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Contralateral Turning Caused by Metabotropic Glutamate Receptor Stimulation in the Dorsal Striatum Is Reversed by MCPG, TTX, and cis-Flupenthixol

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Recent evidence suggests an involvement of metabotropic glutamate receptors in the physiology of the striatum. In this study, rotation was recorded in an automated rotometer for 20 min following dorsal striatal injections (0.5 μl) in cannulated rats. The metabotropic agonist 1-aminocyclopentane-trans-1,3-dicarboxylic acid (1S,3R-ACPD) caused dose-dependent contralateral rotation. Turning caused by 500 μM 1S,3R-ACPD was reversed by coinjections of the metabotropic antagonist +-alpha-methyl-4-carboxyphenylglycine (MCPG, 1 mM) and by tetrodotoxin (100 μM). Injections of MCPG alone (10 μM, 100 μM, 1 mM) failed to elicit turning. Increasing doses of the dopamine antagonist cis-flupenthixol also reversed 1S,3R-ACPD-induced rotation. Thus unilateral striatal metabotropic glutamate receptor stimulation can cause receptor-specific rotation that may result from an increase in neural activity, and is dependent on intact dopamine neurotransmission.

The cortex provides the dorsal striatum with excitatory glutamatergic input (Spencer, 1976) carrying sensory and motor signals (McGeorge & Faull, 1989; Carelli & West, 1991). Striatal processing can affect behavior as this information is sent through intermediate and output nuclei of the basal ganglia to disinhibit thalamic neurons that project back to the cortex (Chevalier & Deniau, 1990). In addition, basal ganglia output is directed toward mesopontine nuclei that in turn project caudally (Garcia-Rill, 1986), providing an additional, descending pathway through which the striatum may alter behavior.

There are several subtypes of glutamate receptors that mediate neurotransmission in the cortico-striatal pathway, including the ionotropic kainate (KA), α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) and *N*-methyl-D-aspartate (NMDA) subtypes, and the metabotropic (mGluR) subtype (Nakanishi & Masu, 1994). Fast synaptic excitation in the striatum caused by glutamate release from corticofugal fibers occurs largely through the kainate or AMPA subtypes, whereas the NMDA receptor seems to be involved in more sustained responses (Calabresi et al., 1991; Herrling, 1985). It has been shown recently that mGluR stimulation also plays a role in striatal physiology, and is necessary for the induction of long-term depression which follows repeated stimulation of cortical afferents (Calabresi, Maj, Pisani, Mercuri, & Bernardi, 1992).

Unilateral stimulation of ionotropic glutamate receptor subtypes in the dorsal striatum has been shown to cause contralateral turning in rats. Thus injections of NMDA (Black et al., 1994; Thanos, Jhamandas, & Beninger, 1992; Toth & Lajtha, 1989), KA (Smith & Beninger, 1992; Taylor et al., 1981), and AMPA (Smith, Mitha, & Beninger, 1993) all induce a directional bias in rats away from the side of the injection. The phenomenon of rotation is also associated with imbalances in striatal dopamine (DA) neurotransmission and was first reported in animals after unilateral depletion of DA (Ungerstedt & Arbuthnott, 1970). It has been demonstrated repeatedly that rats will turn away from the side of higher DA receptor stimulation (Marshall & Ungerstedt, 1977; for review, see Pycock & Kilpatrick, 1989). A clear distinction can be made between the behavior of supersensitive animals that have been depleted of DA and treated with DA agonists or intact animals that have been injected with high concentrations of glutamate agonists, and normosensitive animals that have received intrastriatal injections of lower doses of glutamate agonists. Whereas it is common to observe hundreds of turns during a recording session (e.g., 20 min) with the former treatments (e.g., Marshall & Ungerstedt, 1977; Taylor et al., 1981) DA agonists or low doses of glutamate agonists in normosensitive animals produce a small but statistically reliable bias in motor activity away from the side of striatal injections (e.g., Moore, Merali, & Beninger, 1994; Smith, Mitha, & Beninger, 1993).

It has also been shown that the metabotropic agonist 1-aminocyclopentane-trans-1,3-dicarboxylic acid (1S,3R-ACPD), the active enantiomer or trans-ACPD, injected acutely in high doses into the striatum causes contralateral rotation (Sacaan, Monn, & Schoepp, 1991) that is dependent on DA receptor stimulation (Sacaan, Bymaster, & Schoepp, 1992). These studies point to a role of mGluRs in the striatum in altering unconditioned behavior, and raise questions about the receptor-specificity and cellular mechanisms of the observed turning. The effects of low doses of 1S,3R-ACPD in a chronic preparation on rotation remain to be investigated, as does the involvement of elevated neuronal activity in these effects.

