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Striatal dopamine and glutamate receptors modulate methamphetamine-induced cortical Fos expression

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Abstract

Methamphetamine (mAMPH) is a psychostimulant drug that increases extracellular levels of monoamines throughout the brain. It has previously been observed that a single injection of mAMPH increases immediate early gene (IEG) expression in both the striatum and cerebral cortex. Moreover, this effect is modulated by dopamine and glutamate receptors since systemic administration of dopamine or glutamate antagonists has been found to alter mAMPH-induced striatal and cortical IEG expression. However, because dopamine and glutamate receptors are found in extra-striatal as well as striatal brain regions, studies employing systemic injection of dopamine or glutamate antagonists fail to localize the effects of mAMPH-induced activation. In the present experiments, the roles of striatal dopamine and glutamate receptors in mAMPH-induced gene expression in the striatum and cerebral cortex were examined. The nuclear expression of Fos, the protein product of the IEG *c-fos*, was quantified in both the striatum and the cortex of animals receiving intrastratial dopamine or glutamate antagonist administration. Intrastratial infusion of dopamine (D1 or D2) or glutamate [N-methyl-D-aspartic acid (NMDA) or alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)] antagonists affected not only mAMPH-induced striatal, but also cortical, Fos expression. Overall, the effects of the antagonists occurred dose-dependently, in both the infused and non-infused hemispheres, with greater influences occurring in the infused hemisphere. Finally, unilateral intrastratial infusion of dopamine or glutamate antagonists changed the behavior of the rats from characteristic mAMPH-induced stereotypy to rotation ipsilateral to the infusion. These results demonstrate that mAMPH's actions on striatal dopamine and glutamate receptors modulate the widespread cortical activation induced by mAMPH. It is hypothesized that dopamine release from nigrostriatal terminals modulates activity within striatal efferent pathways, thereby disinhibiting thalamo-cortical circuits. By extension, these results suggest processes through which repeated exposure to mAMPH might influence cortical function in mAMPH abusers.

Keywords

psychostimulant; immediate early gene; SCH23390; sulpiride; AP5; DNQX

The abuse of the psychostimulant drug, mAMPH, results in long-lasting structural and functional abnormalities in the striatum and cerebral cortex (McCann et al., 1998; Volkow et

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al., 2001; Sekine et al., 2001; London et al., 2004). Acutely, mAMPH administration has been shown to increase cellular markers of neuronal activity, including the expression of IEGs, in the striatum and sensorimotor cortex (Wang and McGinty, 1995). Within the striatum, mAMPH initially reverses dopamine transporters causing a Ca^{2+} -independent efflux of cytosolic dopamine into the extracellular space (Schmidt and Gibb, 1985). Elevated extracellular dopamine leads to increased receptor activation, gene expression, and altered activity in striatal efferent pathways. It is thought that mAMPH administration increases extracellular glutamate in the striatum and cortex via activation of the recurrent striato-thalamo-cortico-striatal circuit (Mark et al., 2004, 2007). Increased extracellular dopamine and glutamate represent the initial steps in producing long-term changes in the striatum and cortex (Nash and Yamamoto, 1992; O'Dell et al., 1993; Mark et al., 2004).

The striatum contains high densities of glutamate receptors (Monaghan et al., 1989; Greenamyre and Young, 1989; Albin et al., 1992), and receives major excitatory inputs from all regions of the cortex and from the thalamus (McGeorge and Faull, 1989; Mengual et al., 1999; Smith et al., 2004). In addition, the striatum contains high densities of dopamine (D1 and D2) receptors and receives dopaminergic input from the ventral midbrain (Beckstead et al., 1979; Altar and Marshall, 1985; Boyson et al., 1986; Bolam and Smith, 1990). Striatal projection neurons send efferent fibers to the output nuclei of the basal ganglia [internal globus pallidus and substantia nigra pars reticulata (SNr)], nuclei that control the thalamus, and cortex (Gerfen, 1990; Alexander and Crutcher, 1990; Graybiel et al., 1990).

mAMPH administration alters neuronal activity in the striatum and cortex via activation of dopamine and glutamate receptors. For example, systemic administration of dopamine or glutamate antagonists blocks mAMPH-induced *zif/268* mRNA expression in the striatum, somatosensory cortex, and motor cortex (Wang and McGinty, 1995, 1996), findings that raise the question of the locus of these antagonists' effects on mAMPH-induced alterations in striatal and cortical activity. This question was investigated in a study by Mark et al. (2004), where the effects of mAMPH administration (4×10 mg/kg) on the striatonigral pathway were assessed. The results of this dual-probe microdialysis study suggest that mAMPH administration activates D1 receptor stimulation in the SNr, (i.e. on striatonigral terminals), which modulates GABA release in the SNr to affect thalamo-cortical and cortico-striatal glutamate release. In addition to SNr, striatal D1 receptors also appear to contribute to the effects of dopamine receptor stimulation on cortical activity. In an experiment to investigate the effects of striatal dopamine receptor stimulation on cortical activation, Steiner and Kitai (2000) demonstrated that intrastriatal D1 antagonism blocked the IEG expression in both the striatum and cortex resulting from administration of the directly acting D1/D2 agonist apomorphine. These experiments provide evidence that D1 receptors in the SNr and striatum affect information processing through the striato-nigro-thalamo-cortical circuit. The role of other striatal receptors in regulating neuronal activity in the cortex remains to be elucidated.

In the present study, we examined the role of striatal dopamine (D1 and D2) or glutamate (NMDA and AMPA) receptors on mAMPH-induced gene expression in the striatum and cortex. To investigate the contribution of striatal dopamine or glutamate receptors on mAMPH-induced cortical activity, we assessed Fos expression in both the striatum and cortex after intrastriatal dopamine or glutamate antagonist administration. Rats received pretreatment with either a D1, D2, NMDA, or AMPA receptor antagonist prior to a single systemic injection of mAMPH. The results of these experiments indicate that mAMPH-induced activation of striatal dopamine and glutamate receptors modulates not only striatal, but also cortical Fos expression.

Experimental Procedures

Subjects

Male Sprague-Dawley rats (275–325 g) were obtained from Charles Rivers Labs (Hollister, Ca.) and individually housed, with food and water *ad libitum*, under a standard 12 hr-light/12 hr-dark cycle (lights on 6 am–6 pm). Animals remained in the holding room for four days prior to surgery, and were handled every day following surgery. All experiments were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals. Formal approval to conduct the experiments described has been obtained from the UC Irvine animal subjects review board (IACUC). All efforts were made to minimize the number of animals used and their suffering.

Implantation of guide cannulas

Rats were anesthetized with Equithesin (10 mM sodium pentobarbital, 256.8 mM chloral hydrate, 86 mM MgSO₄, 10.5% propylene glycol, and 12% ethanol, administered at 4.2 mg/kg, i.p.) and placed in a Kopf stereotaxic apparatus. A guide cannula (26 gauge, stainless steel; Plastics One, Roanoke, VA) was lowered into the right striatum and fixed to the skull with acrylic cement. The coordinates used for the tip of the guide cannula were (relative to bregma): AP, +0.4 mm; ML, –3.4 mm; DV, –5.0 mm (Paxinos and Watson, 2003). The guide cannula was occluded with a "dummy cannula" of the same length.

