

Metabotropic Glutamate Agonist-Induced Rotation: A Pharmacological, FOS Immunohistochemical, and [¹⁴C]-2-Deoxyglucose Autoradiographic Study

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Metabotropic glutamate receptors (mGluRs) are a major class of excitatory amino acid receptors. Eight mGluR subtypes, coupled to a variety of effector systems, have been cloned. These receptors have been classified into three groups based on amino acid sequence homology, effector systems, and pharmacological profile. Group I mGluRs increase phosphoinositide turnover, whereas groups II and III mGluRs are negatively coupled to adenylyl cyclase. The striatum possesses a high density of mGluR binding sites, and several mGluR mRNAs and proteins are expressed by striatal neurons. In rats, unilateral striatal injection of the nonsubtype selective mGluR agonist 1-aminocyclopentane-1S,3R-dicarboxylic acid (1S,3R-ACPD) results in contralateral rotation with delayed onset, thought to be secondary to an increase in dopamine release. We sought to determine the mGluR subtype(s) involved, the modulation of the rotation by other basal ganglia neurotransmitter systems, and the functional anatomy underlying the rotational behavior. The group I mGluR agonist 3,5-dihydroxyphenylglycine (DHPG) in-

duced contralateral rotation in a dose-dependent manner, whereas group II and group III agonists were ineffective. Rotation induced by DHPG or 1S,3R-ACPD was attenuated by group I antagonists, but not by group II or group III antagonists. This suggests that the rotation is mediated by group I mGluRs. Rotation induced by DHPG or 1S,3R-ACPD was attenuated by pretreatment with antagonists at muscarinic cholinergic, adenosine A₂, dopamine D₂, or dopamine D₁ receptors. Examination of FOS-like immunoreactivity after group I and group II mGluR agonist administration suggests increased activity in the striatopallidal pathway. However, [¹⁴C]-2-deoxyglucose uptake studies indicate increased activity in nuclei of the striatopallidal (indirect) pathway, particularly in the subthalamic nucleus, only after group I mGluR activation.

Key words: metabotropic glutamate receptor; basal ganglia; subthalamic nucleus; striatum; dopamine; adenosine A₂ receptors; muscarinic receptors

Excitatory amino acids (EAAs) are important neurotransmitters in the basal ganglia, and EAAergic agents are being investigated actively as possible pharmacotherapies for basal ganglia disorders. A major class of EAA receptors are the metabotropic glutamate receptors (mGluRs), coupled to second messenger systems via G-proteins. Eight mGluR subtypes have been cloned, several of which possess splice variants. These mGluR subtypes have been categorized into three groups based on their amino acid sequence homology, effector systems, and pharmacological profile. When expressed and activated in transfection systems, group I receptors (mGluRs 1 and 5) stimulate phosphoinositide hydrolysis. Group II (mGluRs 2 and 3) and group III (mGluRs 4–8) receptors inhibit adenylyl cyclase, although with different pharmacological profiles (for review, see Pin and Duvosin, 1995; Roberts, 1995). Ligand-binding studies have shown the striatum to possess a high density of mGluR binding sites (Albin et al., 1992), and several

mGluR mRNAs and proteins are expressed by striatal neurons, including members of all three mGluR groups (Abe et al., 1992; Martin et al., 1992; Shigemoto et al., 1992, 1993; Fotuhi et al., 1993; Ohishi et al., 1993a,b; Saugstead et al., 1994; Testa et al., 1994; Joly et al., 1995; Romano et al., 1995).

Intrastratial injection of the nonsubtype selective mGluR agonist 1-aminocyclopentane-1S,3R-dicarboxylic acid (1S,3R-ACPD) results in vigorous contralateral rotation in rats (Sacaan et al., 1991, 1992; Kaatz and Albin, 1995). The basis for this behavioral change appears to be increased output of dopaminergic nigrostriatal neurons on the injected side, because there is an increase in dopamine and dopamine metabolites after *in vivo* intrastratial injection of 1S,3R-ACPD (Sacaan et al., 1992). This does not appear to be a direct effect on dopamine release, because 1S,3R-ACPD does not increase dopamine release from striatal slices *in vitro* (Sacaan et al., 1992). Examination of Fos-like immunoreactivity (FLIR), a presumed indicator of neuronal activity changes, suggests that dopamine release occurs secondary to activation of the subthalamic nucleus (STN), and lesions of the STN result in blockade of 1S,3R-ACPD-induced contralateral rotation (Kaatz and Albin, 1995). We hypothesize that activation of striatal mGluRs results in increased activity of striatopallidal projection neurons with consequent disinhibition of the STN. The STN sends a major excitatory projection to the substantia nigra pars compacta (SNc) (Smith and Grace, 1992), and we believe that it is via

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this projection that increased dopamine release occurs after stimulation of striatal mGluRs. We performed a series of experiments with FLIR and mapping of local cerebral glucose metabolism (ICMRglu) with the [14 C]-2-deoxyglucose autoradiographic method (Sokoloff, 1977).

A major feature of Parkinson's Disease (PD) is overactivity of the STN (Albin et al., 1989). The possible STN overactivity seen after 1S,3R-ACPD suggests that mGluR antagonists may be useful for pharmacotherapy in PD. However, a clearer understanding of the pharmacology of mGluR-induced rotation is needed before this knowledge can be applied to pharmacotherapy of PD. In addition to testing our hypothesis about the functional anatomy of the rotational behavior, we addressed two additional questions. First, which mGluR subtype(s) is involved in the rotational behavior? Second, how do other neurotransmitter systems of the basal ganglia modulate mGluR-induced rotation?

MATERIALS AND METHODS

Drugs. 1-Aminocyclopentane-1S,3R-dicarboxylic acid (1S,3R-ACPD), (R,S)-3,5-dihydroxyphenylglycine (DHPG), (2S,3S,4S)-CCG (L-CCG-I), L-(+)-2-amino-4-phosphonobutyric acid (L-AP4), α -methyl-4-phosphonophenylglycine (MPPG), α -methyl-4-tetrazolylphenylglycine (MTPG), 1-aminoindan-1,5-dicarboxylic acid (UPF523), and α -methyl-4-carboxyphenylglycine (MCPG) (Tocris Cookson, St. Louis, MO) were dissolved in 0.1 M phosphate buffer (PB), and pH was adjusted with 4N NaOH. 8-(3-Chlorostyryl) caffeine (CSC) and 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) (RBI, Natick, MA) were dissolved in 40% DMSO and diluted to volume with PB. 2-*p*-(2-carboxyethyl)phenethylamino-5'-*N*-ethylcarboxamido adenosine HCl (CGS 21680) (RBI) was dissolved in PB. SKF38393, SCH23390, eticlopride, quinpirole, and scopolamine (RBI) were dissolved in ddH₂O. Haloperidol, USP (RBI) was dissolved in glacial acetic acid and diluted to volume with PB.

Intrastratial injections. Male Sprague Dawley rats (Harlan, Indianapolis, IN) weighing 200–300 gm were used in all experiments. Rats were anesthetized with ether and mounted in a small animal stereotaxic apparatus (Kopf, Tujunga, CA). Through a burr hole in the skull, a 25 μ l Hamilton syringe was introduced in the striatum, and drug was injected in a 2 μ l volume over a 2 min period. Coordinates for striatal injection relative to bregma with the incisor bar at 0 mm were anterior–posterior, +1.0 mm; medial–lateral, 2.6 mm; dorsal–ventral, –5.7 mm (Paxinos and Watson, 1986). After injection, the syringe was left in place for 5 min and then slowly withdrawn. Animals were allowed to recover for 4 hr in bedded cages before rotational behavior was measured.

Rotational behavior. Four hours after intrastratial injection, rats were placed in a clear hemispherical container and frequency of rotations ipsilateral and contralateral to the side of injection was recorded for a 5 min period. One rotation was defined as a 360° turn without a change of direction. Previous work from our laboratory (Kaatz and Albin, 1995) and others (Sacaan et al., 1991, 1992) has determined this to be a reliable measure of rotational behavior, because contralateral rotations plateau at 3–4 hr after injection and remain constant for several hours. Significant differences in rotational behavior between groups were established by ANOVA in conjunction with Fisher's protected least-significant difference post hoc comparisons ($p < 0.05$; Statview 4.01, Abacus Concepts, Berkeley, CA).