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Manipulations that cause contralateral turning, such as unilateral glutamate agonist or DA agonist injections, would be expected to increase firing of striatal neurons. In one study, intrastriatal injections of amphetamine, a DA agonist, caused elevated firing of striatal output neurons and rotation in rats (Wang & Rebec, 1993). Indeed, the turning caused by both KA and AMPA injections is reversed by the action potential blocker tetrodotoxin (TTX) (Smith, Mitha, & Beninger, 1993).

The current study investigated the effects of intrastriatal injections of an mGluR agonist, 1S,3R-ACPD, on rotational behavior in chronically cannulated rats, and the possible mechanisms through which turning effects could occur. Portions of these data have appeared previously in abstract form (Smith & Beninger, 1994).

Method

Subjects and Surgery

Male Wistar rats (Charles River, Canada) were individually caged in a climate-controlled environment and given free access to food and water. All animal procedures were in accordance with the Guidelines of the Canadian Council on Animal Care, the Animals for Research Act, and relevant university policy, and were approved by the Queen's University Animal Care Committee.

Rats were anesthetized with halothane (1.5–4%) and implanted unilaterally with a 23-gauge guide cannula (0.6 mm o.d.) in the dorsal striatum (A-0.3 mm, L3.0 mm, V3.5 mm) according to Paxinos and Watson (1986). At the same time, an arborite chip was affixed to the skull with dental cement for attachment to a rotometer apparatus. Rats were allowed to recover for 5 days before the 13-day experimental protocol began.

Apparatus

The rotometer consisted of a notched disk that passed through 4 equally spaced infrared beams. A full turn in either direction was counted when 5 beam-breaks occurred in succession. Data were collected on-line by way of an experimenter control board and Macintosh microcomputer, and stored for subsequent off-line analysis. Rat skullcaps were clipped to the rotometer apparatus and animals were placed in a cylindrical chamber (45 cm diam., 30 cm high) inside a sound-attenuating, ventilated and illuminated box for a 20-min recording period. The rotometer hookup was a sliding, pivoting stainless steel shaft that allowed the rat free movement in horizontal and vertical planes, and also permitted the head to pitch without restraint.

Procedure

Independent groups of animals were tested in 4 experiments, including: (a) a dose-response test of the mGLUR agonist 1S,3R-ACPD (5, 50 and 500 μM (0.4, 4.3, 43.3 ng); n = 11); (b) a dose-response test of the mGLUR antagonist (+-alpha-methyl-4-carboxyphenylglycine (MCPG) (10 μM, 100 μM, 1 mM (1.0, 10.4, 104.6 ng); n = 14); (c) a test of the ability of MCPG (1 mM (104.6 ng)), TTX (100 μM (16.0 ng) and the DA antagonist cis-flupenthixol (7.9 mM (2.0 μg)) to reverse 1S,3R-ACPD-induced turning (n = 14); (d) a test of the ability of increasing doses of cis-flupenthixol (0.79 mM (0.2 μg), 7.9 mM (2.0 μg), 79.0 mM (20 μg)) to block 1S,3R-ACPD-induced turning (n = 12). The procedure for each experiment involved 7 sessions separated by 48-hr intervals. The first and seventh session always was preceded by no injection. The second and sixth session, in the case of drug dose-response testing, was preceded by control injections of a saline vehicle, and the middle three sessions were preceded by injections of each drug dose given in an order counterbalanced across rats. In the case of testing antagonist coinjections against a single agonist dose, the saline sessions (2 and 6) were replaced by the agonist injected alone.

Central Injections, Drugs and Histology

Injections of 0.5 μl of fluid were made through vinyl tubing attached to a 30-gauge cannula (0.3 mm o.d., 0.15 mm i.d.) extending 1 mm below the guide cannula (V4.5 mm). Injections were made with an infusion pump over a 30-s period, and the cannula was left in place for an additional 30 s to allow for drug diffusion. Rats were unrestrained during injections.

Drugs were dissolved in either saline (1S-3R-ACPD; Research Biochemicals, Natick, MA), cis-flupenthixol (Lundbeck, Copenhagen, Denmark)), or a saline solution buffered with NaOH (MCPG; Tocris Cookson, St. Louis, MO), TTX (Sigma Chemical, St. Louis, MO)). The pH of all solutions was adjusted to 7–8 with 1 M NaOH or 1 M HCl.

After behavioral testing was completed, rats were sacrificed through inhalation of CO_2. Brains were extracted and stored in a 10% formalin solution for at least 10 days. To verify cannula injection sites, coronal sections of brain tissue (60 μm) were taken on a freezing-stage microtome, mounted, and stained with thionine.