Recovery and infusion preparation procedures

Rats were allowed to recover for 1 week prior to the infusion day. During this recovery period, rats were handled in their holding room. Handling included wrapping a cloth towel around the rats, while leaving their heads uncovered. The rats were mildly restrained in the towel to simulate the infusion experience. One day prior to the infusion, rats were placed in a separate testing room where the dummy cannula was replaced with a longer dummy cannula that protruded 2 mm beyond the tip of the guide cannula. The rats were then returned immediately to the holding room. On the infusion day, rats were again placed in the testing room, and the long dummy cannula replaced with the original dummy cannula. Approximately 2.5 hours following dummy cannula replacement, the dummy cannula was removed and replaced with an infusion cannula (33 gauge, 1 mm longer than the guide cannula), and infusions commenced immediately. This procedure was used to minimize the trauma associated with introduction of the infusion cannula, which can cause robust induction of IEGs in the cortex and striatum on the day of the experiment.

Drugs and injection procedures

The D1 antagonist SCH23390 [*R*(+)-SCH-23390 hydrochloride] (1 µg and 2 µg in 1 µl of saline; Steiner and Kitai, 2000), the D2 antagonist (–)-sulpiride (75 ng and 200 ng in 1 µl saline; Marshall et al., 2001), the NMDA receptor antagonist, (2*R*)-amino-5-phosphonovaleric acid [(AP5); 2 µg and 5 µg in 1 µl of saline; McPherson and Marshall, 1996], or the AMPA receptor antagonist, 6,7-Dinitroquinoxaline-2,3-dione [(DNQX); 0.5 µg and 1.5 µg in 1 µl of phosphate buffer saline (PBS); Schuller and Marshall, 1998], was infused into the right striatum in mildly restrained rats (Fig. 1A). All antagonists were purchased from Sigma-Aldrich, Inc., St. Louis, MO. The infusions were performed at a rate of 0.5 µl/minute. After the infusion, the infusion cannula was removed and replaced with the original dummy cannula after an additional 2 minutes to allow for diffusion of the drug. The rat was then returned to its home cage for observation (see below). Fifteen minutes following the beginning of the intrastriatal infusion the animals received a systemic injection of mAMPH (mAMPH hydrochloride; Sigma, St. Louis, MO) (4 mg/kg, s.c.; in saline) or saline (1 ml/kg). Control infusions consisted of either saline (i.e. 1 µl for the SCH23390, sulpiride, and AP5 experiments) or PBS (i.e. 1 µl for the

DNQX experiment). Additional control infusions consisted of SCH23390 (2 µg in 1 µl of saline), sulpiride (200 ng in 1 µl of saline), AP5 (5 µg in 1 µl of saline), or DNQX (1.5 µg in 1 µl of PBS). The rat was then returned to the home cage which was positioned on ground level in the testing room for behavioral observation. mAMPH-induced stereotypy or rotational behavior was observed. The direction of rotations as well as the time at the beginning and end of the rotational period were noted.

Tissue preparation

Ninety minutes after the mAMPH injection, rats were deeply anesthetized with sodium pentobarbital, then transcardially perfused with PBS (0.1 M phosphate buffer, pH 7.4 with 0.9% NaCl, 50–70 ml), followed by ice-cold 4% paraformaldehyde in PBS (100–140 ml). The perfused brains were removed, post-fixed overnight in cold 4 % paraformaldehyde, and transferred to 30% sucrose for 2 days. Forty µm thick coronal sections were cut on a freezing microtome at two levels (Bregma +0.4mm, +1.92mm) and stored in sodium azide (1 mg/ml) until used for either cannula track reconstruction (Bregma +0.4mm; Fig. 1A) with cresyl violet stain (Fig. 1B) or immunohistochemical localization of Fos-positive nuclei (Bregma +1.92mm; Fig. 1C, Fig. 2).

Fos immunohistochemistry

Sections were initially rinsed in 0.1 M PBS (pH 7.4). Endogenous peroxidase activity was inhibited by treating the tissue in 0.5% H₂O₂ in 70% methanol for 30 min, followed by rinses in PBS. Nonspecific antibody binding was blocked by pretreatment for 1 hr with 5% dry milk in PBS containing 0.2% Triton X-100. Sections were then incubated with a rabbit anti-Fos polyclonal primary antibody [(Santa Cruz Biotechnology, Inc.; sc-52; Santa Cruz, Ca.) (1:8500 dilution in 1% dry milk in PBS with 0.1% Triton X-100)] for 48 hr at 4 °C. After incubation with the primary antibody, sections were rinsed in PBS, and then incubated in a solution containing biotinylated anti-rabbit IgG made in goat (1:200 dilution in 1% dry milk in PBS) for 2 hr at room temperature. Sections were then rinsed in PBS and incubated in a solution containing an avidin-biotin-peroxidase complex (Vectastain Elite ABC) for 1 hr. After rinsing with PBS, immunostaining was visualized by peroxidase reaction with a stabilized, metal-enhanced diaminobenzidine (DAB) complex (10% solution; Pierce Chemical Co.; Rockford, IL) for approximately 3 minutes. The DAB reaction was terminated by rinsing with Tris-buffered saline and the tissue was stored in Tris buffer until mounted onto gelatin-chrom-alum subbed slides.

Fos quantification

Levels of Fos protein expression were determined by counting Fos-immunolabeled nuclei within the striatum and cortex (cingulate, motor, somatosensory, insular and piriform cortices, approximately 2mm anterior to the cannula track, where the medial portions of corpus collosum fuse at the midline of the cerebral hemispheres. Fos-positive nuclei were observed at 100x magnification (Fig. 2) using an Olympus BX60 microscope and were counted using computer-assisted image analysis employing a Roper Cool-Snap digital camera and a grain counting module of an MCID image analysis system (GE Healthcare; Chalfont St. Giles, UK). For each brain region, threshold 'optical density' criterion for measuring Fos+ immunoreactive nuclei was adjusted. Extraneous labeling was excluded by use of minimum and maximum target size criteria, which was based on a specified range calculated around the mean of ten randomly chosen Fos-IR nuclei. Fos immunoreactivity levels were quantified using the "mean proportional area" generated by the MCID program, which represents the proportion of the sampling area containing Fos+ nuclei, as defined by density and size criteria. Values are indicated by mean proportional area multiplied by 100.

Statistics

Main effects of methamphetamine treatment, intrastriatal infusions, and region, as well as the interactions between these factors, were determined using separate three-way ANOVAs for the infused and non-infused hemispheres. These tests were followed by one way ANOVAs and, where applicable, *post hoc* Bonferroni tests to assess differences between treatment groups for each brain region. *P* values less than .05 were considered statistically significant. Data are presented as mean $\pm$ SEM and analyzed using SPSS 13.0 (Chicago, IL).

Results

Effects of intrastriatal infusion of D1, D2, NMDA, or AMPA antagonists on mAMPH-induced striatal and cortical Fos expression

Data obtained from each antagonist experiment were analyzed by 3-way ANOVA, in which the between-subjects factors were systemic injection (mAMPH vs. saline) and intrastriatal infusion (e.g., SCH23390 low dose, high dose, or saline), and region was a within-subjects factor. Results from 3-way ANOVAs are as follows:

In the SCH23390 experiment for the infused hemisphere, significant main effects were found for intrastriatal infusion, $F_{(2,30)} = 5.52, p < .01$ and systemic injection, $F_{(1,30)} = 62.23, p < .001$, and significant interactions were found (intrastriatal infusion x systemic injection, $F_{(1,30)} = 10.49, p < .01$; region x intrastriatal infusion x systemic injection, $F_{(5,30)} = 2.74, p < .05$). For the non-infused hemisphere, a significant main effect was found for systemic injection, $F_{(1,30)} = 89.99, p < .001$, and significant interactions were observed (region x systemic injection, $F_{(5,30)} = 25.75, p < .001$; intrastriatal infusion by systemic injection, $F_{(1,30)} = 6.54, p < .05$).

