

Glutamate receptor agonist injections into the dorsal striatum cause contralateral turning in the rat: involvement of kainate and AMPA receptors

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Abstract

Unilateral stimulation of glutamate receptors in the dorsal striatum of intact rats resulted in contralateral turning. Turning behavior was recorded for 20 min following unilateral intrastratial injections (0.5 μ l) in chronically cannulated rats. Kainate injections caused a dose-dependent increase in contralateral rotation that was blocked by the glutamate receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), the action potential blocker tetrodotoxin, and by increasing doses of the dopamine receptor antagonist *cis*-flupenthixol. Injections of α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) also caused rotation that was blocked with co-injections of CNQX, tetrodotoxin or *cis*-flupenthixol. Neither CNQX nor tetrodotoxin injected alone caused turning. This effect is dopamine-dependent, and may result from a kainate or AMPA-induced increase in dopamine release. Glutamate receptor agonist injections into the striatum may cause contralateral turning by degrading information in ascending cortical projections and may further influence locomotion via basal ganglia output nuclei projections to the brainstem.

Keywords: Glutamate; Turning; Striatum; Tetrodotoxin; CNQX (6-cyano-7-nitroquinoxaline-2,3-dione); *cis*-Flupenthixol

1. Introduction

It has long been established that the cortex provides the striatum with its principal afferent input (Spencer, 1976). This excitatory projection consists of fibers from virtually every cortical area, using either glutamate or aspartate as their neurotransmitter (Spencer, 1976; Godukhin et al., 1980; Perschak and Cuénod, 1990; Palmer et al., 1989). Among the glutamate receptors located in the striatum are the ionotropic subtypes kainate, α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) and *N*-methyl-D-aspartate (NMDA) (Nakanishi and Masu, 1994). A second major projection to the striatum is the dopaminergic tract from the substantia nigra. These and other transmitters function in concert to shape corticofugal signals that, particularly in the dorsal striatum, modulate the timing and course of movement.

Motor abnormalities can result from manipulating either the glutamate or dopamine afferent systems in the striatum. For example, circling behavior in rats after unilateral de-

pletion of striatal dopamine has been used for several decades as a measure of imbalance in the dopamine system. Ungerstedt (1968) first reported that rats tend to rotate towards the side of lower striatal dopamine activity, a result that has formed the basis for hundreds of behavioral studies of striatal pharmacology (for review, see Pycoc and Kilpatrick, 1989). Thus, if overall dopamine transmission in animals depleted of dopamine unilaterally was stimulated by a dopamine releaser such as systemically injected amphetamine, intense ipsilateral rotation was observed (Marshall and Ungerstedt, 1977). Conversely, if a direct dopamine receptor agonist such as apomorphine was administered, the greater activity at supersensitive and up-regulated dopamine receptors on the lesioned side resulted in intense contralateral rotation (Marshall and Ungerstedt, 1977). Furthermore, direct injections of amphetamine into the striatum of non-lesioned animals have been shown to produce contralateral turning (Moore et al., 1994). It is noteworthy that turning behaviour in the intact animal, although showing dose dependency and being highly reliable, was far less intense than in denervated animals.

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More recently, it has been shown that contralateral rotation can be elicited with dorsal striatal microinjections of the glutamate receptor agonists NMDA (Thanos et al., 1992; Toth and Lajtha, 1989; Black et al., 1994) and *trans*-(1*S*,3*R*)-1-amino-1,3-cyclopentanedicarboxylic acid (*trans*-ACPD), a metabotropic glutamate receptor agonist (Sacaan et al., 1992; Smith and Beninger, 1996). It was demonstrated that these effects depended on concurrent dopamine receptor stimulation, suggesting that turning may have been caused by an NMDA- or metabotropic receptor-mediated increase in dopamine transmission (Thanos et al., 1992; Sacaan et al., 1992). AMPA, kainate and NMDA injections into the ventral striatum can also induce contralateral turning as demonstrated by a recent study by Ossowska and Wolfarth (1995).

In neurochemical studies, glutamate receptors in the striatum have been shown to modulate the release of dopamine. Both *in vivo* and *in vitro* studies indicate that dopamine levels are increased indirectly through stimulation of either NMDA receptors (Clow and Jhamandas, 1989; Roberts and Anderson, 1979) or kainate and AMPA receptors (Imperato et al., 1990; Barbeito et al., 1990). It has been noted however, that the doses required to observe many of these effects are often quite high, and may result in non-physiological changes such as toxic effects or spreading depression in the surrounding tissue (Moghadam et al., 1990). Indeed, some reports indicate concurrent seizure activity (see McKenzie et al., 1991) or excitotoxicity (see Taylor et al., 1981) accompanying rotation at high drug doses.

This study examines the effects of unilateral injections of glutamate receptor agonists into the dorsal striatum on turning behavior and activity level in rats. It has been shown that the excitatory post-synaptic potentials in striatal neurons elicited by cortical stimulation or by exogenously applied glutamate are principally mediated by the non-NMDA ionotropic glutamate receptors, kainate or AMPA (Herrling, 1985; Calabresi et al., 1991). It was expected that striatal injections of relatively low doses of these two agonists would cause contralateral rotation. Indeed, high doses of kainate have been reported to cause turning in previous studies (Taylor et al., 1981; Toth and Lajtha, 1989). To determine the pharmacological specificity of this effect, the non-NMDA glutamate receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) was co-injected. To examine the possibility that an increase in dopamine levels contributed to rotation, co-injections of the dopamine receptor antagonist *cis*-flupenthixol were also performed. In addition, the Na⁺ channel blocker tetrodotoxin was co-injected to determine whether this effect was the result of an increase in striatal unit discharge. Finally, CNQX and tetrodotoxin were injected alone to study the effects of changes in tonic glutamate receptor activation and neuronal firing on rotation. Portions of these data have been presented in abstract form (Smith and Beninger, 1992; Smith et al., 1993).