Dose–effect curves for the mGluR agonists 1S,3R-ACPD, DHPG, L-CCG-I, and L-AP4 were obtained by intrastratial injection of these agents. Likewise, dose–effect curves were obtained for the mGluR antagonists MCPG, UPF523, MTPG, and MPPG by intrastratial co-injection of each agent at various concentrations with a constant concentration of DHPG (0.5 μ mol) or 1S,3R-ACPD (0.75 μ mol).

CSC, DPCPX, CGS 21680, SKF 38393, SCH 23390, haloperidol, eticlopride, quinpirole, or scopolamine were administered intraperitoneally 20 min before intrastratial injection of 1S,3R-ACPD or DHPG.

STN lesions. Animals were anesthetized with ketamine/xylazine (10:3, 1 mg/kg, i.p.) and mounted in a small animal stereotaxic apparatus. Ibotenic acid (6 μ g in 0.5 μ l) was injected unilaterally in the STN with a 25 μ l Hamilton syringe. Coordinates for STN injection relative to bregma with the incisor bar at –3.4 mm were anterior–posterior, –3.7 mm; medial–lateral, 2.3 mm; dorsal–ventral, –8.4 mm (Paxinos and Watson, 1986). After injection, the syringe was left in place for 5 min and then

slowly withdrawn. Animals were allowed to recover for 8 d. To select animals with appropriate lesions, animals were challenged with apomorphine (0.5 mg/kg, s.c.) on day 9 to test for ipsilateral rotational behavior (Delfs et al., 1995). Animals demonstrating >10 ipsilateral rotations/5 min were used in this experiment. On day 12, animals underwent the [14 C]-2-deoxyglucose procedure as described below. At the conclusion of the experiment, the extent of STN lesions was determined from Nissl-stained sections by computer-assisted morphometry (Imaging Research Systems, Ontario, Canada), comparing the volume of the STN on the lesioned side versus the nonlesioned side.

Immunohistochemistry. Immediately after measurement of rotational behavior, rats were anesthetized deeply with pentobarbital (100 mg/kg, i.p.) and perfused transcardially with 100 ml PB followed by 250 ml 4% paraformaldehyde in PB. Brains were removed, post-fixed overnight in 4% paraformaldehyde in PB, and cryoprotected in 20% sucrose in PB for 24 hr. Sections (40 μ m) were cut on a sliding microtome, saving sections from the forebrain through the midpons in 0.02% sodium azide in PB. Sections were processed for FLIR with an affinity-purified polyclonal antiserum directed against a conserved portion of the fos protein (Cambridge Biomedical Research, Wilmington, DE). Dilution of the antibody was 1:8000, and FLIR was visualized by the avidin–biotin conjugate technique using a Vectastain kit (Vector, Burlingame, CA) with NiCl₂ enhancement. Sections were mounted on gelatin-coated slides, cover-slipped, and viewed under light microscopy. Adjacent sections were stained with cresyl violet to confirm injection sites.

Histology. After measurement of rotational behavior, animals were anesthetized deeply with 0.4 ml ketamine/xylazine (10:3) and brains were removed rapidly and frozen over dry ice. Brains were sliced in 20 μ m sections and thaw-mounted on gelatin-coated slides. Tissue was fixed over paraformaldehyde vapor for several days, stained with cresyl violet, and examined under light microscopy to confirm injection sites.

[14 C]-2-deoxyglucose autoradiography. For 4 d before surgery, animals were habituated to rodent restrainers. On the day of the experiment, animals were implanted with a femoral vein catheter and then underwent intrastratial injection of DHPG (1 μ mol), L-CCG-I (1 μ mol), or PB vehicle, as described above. After intrastratial injection, animals were placed in rodent restrainers. At 195 min after intrastratial injection, animals received an intravenous injection of [14 C]-2-deoxyglucose (100 μ Ci/kg) (ARC, St. Louis, MO). At 240 min, animals were killed by intravenous injection of pentobarbital (50 mg/kg) followed by 0.1 M KCl (0.5 ml) and brains were removed rapidly and frozen over dry ice. Brains were sliced in 20 μ m sections on a Lipschaw cryostat. Sections were thaw-mounted on gelatin-coated slides and exposed along with calibrated [14 C] plastic standards (ARC) on β -max Hyperfilm (Amersham, Arlington Heights, IL) for 7 d. Sections were then fixed over paraformaldehyde vapor and stained with cresyl violet to confirm injection sites and aid in image analysis. Autoradiograms were analyzed by quantitative densitometry using an MCID-M1 image analysis system (Imaging Research Systems). Optical density measurements for each region were taken bilaterally in a minimum of five brain sections. Measurements of each structure were made in each section in which the structure was visible, and as large an area as possible was sampled. Tissue 14 C concentrations were determined from the optical densities and a calibration curve obtained from co-exposed [14 C] standards. For each animal, ratios of injected/uninjected side were made for each region (Patel et al., 1985). For each condition (vehicle, DHPG, STN lesion + DHPG, or L-CCG-I), the mean ratio for each region was determined. Mean ratios were compared by repeated-measures ANOVA (region, within-subjects factor) in conjunction with Bonferroni–Dunn post hoc comparisons (Statview 4.01, Abacus Concepts). Significant differences between drug treatments within each region were determined with 95% confidence intervals. Additional comparisons were made by determining the relative glucose utilization in each region as a ratio of the 14 C concentration of the region to the mean 14 C concentration of whole brain uptake for that animal (relative 2-deoxyglucose uptake) (Mitchell and Crossman, 1984; Sharp et al., 1993; Trugman and James, 1993). The ratio of region/whole brain uptake could then be used for comparisons with Student's *t* tests. Statistical significance was taken as $p < 0.05$.

RESULTS

Pharmacology

Ipsilateral rotations were infrequent, and there were no significant differences between groups in all the rotational behavior experiments. Additionally, administration of the mGluR antagonists

UPF523, MCPG, MTPG, or MPPG alone produced no significant level of rotation. None of the mGluR agonists or antagonists produced histological evidence of toxicity at 24–48 hr after acute intrastriatal injection at any of the doses administered.

Intrastriatal administration of the selective type I mGluR agonist DHPG induces contralateral rotation in a dose-dependent manner. In addition, it appears to be more potent than the nonselective mGluR agonist 1S,3R-ACPD (Fig. 1). L-CCG-I (group II \gg group I) does not elicit any significant level of rotation at any of the doses administered, nor does the group III agonist L-AP4 (Fig. 1). The group I mGluR antagonists UPF523 and MCPG (group I \geq group II) attenuate contralateral rotation induced by DHPG or 1S,3R-ACPD, whereas the group II/III antagonists MTPG and MPPG have no effect at any of the doses administered (Fig. 2).

Purinergic, cholinergic, and dopaminergic modulation

Pretreatment with the adenosine A_2 receptor antagonist CSC (3 mg/kg, i.p.) significantly reduced contralateral rotation induced by intrastriatal DHPG, whereas pretreatment with the adenosine A_1 receptor antagonist DPCPX (5 mg/kg, i.p.) had no effect. Additionally, pretreatment with the adenosine A_2 receptor agonist CGS 21680 (0.5 mg/kg, i.p.) significantly potentiated DHPG-induced contralateral rotation (Fig. 3). These results are similar to those obtained previously in our lab with 1S,3R-ACPD-induced contralateral rotation (Kearney and Albin, 1995).

Pretreatment with the muscarinic cholinergic antagonist scopolamine (5 mg/kg, i.p.) significantly reduced contralateral rotation induced by striatal 1S,3R-ACPD (1 μ mol) or DHPG (1 μ mol) (Fig. 4) by 35 and 42%, respectively.

