies from this laboratory<sup>3,18,25,26,37</sup>. It is noteworthy that the highest dose of NMDA (5.0  $\mu$ g) may have been excitotoxic<sup>11</sup> producing a striatal lesion. Such an effect might have been expected to lead to a change in directional bias from the first to the second no-injection session. No such effect was detected.

The series of 3 central injections of glutamatergic agents in experiments 1 and 2 also was bracketed by unilateral central injections of saline. Ratio values were near 0.5 and did not differ significantly from the pre- to post-drug sessions. These results showed that turning did not result simply from mechanical stimulation of striatal tissue caused by fluid infusion. Nor could the results be attributed to a sensitization to the effects of repeated injections as the pre- and post-drug turning ratios did not differ significantly. This observation also

might suggest that NMDA in the dose range used here did not produce behaviourally-relevant lesions of striatal tissue. These results are consistent with those of previous control studies<sup>18</sup>.

The possibility that the present results were due to diffusion of the drug to the nucleus accumbens seems unlikely. It has been shown that a 1.0  $\mu$ l injection volume diffuses into a sphere of approximately 1.0 mm diameter<sup>27,39</sup> and an even smaller volume (0.5  $\mu$ l) was used here. Furthermore, if the drug effect was due to diffusion to a remote site, a delay in its onset of action would be expected. In the present study the maximal effects on turning were seen in the first 5-min observation period making a remote site of action less likely.

In this and in previous studies from this laboratory (e.g. ref. 18) it has been found that turning does not occur if cannulae tips are in or dorsal to the corpus callosum dorsal to the striatal target site. However, as shown in Fig. 1, turning is seen with a variety of placements within the dorsal anterior portion of the striatum. Whether placements in other regions of the striatum can produce similar effects remains to be investigated.

In each experiment, directionally and total turns were evaluated. However, it appears that only directionality was influenced in a consistent manner. Total turns in experiment 1 were seen to increase with increasing dose of NMDA. In experiments 2 and 3, there was no significant change in total turns associated with different doses of APH or repeated doses of NMDA, respectively. The 5.0  $\mu$ g dose of NMDA that produced a significant increase in total turns in experiment 1 failed to do so in experiment 3. The reason for this discrepancy is unclear. However, it is noteworthy that the effects of NMDA on the *direction* of turning were consistent across the two experiments. Furthermore, these consistent effects were seen in spite of a small overall total number of turns. The finding of inconsistent effects of centrally infused pharmacological compounds on total turns is in accord with previous studies from this laboratory as is the finding of consistent effects on the directional bias of the animal<sup>3,18</sup>. These findings underscore the utility of directional bias or relative turning as a sensitive dependent measure reflecting bilateral differences in striatal function.

The observation that unilateral intrastriatal microinjections of NMDA produced contralateral turning in a dose-dependent manner is consistent with the preliminary observations reported by Toth and Lajtha<sup>39</sup>. These researchers reported contralateral rotation following L-glutamate, L-aspartate, NMDA, quisqualate or KA. Others also have reported contralateral turning following unilateral intrastriatal injections of KA<sup>20,38</sup>.

Dopaminergic agonists injected unilaterally into the otherwise intact striatum produce contralateral rotation<sup>30,31</sup> and bilateral injections of these agents produce hyperactivity<sup>7</sup>. This suggests a relationship between the effects of unilateral and bilateral injections. Thus, it would appear that agents that produce contralateral rotation when centrally administered unilaterally produce locomotor enhancement when centrally administered bilaterally. From this point of view, the observation of contralateral circling following unilateral NMDA would suggest that glutamate acting at striatal NMDA receptors enhances locomotor activity. This is consistent with previous reports of hyperactivity following bilateral intraaccumbens injections of NMDA<sup>1,8-10,13,28</sup>. In one paper, Schmidt and Bury<sup>34</sup> reported that bilateral injections of NMDA into a region of the striatum anterior and ventral to the site employed here and dorsal to the nucleus accumbens produced *decreases* in locomotor activity. Whether this result indicates regional differences in the locomotor function of striatal NMDA receptors or reflects different methodologies used to assess locomotor activity awaits further study.

The observation that unilateral intrastratal microinjections of the specific NMDA receptor antagonist, APH produced ipsilateral turning in a dose-dependent manner has not been reported previously. This result suggests that glutamate acting at NMDA receptors may produce a tonic effect on motor output.