2. 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.

2.1. Animals and surgery

Male Wistar rats (Charles River, Canada) were individually caged in a climate-controlled environment and given free access to food and water.

At least 12 rats were used for each experiment. Rats (225–275 g) were anesthetized with either halothane (1.5–4%) or pentobarbital (60 mg/kg *i.p.*) and surgically implanted with a 23 gauge (0.6 mm o.d.) guide cannula in the dorsal striatum at coordinates anterior –0.3 mm from bregma, lateral 3.0 mm, ventral 3.5 mm according to Paxinos and Watson (1986). At the same time, an arborite chip for attachment to a rotometer was affixed to the skull with dental acrylic and 4 skull screws.

2.2. Apparatus

The rotometer was a rotating disk with a single slot that moved past 4 infrared beams oriented in equal 90° intervals. Photocell beam breaks were recorded on an experimenter-controlled circuit board connected to a Macintosh microcomputer. A sliding, pivoting stainless steel rotometer lead was clipped to the arborite chip in the rat's skull mount, allowing free movement around the experimental chamber. Any postural turns involving the head or circling locomotor activity were registered and recorded as beam breaks. Data were stored as the number of full turns in each direction occurring in 1-min bins over a 20-min recording session. To register a full turn, a sequence of 5 beam breaks must have occurred in one direction. The rat and rotometer was located in a 45 cm (diameter) by 30 cm (height) plastic cylinder inside a sound-attenuating, ventilated and illuminated box.

2.3. Procedure

After at least 5 days of recovery from surgery, in 7 different experiments, rats began a 13 day protocol of 7 turning activity recording sessions, with each session separated by 2 days. For dose-response tests of drug effects, there were no injections before the first and seventh sessions, and saline injections before the second and sixth sessions. Three drug doses were given in a counterbalanced order over the third, fourth and fifth sessions. For experiments testing drug effect reversal, the saline sessions (second and sixth) were replaced by a single dose of the agonist alone, and a mixture of the agonist and antagonists was administered in a counterbalanced order over the third,

fourth and fifth sessions. In the experiment testing the effects of tetrodotoxin injected alone, 2 doses of tetrodotoxin were tested resulting in only 6 experimental sessions. The treatments for each session in 7 experiments, including doses of the various drugs administered, are summarized in Table 1.

In experiment 5, involving co-injections of AMPA and various antagonists, rats were placed in similar recording cylinders (45 cm diameter) and their behavior was videotaped for a 20-min period following injections. The number and direction of rotations subsequently was counted visually and recorded by the experimenter. Unpublished data from this laboratory show that this method of data collection results in activity and turning dose-response profiles, as indicated by full turns per minute, that are in good agreement with those obtained in the rotometer apparatus for kainate injections. In addition, an observer blind to the experimental condition viewed sections of videotape in order to obtain an inter-rater reliability measure. This altered procedure allowed for closer visual observation of behavior following injections, and provided further data on the validity of the rotometer apparatus to be incorporated into a detailed methodology paper currently in preparation.

As an index of rotational behavior, a turning ratio was calculated for each animal over each session. This measure is the number of full turns made ipsilateral to the injection side divided by the total number of full turns. Thus, a ratio of 0.5 would indicate a non-directionally biased session and a lower or higher ratio would indicate contralateral or ipsilateral turning, respectively. The total number of full turns over a session was analyzed as a measure of overall motor activity.

2.4. Central injections, drugs and histology

Drug and saline injections were made through a 30 gauge (0.3 mm o.d., 0.15 mm i.d.) cannula attached via polyethylene tubing to a Hamilton micro-syringe mounted in an infusion pump. The injection cannula was inserted

into the guide cannula such that it extended 1 mm further into the striatum to ventral 4.5. Injections of 0.5 μ l volume were made over 30 s and the cannula was left in place for an additional 30 s to allow for further drug diffusion. Drugs were dissolved as follows: *cis*-flupenthixol (H. Lundbeck) and kainate (Sigma) in physiological saline, CNQX (Tocris Cookson) and AMPA (Tocris Cookson) in dilute aqueous HCl, and tetrodotoxin (Sigma) in NaOH. The pH of solutions was adjusted to between 6.0 and 8.2 before injections were made. Following behavioral testing, rats were killed by CO₂ inhalation. Brains were extracted and stored in a 10% sucrose/formalin solution. Coronal sections (60 μ m) were mounted and stained with thionine for histological evaluation of injection sites.

2.5. Statistics

Statistical analyses for each experiment consisted of a single-factor repeated measures analysis of variance (ANOVA) for overall treatment effects. Changes in both turning ratio and total turns were analyzed in the same manner. The Geisser-Greenhouse adjusted degrees of freedom for repeated measure designs were used, although for clarity, unadjusted degrees of freedom are presented in Results. Changes in behavioral measures between the first and final no-injection sessions were examined with a correlated *t*-test. If no difference was found in these measures, values were averaged before the ANOVA was performed. To test drug doses against a single 'control' measure, the two saline injections were also compared using a correlated *t*-test and collapsed into a single mean if no difference was found. Similarly, the two baseline agonist sessions in the reversal studies (experiments 2,3 and 5) were compared and, if no differences were found, collapsed into a single control mean against which antagonist co-injections were tested. Dunnett's *t*-tests were used as post-hoc tests of drug effects, comparing 'control' conditions against subsequent agonist injections or agonist/antagonist co-injections. In the cases of testing the efficacy