Pretreatment with the nonsubtype selective dopamine antagonist haloperidol (0.3 mg/kg, i.p.) reduced contralateral rotation induced by DHPG (1 μ mol) by 40% (Fig. 5A). This is similar to a previous result from Sacaan et al. (1992) that showed a similar reduction in 1S,3R-ACPD-induced rotation by haloperidol. Pretreatment with the dopamine D_2 antagonist eticlopride (0.5 mg/kg, i.p.) significantly attenuated contralateral rotation induced by DHPG (0.5 μ mol) or 1S,3R-ACPD (0.75 μ mol), whereas pretreatment with the dopamine D_2 agonist quinpirole (1 mg/kg, i.p.) had no effect on DHPG- or 1S,3R-ACPD-induced contralateral rotation (Fig. 5C). Pretreatment with the dopamine D_1 antagonist SCH 23390 (0.1 mg/kg, i.p.) attenuated rotation induced by DHPG, whereas it completely blocked rotation induced by 1S,3R-ACPD. The dopamine D_1 agonist SKF 38393 (5 mg/kg) significantly attenuated rotation induced by 1S,3R-ACPD, but only reduced moderately DHPG-induced rotation (Fig. 5B).

Immunohistochemistry

Intrastriatal injection of vehicle produces virtually no FLIR in the basal ganglia, although there is some FLIR in the parafascicular nucleus of the thalamus and hypothalamus (data not shown). Intrastriatal injection of DHPG produced a pattern of FLIR identical to that seen after 1S,3R-ACPD administration (Kaatz and Albin, 1995). Although there was virtually no FLIR in the striatum and SNc, there was a marked increase in FLIR in the globus pallidus (GP), entopeduncular nucleus (EP), STN, and substantia nigra pars reticulata (SNr) on the injected side (Fig. 6), as well as in the ventrobasal thalamus (data not shown). Intrastriatal injection of L-CCG-I produced a pattern of FLIR similar to that seen after 1S,3R-ACPD and DHPG (Fig. 6), although the magnitude of FLIR in the STN is not as great as that seen after DHPG (Fig. 6C). After intrastriatal DHPG, virtually every neu-

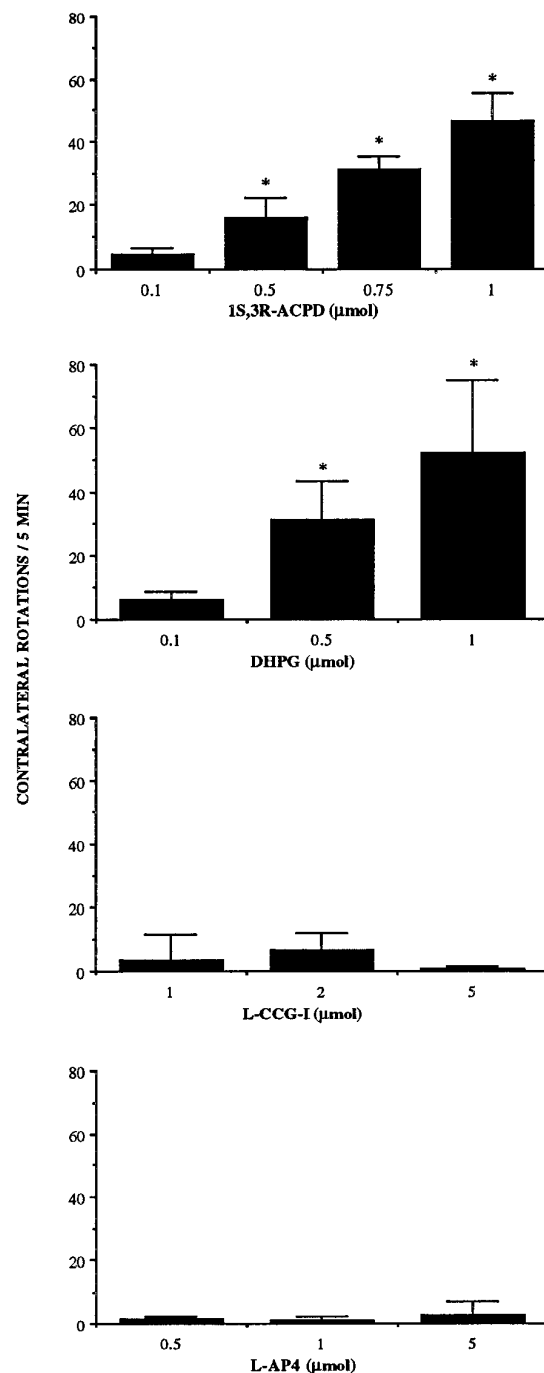


Figure 1. Dose-effect studies on contralateral rotation induced by intrastriatal administration of selective mGluR agonists. 1S,3R-ACPD (0.1, 0.5, 0.75, 1 μ mol), DHPG (0.1, 0.5, 1 μ mol), L-CCG-I (1, 2, 5 μ mol), or L-AP4 (1, 2, 5 μ mol) was injected unilaterally in the striatum (total volume, 2 μ l). At 4 hr, rotations ipsilateral and contralateral to the side of injection were measured for a 5 min period. Data are mean \pm SD; * p < 0.05 compared with vehicle-injected controls (ANOVA with Fisher's PLSD) (n = 5–6 per group).

ron in the STN is labeled. However, after intrastriatal L-CCG-I, there are STN neurons that do not show FLIR.

Intrastriatal injection of L-AP4 produced a distinctive pattern of FLIR. There was a marked increase in FLIR in the striatum and nucleus accumbens. However, there was little FLIR in the GP, EP, STN, SNr, and SNc (Fig. 7).

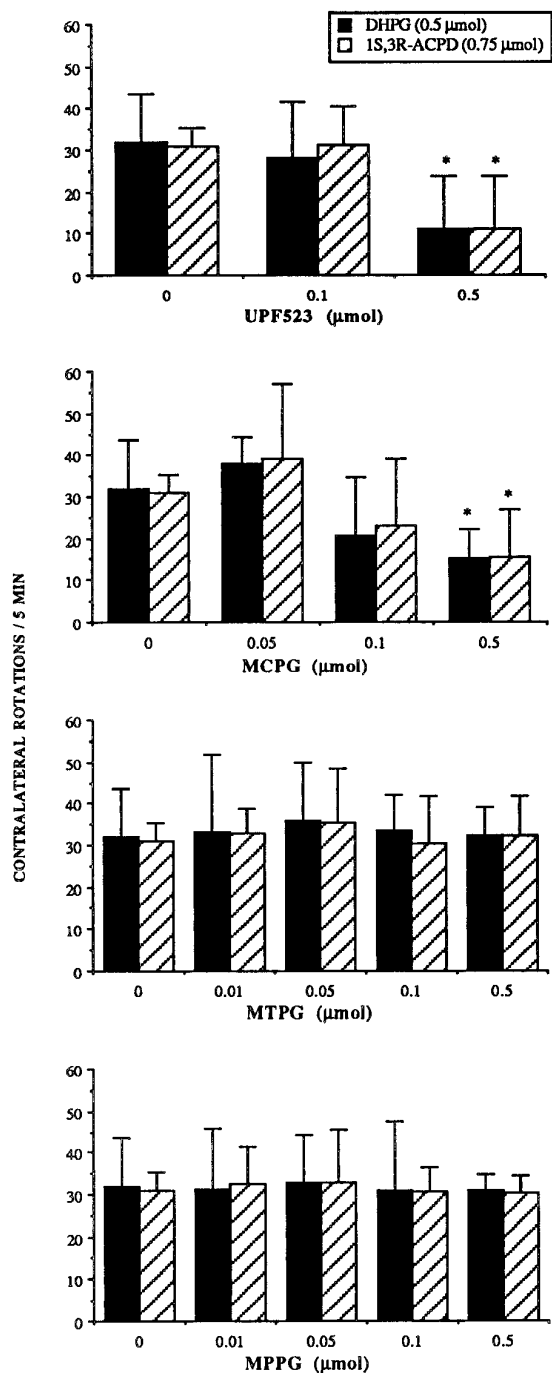


Figure 2. Attenuation of DHPG- or 1S,3R-ACPD-induced contralateral rotation by selective mGluR antagonists. UPF523 (0.1, 0.5 μmol), MCPG (0.05, 0.1, 0.5 μmol), MTPG (0.01, 0.05, 0.1, 0.5 μmol), or MPPG (0.01, 0.05, 0.1, 0.5 μmol) was co-injected with DHPG (0.5 μmol) or 1S,3R-ACPD (0.75 μmol) unilaterally in the striatum (total volume, 2 μl). The 0 μmol dose of antagonist represents DHPG (0.5 μmol) or 1S,3R-ACPD (0.75 μmol) alone. At 4 hr, rotations ipsilateral and contralateral to the side of injection were measured for a 5 min period. Data are mean ± SD; **p* < 0.05 when compared with DHPG or 1S,3R-ACPD alone (ANOVA with Fisher's PLSD) (*n* = 5–6 per group).