The results of the present experiments showed that a 0.1  $\mu$ g dose of APH that was ineffective at producing turning when given alone, antagonized the turning response to NMDA. As APH is a relatively specific NMDA antagonist<sup>12</sup> this confirms that turning produced by NMDA was due to its action at the NMDA receptor.

Schmidt<sup>32,33</sup> reported that bilateral intrastratal injections of the NMDA receptor antagonists, DL-2-amino-5-phosphonovaleric acid (AP-5) or the glutamate receptor antagonist, kynurenic acid produced hyperactivity. If agents that produce ipsilateral turning when injected unilaterally produce decreases in locomotor activity when injected bilaterally, the converse to the relationship for contraversive turning and increased activity discussed above, then the results of Schmidt<sup>32,33</sup> are inconsistent with the present findings. However, Schmidt<sup>32</sup> stated that unilateral injections of kynurenic acid failed to produce a turning response although no data were presented. Further experiments are needed to determine possible regional heterogeneity in the function of striatal NMDA and other glutamate receptor subtypes that can reconcile these findings.

The observation that the DA receptor antagonist, *cis*-flupenthixol blocked turning produced by intrastratal NMDA has not been reported previously. This result suggests that the effects of NMDA require intact DA neurotransmission. Studies in this laboratory<sup>18</sup> and elsewhere<sup>6</sup> have shown that *cis*-flupenthixol fails to produce turning when injected alone and blocks turning produced by DA agents. Thus, the present finding probably did not result simply from two opposite effects cancelling one another. Taylor et al.<sup>38</sup> reported that the turning response produced by intrastratal KA, also known to produce DA release<sup>5</sup> was attenuated by systemic injections of the DA D<sub>2</sub> antagonist, haloperidol. This result is consistent with the present finding. In contrast, Toth and Lajtha<sup>39</sup> failed to observe a blockade of L-glutamate-produced contralateral turning by haloperidol. However, the absence of pertinent data in their paper makes it difficult to evaluate the significance of this effect.

Carlsson and Carlsson<sup>4</sup> have proposed that central glutamatergic and dopaminergic projections to the striatum may be functionally opposed with regard to the control of locomotor activity. They base their model largely on the observation that systemic treatment with the NMDA receptor antagonist, MK-801 leads to an increase in locomotor activity in monoamine-depleted mice. Perhaps the motor influences of this compound, when administered systematically, take place at central sites that are downstream from the striatum. Further studies are needed to reconcile the interesting observations reported by Carlsson and Carlsson<sup>4</sup> with the results of studies using intrastratal delivery of glutamatergic agents.

Others have reported that bilateral intraaccumbens injections of NMDA or the other glutamate receptor-subtype specific agonists, KA or AMPA produced hyperactivity<sup>1,8-10,13,28</sup>. This response was blocked by haloperidol or *cis*-flupenthixol suggesting mediation by DA<sup>1,8</sup>. These results from the nucleus accumbens are consistent with the present findings from the dorsal striatum. It appears that the motor excitatory influence of glutamate at the NMDA receptor in the striatum requires concurrent stimulation of DA receptors in the same region.

**Acknowledgements.** We wish to thank H. Lundbeck for the generous gift of *cis*-flupenthixol. This research was funded by grants from the Medical Research Council of Canada to K.J. and the Natural Sciences and Engineering Research Council of Canada and the Ontario Ministry of Health to R.J.B.

## REFERENCES

- 1 Arnt, J., Hyperactivity following injection of glutamate agonists in the rat nucleus accumbens, *Life Sci.*, 28 (1981) 1597-1603.