In the sulpiride experiment for the infused hemisphere, a significant main effect was found for systemic injection, $F_{(1,23)} = 72.37, p < .001$, and significant interactions were observed (intrastriatal infusion x systemic injection, $F_{(1,23)} = 8.30, p < .01$; region x intrastriatal infusion x systemic injection, $F_{(5,23)} = 5.20, p < .001$). For the non-infused hemisphere, a significant main effect was found for systemic injection, $F_{(1,23)} = 105.91, p < .001$ and a significant interaction was observed (region x intrastriatal infusion x systemic injection, $F_{(5,23)} = 4.15, p < .01$).

In the AP5 experiment for the infused hemisphere, significant main effects were found for intrastriatal infusion, $F_{(2,22)} = 6.43, p < .01$ and systemic injection, $F_{(1,22)} = 61.99, p < .001$, and significant interactions were observed (intrastriatal infusion x systemic injection, $F_{(1,22)} = 11.70, p < .01$; region x intrastriatal infusion x systemic injection, $F_{(5,22)} = 4.85, p < .001$). For the non-infused hemisphere, a significant main effect was found for systemic injection, $F_{(1,22)} = 95.70, p < .001$, and a significant interaction was found (region x intrastriatal infusion x systemic injection, $F_{(5,22)} = 2.96, p < .05$).

In the DNQX experiment for the infused hemisphere, significant main effects were found for intrastriatal infusion, $F_{(2,17)} = 25.26, p < .001$ and systemic injection, $F_{(1,17)} = 175.37, p < .001$, and significant interactions occurred (intrastriatal infusion x systemic injection, $F_{(1,17)} = 49.60, p < .001$; region x intrastriatal infusion x systemic injection, $F_{(5,17)} = 5.26, p < .001$). For the non-infused hemisphere, significant main effects were found for intrastriatal infusion, $F_{(2,17)} = 9.04, p < .01$ and systemic injection, $F_{(1,17)} = 163.58, p < .001$, and significant interactions were found (intrastriatal infusion x systemic injection, $F_{(1,17)} = 13.05, p < .01$; region x intrastriatal infusion x systemic injection, $F_{(5,17)} = 4.45, p < .01$).

Where the 3-way ANOVAs permitted, one-way ANOVAs were performed to test for group differences in Fos expression of the regions. Significant one-way ANOVAs were followed by

post-hoc tests to evaluate assess three comparisons, (1) saline-infusion / saline-injection vs. high dose antagonist-infusion (e.g. SCH23390 2 µg/µl) /saline-injection, to test for effects of antagonist alone, (2) saline-infusion / saline-injection vs. saline-infusion / mAMPH-injection, to test for effects of systemic mAMPH administration, and (3) saline-infusion / mAMPH-injection vs. antagonist-infusion (low and high doses) /mAMPH-injection to test for effects of antagonist infusion on mAMPH-induced Fos expression.

For all antagonist experiments (infused and non-infused hemispheres), there was no significant effect of antagonist-infusion / saline-injection compared to saline-infusion / saline-injection, $p's > .05$ in all cases (except for sulpiride-infusion / saline-injection, see below). Also, there was always a significant increase in all regions examined (infused and non-infused hemispheres) for all antagonist experiments in saline-infusion / mAMPH-injection compared to saline-infusion / saline-injection, $p's < .05$.

SCH23390—The treatment groups used in this experiment include: saline / saline (n=4), SCH23390 (2 µg/µl) / saline (n=5), saline / mAMPH (n=8), SCH23390 (1 µg/µl) / mAMPH (n=5), SCH23390 (2 µg/µl) / mAMPH (n=13) (intraatrial infusion/systemic injection). The effects of intraatrial SCH23390 infusions on mAMPH injection were determined by comparing Fos expression in animals of the saline/mAMPH group with that of the SCH23390 (1 µg) / mAMPH or SCH23390 (2 µg) / mAMPH groups. These comparisons revealed that SCH23390 reduced mAMPH-induced Fos immunoreactivity of the infused hemisphere in a dose-dependent manner in striatum (−72%, −95%, for 1 µg and 2 µg doses respectively), motor cortex (−47%, −55%), somatosensory cortex (−42%, −84%), piriform cortex (−44%, −51%), and insular cortex (−28%, −49%), while no significant effects occurred in cingulate cortex (Fig. 3, top left). SCH23390 infusions also reduced mAMPH-induced Fos immunoreactivity in the non-infused hemisphere, although these effects were generally less extensive and less frequently significant than for the infused hemisphere (Fig. 3, top right). For example, in the non-infused hemisphere, each dose of SCH23390 significantly reduced mAMPH-induced Fos immunoreactivity in striatum (−55%, −84%), but only the higher SCH23390 dose (2 µg) significantly reduced Fos in somatosensory cortex (−69%), piriform cortex (−37%), and insular cortex (−33%).

In addition, systemic mAMPH administration produced stereotypic behaviors, characterized by intense, focused sniffing. Approximately 25 minutes post mAMPH injection, most animals that received SCH23390 infusions rotated ipsilateral to the infusion (3/5 rats given 1 µg; 7/13 rats given 2 µg) for a brief period of time (20–30 minutes), whereas saline infusion caused no rotation.

Sulpiride—The treatment groups used in this experiment include: saline / saline (n=4), sulpiride (200 ng/µl) / saline (n=4), saline / mAMPH (n=8), sulpiride (75 ng/µl) / mAMPH (n=6), sulpiride (200 ng/µl) / mAMPH (n=6). Contrary to the other antagonists used in this study, sulpiride (200 ng/µl) / saline significantly increased Fos expression in the striatum of the infused hemisphere compared to saline / saline, $F_{(1,7)} = 72.08, p < .001$.

The effects of intraatrial sulpiride infusions on mAMPH injection were determined by comparing Fos expression in animals of the saline/mAMPH group with that of the sulpiride (75 ng) / mAMPH or sulpiride (200 ng) / mAMPH groups. These comparisons revealed that sulpiride increased mAMPH-induced Fos immunoreactivity of the infused hemisphere in a dose-dependent manner in striatum (+39%, +56%), but reduced mAMPH-induced Fos immunoreactivity in motor cortex (−43%, −66%), somatosensory cortex (−33%, −67%), piriform cortex (−36%, −58%), and insular cortex (−25%, −43%), while no significant effects occurred in cingulate cortex (Fig. 3, bottom left). Sulpiride infusions had minimal effect on mAMPH-induced Fos immunoreactivity in the non-infused hemisphere (Fig. 3, bottom right),

the only significant change being a reduction in mAMPH-induced Fos of piriform cortex (−47%) from the higher sulpiride (200 ng) dose.

Approximately 25 minutes post mAMPH injection, all 12 animals that received either dose of sulpiride rotated ipsilateral to the infused hemisphere for an extended period of time (60–90 minutes), whereas saline infusion produced no rotation.

AP5—The treatment groups used in this experiment include: saline / saline (n=4), AP5 (5 µg/µl) / saline (n=5), saline / mAMPH (n=8), AP5 (2 µg/µl) / mAMPH (n=5), AP5 (5 µg/µl) / mAMPH (n=5). The effects of intrastriatal AP5 infusions on mAMPH injection were determined by comparing Fos expression in animals of the saline / mAMPH group with that of the AP5 (2 µg) / mAMPH or AP5 (5 µg) / mAMPH groups. These comparisons revealed that AP5 reduced mAMPH-induced Fos immunoreactivity of the infused hemisphere in a dose-dependent manner in striatum (−70%, −92%), motor cortex (−50%, −65%), somatosensory cortex (−47%, −58%), piriform cortex (−40%, −63%), and insular cortex (−29%, −45%), while no significant effects occurred in cingulate cortex (Fig. 4, top left). AP5 infusions less extensively reduced mAMPH-induced Fos immunoreactivity in the non-infused hemisphere (Fig. 4, top right). For example, in the non-infused hemisphere, the higher dose of AP5 (5 µg) significantly reduced mAMPH-induced Fos immunoreactivity only in striatum (−51%), somatosensory cortex (−16%), and piriform cortex (−44%).