Local cerebral glucose metabolism

Animals receiving a unilateral, striatal injection of DHPG demonstrated asymmetric increased ICMRglu in the following basal ganglia structures: GP (+12%), STN (+44%), SNr (+24%), SNc

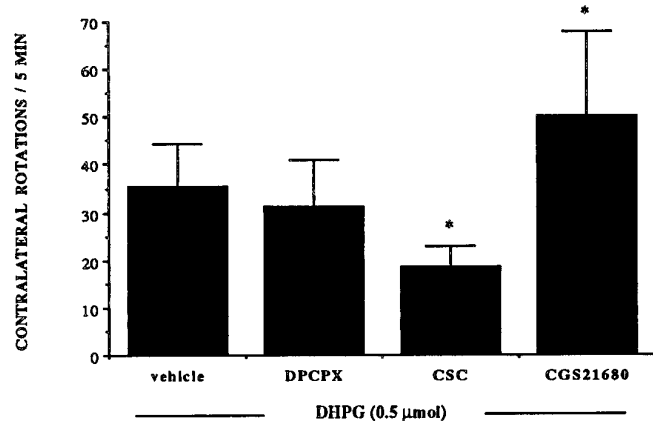


Figure 3. Effect of adenosine receptor agents on contralateral rotation induced by DHPG. CSC (3 mg/kg, i.p.), DPCPX (5 mg/kg, i.p.), or CGS 21680 (0.5 mg/kg, i.p.) was administered 20 min before unilateral striatal injection of DHPG (0.5 μmol). At 4 hr, rotations ipsilateral and contralateral to the side of injection were measured for a 5 min period. Data are mean ± SD; **p* < 0.05 when compared with DHPG or 1S,3R-ACPD alone (ANOVA with Fisher's PLSD) (*n* = 5–6 per group).

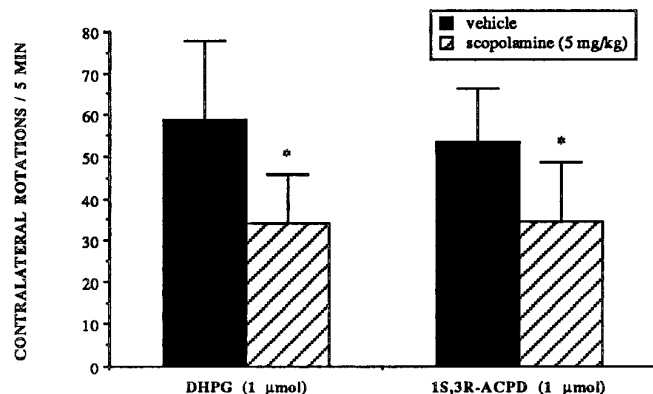


Figure 4. Effect of the muscarinic cholinergic antagonist scopolamine on contralateral rotation induced by DHPG or 1S,3R-ACPD. Scopolamine HBr (5 mg/kg, i.p.) was administered 20 min before unilateral striatal injection of DHPG (1 μmol) or 1S,3R-ACPD (1 μmol). At 4 hr, rotations ipsilateral and contralateral to the side of injection were measured for a 5 min period. Data are mean ± SD; **p* < 0.05 when compared with DHPG or 1S,3R-ACPD alone (ANOVA with Fisher's PLSD) (*n* = 5–6 per group).

(+49%), and EP (+42%). In addition, increased ICMRglu was seen in the following basal ganglia projection regions: ventroanterior nuclei (VA, +25%), intralaminar nuclei (IL, +12%), and ventrolateral nuclei (VL, +16%) of the thalamus; lateral habenula (+15%); and the intermediate (SCint, 35%) and deep layers (SCdp, +26%) of the superior colliculus (Figs. 8, 9). Vehicle-injected controls showed no significant side-to-side differences. There was a small decrease in ICMRglu in the striatum on the injected side in both DHPG (−8%) and vehicle (−10%) injected animals, which is most likely the result of mechanical damage caused by injection.

Ibotenic acid lesion of the STN resulted in 65% loss of STN volume (Fig. 10). STN lesions decreased the mGluR stimulation effects on basal ganglia and projection area ICMRglu. The only areas that still showed significant increased ICMRglu after DHPG administration are the LH (+12%), SNc (+30%), and the EP (+23%) (Figs. 8, 9). After vehicle administration in animals with

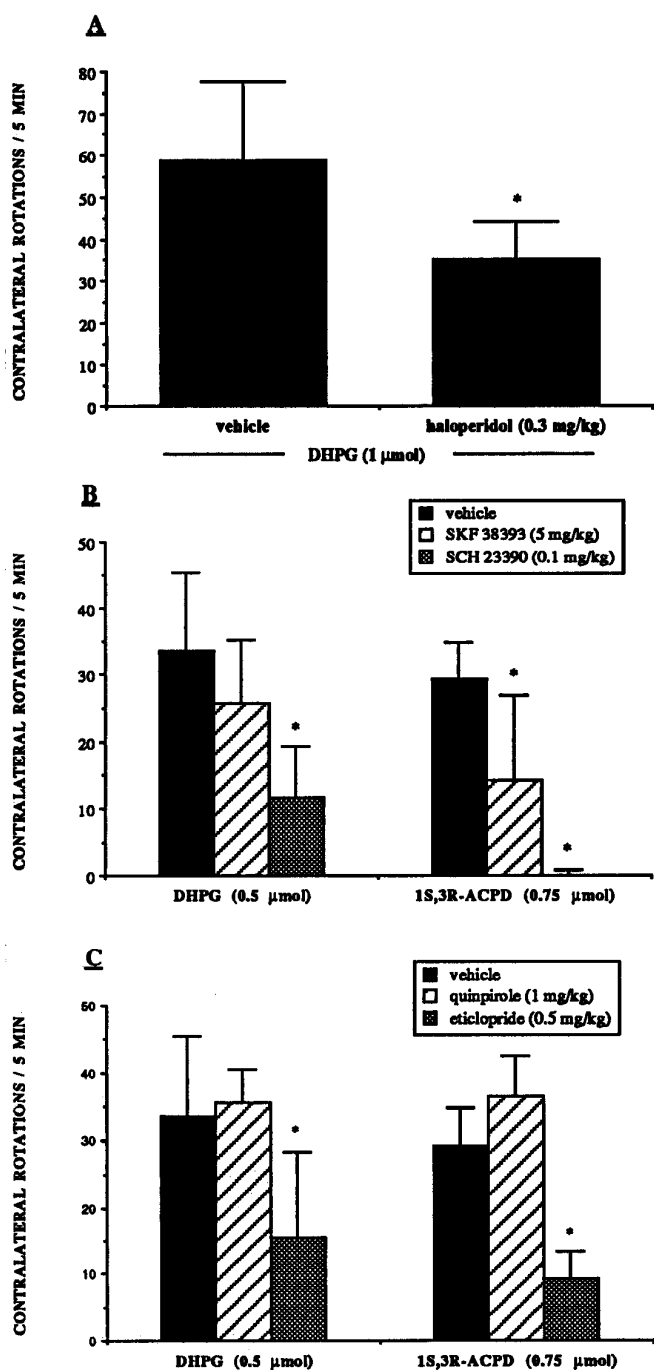


Figure 5. Effect of dopamine receptor agents on contralateral rotation induced by DHPG or 1S,3R-ACPD. Haloperidol (0.3 mg/kg, i.p.) was administered 20 min before unilateral striatal administration of DHPG (1 μmol) (A). SCH23390 (0.1 mg/kg, i.p.) or SKF38393 (5 mg/kg, i.p.) (B), eticlopride (0.5 mg/kg, i.p.) or quinpirole (1 mg/kg, i.p.) (C) was administered 20 min before unilateral striatal injection of DHPG (0.5 μmol) or 1S,3R-ACPD (0.75 μmol). At 4 hr, rotations ipsilateral and contralateral to the side of injection were measured for a 5 min period. Data are mean ± SD; **p* < 0.05 when compared with DHPG or 1S,3R-ACPD alone (ANOVA with Fisher's PLSD) (*n* = 5–6 per group).