- 2 Beninger, R.J., The role of dopamine in locomotor activity and learning. *Brain Res. Rev.*, 6 (1983) 173-196.
- 3 Beninger, R.J., Musgrave, M.A. and Dickson, P.R., Unilateral injections of a D2 but not D1 agonist into the frontal cortex of rats produce a contralateral directional bias. *Pharmacol. Biochem. Behav.*, 37 (1990) 387-392.
- 4 Carlsson, M. and Carlsson, A., Interactions between glutamatergic and monoaminergic systems within the basal ganglia - implications for schizophrenia and Parkinson's disease. *Trends Neurosci.*, 13 (1990) 272-276.
- 5 Clow, D.W. and Jhamandas, K., Characterization of L-glutamate action on the release of endogenous dopamine from the rat caudate-putamen. *J. Pharmacol. Exp. Ther.*, 248 (1989) 722-728.
- 6 Costall, B., Kelly, M.E. and Naylor, R.J., The production of asymmetry and circling behaviour following unilateral, intrastriatal administration of neuroleptic agents: a comparison of abilities to antagonise striatal function. *Eur. J. Pharmacol.*, 96 (1983) 79-86.
- 7 Costall, B. and Naylor, R.J., A comparison of the abilities of typical neuroleptic agents and of thioridazine, clozapine, sulpiride and metoclopramide to antagonise the hyperactivity induced by dopamine applied intracerebrally to areas of the extrapyramidal and mesolimbic systems. *Eur. J. Pharmacol.*, 40 (1976) 9-19.
- 8 Donzanti, B.A. and Uretsky, N.J., Effects of excitatory amino acids in locomotor activity after bilateral microinjection into the rat nucleus accumbens: possible dependence on dopaminergic mechanisms. *Neuropharmacology*, 22 (1983) 971-981.
- 9 Donzanti, B.A. and Uretsky, N.J., Antagonism of the hypermotility response induced by excitatory amino acids in the rat nucleus accumbens. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, 325 (1984) 1-7.
- 10 Donzanti, B.A. and Uretsky, N.J., Magnesium selectively inhibits N-methyl-aspartic acid-induced hypermotility after intra-accumbens injection. *Pharmacol. Biochem. Behav.*, 20 (1984) 243-246.
- 11 Dunnett, S.B., Whishaw, I.Q., Jones, G.H. and Bunch, S.T., Behavioural, biochemical and histochemical effects of different neurotoxic amino acids injected into nucleus basalis magnocellularis of rats. *Neuroscience*, 20 (1987) 653-669.
- 12 Evans, R.H. and Watkins, J.C., Pharmacological antagonists of excitant amino acid action. *Life Sci.*, 28 (1981) 1303-1308.
- 13 Hamilton, M.H., De Belleroche, J.S., Gardiner, I.M. and Herberg, L.J., Stimulatory effect of N-methyl aspartate on locomotor activity and transmitter release from rat nucleus accumbens. *Pharmacol. Biochem. Behav.*, 25 (1986) 943-948.
- 14 Imperato, A., Honoré, T. and Jensen, L.H., Dopamine release in the nucleus caudatus and in the nucleus accumbens is under glutamatergic control through non-NMDA receptors: a study in freely-moving rats. *Brain Res.*, 530 (1990) 223-228.
- 15 Imperato, A., Scrocco, M.G., Bacchi, S. and Angelucci, L., NMDA receptors and in vivo dopamine release in the nucleus accumbens and caudatus. *Eur. J. Pharmacol.*, 187 (1990) 555-556.
- 16 Jhamandas, K. and Marien, M., Glutamate-evoked release of endogenous brain dopamine: inhibition by an excitatory amino acid antagonist and an enkephalin analogue. *Br. J. Pharmacol.*, 90 (1987) 641-650.
- 17 Jones, S.M., Snell, L.D., Johnson, K.M., Inhibition by phenylcyclidine of excitatory amino acid-stimulated release of neurotransmitter in the nucleus accumbens. *Neuropharmacology*, 26 (1987) 173-179.
- 18 Josselyn, S.A. and Beninger, R.J., Behavioural effects of intrastriatal caffeine mediated by adenosinergic modulation of dopamine. *Pharmacol. Biochem. Behav.*, 39 (1991) 91-103.
- 19 Krebs, M.O., Desce, J.M., Kemel, M.L., Gauchy, C., Godeheu, G., Cheramy, A. and Glowinski, J., Glutamatergic control of dopamine release in rat striatum: Evidence for presynaptic N-Methyl-D-aspartate receptors on dopaminergic nerve terminals. *J. Neurochem.*, 56 (1991) 81-85.
- 20 Kuriyama, K. and Kurihara, E., Correlation between rotational behaviours and neurochemical changes associated with damage of rat striatum: analysis using unilateral microinjection of kainic acid. *Neurochem. Int.*, 84 (1982) 42-47.