Approximately 25 minutes post mAMPH injection, all 10 animals that received infusions of either dose of AP5 rotated ipsilateral to the infusion for an extended period of time (60–70 minutes), whereas saline infusion produced no rotation.

DNQX—The treatment groups used in this experiment include: PBS / saline (n=3), DNQX (1.5 µg/µl) / saline (n=3), PBS / mAMPH (n=6), DNQX (0.5 µg/µl) / mAMPH (n=4), DNQX (1.5 µg/µl) / mAMPH (n=6). The effects of intrastriatal DNQX infusions on mAMPH injection were determined by comparing Fos expression in animals of the PBS/mAMPH group with that of the DNQX (0.5 µg) / mAMPH or DNQX (1.5 µg) / mAMPH groups. These comparisons revealed that DNQX reduced mAMPH-induced Fos immunoreactivity of the infused hemisphere in a dose-dependent manner in striatum (−68%, −98%), motor cortex (−42%, −73%), somatosensory cortex (−53%, −75%), piriform cortex (−23%, −57%), and insular cortex (−28%, −65%), while no significant effects occurred in cingulate cortex (Fig. 4, bottom left). DNQX infusions also reduced mAMPH-induced Fos immunoreactivity in the non-infused hemisphere, although these effects were only observed for the higher dose of DNQX (Fig. 4, bottom right). For example, in the non-infused hemisphere, DNQX (1.5 µg) significantly reduced mAMPH-induced Fos immunoreactivity in striatum (−59%), motor cortex (−43%), somatosensory cortex (−58%), piriform cortex (−43%), and insular cortex (−44%).

Approximately 25 minutes post mAMPH injection, all 10 animals that received infusions of either dose of DNQX rotated ipsilateral to the infusion for an extended period of time (60–90 minutes), whereas PBS infusion produced no rotation.

Discussion

The present experiments demonstrate an important role of striatal dopamine (D1 and D2) and ionotropic glutamate (NMDA and AMPA) receptors in the striatal and cortical immediate early gene responses arising from a single systemic administration of mAMPH. Intrastriatal infusion of dopamine or glutamate antagonists affected not only mAMPH-induced striatal, but also cortical, Fos expression. In general, the effects of the antagonists occurred dose-dependently, affecting both the infused and non-infused hemispheres, with greater influences occurring in

the infused hemisphere. Finally, unilateral intrastratial infusion of dopamine or glutamate antagonists changed the behavior of the rats from characteristic mAMPH-induced stereotypy to rotating ipsilateral to the infusion.

Dopamine receptor modulation of mAMPH-induced striatal Fos expression

Unilateral intrastratial infusion of D1- and D2-receptor antagonists produced significant changes to mAMPH-induced Fos expression in the striatum and cerebral cortex. The activation and localization of IEGs, especially *c-fos*, which has low basal expression, has become a tool for investigating neural circuits that are biochemically activated in response to specific stimuli, including drugs of abuse (reviewed in Hughes and Dragunow; 1995; Harlan and Garcia, 1998). It should be noted, however, that neuronal Fos expression signifies biochemical activity in a single neuron in response to stimulation of one or several signal transduction pathways. An additional caveat is that IEG expression does not imply electrophysiological neuronal activation, since electrophysiological responses have sometimes been shown to be opposite to the neuronal IEG response (Labiner et al., 1993). Despite these limitations, the induction of IEGs can be used for determining region-specific activity patterns across multiple neuronal populations. In this study, changes in Fos protein expression provide useful information about the numbers of cells that are recruited into the activity patterns within a particular brain region.

Several lines of evidence indicate that administration of psychostimulant drugs induces IEG expression primarily in striatonigral neurons that express mRNAs for D1 receptors, preprodynorphin and preprotachykin (Gerfen et al., 1990; Graybiel et al., 1990; Berretta et al., 1992; Ruskin and Marshall, 1994). Our finding that blockade of striatal D1 receptors profoundly reduced mAMPH-induced striatal Fos protein expression is consistent with the view that striatal D1 antagonism inhibited Fos expression in striatonigral D1-containing neurons, but it has been shown that D1 receptor blockade can also inhibit psychostimulant-induced IEG mRNA expression in striatal neurons that contain mRNA for enkephalin (Enk+), indicating a possible intra- or inter-cellular interaction between D1- and D2-containing neurons (Surmeier et al., 1992; Ferguson et al., 2003).

In contrast with the results obtained with infusion of the D1 antagonist, SCH23390, unilateral intrastratial infusion of the D2 antagonist, sulpiride, 15 minutes prior to systemic injection of mAMPH, dose-dependently increased striatal Fos-immunoreactivity. Consistent with previous studies, we found that intrastratial D2 receptor blockade alone increased striatal Fos expression (Roberston et al., 1992; Marshall et al., 2001). However, differing effects of D2 receptor antagonism on psychostimulant-induced IEG expression in this region have been reported. For example, Wang and McGinty (1995) demonstrated that pretreatment with the D2 receptor antagonist, eticlopride, prior to mAMPH, augmented striatal *zif 268* mRNA expression at an early time point following mAMPH administration (45 minutes), but reduced it at a later time point (3 hours). Other research has demonstrated that D2 receptor blockade inhibits *c-fos* mRNA and Fos protein expression resulting from psychostimulant or combined D1/D2 agonist administration (Ruskin and Marshall, 1994; Keefe and Gerfen, 1995; Ferguson et al., 2003). This inhibition occurs in neurons that express mRNA for dynorphin/substance P (Dyn/SP+) and that project to the SNr.

In our experiment, intrastratial infusion of the D2 antagonist, sulpiride, prior to a single injection of mAMPH, dose-dependently increased striatal Fos-immunoreactivity. We interpret these results in the following ways. To begin with, the environment in which the animals are administered psychostimulants greatly affects which striatal cell populations are activated. For example, psychostimulant drugs evoke *c-fos* expression primarily in Dyn/SP+ neurons, but when given in a novel environment, psychostimulant-induced *c-fos* expression occurs in both Dyn/SP+ and Enk+ neurons in the striatum (Badiani et al., 1999; Uslaner et al., 2001; Ferguson et al., 2003). Exposure to a novel environment significantly activates the neocortex, presumably

increasing corticostriatal glutamate release (Uslaner et al., 2001) to selectively engage Enk+ neurons (Berretta et al., 1997). In addition, intrastratial D2 receptor blockade has been shown to amplify cortically-evoked striatal Fos protein expression (Berretta et al., 1999). In our experiment, the testing conditions entailed administering mAMPH in a room distinct from the animals' holding room, which perhaps enhanced the novelty associated with the drug injection environment. In addition, the animals were restrained during intrastratial antagonist infusion and restraint stress has been shown to produce increases in cortical IEG expression (Cullinan et al., 1995; Weinberg et al., 2007). It is possible that even the mild restraint associated with intracranial infusions primed corticostriatal glutamate pathways and altered their response to mAMPH administration. It is particularly noteworthy that intrastratial sulpiride infusion prior to a systemic injection of mAMPH resulted in an increase in striatal Fos expression above that of vehicle-infused animals that received a systemic injection of mAMPH, and also that of sulpiride-infused animals that received a systemic injection of saline. Firstly, D2 receptor blockade likely increased the number of Fos-labeled Enk+ neurons. And, second, because mAMPH normally induces Fos immunoreactivity in Enk- neurons, we propose that the overall increase in striatal Fos expression resulting from sulpiride infusion was an additive effect. Thus, intrastratial sulpiride infusion increased striatal Fos expression, adding Fos-labeled D2 receptor-containing neurons to the mAMPH-induced D1 receptor-containing neurons.