STN lesions, there were no significant differences versus intact, vehicle-injected controls (data not shown).

L-CCG-I-injected animals showed significant increased ICMRglu in the SNc (+11%). However, they show decreased ICMRglu in the striatum (−30%), VA thalamus (−23%), IL thalamus

(−13%), VL thalamus (−19%), and the SCint (−7%) and SCdp (−4%) (Figs. 8, 9). Comparison of the relative 2-deoxyglucose uptake of the uninjected side in STN-lesioned, DHPG-injected rats versus intact vehicle-injected controls revealed that these changes represent actual decreases in ICMRglu on the injected side, and not increased ICMRglu on the uninjected side (Student's *t* test, *p* < 0.05).

DISCUSSION

Pharmacology

Our results suggest that contralateral rotation induced by unilateral striatal mGluR activation is mediated by group I mGluRs. First, the group I agonist DHPG (Ito et al., 1992; Schoepp et al., 1995) elicits dose-dependent rotation and is more potent than the nonselective agonist 1S,3R-ACPD, whereas group II and group III agonists induced no rotation. Second, group I antagonists UPF523 (Pellicciari et al., 1995; Sacaan et al., 1996) and MCPG (Hayashi et al., 1992) attenuate rotation induced by 1S,3R-ACPD or DHPG. The Group II/III antagonists MTPG and MPPG (Jane et al., 1995; Beddingfield et al., 1996) have no effect, even at higher doses, although they are more potent at their respective receptors than UPF523 and MCPG are at group I receptors (Jane et al., 1995; Pellicciari et al., 1995; Beddingfield et al., 1996). Additionally, chronic lithium treatment, which impairs mGluR-mediated PI signaling, suppresses 1S,3R-ACPD-induced rotational behavior (K. Kaatz and R. Albin, unpublished observations).

Rotation induced by intrastriatal DHPG or 1S,3R-ACPD is modulated similarly by agents that interact with other neurotransmitter systems of the basal ganglia. Pretreatment with a low dose of haloperidol (0.3 mg/kg) resulted in an ~50% reduction of contralateral rotation induced by 1S,3R-ACPD (Sacaan et al., 1992) or DHPG, supporting the role of dopamine in mediating this behavior. To investigate further mGluR/dopamine receptor interactions, we examined the role of the D₁ and D₂ dopamine receptor subtypes. The D₂ antagonist eticlopride decreased contralateral rotation induced by both 1S,3R-ACPD and DHPG to approximately the same extent, whereas the D₂ agonist quinpirole had no effect. The D₁ antagonist SCH23390 decreased both 1S,3R-ACPD- and DHPG-induced rotation. However, whereas SCH23390 reduced DHPG-induced rotation by ~65%, it blocked 1S,3R-ACPD-induced rotation completely. The D₁ agonist SKF38393 moderately reduced DHPG-induced rotation, whereas it reduced 1S,3R-ACPD-induced rotation by ~50%. We propose that this difference is the result of an interaction between dopamine D₁ and group II mGluRs. Preliminary results from our laboratory show that when rats are pretreated with SCH23390 or SKF 38393, intrastriatal L-CCG-I induces modest ipsilateral rotation (L. Darling, J. Kearney, and R. Albin, unpublished observations).

We reported previously on adenosine A₂ receptor-mediated modulation of 1S,3R-ACPD-induced contralateral rotation (Kearney and Albin, 1995). We found similar adenosine A₂ receptor-mediated modulation of DHPG-induced contralateral rotation. Specifically, the A₂ antagonist CSC attenuates DHPG-induced contralateral rotation, whereas the A₂ agonist CGS 21680 potentiates rotation. The adenosine A₁ antagonist DPCPX had no effect on rotation induced by either DHPG or 1S,3R-ACPD. Our results suggest that an interaction occurs between mGluRs and adenosine A₂ receptors. The distribution of adenosine A₂ receptors in the striatum is restricted primarily to striatopallidum neurons (Schiffman et al., 1991). A potential site of action of mGluR-

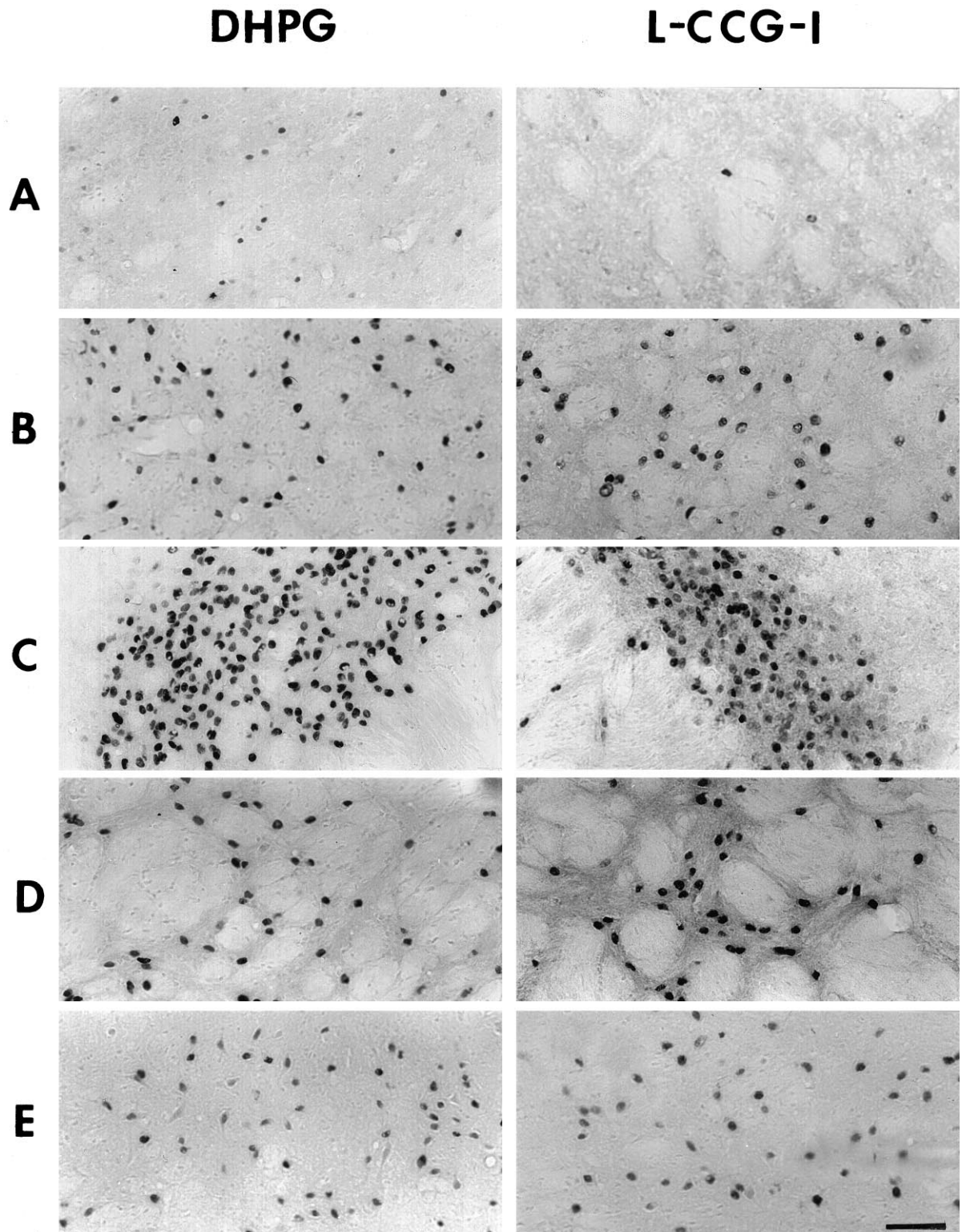


Figure 6. Basal ganglia FLIR after intrastriatal injection of group I and group II mGluR agonists DHPG (1 μ mol), or L-CCG-I (1 μ mol), respectively. With both DHPG and L-CCG-I, little FLIR is seen in the striatum (*A*), but marked FLIR is seen in the GP (*B*), STN (*C*), EP (*D*), and SNr (*E*). Scale bar, 50 μ m.

adenosine interaction is striatopallidal neurons, which also express mGluRs 1a and 5 (S. Tallaksen-Greene, K. Kaatz, C. Romano, and R. Albin, unpublished observations).