- 21 Leviel, V., Gobert, A. and Guibert, B., The glutamate-mediated release of dopamine in the rat striatum: further characterization of the dual excitatory-inhibitory function. *Neuroscience*, 39 (1990) 305-312.
- 22 Lonart, Gyorgy and Zigmond, M.J., High glutamate concentrations evoke  $Ca^{2+}$ -independent dopamine release from striatal slices: a possible role of reverse dopamine transport. *J. Pharmacol. Exp. Ther.*, 256 (1991) 1132-1138.
- 23 Miller, R. and Beninger, R.J., On the interpretation of asymmetries of posture and locomotion produced with dopamine agonists in animals with unilateral depletion of striatal dopamine. *Prog. Neurobiol.*, 36 (1991) 229-256.
- 24 Moghaddam, B., Gruen, R.J., Roth, R.H., Bunney, B.S. and Adams, R.N., Effect of L-glutamate on the release of striatal dopamine: in vivo dialysis and electrochemical studies. *Brain Res.*, 518 (1990) 55-60.
- 25 Morency, M.A., Stewart, R.J. and Beninger, R.J., Effects of unilateral microinjections of sulpiride into the medial prefrontal cortex on circling behavior of rats. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry*, 9 (1985) 735-738.
- 26 Morency, M.A., Stewart, R.J. and Beninger, R.J., Circling behavior following unilateral microinjections of cocaine into the medial prefrontal cortex: dopaminergic or local aesthetic effect? *J. Neurosci.*, 7 (1987) 812-818.
- 27 Myers, R.D., *Handbook of Drug and Chemical Stimulation of the Brain: Behavioral, Pharmacological and Physiological Aspects*, Van Nostrand, New York, 1974.
- 28 O'Neill, K.A., Carelli, R.M., Jarvis, M.F. and Liebman, J.M., Hyperactivity induced by N-methyl-D-aspartate injections into nucleus accumbens: lack of evidence for mediation by dopaminergic neurons. *Pharmacol. Biochem. Behav.*, 34 (1989) 739-745.
- 29 Paxinos, G. and Watson, C., *The Rat Brain in Stereotaxic Coordinates*, 2nd edn., Academic Press, New York, 1986.
- 30 Pycock, C.J., Turning behaviour in animals. *Neuroscience*, 5 (1980) 461-514.
- 31 Pycock, C.J. and Kilpatrick, I.C., Motor asymmetries and drug effects: Behavioral analyses of receptor activation. In A.A. Boulton, G.B. Baker and A.J. Greenshaw (Eds.), *Neuromethods: Vol. 13. Psychopharmacology*, Humana Press, Clifton, NJ, 1989, pp. 1-94.
- 32 Schmidt, W.J., Striatal glutamic acid and behaviour. *Neurosci. Lett.*, Suppl. 22 (1985) 93.
- 33 Schmidt, W.J., Intrastriatal injection of D,L-2-amino-5-phosphonovaleric acid (AP-5) induces sniffing stereotypy that is antagonized by haloperidol and clozapine. *Psychopharmacology*, 90 (1986) 123-130.
- 34 Schmidt, W.J. and Bury, D., Behavioural effects of N-methyl-D-aspartate in the anterodorsal striatum of the rat. *Life Sci.*, 43 (1988) 545-549.
- 35 Shimizu, N., Duan, S., Hori, T. and Oomura, Y., Glutamate modulates dopamine release in the striatum as measured by brain microdialysis. *Brain Res. Bull.*, 25 (1990) 99-102.
- 36 Spencer, H.J., Antagonism of cortical excitation of striatal neurons by glutamic acid diethyl ester: evidence for glutamic acid as an excitatory transmitter in the rat striatum. *Brain Res.*, 102 (1976) 91-101.
- 37 Stewart, R.J., Morency, M.A. and Beninger, R.J., Differential effects of intrafrontocortical microinjections of dopamine agonists and antagonists on circling behavior of rats. *Behav. Brain Res.*, 17 (1985) 67-72.
- 38 Taylor, R.J., Reavill, C., Jenner, P. and Marsden, D., Circling behaviour following unilateral kainic acid injections into rat striatum. *Eur. J. Pharmacol.*, 76 (1981) 211-222.
- 39 Toth, E. and Lajtha, A., Motor effects of intracaudate injection of excitatory amino acids. *Pharmacol. Biochem. Behav.*, 33 (1989) 175-179.
- 40 Ungerstedt, U., Histochemical studies on the effect of intracerebral and intraventricular injections of 6-hydroxydopamine on monoamine neurons in the rat brain. In T. Malme fors and H. Thoenen (Eds.), *6-Hydroxydopamine and Catecholamine Neurons*, Elsevier, Amsterdam, 1971, pp. 101-127.