Glutamate receptor modulation of mAMPH-induced striatal Fos expression

Unilateral intrastratial infusion of NMDA or AMPA receptor antagonists significantly inhibited mAMPH-induced Fos expression in both the striatum and cerebral cortex. Consistent with previous research, this experiment demonstrated a role for both NMDA and AMPA classes of ionotropic glutamate receptors in psychostimulant-induced IEG expression. Systemic administration of both NMDA and AMPA receptor antagonists have been shown previously to decrease psychostimulant-evoked IEG expression in the striatum (Dragunow et al., 1991; Wang and McGinty, 1994; Konradi et al., 1996). It has been shown that systemic administration of NMDA antagonists inhibit amphetamine-induced *c-fos* mRNA expression in Dyn/SP+ neurons, and depending on the environment, in Enk+ neurons (Ferguson et al., 2003). Interestingly, stimulation of corticostriatal afferents or intrastratial infusion of glutamate agonists induces *c-fos* preferentially in Enk+ neurons, an effect that is blocked by an NMDA receptor antagonist (Berretta et al., 1992, 1997). Also, transection of corticostriatal projections has been shown to reduce, whereas a positive modulator of AMPA receptors (i.e. ampakine) has been shown to enhance, amphetamine-induced *c-fos* expression in Enk+ neurons, specifically in striatal regions receiving enhanced corticostriatal innervation (Hess et al., 2003; Ferguson and Robinson, 2004). These experiments demonstrate that amphetamine administration can lead to glutamate receptor stimulation in distinct neuronal populations, and that changes in corticostriatal glutamate transmission more directly determine the IEG expression of striatal Enk+ than Dyn/SP+ neurons.

Striatal dopamine and glutamate receptor modulation of cortical IEG expression

In addition to its effects on striatal Fos expression, intrastratial SCH23390 infusion reduced mAMPH-induced cortical Fos expression in a region-specific manner, including motor, somatosensory, insular, and piriform cortex, but not in the cingulate cortex. An issue in interpreting effects of intrastratial antagonist administration on cortical function is the possible spread of drug from the striatum to the cortex. For example, Steiner and Kitai (2000) showed that intrastratial infusion of SCH23390 (0.5 µg/µl) blocked gene expression in a distinct area around the cannula track, demonstrating diffusion of SCH23390 along the outer walls of the cannula. This type of small drug diffusion, however, does not explain the widespread reductions in IEG responses occurring in cortical regions distant from the cannula track. Another issue in interpreting intrastratial infusion effects on cortical activity is that local drug infusions may have resulted in diffusion gradients between different topographic areas of the striatum.

Numerous studies have described the topographic organization of the recurrent cortico-basal ganglia-thalamo-cortical pathway (Beckstead, 1979; Selemon and Goldman-Rakic, 1985; McGeorge and Faull, 1989; Parent, 1995). For example, the association, sensorimotor, and limbic cortical areas project onto three distinct striatal regions referred to as associative, sensorimotor and limbic striatal subdivisions (Parent et al., 1995). In our study, striatal infusions were targeted for the ventro-lateral striatum, which includes sensorimotor and associative striatal subdivisions (McGeorge and Faull, 1989; Willuhn et al., 2003). One of the consequences of targeting the ventrolateral quadrant of the striatum is the possibility that the differences in cortical IEG expression may have resulted from concentration gradients due to drug diffusion between different striatal subregions. As previously mentioned, intrastriatal antagonist infusion did not block mAMPH-induced IEG expression in the cingulate cortex, part of the limbic subdivision. The infusion of small drug amounts into the ventro-lateral striatum failed to affect the cingulate cortex, a region of cortex that projects to the medial striatum, and likely not topographically affected by manipulations to the ventro-lateral striatum. An alternative explanation is that dopaminergic agonists, like mAMPH, or apomorphine (Steiner and Kitai, 2000) can modulate neuronal activity in the cingulate cortex independent of the striatum due to local dopaminergic transmission within the cingulate cortex (Gaspar et al., 1995; Stephans and Yamamoto, 1995; Nishijima et al., 1996).

Previously, it has been demonstrated that mAMPH administration activates the striato-nigro-thalamo-cortical circuit (Mark et al., 2004). D1 receptor activation modulates activity in this circuit, since D1 receptor blockade in the SNr affects GABA release in the SNr and ventromedial thalamus to reduce corticostriatal glutamate release. This circuit has also been shown to be regulated by striatal D1 receptor activation during acute apomorphine treatment (Steiner and Kitai, 2000). Therefore, our results suggest that following acute mAMPH administration, activation of striatal D1 receptors leads to increased Fos expression in the striatum and in widespread portions of the cortex, an effect presumably mediated by the striato-nigro-thalamo-cortical circuit.

We demonstrated that intrastriatal sulpiride infusion inhibited mAMPH-induced cortical Fos expression in the infused hemisphere. Our results are consistent with Wang and McGinty's (1995) observation that D2 receptor blockade, during mAMPH administration, can increase striatal *zif268* expression, while reducing mAMPH-induced cortical *zif268* expression. Consistent with the idea that psychostimulant drugs administered in a novel environment engage Enk+ (D2-containing neurons), and enhance *c-fos* mRNA levels in the subthalamic nucleus of the indirect pathway (Uslaner et al., 2001), it is possible that the intrastriatal infusion of sulpiride reduced the magnitude of cortical Fos expression by means of activation of the indirect pathway.

The results from our experiments show that both NMDA- and AMPA-class glutamate receptors modulate striatal Fos expression, likely in striatonigral and striatopallidal neurons, to affect cortical Fos expression. The extent to which striatal glutamate receptor blockade reduces mAMPH-induced activity in direct or indirect pathway neurons probably depends on 1.) the degree to which mAMPH activated the recurrent cortico-striatal-thalamo-cortical circuit to increase corticostriatal and/or thalamostriatal glutamate transmission and 2.) the ability of the testing conditions to engage corticostriatal glutamate projections.

Bilateral effects resulting from unilateral striatal receptor modulation of cortical activity and relationship to rotational behavior

The unilateral intrastriatal infusion of dopamine or glutamate antagonists induced hemispheric asymmetries in striatal and cortical Fos expression, with more pronounced effects in the infused hemisphere. Several pathways could mediate the bilateral effects we observed in the striatum and cortex. For example, the SNr, which densely projects to the ipsilateral ventromedial nucleus

of the thalamus, also has sparse projections to the contralateral ventromedial nucleus (Gerfen et al., 1982). The ventromedial thalamic nucleus then projects ipsilaterally to layer 1 of most of the neocortex (Herkenham, 1979). Electrical or chemical stimulation of the cortex and anatomical tracing experiments have demonstrated the topographic organization of crossed and uncrossed corticostriatal and crossed corticocortical projections (McGeorge and Faull, 1989; Berretta et al., 1997; Sgambato et al., 1999). Thus, unilateral blockade of striatal dopamine and glutamate receptors could bilaterally affect striatal and cortical IEG expression via projections of basal ganglia output nuclei, thalamus, and cortex.