Data Analysis

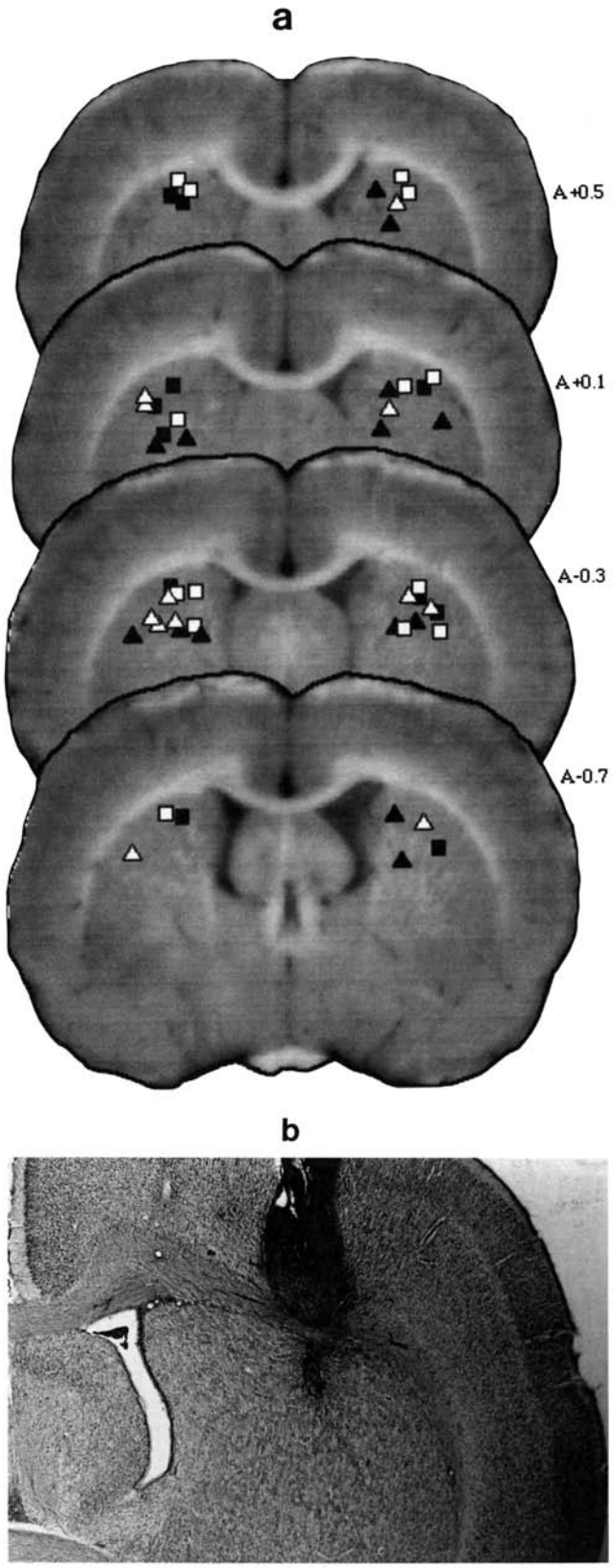
Data were analyzed by calculating a turning ratio over the 20-min recording session. The ratio was the number of full turns ipsilateral to the cannula divided by the total number of full turns. Thus a nondirectionally biased rat would exhibit a ratio of 0.5, whereas contralateral and ipsilateral circling would result in lower and higher values, respectively. The total number of turns made during the experimental session was also recorded and analyzed.

Statistical analyses proceeded as follows: If no significant differences were found with paired t-tests between initial and final no-injection, or the initial and final saline or agonist conditions, then the two no-injection values were averaged as were the two saline or agonist values in order to obtain single "control" measures. This resulted in 5 levels of a single treatment factor, including two collapsed "control" measures and three "experimental" measures. One-way repeated measures ANOVAs were performed for treatment, using the Geisser-Greenhouse corrected degrees of freedom, though the actual degrees of freedom are given in the text in the interest of clarity. Post hoc comparisons between control sessions and experimental sessions were performed with Dunnett's t-tests, and Tukey's HSD tests between high and low doses were used to evaluate dose-dependency.

Results

Histological analysis indicated that all injection sites were within the dorsal striatum near the intended region (A-0.3 from bregma, L3.0, V4.5) and did not result in extensive tissue damage. Figure 1 includes a schematic representation of the injection sites used in the 4 experiments, and a photograph showing an example of a cannula tract and injection site.

For each of the four experiments, the turning ratios of the initial and final no-injection sessions (sessions 1 and 7) and the initial and final saline sessions or 1S,3R-ACPD sessions (sessions 2 and 6) were compared with paired t-tests. In no case did these measures differ significantly from each other (Table 1). The mean value between these sessions was calcu-



lated for each rat and used in the ANOVAs to test for significant treatment effects. These collapsed values are presented in Figure 2 showing turning ratios for the 4 experiments.