Table 1
Treatments for each session for 7 experiments

Exp. # (n)	Treatment session						
	1	2	3	4	5	6	7
1. (25)	NI ^a	Saline	KA 5 μ M (0.53 ng) ^b	KA 50 μ M (5.3 ng)	KA 250 μ M (26.6 ng)	Saline	NI
2. (11)	NI	KA 250 μ M	KA + CNQX 500 μ M (58 ng)	KA + TTX 100 μ M (16 ng)	KA + c-flu ^c 7.9 mM (2.0 μ g)	KA 250 μ M	NI
3. (11)	NI	KA 250 μ M	KA + c-flu 0.79 mM (0.2 μ g)	KA + c-flu 7.9 mM (2.0 μ g)	KA + c-flu 79.0 mM (20 μ g)	KA 250 μ M	NI
4. (14)	NI	Saline	AMPA 50 μ M (4.7 ng)	AMPA 200 μ M (18.6 ng)	AMPA 400 μ M (37.2 ng)	Saline	NI
5. (21)	NI	AMPA 200 μ M	AMPA + CNQX 500 μ M (58 ng)	AMPA + TTX 100 μ M (16 ng)	AMPA + c-flu 7.9 mM (2.0 μ g)	AMPA 200 μ M	NI
6. (12)	NI	Saline	CNQX 100 μ M (11.6 ng)	CNQX 500 μ M (58.0 ng)	CNQX 2.5 mM (290.3 ng)	Saline	NI
7. (14)	NI	Saline	TTX 10 μ M (1.6 ng)	TTX 100 μ M (16.0 ng)	Saline	NI	

^a NI, no injection. ^b Drug doses indicated as concentration and as (absolute quantity injected in 0.5 μ l). ^c c-flu, *cis*-flupenthixol.

of initial drug doses (kainate, AMPA, tetrodotoxin, CNQX), Dunnett's tests incorporated the saline vehicle session as the control. Experiments testing the reversal of agonist effects used the agonist session as the control. Tukey post-hoc tests between each drug dose were used to

examine the dose dependency of glutamate receptor agonist injections. A correlation coefficient was calculated between turning ratios for the rats of the single observational study (experiment 5) and tested for significance.