The degree to which these antagonists produced hemispheric differences in Fos expression related to the rotational behavior observed. Intrastratial infusion of the D1 antagonist produced the strongest bilateral effects in the striatum compared to intrastratial infusion of the other antagonists while the animals that received intrastratial SCH23390 infusion rotated less than animals receiving the other antagonists. The intrastratial infusion of sulpiride, AP5, or DNQX produced a larger asymmetry in Fos expression between the infused and non-infused striatum, with the infused striatum having a more pronounced effect than the non-infused striatum. The rotational behavior we observed from these groups lasted for longer, was more robust, and more reliable within the groups when compared to the SCH23390-infused animals. Although intrastratial antagonist infusion prior to mAMPH injection produced rotational behavior and changes in IEG expression, it is uncertain whether the rotational behavioral contributed to these changes in IEG expression. Therefore, additional research will be required to assess the contribution of rotation behavior to the effects of intrastratial antagonist infusion on IEG expression.

Summary and conclusions

The systemic administration of the psychostimulant drug mAMPH stimulated Fos protein expression throughout the striatum and cerebral cortex, and intrastratial blockade of dopamine or glutamate receptors inhibited striatal and cortical Fos protein expression. Our results demonstrate the important role of striatal dopamine and glutamate receptors in modulating striatal and cortical IEG activation and suggest that these effects are mediated through basal-ganglia-thalamo-cortical circuits. Although we observed striatal modulation of cortical IEG activity, more research is needed to characterize the specific neuronal populations and circuitry responsible for this trans-synaptic regulation of IEG expression. The elucidation of mAMPH-induced alterations to direct and indirect pathway dynamics that affect cortical processing may be an important step in understanding the potential risks to individuals who abuse mAMPH.

Abbreviations

mAMPH, methamphetamine
IEG, immediate early gene
NMDA, N-methyl-D-aspartic acid
AMPA, alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
SNr, substantia nigra pars reticulata
AP5, (2R)-amino-5-phosphonovaleric acid
DNQX, 6,7-Dinitroquinoxaline-2,3-dione
PBS, phosphate buffer saline
DAB, diaminobenzidine
Enk, enkephalin
Dyn/SP, dynorphin/substance P

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REFERENCES

- Albin RL, Makowiec RL, Hollingsworth ZR, Dure LSt, Penney JB, Young AB. Excitatory amino acid binding sites in the basal ganglia of the rat: a quantitative autoradiographic study. *Neuroscience* 1992;46:35–48. [PubMed: 1317515]
- Alburges ME, Hunt ME, McQuade RD, Wamsley JK. D1-receptor antagonists: comparison of [3H] SCH39166 to [3H]SCH23390. *J Chem Neuroanat* 1992;5:357–366. [PubMed: 1358117]
- Alexander GE, Crutcher MD. Functional architecture of basal ganglia circuits: neural substrates of parallel processing. *Trends Neurosci* 1990;13:266–271. [PubMed: 1695401]
- Altar CA, O'Neil S, Walter RJ Jr, Marshall JF. Brain dopamine and serotonin receptor sites revealed by digital subtraction autoradiography. *Science* 1985;228:597–600. [PubMed: 2580352]
- Badiani A, Oates MM, Day HE, Watson SJ, Akil H, Robinson TE. Environmental modulation of amphetamine-induced c-fos expression in D1 versus D2 striatal neurons. *Behav Brain Res* 1999;103:203–209. [PubMed: 10513588]
- Beckstead RM, Domesick VB, Nauta WJ. Efferent connections of the substantia nigra and ventral tegmental area in the rat. *Brain Res* 1979;175:191–217. [PubMed: 314832]
- Berretta S, Parthasarathy HB, Graybiel AM. Local release of GABAergic inhibition in the motor cortex induces immediate-early gene expression in indirect pathway neurons of the striatum. *J Neurosci* 1997;17:4752–4763. [PubMed: 9169535]
- Berretta S, Robertson HA, Graybiel AM. Dopamine and glutamate agonists stimulate neuron-specific expression of Fos-like protein in the striatum. *J Neurophysiol* 1992;68:767–777. [PubMed: 1359024]
- Berretta S, Sachs Z, Graybiel AM. Cortically driven Fos induction in the striatum is amplified by local dopamine D2-class receptor blockade. *Eur J Neurosci* 1999;11:4309–4319. [PubMed: 10594656]
- Bischoff S, Heinrich M, Krauss J, Sills MA, Williams M, Vassout A. Interaction of the D1 receptor antagonist SCH 23390 with the central 5-HT system: radioligand binding studies, measurements of biochemical parameters and effects on L-5-HTP syndrome. *J Recept Res* 1988;8:107–120. [PubMed: 3290470]
- Blandini F, Fancellu R, Orzi F, Conti G, Greco R, Tassorelli C, Nappi G. Selective stimulation of striatal dopamine receptors of the D1- or D2-class causes opposite changes of fos expression in the rat cerebral cortex. *Eur J Neurosci* 2003;17:763–770. [PubMed: 12603266]
- Boyson SJ, McGonigle P, Molinoff PB. Quantitative autoradiographic localization of the D1 and D2 subtypes of dopamine receptors in rat brain. *J Neurosci* 1986;6:3177–3188. [PubMed: 3534157]
- Cullinan WE, Herman JP, Battaglia DF, Akil H, Watson SJ. Pattern and time course of immediate early gene expression in rat brain following acute stress. *Neuroscience* 1995;64:477–505. [PubMed: 7700534]
- Dragunow M, Logan B, Lavery R. 3,4-Methylenedioxymethamphetamine induces Fos-like proteins in rat basal ganglia: reversal with MK 801. *Eur J Pharmacol* 1991;206:255–258. [PubMed: 1677338]
- Ferguson SM, Norton CS, Watson SJ, Akil H, Robinson TE. Amphetamine-evoked c-fos mRNA expression in the caudate-putamen: the effects of DA and NMDA receptor antagonists vary as a function of neuronal phenotype and environmental context. *J Neurochem* 2003;86:33–44. [PubMed: 12807422]
- Ferguson SM, Robinson TE. Amphetamine-evoked gene expression in striatopallidal neurons: regulation by corticostriatal afferents and the ERK/MAPK signaling cascade. *J Neurochem* 2004;91:337–348. [PubMed: 15447667]
- Gaspar P, Bloch B, Le Moine C. D1 and D2 receptor gene expression in the rat frontal cortex: cellular localization in different classes of efferent neurons. *Eur J Neurosci* 1995;7:1050–1063. [PubMed: 7613610]