Sacaan et al. (1992) reported an increase in striatal acetylcho-

line release, which occurs after striatal 1S,3R-ACPD, approximately coincident with the onset of contralateral rotation. This suggests that striatal acetylcholine may be important for induction of rotation. The density of presynaptic striatal cholinergic markers

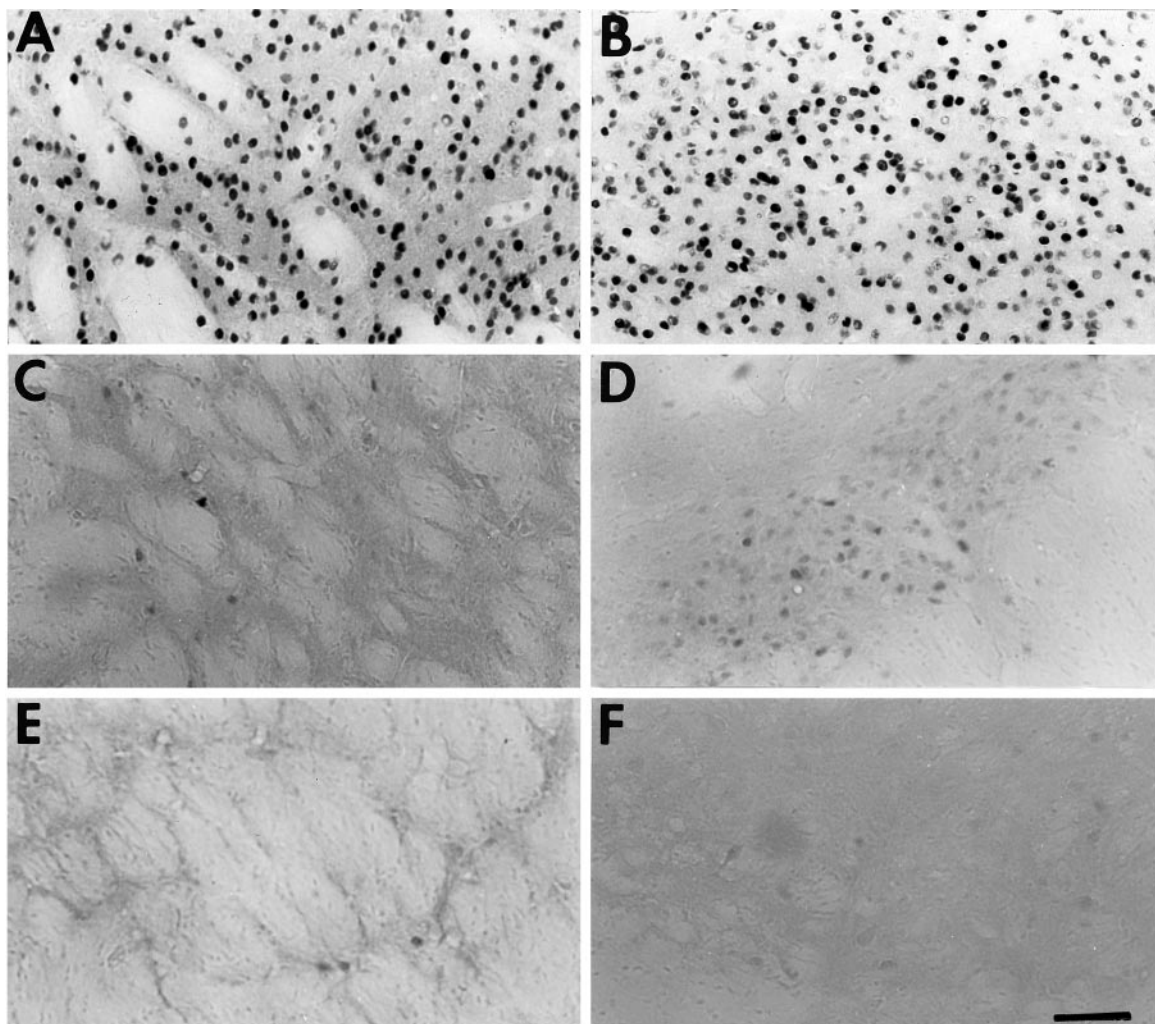


Figure 7. Basal ganglia FLIR after intrastriatal injection of the group III agonist L-AP4 (1 μ mol). Marked FLIR is seen in the striatum (*A*) and nucleus accumbens (*B*), whereas there is little FLIR in the GP (*C*), STN (*D*), EP (*E*), and SNr (*F*). Scale bar, 50 μ m.

is among the highest of any brain region, and cholinergic effects within the striatum are thought to be mediated primarily by muscarinic receptors. Additionally, M_1 , M_3 , and M_5 muscarinic cholinergic receptors share a major signal transduction pathway with group I mGluRs, PLC activation. The muscarinic cholinergic antagonist scopolamine attenuated contralateral rotation induced by 1S,3R-ACPD or DHPG. This could be the result of activation of group I mGluRs on cholinergic interneurons. Takeshita et al. (1996) reported mGluRs 1 and 5 on striatal cholinergic neurons suppress leak K^+ conductance, thereby playing a role in controlling the membrane potential of the cholinergic neurons. Immunocolocalization studies have shown co-localization of cholinergic neurons with mGluR1a and mGluR5 in the striatum (Tallaksen-Greene, Kaatz, Romano, and Albin, unpublished observations).

Functional anatomical correlates

To examine the functional anatomical correlates of mGluR agonist-induced rotation, we examined FLIR as well as ICMRglu mapping. Although FLIR is often used as a marker of neuronal activation, some caution must be used in the interpretation of FLIR results. FLIR changes may be described more accurately as reflecting a *change* in the basal level of neuronal activity at the level of gene transcription (Morgan and Curran, 1991). There is

evidence that some mismatch may occur for FLIR and 2-deoxyglucose in some brain regions, and neurons in some areas do not show FLIR under any stimulus conditions (Dragunow and Faull, 1989). For example, in the kindling model of epilepsy, the SNc showed no increase in *c-fos* mRNA, although it has an increased firing rate (Labiner et al., 1993). Several characteristics of the [14 C]-2-deoxyglucose technique complement FLIR results and their interpretation. First, it is quantitative, which allowed us to make comparisons between different conditions such as intact versus STN-lesioned conditions. Second, it allowed us to determine the *direction* of regional neuronal activity changes (i.e., inhibition vs excitation).

The pattern of FLIR seen after 1S,3R-ACPD (Kaatz and Albin, 1995) and DHPG administration is identical. There is increased FLIR in the GP, EP, STN, SNr, and ventrobasal thalamus. The same pattern of FLIR is also seen after administration of L-CCG-I (group II \gg group I). However, L-CCG-I does not produce rotational behavior. This suggests that there may be a lower threshold for generation of FLIR than for rotational behavior. Labiner et al. (1993) showed that there is little correlation between the magnitude of increase in FOS expression and change in neuronal activity, suggesting that there is a threshold for induc-