Intrastriatal injections of the mGluR agonist 1S,3R-ACPD caused dose-dependent contralateral rotation (Figure 2a). In both no-injection and saline conditions, rats exhibited turning ratios near 0.5, indicating a lack of rotational bias for each condition. Doses of 5, 50, and 500 μM (0.4, 4.3, 43.3 ng) 1S,3R-ACPD produced a mean ± *SEM* turning ratio of 0.32 ± .06 at the highest dose. There was a significant treatment effect, $F(4, 40) = 6.04$, $p < .01$, across experimental conditions. Turning ratios for both the 50 μM and 500 μM doses of 1S,3R-ACPD differed significantly from the saline control condition ($p < .01$), and the dose-dependence of this effect was indicated by a significant difference between the lowest and highest 1S,3R-ACPD doses ($p < .01$).

Injections of the mGluR antagonist MCPG alone at 3 doses (10 μM, 100 μM, 1 mM (1.0, 10.4, 104.6 ng)) did not significantly affect turning behavior (Figure 2b).

An experiment was carried out to test the receptor specificity and evaluate possible mechanisms through which rotation may occur. 1S,3R-ACPD-induced rotation was blocked by coinjections of MCPG or TTX, but was not significantly altered by cis-flupenthixol (Figure 2c). An ANOVA revealed a significant overall treatment effect, $F(4, 52) = 3.87$, $p < .01$. The turning effect of 500 μM 1S,3R-ACPD, seen in the first experiment, was replicated when compared to the no-injection mean ($p < .05$). When co-injected with 1S,3R-ACPD, the antagonist MCPG at a dose that did not significantly affect behavior alone (1 mM (104.6 ng) reversed the rotation caused by the agonist ($p < .05$). In addition, the action potential blocker TTX (100 μM (16.0 ng)) reversed 1S,3R-ACPD-induced turning at a dose that, based on unpublished data from this laboratory, also failed to affect rotation ($p < .05$). Finally, co-injections of the DA antagonist cis-flupenthixol (7.9 mM (2.0 μg)) failed to significantly block circling caused by the mGluR agonist.

To further investigate the DA-dependence of 1S,3R-ACPD-induced turning, three doses of cis-flupenthixol (0.79 mM (0.2 μg), 7.9 mM (2.0 μg), 79.0 mM (20 μg)) were coinjected with 500 μM of the agonist. Rotation was blocked with progressively increasing doses of cis-flupenthixol. An ANOVA revealed an overall treatment effect, $F(4, 44) = 5.29$, $p < .01$, Figure 2d, and the contralateral rotation caused by 500 μM 1S,3R-ACPD was replicated ($p < .01$). The turning ratio for

Figure 1. (a) Cannula placements for all 4 experiments were located in the dorsal striatum. Filled squares = 1S,3R-ACPD alone; open squares = MCPG alone; filled triangles = 1S,3R-ACPD and antagonists; open triangles = 1S,3R-ACPD and cis-flupenthixol. (b) Representative coronal section of rat brain near Anterior −0.3 from bregma, showing guide cannula track through the cortex and narrower injection cannula track in the dorsal striatum below the corpus callosum. This section was taken from a rat in which 2 0.5 μl saline injections and 3 0.5 μl injections of 1S,3R-ACPD at 3 doses were made with 1 day between injections. ACPD = 1-aminocyclopentane-trans-1,3-dicarboxylic acid; MCPG = +-alpha-methyl-4-carboxyphenylglycine.

Table 1
Turning Ratios (Mean ± SEM) for No-Injection, or Saline or 1S,3R-ACPD Alone Treatment Sessions

Experiment	*n*	No-Injection		Saline or 1S,3R-ACPD	
		Session 1	Session 7	Session 2	Session 6
*1. ACPD	11	0.43 ± .099	0.50 ± .073	0.57 ± .056	0.52 ± .057
*2. MCPG	14	0.45 ± .062	0.60 ± .076	0.55 ± .067	0.51 ± .058
†3. ACPD + Antagonists	14	0.59 ± .071	0.53 ± .053	0.37 ± .058	0.35 ± .060
†4. ACPD + cis-flupenthixol	12	0.54 ± .070	0.53 ± .079	0.32 ± .053	0.28 ± .064

Note. ACPD = 1 aminocyclopentane-trans,1,3-dicarboxylic acid; MCPG = +-alpha-methyl-4-carboxyphenylglycine.
* = Saline. † = 1S,3R-ACPD.

1S,3R-ACPD plus the highest dose of cis-flupenthixol was significantly different from 1S,3R-ACPD injected alone ($p < .01$).

Figure 3 shows the average number of total turns exhibited each minute of the recording session after injections of 500 μM 1S,3R-ACPD. The values from both the initial and final agonist sessions in the last experiment described above have been averaged for each rat. Activity was highest when the rat was first placed in the experimental chamber, and then rapidly decreased to less than a single turn per minute.