Although the activity data were converted into turning

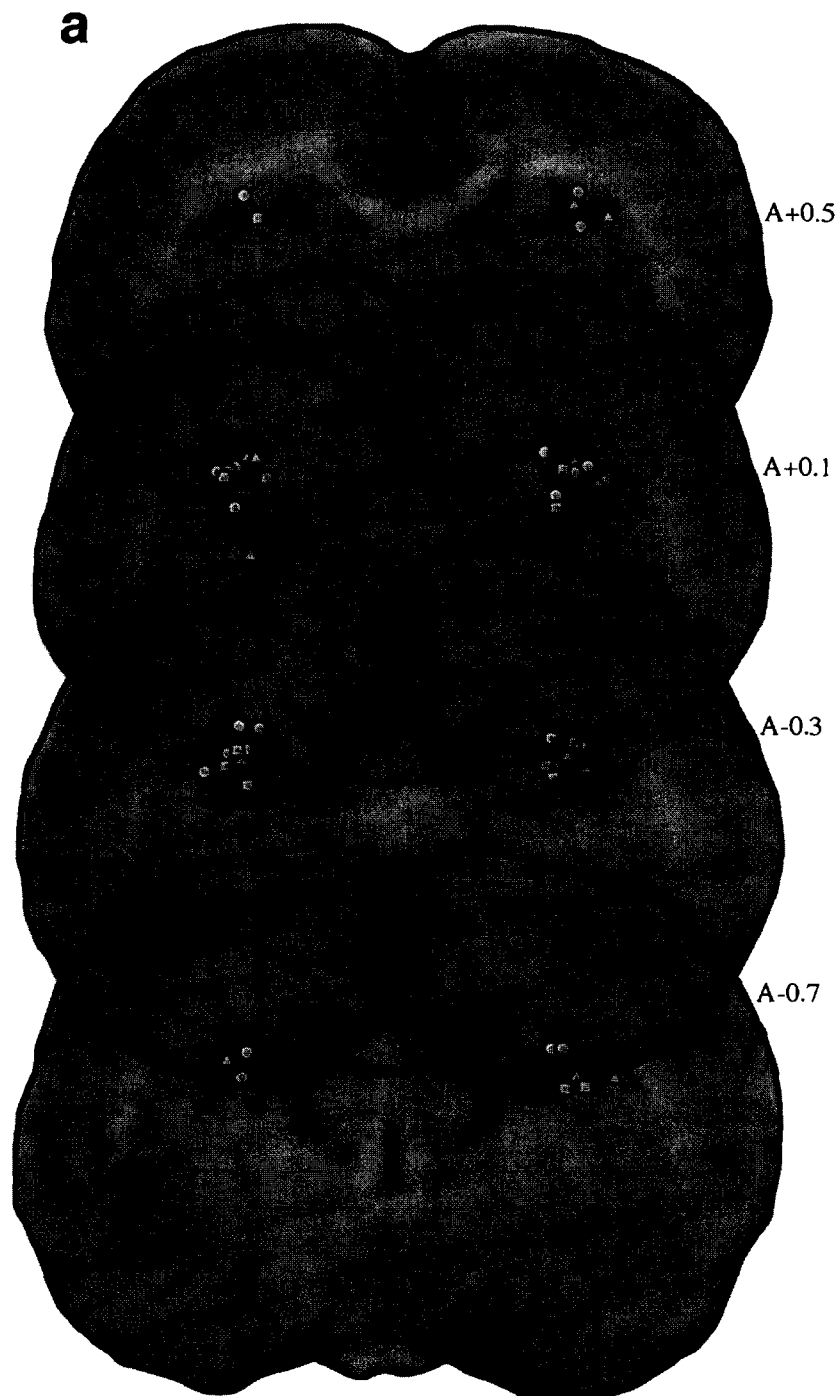


Fig. 1. (a) Cannula placements for all 7 experiments were located in the dorsal striatum. Both left and right-side cannulations were performed. (b) Representative coronal section of rat brain near anterior -0.3 from bregma, showing guide cannula tract through the cortex and narrower injection cannula tract in the dorsal striatum below the corpus callosum. This section taken from a rat in experiment 6 in which 2 $0.5 \mu\text{l}$ saline injections and 3 $0.5 \mu\text{l}$ injections of kainate (KA) at 3 doses were made with 1 day between each injection. Damage to the overlying cortex was comparable for all experimental groups. \circ Kainate alone (Exp. 1); \bullet kainate and antagonist (Exp. 2); \times kainate and *cis*-flupenthixol (Exp. 3); \triangle AMPA alone (Exp. 4); \blacktriangle AMPA and antagonists (Exp. 5); \square CNQX alone (Exp. 6); \blacksquare TTX alone (Exp. 7).



Fig. 1 (continued).

ratios to analyze directional biases, parallel analyses were carried out with total contralateral turns as the dependent measure. In general, the statistical significance of treatment effects (data not shown) matched those found with the turning ratio analysis shown below.

3. Results

Histological examination of brain slices showed that injection sites were located within the dorsal part of the striatum as indicated in Fig. 1a. An example of such a site is shown in the photograph of a coronal section through the striatum in Fig. 1b. A small proportion of animals received injections dorsal to the striatum within the overlying corpus callosum (2 rats), or exhibited substantial tissue

damage at the injection site (3 rats), and were discarded from the study.

For each experiment, the turning ratios (Table 2) of the initial and final no-injection sessions (sessions 1 and 7) and the initial and final saline sessions or agonist sessions (sessions 2 and 6) were compared with paired *t*-tests. In no case did these measures differ significantly from each other. The mean value between these sessions was calculated for each animal and used in the ANOVAs to test for significant treatment effects. These collapsed values are also presented in figures showing turning ratios for the 7 experiments. Initial and final no-injection and saline turning ratios generally were close to 0.5, indicating no directional bias, whereas glutamate receptor agonists produced contralateral turning following each injection.

Intra-striatal injections of the glutamate receptor agonist

Table 2

Turning ratios (mean \pm S.E.M.) for no injection or saline or agonist alone treatment sessions for all experiments

Experiment	Treatment session			
	No Inj. 1 (1) ^a	No Inj. 2 (7)	Saline 1 or ^b agonist 1 (2)	Saline 2 or agonist 2 (6)
1.	0.52 \pm 0.024	0.54 \pm 0.023	0.53 \pm 0.029	0.54 \pm 0.030
2.	0.47 \pm 0.065	0.47 \pm 0.029	0.29 \pm 0.047	0.41 \pm 0.027
3.	0.47 \pm 0.051	0.50 \pm 0.059	0.27 \pm 0.069	0.38 \pm 0.081
4.	0.58 \pm 0.079	0.55 \pm 0.042	0.51 \pm 0.048	0.45 \pm 0.045
5.	0.49 \pm 0.024	0.51 \pm 0.026	0.38 \pm 0.038	0.38 \pm 0.039
6.	0.43 \pm 0.049	0.51 \pm 0.037	0.54 \pm 0.066	0.50 \pm 0.042
7.	0.59 \pm 0.056	0.53 \pm 0.035	0.53 \pm 0.056	0.48 \pm 0.051

^a Experimental session number indicated in parentheses. No Inj.: no injection. ^b Agonist sessions for experiments 2 and 3: KA (kainate, 250 μ M); experiment 5: AMPA (200 μ M).