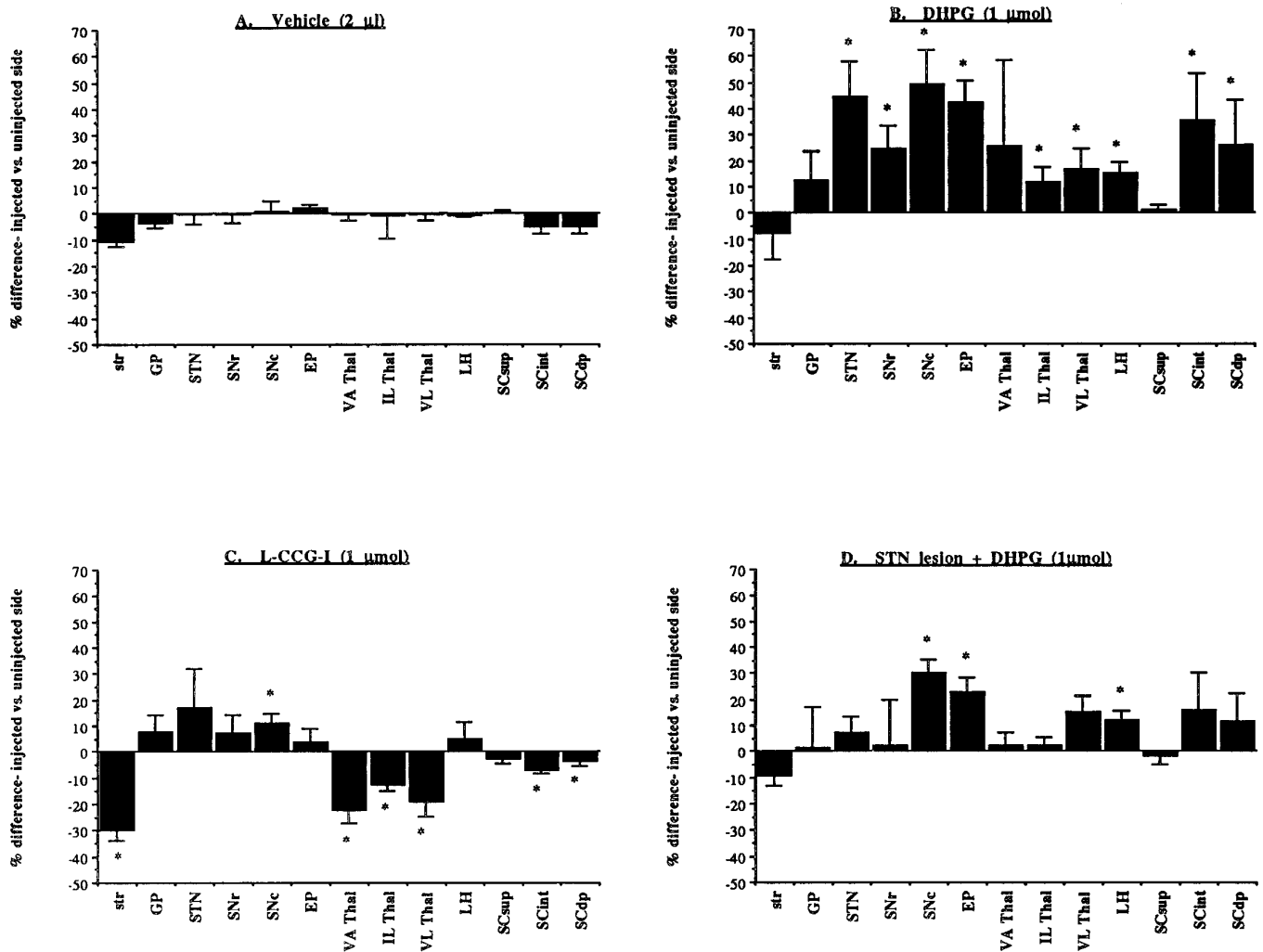


Figure 8. Asymmetry of ICMRglu after unilateral striatal administration of vehicle (2 μ l) (*A*), DHPG (1 μ mol) (*B*), or L-CCG-I (1 μ mol) (*C*) in intact rats, or DHPG (1 μ mol) in STN-lesioned rats (*D*). Data are mean \pm SD of the percent difference in ICMRglu on the injected versus uninjected side; * p < 0.05 compared with uninjected side (95% confidence intervals) (n = 4–5 per group).

tion of FOS, beyond which there is little relationship between expression and neuronal activity.

Although L-CCG-I and DHPG produced the same pattern of FLIR, they produced very different patterns of ICMRglu. L-CCG-I administration resulted in *decreased* ICMRglu in basal ganglia projection areas, including the VA, VL and IL thalamus, SCint, and SCdp. The small increases in ICMRglu in some basal ganglia regions after intrastriatal L-CCG-I in the basal ganglia are most likely attributable to weak stimulation of group I mGluRs by L-CCG-I.

After intrastriatal DHPG administration, there was increased ICMRglu in the GP, STN, EP, SNr, and SNc. Additionally, we saw increases in ICMRglu in downstream projection areas, including VA, VL and IL thalamus, LH, SCint, and SCdp, suggesting increased basal ganglia output on the injected side. Conventionally, ICMRglu reflects increased glucose utilization in synaptic terminals and dendrites. However, in some cases in which neurons are extremely active, somal metabolism may also be evident (Di Rocco et al., 1989). We propose that this is the case within the STN. STN integrity is essential for rotational behavior after intrastriatal injection of mGluR agonists, and virtually every neuron in the STN shows FLIR (Kaatz and Albin, 1995). Additionally, regions receiving STN afferents such as

the GP, EP, SNr, and SNc show increased ICMRglu, suggesting that the STN terminals are more active after stimulation of striatal group I mGluRs. In particular, the SNc, which receives an excitatory input from the STN, shows a 50% increase in ICMRglu. This suggests that the SNc is being activated, which may result in increased dopamine release in the striatum. One would also predict that the SNr/EP, which receives an excitatory input from the STN, would be activated as well. This prediction is borne out in that we observed increased ICMRglu in the SNr/EP as well as in their projection regions, including the LH, VA, VL and IL thalamus, SCint, and SCdp.

Lesions of the STN completely block rotational behavior after unilateral striatal 1S,3R-ACPD administration (Kaatz and Albin, 1995). STN lesions abolished ICMRglu increases in the STN, SNr, and basal ganglia projection areas including the thalamus, SCint, and SCdp. This supports the crucial role that the STN plays in the induction of rotational behavior. The only regions that still had significant increased ICMRglu were the EP, SNc, and LH. However, the magnitude of these increases was half that seen in intact rats. The lesions of the STN were not complete, and \sim 35% of STN neurons remained. The incompleteness of the lesions would also account for some of the small, but nonsignificant, increases seen in the VL thalamus, SCint, and SCdp as well.