The total number of turns over the 20-min recording session was taken as a measure of overall activity level. There were no reliable differences in the amount of total turns averaged over sessions exhibited across any of the treatment conditions (data not shown). The mean number of turns also varied little among experimental groups (Figure 4), with a grand mean ± *SEM* of 12.9 ± 2.0 turns per 20 min. During testing, some rats were observed visually. Their overt behavior included normal grooming activity and exploration of the experimental chamber. In rats 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 reported in rats with unilateral lesions of the DA system (Marshall & Ungerstedt, 1977).

Discussion

This study demonstrated that unilateral stimulation of metabotropic glutamate receptors in the dorsal striatum with 1S,3R-ACPD results in receptor-specific, dose-dependent contralateral rotation. Evidence that the agonist was acting at its own receptor site was provided by the reversal of turning with the mGluR antagonist, MCPG. Furthermore, turning seems to be dependent on an increase in striatal neural activity, given that the effect was reversed by the action potential blocker, TTX. 1S,3R-ACPD-induced circling appears also to be dependent on DA neurotransmission, as the effect was reversed with coinjections of a DA antagonist. Finally, normal motor activity, as indicated by nondirectionally biased patterns of movement, did not seem to be dependent on tonic mGluR stimulation in the striatum as MCPG failed to produce rotational behavior at any dose administered.

Stimulation of metabotropic glutamate receptors in vitro causes the hydrolysis of the intracellular messenger phosphoinositide. It has been shown that 1S,3R-ACPD is also capable of binding NMDA receptors at high concentrations. Although this compound is 30 times more potent at mGLUR sites than at NMDA receptors (Schoepp, Johnson, True, & Monn, 1991), the effective dose used in this experiment (500 μM) may still be expected to activate NMDA receptors if applied in vitro. The diffusion of 1S,3R-ACPD from an injection site in vivo, however, may be expected to dilute the agonist considerably (Klockgether & Turski, 1993), allowing for receptor-specific action in tissue somewhat distal to the tip of the injection cannula. Furthermore, excitotoxicity related to over-stimulation of NMDA receptors by trans-ACPD injections has not been reported following intrastriatal injections of concentrations 3 orders of magnitude higher than the highest dose used here (Sacaan et al., 1991).

In other studies of the effects of intracerebral injections of trans-ACPD, Koch (1993) reported an increase in the acoustic startle response after a high-dose injection into the amygdala. Interestingly, a turning effect after unilateral microinjections into the hippocampus was also reported. It is noted, however, that these behavioral changes occurred 4 hr after the injections, were accompanied by seizure activity, and were a result of doses more than 100 times higher than those used in the present investigation. In addition, intracerebral ventricular infusion of high doses of trans-ACPD induced behavioral activation in mice (Laudrup & Klitgaard, 1993). In contrast to the present results, coinjections of neither a DA antagonist nor the putative mGluR antagonist L-2-amino-3-phosphonopropionate (L-AP3) were effective in reversing this activation.

The present results can be compared with the work of Sacaan and colleagues (1991; 1992) who investigated the effects of intrastriatal injections of 1S,3R-ACPD on turning behavior. Although conceptually similar, the methodology employed in the present study was different in several ways: (a) Chronic cannulation of the striatum, as compared with the acute preparation used by Sacaan and colleagues, allowed for immediate recording of behavior following drug injections, and permitted observation of different drug effects within the same subjects. (b) The time course of turning observed in this study was over a period of minutes rather than hours. (c) The dependence of the turning behavior on intact DA neurotransmission was verified with a central co-injection of the DA antagonist cis-flupenthixol rather than a systemic haloperidol injection or a DA-depleting pretreatment. (d) The highest concentration of 1S,3R-ACPD employed in the present study