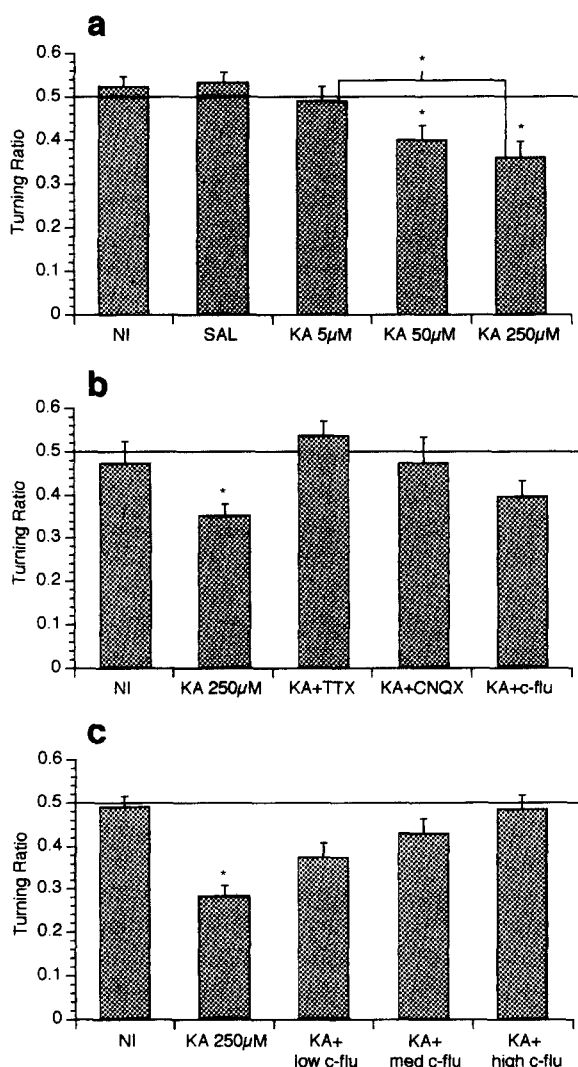


Fig. 2. Effects of kainate (KA) and kainate with different antagonists injected into the dorsal striatum on turning ratio (total ipsilateral/total turns). (a) In experiment 1, kainate induced a contralateral bias in turning. Mean (+S.E.M.) turning ratios calculated over 20 min following no injection (NI) sessions, 0.5 μ l injections of saline (SAL) or 3 doses of kainate. The 2 no injection sessions and 2 saline sessions are averaged. * Significant difference between mean of 50 μ M and 250 μ M doses of kainate and mean of saline session; significant difference between 5 μ M and 250 μ M injections indicating dose dependence of the effect. (b) In experiment 2, contralateral turning caused by 250 μ M kainate is reversed with co-injections of TTX (100 μ M), CNQX (500 μ M) or *cis*-flupenthixol (c-flu, 7.9 mM). The 2 no injection and 2 kainate sessions are averaged. * Significant difference between mean of kainate sessions and mean of no injection sessions. (c) In experiment 3, there is a progressive blockade of kainate-induced contralateral rotation with low (0.79 mM), medium (7.9 mM) and high (79.0 mM) concentrations of *cis*-flupenthixol. The 2 no injection and 2 kainate sessions are averaged. * Significant difference between mean of kainate sessions and mean of no injection sessions.

kainate caused a contralateral rotational bias (Fig. 2a). An ANOVA of 3 kainate doses and of the mean no-injection and saline sessions revealed a highly significant treatment effect of drug injection on turning ratio ($F(4,96) = 9.08$, $P < 0.001$). Dunnett's tests of each dose against the saline

vehicle indicated significant differences at the 50 μ M and 250 μ M doses ($P < 0.01$). A Tukey test revealed that the rotation was dose-dependent, with the high dose of kainate producing significantly more contralateral turning than the low dose ($P < 0.01$).

Fig. 2b illustrates the effects of coinjections of various drugs with kainate, showing a blockade of kainate-induced turning by both tetrodotoxin and CNQX. The ANOVA comparing all 5 experimental treatments revealed a significant main effect ($F(4,40) = 3.56$, $P < 0.05$). Kainate injected alone produced contralateral turning when compared against the no-injection condition ($P < 0.05$), replicating the effect of 250 μ M kainate described in the previous experiment. Co-injection of the kainate/AMPA receptor antagonist CNQX completely reversed the directional bias induced by kainate ($P < 0.05$). In addition, the action potential blocker tetrodotoxin eliminated the contralateral turning ($P < 0.01$). In contrast, co-injection of the dopamine receptor antagonist *cis*-flupenthixol at a dose of 7.9 mM failed to significantly reverse the kainate effect.

A further experiment was performed to examine the dependency of the 250 μ M kainate effect on dopamine receptor stimulation, revealing a progressive blockade of turning by coinjections of increasing doses of *cis*-flupenthixol (Fig. 2c). An ANOVA comparing collapsed no-injection and kainate means and *cis*-flupenthixol doses revealed a significant treatment effect ($F(4,40) = 3.20$, $P < 0.05$). Post-hoc Dunnett's tests indicate a further replication of the kainate-induced turning compared with no-injection ($P < 0.01$), and show a reversal of this effect with a co-injection of the 79 mM dose of *cis*-flupenthixol ($P < 0.05$).

Injections of the glutamate receptor agonist AMPA into the dorsal striatum also caused contralateral rotation in rats (Fig. 3a). The ANOVA comparing all treatments showed a significant main effect ($F(4,52) = 3.86$, $P < 0.01$, Fig. 3a). Dunnett's tests failed however, to reveal significant differences between the saline condition and AMPA conditions.

Co-injections of AMPA and blocking compounds resulted in an overall significant treatment effect ($F(4,80) = 7.85$, $P < 0.01$, Fig. 3b). The injection of 200 μ M AMPA alone again caused a significant contralateral turning bias when compared with no-injection sessions ($P < 0.01$), replicating the effect seen in the previous experiment. Furthermore, this effect was reversed by co-injection of the kainate/AMPA receptor antagonist CNQX ($P < 0.01$), the Na^+ channel blocker tetrodotoxin ($P < 0.01$), and the dopamine receptor antagonist *cis*-flupenthixol ($P < 0.01$). As this experiment was scored visually, an inter-rater reliability coefficient was obtained for two raters blind to the experimental condition. Over 8 viewing sections of 5 min each, turning ratios were correlated at a highly significant level ($r = 0.87$, $P < 0.01$).

Intrastriatal injections of 3 doses of CNQX alone failed to produce a turning bias in rats (Fig. 4a). In addition, no