- Gerfen CR, Engber TM, Mahan LC, Susel Z, Chase TN, Monsma FJ Jr, Sibley DR. D1 and D2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. *Science* 1990;250:1429–1432. [PubMed: 2147780]
- Gerfen CR, Staines WA, Arbuthnott GW, Fibiger HC. Crossed connections of the substantia nigra in the rat. *J Comp Neurol* 1982;207:283–303. [PubMed: 7107988]
- Greenamyre JT, Young AB. Synaptic localization of striatal NMDA, quisqualate and kainate receptors. *Neurosci Lett* 1989;101:133–137. [PubMed: 2549458]
- Harlan RE, Garcia MM. Drugs of abuse and immediate-early genes in the forebrain. *Mol Neurobiol* 1998;16:221–267. [PubMed: 9626665]
- Herkenham M. The afferent and efferent connections of the ventromedial thalamic nucleus in the rat. *J Comp Neurol* 1979;183:487–517. [PubMed: 759445]
- Hess US, Whalen SP, Sandoval LM, Lynch G, Gall CM. Ampakines reduce methamphetamine-driven rotation and activate neocortex in a regionally selective fashion. *Neuroscience* 2003;121:509–521. [PubMed: 14522010]
- Hughes P, Dragunow M. Induction of immediate-early genes and the control of neurotransmitter-regulated gene expression within the nervous system. *Pharmacol Rev* 1995;47:133–178. [PubMed: 7784478]
- Keefe KA, Gerfen CR. D1-D2 dopamine receptor synergy in striatum: effects of intrastriatal infusions of dopamine agonists and antagonists on immediate early gene expression. *Neuroscience* 1995;66:903–913. [PubMed: 7651617]
- Konradi C, Leveque JC, Hyman SE. Amphetamine and dopamine-induced immediate early gene expression in striatal neurons depends on postsynaptic NMDA receptors and calcium. *J Neurosci* 1996;16:4231–4239. [PubMed: 8753884]
- Labiner DM, Butler LS, Cao Z, Hosford DA, Shin C, McNamara JO. Induction of c-fos mRNA by kindled seizures: complex relationship with neuronal burst firing. *J Neurosci* 1993;13:744–751. [PubMed: 8381172]
- London ED, Simon SL, Berman SM, Mandelkern MA, Lichtman AM, Bramen J, Shinn AK, Miotto K, Learn J, Dong Y, Matochik JA, Kurian V, Newton T, Woods R, Rawson R, Ling W. Mood disturbances and regional cerebral metabolic abnormalities in recently abstinent methamphetamine abusers. *Arch Gen Psychiatry* 2004;61:73–84. [PubMed: 14706946]
- Mark KA, Quinton MS, Russek SJ, Yamamoto BK. Dynamic changes in vesicular glutamate transporter 1 function and expression related to methamphetamine-induced glutamate release. *J Neurosci* 2007;27:6823–6831. [PubMed: 17581970]
- Mark KA, Soghomonian JJ, Yamamoto BK. High-dose methamphetamine acutely activates the striatonigral pathway to increase striatal glutamate and mediate long-term dopamine toxicity. *J Neurosci* 2004;24:11449–11456. [PubMed: 15601951]
- Marshall JF, Henry BL, Billings LM, Hoover BR. The role of the globus pallidus D2 subfamily of dopamine receptors in pallidal immediate early gene expression. *Neuroscience* 2001;105:365–378. [PubMed: 11672604]
- McCann UD, Wong DF, Yokoi F, Villemagne V, Dannals RF, Ricaurte GA. Reduced striatal dopamine transporter density in abstinent methamphetamine and methcathinone users: evidence from positron emission tomography studies with [¹¹C]WIN-35,428. *J Neurosci* 1998;18:8417–8422. [PubMed: 9763484]
- McGeorge AJ, Faull RL. The organization of the projection from the cerebral cortex to the striatum in the rat. *Neuroscience* 1989;29:503–537. [PubMed: 2472578]
- McPherson RJ, Marshall JF. Intrastriatal AP5 differentially affects behaviors induced by local infusions of D1 vs. D2 dopamine agonists. *Brain Res* 1996;739:19–25. [PubMed: 8955920]
- Mengual E, de las Heras S, Erro E, Lanciego JL, Gimenez-Amaya JM. Thalamic interaction between the input and the output systems of the basal ganglia. *J Chem Neuroanat* 1999;16:187–200. [PubMed: 10422738]
- Monaghan DT, Bridges RJ, Cotman CW. The excitatory amino acid receptors: their classes, pharmacology, and distinct properties in the function of the central nervous system. *Annu Rev Pharmacol Toxicol* 1989;29:365–402. [PubMed: 2543272]

- Nash JF, Yamamoto BK. Methamphetamine neurotoxicity and striatal glutamate release: comparison to 3,4-methylenedioxymethamphetamine. *Brain Res* 1992;581:237–243. [PubMed: 1356579]
- Nishijima K, Kashiwa A, Hashimoto A, Iwama H, Umino A, Nishikawa T. Differential effects of phencyclidine and methamphetamine on dopamine metabolism in rat frontal cortex and striatum as revealed by in vivo dialysis. *Synapse* 1996;22:304–312. [PubMed: 8867025]
- O'Del SJ, Weihmuller FB, Marshall JF. Methamphetamine-induced dopamine overflow and injury to striatal dopamine terminals: attenuation by dopamine D1 or D2 antagonists. *J Neurochem* 1993;60:1792–1799. [PubMed: 8473897]
- Parent A, Hazrati LN. Functional anatomy of the basal ganglia. I. The cortico-basal ganglia-thalamo-cortical loop. *Brain Res Brain Res Rev* 1995;20:91–127. [PubMed: 7711769]
- Paxinos, G.; Watson, C. *The Rat Brain Atlas in Stereotaxic Coordinates*. Vol. Ed. 5. Sydney, Australia: Academic Press; 2003.
- Robertson GS, Vincent SR, Fibiger HC. D1 and D2 dopamine receptors differentially regulate c-fos expression in striatonigral and striatopallidal neurons. *Neuroscience* 1992;49:285–296. [PubMed: 1359451]
- Ruskin DN, Marshall JF. Amphetamine- and cocaine-induced fos in the rat striatum depends on D2 dopamine receptor activation. *Synapse* 1994;18:233–240. [PubMed: 7855736]
- Schmidt CJ, Gibb JW. Role of the dopamine uptake carrier in the neurochemical response to methamphetamine: effects of amfonelic acid. *Eur J Pharmacol* 1985;109:73–80. [PubMed: 2581794]
- Schuller JJ, Tran DD, Marshall JF. Striatal glutamate antagonism induces contralateral neglect. *Brain Res* 1998;788:315–319. [PubMed: 9555073]
- Sekine Y, Iyo M, Ouchi Y, Matsunaga T, Tsukada H, Okada H, Yoshikawa E, Futatsubashi M, Takei N, Mori N. Methamphetamine-related psychiatric symptoms and reduced brain dopamine transporters studied with PET. *Am J Psychiatry* 2001;158:1206–1214. [PubMed: 11481152]
- Selemon LD, Goldman-Rakic PS. Longitudinal topography and interdigitation of corticostriatal projections in the rhesus monkey. *J Neurosci* 1985;5:776–794. [PubMed: 2983048]
- Sgambato V, Maurice N, Besson MJ, Thierry AM, Deniau JM. Effect of a functional impairment of corticostriatal transmission on cortically evoked expression of c-Fos and zif 268 in the rat basal ganglia. *Neuroscience* 1999;93:1313–1321. [PubMed: 10501455]
- Smith AD, Bolam JP. The neural network of the basal ganglia as revealed by the study of synaptic connections of identified neurones. *Trends Neurosci* 1990;13:259–265. [PubMed: 1695400]
- Smith Y, Raju DV, Pare JF, Sidibe M. The thalamostriatal system: a highly specific network of the basal ganglia circuitry. *Trends Neurosci* 2004;27:520–527. [PubMed: 15331233]
- Steiner H, Kitai ST. Regulation of rat cortex function by D1 dopamine receptors in the striatum. *J Neurosci* 2000;20:5449–5460. [PubMed: 10884328]
- Stephans SE, Yamamoto BY. Effect of repeated methamphetamine administrations on dopamine and glutamate efflux in rat prefrontal cortex. *Brain Res* 1995;700:99–106. [PubMed: 8624733]
- Surmeier DJ, Xu ZC, Wilson CJ, Stefani A, Kitai ST. Grafted neostriatal neurons express a late-developing transient potassium current. *Neuroscience* 1992;48:849–856. [PubMed: 1378575]
- Uslaner J, Badiani A, Day HE, Watson SJ, Akil H, Robinson TE. Environmental context modulates the ability of cocaine and amphetamine to induce c-fos mRNA expression in the neocortex, caudate nucleus, and nucleus accumbens. *Brain Res* 2001;920:106–116. [PubMed: 11716816]
- Uslaner J, Badiani A, Norton CS, Day HE, Watson SJ, Akil H, Robinson TE. Amphetamine and cocaine induce different patterns of c-fos mRNA expression in the striatum and subthalamic nucleus depending on environmental context. *Eur J Neurosci* 2001;13:1977–1983. [PubMed: 11403691]
- Volkow ND, Chang L, Wang GJ, Fowler JS, Leonido-Yee M, Franceschi D, Sedler MJ, Gatley SJ, Hitzemann R, Ding YS, Logan J, Wong C, Miller EN. Association of dopamine transporter reduction with psychomotor impairment in methamphetamine abusers. *Am J Psychiatry* 2001;158:377–382. [PubMed: 11229977]
- Wang JQ, Daunais JB, McGinty JF. NMDA receptors mediate amphetamine-induced upregulation of zif/268 and preprodynorphin mRNA expression in rat striatum. *Synapse* 1994;18:343–353. [PubMed: 7886627]