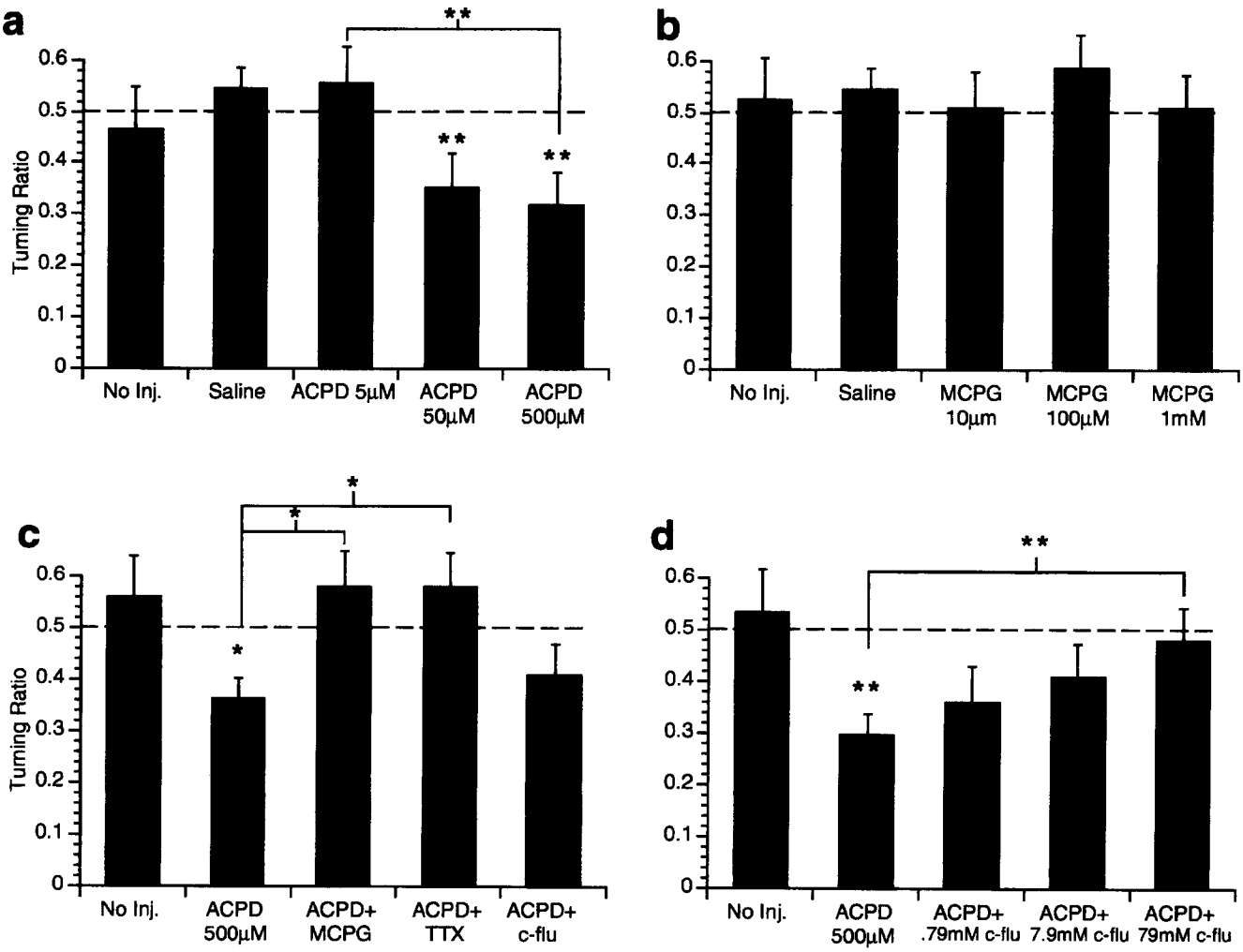


Figure 2. Effects of the mGluR agonist 1S,3R-ACPD and various antagonists injected into the dorsal striatum on turning ratio (total ipsilateral divided by total turns). In each figure, the broken horizontal line at 0.5 indicates no directional bias, whereas lower numbers and higher numbers indicate a contralateral and ipsilateral bias, respectively. (a) 1S,3R-ACPD (ACPD) induced a contralateral bias in turning. Mean (+*SEM*) turning ratios calculated over 20 min following no injection (No inj.) sessions, 0.5 μl injections of saline or 3 doses of 1S,3R-ACPD are shown. The 2 no injection sessions and 2 saline sessions are averaged. **Significant difference between 50 μM and 500 μM doses of 1S,3R-ACPD and saline; Significant difference between 5 μM and 500 μM injections indicating dose-dependency of the effect ($p < .01$). (b) Injections of the mGluR antagonist MCPG alone did not affect rotation. (c) Contralateral turning caused by 500 μM 1S,3R-ACPD is reversed with co-injections of TTX (100 μM) or MCPG (1 mM), but not by 7.9 mM cis-flupenthixol (c-flu). *Significant difference between mean of 1S,3R-ACPD sessions and mean of no injection sessions, and between 1S,3R-ACPD and co-injections of 1S,3R-ACPD with MCPG or TTX ($p < .05$). (d) Progressive blockade of 1S,3R-ACPD-induced contralateral rotation with low (0.79 mM), medium (7.9 mM), and high (79.0 mM) concentrations of the DA antagonist cis-flupenthixol (c-flu). **Significant difference between mean of 1S,3R-ACPD sessions and mean of no injection sessions ($p < .01$), and between 1S,2R-ACPD and co-injection of 1S,3R-ACPD with cis-flupenthixol (79.0 mM) ($p < .01$).

was 1,000 times lower than that of Sacaan et al., (1992) and the injection volume was one fourth as large.