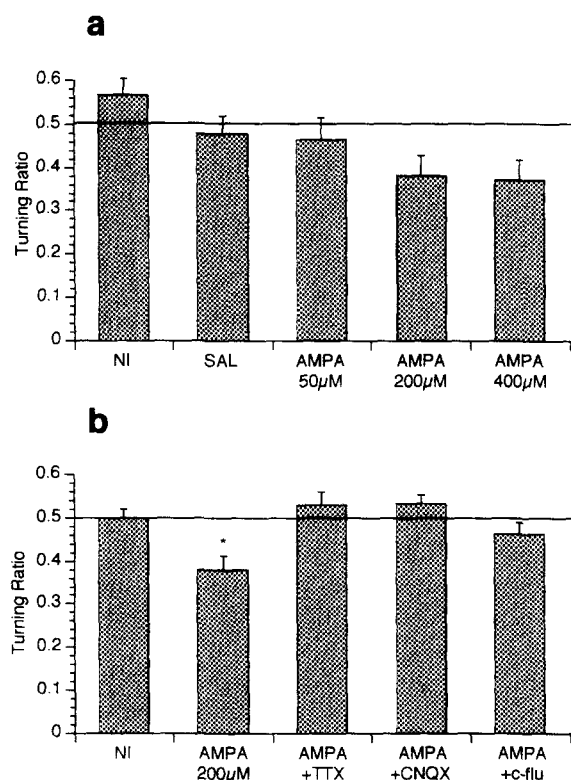


Fig. 3. Effects of injections of AMPA and AMPA with 3 different antagonists into the dorsal striatum on turning ratio. (a) In experiment 4, AMPA injections cause contralateral rotation. Mean (+S.E.M.) turning ratios calculated over 20 min following no injection (NI) sessions, 0.5 μl injections of saline (SAL) or 3 doses of AMPA (50, 200, 400 μM). An ANOVA revealed a significant main effect of treatment ($P < 0.05$). (b) In experiment 5, co-injections of TTX (100 μM), CNQX (500 μM) or *cis*-flupenthixol (*c*-flu, 7.9 μM) reversed the contralateral turning caused by 200 μM AMPA injections. The 2 no injection and 2 AMPA sessions are averaged. * Significant difference between mean of AMPA sessions and mean of no injection sessions.

significant effect on turning behavior was produced by injections of 2 doses of the action potential blocker tetrodotoxin (Fig. 4b).

The number of full turns exhibited over the 20-min recording session was taken as a measure of the overall level of motor activity. In general, activity levels remained stable across different sessions within studies, despite the presence of reliable turning effects in some experiments. The only reliable difference in activity level between experimental sessions in any of the 7 studies occurred in rats being tested for a blockade of AMPA-induced turning ($F(20,6) = 5.53$, $P < 0.001$). This difference emerged due to lower mean ± S.E.M. activity in the final AMPA injection session (12.2 ± 0.87 turns/20 min, overall mean ± S.E.M. = 16.2 ± 1.50).

The mean number of turns varied little among experiments over all treatments, with a grand mean of 19.1 ± 2.08 full turns per 20-min session. Fig. 5 shows the mean ± S.E.M. number of turns averaged over all sessions for each experiment. During this time, some rats were observed visually. Locomotion was at its highest level immediately

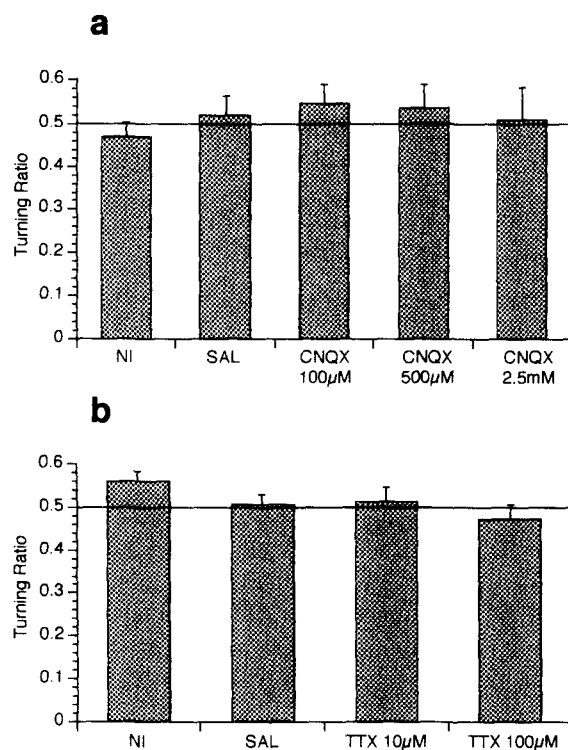


Fig. 4. Effects of CNQX and TTX injected alone into the dorsal striatum on turning ratio (mean + S.E.M. over 20 min following 0.5 μl injections). (a) In experiment 6, 3 doses of CNQX (100 μM, 500 μM, 2.5 mM) injected alone failed to cause a directional bias. The 2 no injection sessions and 2 saline sessions are averaged. (b) In experiment 7, 2 doses of TTX (10, 100 μM) injected alone failed to cause rotation. The 2 no injection sessions and 2 saline sessions are averaged.

after rats were placed in the recording chamber, and diminished progressively over the session, as demonstrated previously with metabotropic glutamate receptor agonist-induced contralateral turning (Smith and Beninger, 1996).

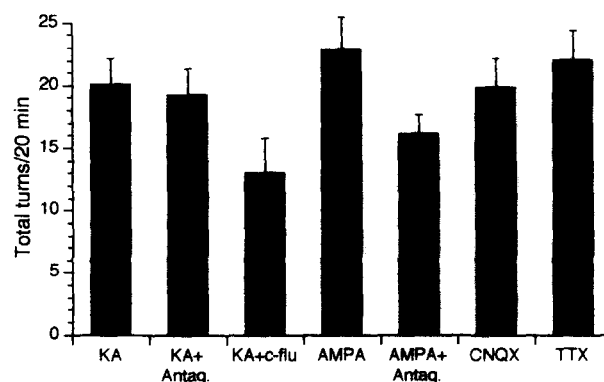


Fig. 5. The total number of full turns averaged over all treatment sessions for each experiment. In all but 1 experiment, no significant differences were recorded between the average total turns in each of 7 treatment sessions. No significant differences in total full turns between each experiment were revealed. The grand mean ± S.E.M. total number of full turns over 20 min for all treatment sessions in each experiment was 19.1 ± 2.08 . Exp. 1: KA (kainate); Exp. 2: KA + Antag. (antagonists); Exp. 3: KA + *c*-flu (*cis*-flupenthixol) Exp. 4: AMPA; Exp. 5: AMPA + Antag.; Exp. 6: CNQX; Exp. 7: TTX.