- Wang JQ, Daunais JB, McGinty JF. Role of kainate/AMPA receptors in induction of striatal zif/268 and preprodynorphin mRNA by a single injection of amphetamine. *Brain Res Mol Brain Res* 1994;27:118–126. [PubMed: 7877442]
- Wang JQ, McGinty JF. Differential effects of D1 and D2 dopamine receptor antagonists on acute amphetamine- or methamphetamine-induced up-regulation of zif/268 mRNA expression in rat forebrain. *J Neurochem* 1995;65:2706–2715. [PubMed: 7595569]
- Wang JQ, McGinty JF. Dose-dependent alteration in zif/268 and preprodynorphin mRNA expression induced by amphetamine or methamphetamine in rat forebrain. *J Pharmacol Exp Ther* 1995;273:909–917. [PubMed: 7752096]
- Wang JQ, McGinty JF. Acute methamphetamine-induced zif/268, preprodynorphin, and preproenkephalin mRNA expression in rat striatum depends on activation of NMDA and kainate/AMPA receptors. *Brain Res Bull* 1996;39:349–357. [PubMed: 9138744]
- Wang JQ, Smith AJ, McGinty JF. A single injection of amphetamine or methamphetamine induces dynamic alterations in c-fos, zif/268 and preprodynorphin messenger RNA expression in rat forebrain. *Neuroscience* 1995;68:83–95. [PubMed: 7477938]
- Weinberg MS, Girotti M, Spencer RL. Restraint-induced fra-2 and c-fos expression in the rat forebrain: relationship to stress duration. *Neuroscience* 2007;150:478–486. [PubMed: 17936518]
- Willuhn I, Sun W, Steiner H. Topography of cocaine-induced gene regulation in the rat striatum: relationship to cortical inputs and role of behavioural context. *Eur J Neurosci* 2003;17:1053–1066. [PubMed: 12653981]

Figure 1.

Schematic diagrams depicting cannula track and sampling areas in which Fos protein expression was measured. A.) Schematic diagram depicting guide cannula and infusion cannula placement. Tips of infusion cannula are denoted by “*” and illustrate the representative distribution of infusion tips from multiple treatment groups that were verified by cresyl violet histological examination. B.) A representative cresyl violet-stained coronal section depicting cannula placement in the right striatum at a middle striatal level (0.4 mm anterior to Bregma, Paxinos and Watson, 2003). C.) Schematic diagram of a coronal section depicting approximate sampling areas used for Fos quantification. Fos protein expression was measured in the cingulate (1), motor (2), somatosensory (3), insular (4) and piriform (5) cortices, and the

striatum (6) at a rostral striatal level (ca. 2.0 mm anterior to Bregma). For panel B, background staining brightness/contrast were adjusted to optimize visualization of corpus collosum and infusion site (Adobe Photoshop CS2, Adobe Systems, Inc.).

Figure 2.

Representative Fos photomicrographs (100x magnification) depicting Fos+ immunoreactive nuclei in the striatum, motor cortex, insular cortex, and cingulate cortex in rats that received an intrastriatal infusion of saline (1 μ l; left column), SCH23390 (2 μ g/ μ l; middle column), or AP5 (5 μ g/ μ l; right column) followed by a systemic injection of mAMPH (4 mg/kg). Scale bar = 200 μ m. Fos immunoreactive images were adjusted for brightness and contrast to normalize background and enhance visualization of Fos-positive nuclei (Adobe Photoshop CS2, Adobe Systems, Inc.).

Figure 3.

Fos protein expression levels in the striatum and cerebral cortex after intrastratial infusion of the D1 receptor antagonist SCH23390, or the D2 receptor antagonist sulpiride, followed by mAMPH injection. Mean ($\pm$ SEM) proportional area (x 100) measured in the striatum, the cingulate cortex, motor cortex, somatosensory cortex, piriform cortex, and insular cortex, in the infused (left panels) and non-infused (right panels) hemispheres. Rats received an intrastratial infusion of saline (1 μ l), SCH23390 (1 μ g/ μ l or 2 μ g/ μ l; top panels), or sulpiride (75 ng/ μ l or 200 ng/ μ l; lower panels), before an injection of mAMPH (1 injection x 4 mg/kg). SCH23390 treatment groups include (intrastratial infusion/systemic injection): saline/saline (n=4), SCH23390 (2 μ g/ μ l)/saline (n=5), saline/mAMPH (n=8), SCH23390 (1 μ g/ μ l)/mAMPH

(n=5), SCH23390(2 μ g/ μ l)/mAMPH (n=13); sulpiride treatment groups include: saline/saline (n=4), sulpiride(200 ng/ μ l)/saline (n=4), saline/mAMPH (n=8), sulpiride(75 ng/ μ l)/mAMPH (n=6), sulpiride(200 ng/1 μ l)/mAMPH (n=6). * p < .05, ** p < .01, *** p < .001.

Figure 4.

Fos protein expression levels in the striatum and cerebral cortex after intrastratial infusion of the NMDA receptor antagonist AP5, or the AMPA receptor antagonist DNQX, followed by mAMPH injection. Mean ($\pm$ SEM) proportional area (x 100) measured in the striatum, the cingulate cortex, the motor cortex, sensory cortex, piriform cortex, and insular cortex, in the infused (left panels) and non-infused (right panels) hemispheres. Rats received an intrastratial infusion of saline (1 μ l), AP5 (2 μ g/ μ l or 5 μ g/ μ l; top panels), or DNQX (0.5 μ g/ μ l or 1.5 μ g/ μ l; lower panels), before an injection of mAMPH (1 injection x 4 mg/kg). AP5 treatment groups include: saline/saline (n=4), AP5 (5 μ g/ μ l)/saline (n=5), saline/mAMPH (n=8), AP5 (2 μ g/ μ l)/mAMPH (n=5), AP5 (5 μ g/ μ l)/mAMPH (n=5); DNQX treatment groups include: PBS/saline

(n=3), DNQX (1.5 $\mu\text{g}/\mu\text{l}$)/saline (n=3), PBS/mAMPH (n=6), DNQX (0.5 $\mu\text{g}/\mu\text{l}$)/mAMPH (n=4), DNQX (1.5 $\mu\text{g}/\mu\text{l}$)/mAMPH (n=6). $*p < .05$, $**p < .01$, $***p < .001$.