The major result of the present experiments and those of Sacaan et al. (1991, 1992) are similar in that 1S,3R-ACPD caused contralateral rotation that was reversed by interrupting DA neurotransmission. In accordance with the above methodological differences however, the results of the present study are also different in some significant ways: (a) In the study of Sacaan and colleagues (1992), significant contralateral turning was not observed until 3 hr after the injection, with a peak effect at 5 hr. Furthermore, significant *ipsilateral* turning was observed during the first hour. In contrast, contralateral

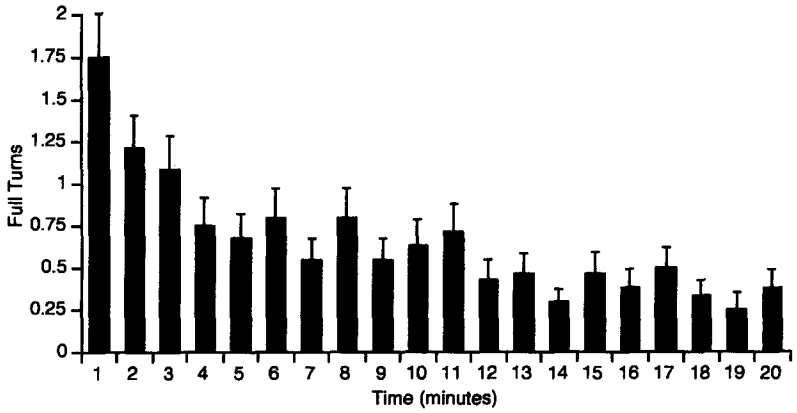


Figure 3. Average number of turns per minute (mean + *SEM*) following intrastriatal injections of the mGluR agonist 1S,3R-ACPD (500 μM in 0.5 μl), an effect that produced a significant contralateral bias. Two agonist sessions, separated by 3 cis-flupenthixol co-injections, have been averaged (n = 12). Activity was highest when the rat was first placed in the rotometer chamber and steadily decreased over 20 min. There was no significant difference in total turns compared with no-injection sessions.

rotation observed here began immediately following intrastriatal injections and decreased progressively over 20 min. (b) The blockade of rotation with cis-flupenthixol co-injections did not affect overall activity level. It is unclear whether the reduction in turning reported by Sacaan and co-workers after systemic haloperidol injection or pretreatment with alpha-methyl-paratyrosine was due to an overall decrease in activity level due to the cataleptic effects of these compounds (Wanibuchi & Usda, 1990). (c) The number of uninterrupted full turns exhibited by rats in this study was a modest 12.9 turns per 20 min, whereas in the previous experiments, rats turned more than 150 times in 20 min. We suggest that this behavior is similar in magnitude to that expressed in rats with lesions of the nigrostriatal DA system after a challenge with a DA agonist, and thus may 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 nondrugged, nonlesioned animal, suggesting an effect more within the realm of normal behavior.

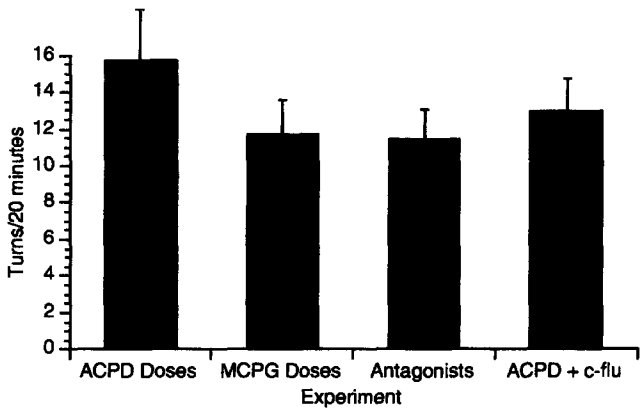


Figure 4. Total turns (mean + *SEM*) were calculated for 20-min recording sessions averaged over all 7 sessions for each of 4 experiments as a measure of activity level. No significant differences were observed, either between individual treatment sessions or between experimental conditions. The grand mean is 12.9 ± 2.0 turns per 20 min.

It could be argued that stimulating mGluRs in the dorsal striatum resulted in contralateral muscle rigidity due to alterations in basal ganglia outflow, thus causing turning toward the affected side of the body. In an investigation of the role of glutamate receptor subtypes in experimental Parkinsonism, Klockgether and Turski (1993) measured muscle tone in rats after bilateral injections of trans-ACPD into the striatum. An increase in tone, thought to correspond to akinesia, was reported at only high doses (20 mM) of trans-ACPD, whereas lower concentrations were ineffective. Although generalization between bilateral and unilateral manipulations is difficult, it appears that the 1S,3R-ACPD injections made in the present study would not result in muscular rigidity as measured in the above report.