Their overt behavior included normal exploration of the experimental chamber and grooming activity. In animals that exhibited a turning bias, there was no evidence of unusual motor activity such as seizure-related clonus, or the stereotyped nose-to-tail turning and postural asymmetry reported in rats with unilateral lesions of the dopamine system (Marshall and Ungerstedt, 1977). It was noted that the behavior of drug-treated animals was to a large extent indistinguishable from that of no-injection sessions, apart from reliable directional biases as described above.

4. Discussion

The results show that microinjections of glutamate receptor agonists into the dorsal striatum cause contralateral rotation. Both kainate and AMPA injections resulted in turning away from the cannulated side, effects that were blocked by the kainate and AMPA receptor antagonist CNQX, by the action potential blocker tetrodotoxin, and by the dopamine receptor antagonist *cis*-flupenthixol. The region selected for intrastriatal injections has been shown to receive projections from sensory and motor areas of the cortex (McGeorge and Faull, 1989).

These results are in agreement with a recent study by Ossowska and Wolfarth (1995) showing contralateral turning following kainate and AMPA injections into the ventral striatum that is blocked by the antagonist DNQX. These authors report a preponderance of contraversive head turns that account for much of the observed behavior. In contrast, the pattern of movement observed in the present study consisted of an overall contralateral locomotor bias with little discernable head turning. Interestingly, recordings in freely moving rats indicate that neurons in the dorsal striatum fire during both forelimb and hind limb movement in freely moving rats, whereas more ventral neurons respond primarily to head, neck and vibrissa movement (Carelli and West, 1991; West et al., 1990).

The average number of uninterrupted full turns exhibited by rats in this study was a modest 19 turns per 20 min. In contrast, rats with lesions of the nigrostriatal dopamine system often exhibit hundreds of rotations in the same period after a challenge with a dopamine receptor agonist (Marshall and Ungerstedt, 1977). This dramatic change in motor activity clearly does not represent a physiological process of the normally functioning basal ganglia output system. In contrast, the modest yet quantifiable and reliable rotation exhibited in the present study occurred at an activity level similar to the non-drugged, non-lesioned animal. Furthermore, it was noted that normal exploratory and grooming behaviour followed all injections, suggesting that the turning described represents an effect more within the realm of normal behaviour.

A correlation has been found between changes in unit activity in the striatum and directional biases exhibited by experimental animals. Intrastriatal injections of am-

phetamine that cause contralateral rotation (Moore et al., 1994) also resulted in an increase in baseline firing rate of motor-related projection neurons (Haracz et al., 1989; Wang and Rebec, 1993). Similarly, glutamate receptor agonists and GABA_A receptor antagonists that cause contralateral turning (Thanos et al., 1992; Toth and Lajtha, 1989; Smith et al., 1993; Taylor et al., 1981; McKenzie et al., 1991; Smith and Beninger, 1992; Black et al., 1994) also elevate striatal cell discharge (Herrling, 1985; Calabresi et al., 1991). Consistent with the conclusion that an increase in striatal cell firing results in contralateral turning, kainate- and AMPA-induced turning was reversed by tetrodotoxin. When injected alone, tetrodotoxin did not cause a bias in locomotor activity, suggesting that a simple imbalance of bilateral output signals from this region is insufficient to produce turning. In the present study, the only reliable effects were produced by manipulations that have been associated with an elevation in neural activity above normal levels; perhaps this is a prerequisite for eliciting a directional bias in intact rats.

It has been suggested that high doses of glutamate receptor agonists injected into the striatum may lead to spreading depression (Moghaddam et al., 1990). The tetrodotoxin blockade of agonist-induced turning reported here suggests that spreading depression, which is insensitive to tetrodotoxin (Van Harreveld, 1978; Moghaddam et al., 1987) is an insufficient explanation for the present results. Alternatively, glutamate receptor agonist injections into the striatum may lead to excitotoxicity (Taylor et al., 1981). The possibility that tissue damage, to the overlying cortex or to the striatal injection site, caused these effects is unlikely given that histological analysis showed no evidence of extensive tissue damage surrounding the injection site, and that there were no observable directional biases during either initial or final vehicle injection sessions.

When compared with *in vitro* affinity studies (Hansen and Krogsgaard-Larsen, 1990), the agonist concentrations in the present experiments are high enough to question whether the effects were limited to specific interactions with postsynaptic kainate or AMPA receptors. However, factors such as diffusion from the injection site and metabolic degradation that would occur with intra-cerebral injections likely would reduce the drug concentrations rapidly. The blockade by CNQX suggests that the observed turning was a receptor-specific effect, and the dose dependency of the kainate effect argues further for the presence of an agonist-receptor interaction. The possibility of presynaptically mediated changes in endogenous glutamate concentrations, or postsynaptic action at NMDA receptors following kainate or AMPA injections remains to be investigated.

The AMPA/kainate receptor antagonist CNQX failed to affect rotation when injected alone. This is in good agreement with the finding that suggests that DNQX injected into the nucleus accumbens does not affect locomo-

tion (Donzanti and Uretsky, 1984; Kaddis et al., 1993) and suggests that tonic stimulation of these receptors by endogenous glutamate is not necessary to maintain symmetrical motor activity. In the ventral striatum however, unilateral injections of DNQX alone cause ipsilateral rotation (Ossowska and Wolfarth, 1995). Furthermore, intrastriatal injections (Thanos et al., 1992) and intra-accumbens injections (Svensson et al., 1994) of NMDA receptor antagonists have produced ipsilateral turning effects in rats and in mice, respectively. It has also been reported however, that NMDA receptor antagonists injected into the ventral anterior striatum cause hyperlocomotion (Schmidt and Bury, 1988; Schmidt et al., 1992) whereas agonists cause muscular rigidity (Klockgether and Turski, 1993), suggesting an opposing effect of NMDA and kainate/AMPA receptor activation. The discrepancies between these and the present results remain to be explained, though they may depend on regional variability in receptor subtype density in striatal output pathways.