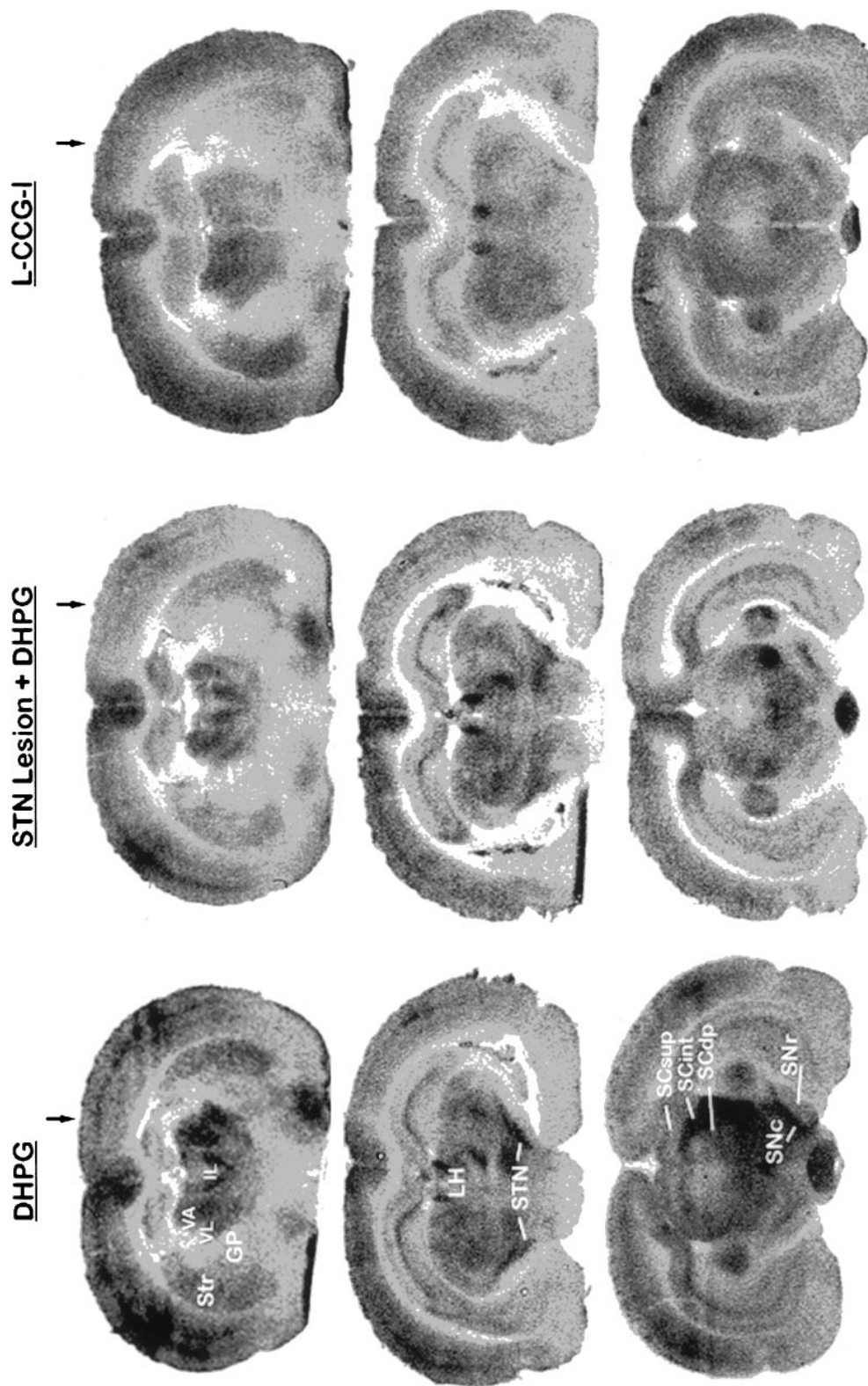


Figure 9. Autoradiographs depicting ICMRglu at the level of the GP, STN, and substantia nigra after intrastriatal injection of DHPG ($1 \mu\text{mol}$) or L-CCG-I ($1 \mu\text{mol}$) in intact rats, or DHPG ($1 \mu\text{mol}$) in STN-lesioned rats. Arrows indicate the injected side. Note the increased ICMRglu in the basal ganglia and basal ganglia projection regions after DHPG administration. These increases can be attenuated by STN lesion. Administration of L-CCG-I produces a different pattern of ICMRglu. Str, Striatum; GP, globus pallidus; VA, ventral anterior thalamus; VL, ventral lateral thalamus; IL, intralaminar thalamus; LH, lateral habenula; STN, subthalamic nucleus; SCsup, superior colliculus-superficial layers; SCint, superior colliculus-intermediate layers; SCdp, superior colliculus-deep layers; SNc, substantia nigra pars compacta; SNr, substantia nigra pars reticulata.

Previous results suggested that mGluR agonist-induced rotation occurs via a complex, multisynaptic mechanism that ultimately results in increased dopamine release on the injected side (Sacaan et al., 1992). Studies of FLIR suggested that the increase in dopamine release occurs secondary to activation of the STN (Kaatz and Albin, 1995), and lesions of the STN block 1S,3R-

ACPD-induced rotational behavior (Kaatz and Albin, 1995), suggesting that the STN plays a *crucial* role in the induction of mGluR-induced rotation. The SNc receives an excitatory afferent from the STN (Smith and Grace, 1993), and we hypothesize that the STN induces the SNc to increase dopamine release through this projection. The level of STN activity is regulated indirectly by

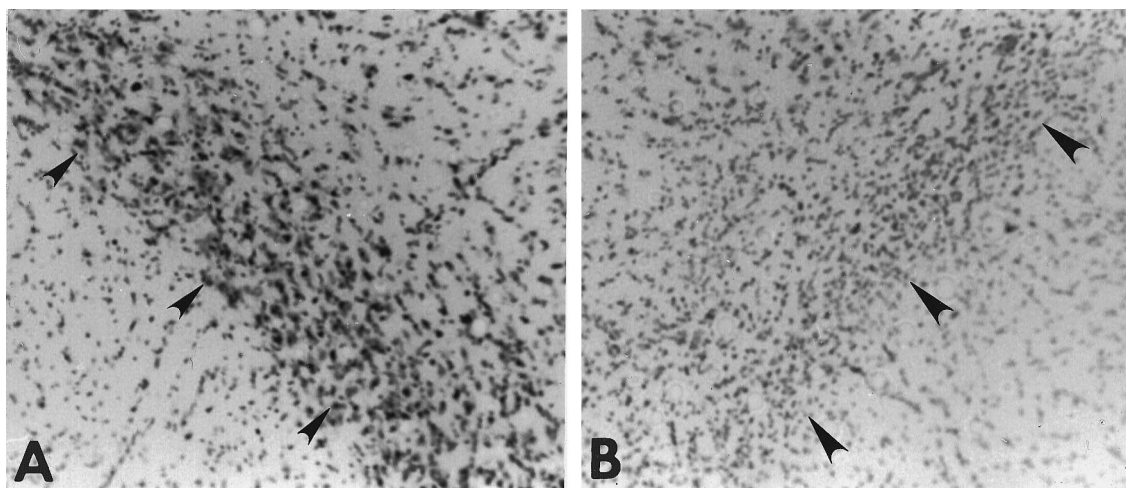


Figure 10. Effect of lesioning the STN with ibotenic acid. Twelve days after ibotenic acid (6 μ g) injection, the injected STN exhibits a marked loss of neurons (*B*) compared with the uninjected side (*A*).

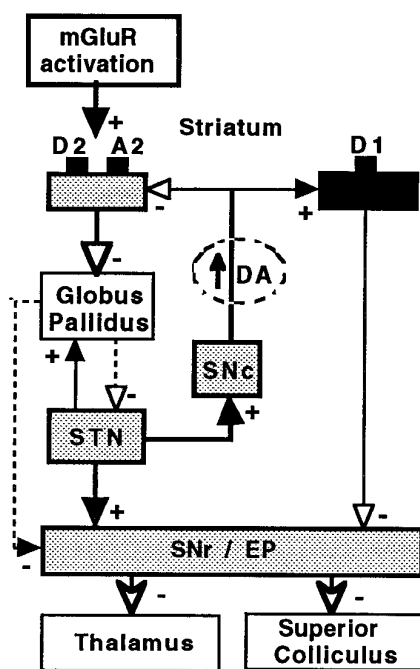


Figure 11. Diagram of the striatal direct and indirect pathways showing a potential mechanism for group I mGluR-mediated contralateral rotation (modified from Albin et al., 1989). The “direct pathway” arises from striatal GABA/substance P/dynorphin neurons, which express dopamine D1 receptors. The “indirect pathway” arises from striatal GABA/enkephalin neurons, which express dopamine D₂ receptors and adenosine A₂ receptors. Unilateral striatal group I mGluR activation may increase the activity of striatopallidal neurons, resulting in increased activity of the STN and subsequent increase in dopamine release from the SNc.

the striatum by modulation of GP neuron activity via the striatopallidal projection. Our functional anatomy studies suggest that activation of group I mGluRs in the striatum results in increased activity of the striatopallidal projection, which results in disinhibition of the STN with consequent excitation of the SNc as well as of the SNr/EP (Fig. 11).

Conclusions

We report evidence to support the role of group I mGluRs in mediating rotational behavior after intrastriatal administration of

mGluR agonists. However, at this point, additional elucidation of the group I subtype involved is impossible, because there are no suitable pharmacological agents to differentiate between mGluRs 1 and 5. Recently, data from genetic knock-out experiments have distinguished between mGluRs 1 and 5 in cerebellar function. Mice deficient in mGluR1 show severe motor coordination problems and ataxia (Aiba et al., 1994; Conquet et al., 1994), whereas motor coordination appears normal in mice lacking mGluR5 (Wojtowicz et al., 1996). Basal ganglia function in these knock-out mice has not been examined as yet. Intra-striatal administration of DHPG in these knock-out mice may provide definitive evidence for involvement of mGluR1 and/or mGluR5 in mGluR-mediated rotational behavior.

mGluRs have been suggested as useful targets for pharmacotherapy in PD (Schoepp and Conn, 1993; Kaatz and Albin, 1995; Nicoletti et al., 1996). A hallmark feature of PD is overactivity of the STN, which may lead to progressive excitotoxic death of the remaining dopamine-containing neurons of the SNc (Saji et al., 1996) and hyperstimulation of the GPi/SNr (GPi = EP in rodents) resulting in a reduction in motor activity (Albin et al., 1989). We have shown that stimulation of striatal group I mGluRs results in probable STN hyperactivity. This suggests that group I mGluR antagonists may alleviate STN hyperactivity and may be useful for pharmacotherapy of PD. However, a clearer understanding of the physiological role of mGluRs within the basal ganglia is needed before this knowledge can be applied to PD pharmacotherapy.

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