Some studies have suggested a primary presynaptic role of metabotropic receptors in regulating glutamate neurotransmission in the brain, though it has been shown that mGluR localization in the striatum is at least partially postsynaptic, given the reduction of agonist binding following striatal lesions (Schoepp, Bockaert, & Sladeczek, 1990; Tallaksen-Greene, Wiley & Albin, 1992). Recently, studies using striatal slices have reported both pre and postsynaptic physiological effects of metabotropic receptors. Low doses (1–30 μM) of trans-ACPD reduced glutamatergic excitation of projection neurons, whereas at high doses (50–100 μM) a direct postsynaptic excitation was observed (Calabresi, Mercuri, & Bernardi, 1992). Thus it could be suggested that the doses of 1S,3R-ACPD that caused rotation in the present study (50 and 500 μM) elevated neuronal discharge, a suggestion that is substantiated by the ability of TTX to block the effect.

The route through which increases in striatal glutamate or dopamine receptor activity cause contralateral turning remains unknown. It is possible that an overall increase in striatal unit discharge would alter signal throughput that is

usually topographically organized in discrete channels (Alexander & Crutcher, 1990). Increased nonspecific striatal firing could result in an overall inhibition of basal ganglia output nuclei. This presupposes a principal contribution of "direct" striatal projections to basal ganglia output nuclei, as opposed to the "indirect" pathway through the subthalamic nucleus that is thought to result in net excitation (Albin, Young, & Penney, 1989; Gerfen, 1992). Nonspecific striatal inhibition of these nuclei could in turn affect the integration of cortical motor signals through disinhibition of thalamic projections to the cortex. This in turn could compromise the ability of the animal to execute normal contralateral motor programs. This treatment may further influence motor function through descending output nuclei projections to pontine locomotor regions (Garcia-Rill, 1986) and to the superior colliculus.

It appears that the maintenance of nonbiased locomotor activity is not dependent on tonic mGluR stimulation, given the lack of effect with injections of the antagonist MCPG. It has been noted that unilateral elevations in electrophysiological activity level are associated with rotation (Wang & Rebec, 1993). Because a blockade of presynaptic inhibition of glutamate release would likely increase striatal cell firing rates, the lack of effect with MCPG also fails to support a tonic inhibition of glutamate release by the mGluR. Antagonists of other glutamate receptors injected into the striatum have yielded disparate results. In the dorsal striatum, the AMPA/kainate antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) failed to elicit rotation (Smith et al., 1993), whereas high doses of the NMDA receptor antagonist amino-phosphono-heptanoate (AP7) caused a reliable ipsilateral circling bias (Thanos, Jhamandas, & Beninger, 1992). In contrast, NMDA antagonists injected into the anterior striatum cause hyperlocomotion (Schmidt & Bury, 1988; Schmidt et al., 1992) whereas agonists cause muscular rigidity (Klockgether & Turski, 1993). The discrepancies between these and the present results remain to be explained, though they may depend on regional variability in striatal output pathways, or on regional heterogeneity in glutamate receptor subtype distribution (Tallaksen-Greene et al., 1992).

The rotation produced by 1S,3R-ACPD was reduced by progressively increasing doses of the DA antagonist cis-flupenthixol. Similarly, rotation induced by KA, AMPA, and NMDA injections in the same location is also blocked by DA receptor antagonism (Smith et al., 1993; Thanos et al., 1992). These results suggest that the behavioral effects of glutamate agonist injections require concurrent DA receptor activity and raise the possibility that a glutamate agonist-induced stimulation of DA release contributed to these effects. Increases in DA levels have been associated with elevated glutamate neurotransmission in several paradigms (Barbeito, Chéramy, Godeheu, Desce, & Glowinski, 1990; Chéramy et al., 1986; Clow & Jhamandas, 1989; Imperato, Honoré & Jensen, 1990; Roberts & Anderson, 1979). Sacaan et al. (1992) reported that high doses of 1S,3R-ACPD did not alter the spontaneous or evoked release of radiolabeled DA from striatal slices, but did increase striatal DA metabolites in vivo. This suggests that the mGluR may increase DA release and turnover in vivo, offering a possible explanation for the dependence of the rotation on intact DA receptor tone.

The striatum has been considered a site of incentive learning (Beninger, 1983), and numerous studies investigating DA receptors in this region have strongly supported this view (see Beninger, 1993 for review). Indeed, single-unit recordings show that neurons in the striatum can acquire and maintain responses that correspond to the CS in a sensorimotor conditioning task (Aosaki et al., 1994). The role of striatal mGluR activity in learning has received limited experimental attention until recently. Metabotropic receptors in the striatum appear to be involved in long-term depression of excitatory cortical input (Calabresi, Mercuri, & Bernardi, 1992), a phenomenon that can be considered a cellular model of learning. It remains to be determined whether long-term alterations in striatally mediated behaviors such as rotation or incentive learning could be effected through manipulation of this receptor site.

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