The dopamine receptor antagonist *cis*-flupenthixol reversed turning caused by both AMPA and kainate. There is little possibility that this occurred through behavioral competition with an ipsilateral turning bias caused by *cis*-flupenthixol given results showing no effects after striatal injections of this drug alone at the same concentrations as those used here (Costall et al., 1983; Josselyn and Beninger, 1991). Thus, concurrent stimulation of dopamine receptors is required for kainate- or AMPA-induced turning behaviour. In agreement with the present findings, others have found that *cis*-flupenthixol blocks turning following intrastriatal NMDA (Thanos et al., 1992), caffeine (Josselyn and Beninger, 1991), amphetamine or neuropeptide Y (Moore et al., 1994), and systemic haloperidol blocks turning following intrastriatal trans-ACPD (Sacaan et al., 1992). Perhaps glutamate receptor-mediated increases in presynaptic dopamine release contribute to the observed rotation as suggested by microdialysis experiments showing that local infusions of kainate (50–500 μ M) elevate extracellular dopamine in the rat striatum, and that this effect is sensitive to reversal with CNQX or DNQX (Imperato et al., 1990; Keefe et al., 1992). Some studies indicate that glutamate-evoked increases in dopamine efflux are insensitive to the effects of tetrodotoxin co-application (Clow and Jhamandas, 1989; Barbeito et al., 1990). Considering that the present turning effects were blocked by tetrodotoxin, it remains possible that the observed rotational behavior required both increased dopamine release and elevation of striatal unit activity.

When considering the effects of changes in basal ganglia output on motor activity, it remains unclear how directional biases are generated. It has been suggested that there are two output pathways through which separate populations of striatal cells can affect behaviour. A 'direct' inhibitory projection to output nuclei would disinhibit thalamic and brainstem projection sites, whereas an 'indirect' projection through the external globus pallidus and subtha-

lamic nucleus would be expected to have an opposing effect (Albin et al., 1989; Gerfen, 1992). Bolus injections of drugs would be expected to non-specifically excite cells in both pathways, making speculation about shifts in output pathway dominance difficult. Recent anatomical evidence suggests however, that direct striatonigral projections are arranged such that a powerful somato-dendritic inhibition of output neurons is achieved. In contrast, excitatory subthalamonigral fibres provide a more diffuse projection consisting of multiple *en-passant* contacts (Parent and Hazrati, 1995). It is possible that global excitation of striatal neurons had a greater net inhibitory effect on output neurons through striatonigral GABAergic projections.

The role of basal ganglia glutamate receptors in Parkinson disease has been studied recently. It has been shown that systemic glutamate receptor antagonists act synergistically with dopamine receptor agonists to improve dyskinesias in dopamine-depleted rodents and primates (Wachtel et al., 1992; Greenamyre et al., 1994). This effect occurs presumably through blockade of glutamate receptors in output nuclei following dopamine lesion-induced overactivation of the subthalamic nucleus. In intact rats, the effects of bilateral injections of glutamate receptor agonists into various basal ganglia regions on muscle tone has been studied (Klockgether and Turski, 1993). A slight increase in tone, thought to correspond to Parkinsonian akinesia, was reported at only high doses of AMPA (20 mM) or kainate (2 mM) in the striatum, whereas lower concentrations like those used here were ineffective, suggesting that the agonist injections made in the present study would not result in contralateral muscular rigidity. NMDA in the striatum on the other hand was effective in producing rigidity at lower doses, indeed below the range required to elicit rotation following unilateral injections (Thanos et al., 1992). Interestingly, electrophysiological evidence suggests that striatal neurons exhibit a greater sensitivity to NMDA-mediated changes in synaptic strength than to either AMPA or kainate receptors (Cepeda et al., 1993), indicating a significant role for this subtype in mediating cortical signals in the striatum.

Decortication does not abolish turning in animals with unilateral dopamine depletions (Crossman et al., 1977), suggesting that changes in thalamocortical basal ganglia output is insufficient to elicit this behavior. This points to descending striatal efferents as possible mediators of circling (Miller and Beninger, 1991). Direct descending basal ganglia output to locomotor areas such as the pedunclopontine nucleus (Garcia-Rill, 1986) and superior colliculus may contribute to contralateral rotation caused by changes in basal ganglia output in the intact rat. Behavioral studies show that GABA receptor blockade in the superior colliculus causes contralateral rotation in rats (Imperato and DiChiara, 1981) and in cats (Jaspers and Cools, 1985). It is notable that intra-collicular GABA receptor antagonist treatment can be considered similar to a decrease in GABA

input from the substantia nigra zona reticulata. This is consistent with the idea that the striatal glutamate receptor agonist injections in the present study resulted in an overall inhibition of basal ganglia output through direct striatonigral projections.

We show here that the glutamate receptor agonists kainate and AMPA injected into the dorsal striatum cause contralateral rotation that is blocked by CNQX, and that depends on concurrent dopamine receptor stimulation. In addition, this rotation appears to be dependent on an increase in striatal cell discharge. Finally, a unilateral decrease in striatal activity through blockade of tonic AMPA/kainate input or through inactivation of neuronal firing is insufficient to produce a motor asymmetry.

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