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Amphetamine-evoked gene expression in striatopallidal neurons: regulation by corticostriatal afferents and the ERK/MAPK signaling cascade

Susan M. Ferguson* and Terry E. Robinson*,†

**Neuroscience Program and †Department of Psychology, University of Michigan, Ann Arbor, Michigan, USA*

Abstract

The environmental context in which psychostimulant drugs are experienced influences their ability to induce immediate early genes (IEGs) in the striatum. When given in the home cage amphetamine induces IEGs predominately in striatonigral neurons, but when given in a novel test environment amphetamine also induces IEGs in striatopallidal neurons. The source of the striatopetal projections that regulate the ability of amphetamine to differentially engage these two striatofugal circuits has never been described. We report that transection of corticostriatal afferents selectively blocks, whereas enhancement of cortical activity with an ampakine selectively augments, the number of amphetamine-evoked c-*fos*-positive striatopallidal (but not striatonigral) neurons. In addition, blockade of the extracellular signal-regulated kinase (ERK)/mitogen-activated protein kinase (MAPK) signaling cascade preferentially inhibits the number of amphetamine-evoked c-*fos*-positive striatopallidal neurons. These results suggest that glutamate released from corticostriatal afferents modulates the ability of amphetamine to engage striatopallidal neurons through an ERK/MAPK signaling-dependent mechanism. We speculate that this may be one mechanism by which environmental context facilitates some forms of drug experience-dependent plasticity, such as psychomotor sensitization.

Keywords: caudate nucleus, dopamine, immediate early genes, *in situ* hybridization, psychostimulants, rat.

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Psychostimulant drugs induce a complex pattern of gene expression in the striatum (Graybiel *et al*. 1990; Berke *et al*. 1998; see Harlan and Garcia 1998 for review), which represents an initial step in the induction of long-term changes in brain and behavior produced by drugs of abuse (Nestler *et al*. 1993; Chiasson *et al*. 1997; Sommer and Fuxe 1997; Hyman and Malenka 2001; Nestler 2001). However, the environmental context in which psychostimulant drugs are experienced modulates both their ability to induce forms of drug experience-dependent plasticity, such as psychomotor sensitization (Badiani *et al*. 1995a, 1995b; Crombag *et al*. 1996), and their ability to induce immediate early genes (IEGs) (Badiani *et al*. 1999; Uslaner *et al*. 2001b, 2003b). For example, when amphetamine is given in the home cage it induces IEGs almost exclusively in striatal neurons that co-express mRNA for dopamine D1 receptors, preprodynorphin and preprotachykinin, and form the striatonigral pathway (Berretta *et al*. 1992; Cenci *et al*. 1992; Johansson *et al*. 1994; Ruskin and Marshall 1994). When given in a novel test environment, however, amphetamine also induces IEGs in striatal neurons that co-express mRNA for dopamine D2 receptors and preproenkephalin, and form the striatopallidal pathway (Jaber *et al*. 1995; Badiani *et al*. 1999; Uslaner *et al*. 2001b, 2003b). Thus, the cells and circuits engaged by amphetamine vary according to where amphetamine is experienced, as does susceptibility to psychomotor sensitization.

The ability of amphetamine to engage IEGs in the striatum is dependent on both dopamine (Graybiel *et al*. 1990; Ruskin

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Address correspondence and reprint requests to Terry E. Robinson, Department of Psychology (Biopsychology), University of Michigan, 525 East University Avenue, Ann Arbor, MI 48109–1109, USA.
E-mail: ter@umich.edu

Abbreviations used: AMPA, α-amino-3-hydroxy-5-methylisoxazole-4-proprionate; DMSO, dimethyl sulfoxide; ERK, extracellular signal-regulated kinase; IEG, immediate early gene; MAPK, mitogen-activated protein kinase; SSC, saline sodium citrate buffer.

and Marshall 1994; LaHoste *et al*. 2000) and glutamate (Dragunow *et al*. 1991; Snyder-Keller 1991; Wang *et al*. 1994; Konradi *et al*. 1996), although amphetamine-evoked IEG expression in striatopallidal neurons is especially susceptible to glutamatergic regulation (Ferguson *et al*. 2003). Nonetheless, the striatopetal projections that regulate amphetamine-induced IEG expression in specific striatal cell populations have not been identified. A likely source is the neocortex, because it provides the major glutamatergic input into the striatum (Spencer 1976; Girault *et al*. 1986; McGeorge and Faull 1989), and the neocortex is strongly activated after amphetamine administration in a novel test environment, that is under conditions that also induce IEGs in striatopallidal neurons (Badiani *et al*. 1998). In addition, activation of the neocortex preferentially induces IEGs in striatopallidal neurons (Berretta *et al*. 1997, 1999; Parthasarathy and Graybiel 1997; Sgambato *et al*. 1997), an effect that occurs via the extracellular signal-regulated kinase (ERK)/mitogen-activated protein kinase (MAPK) signal transduction cascade (Sgambato *et al*. 1998; Gerfen *et al*. 2002).

The present experiments were designed to determine the source of striatopetal inputs that modulate the ability of amphetamine to engage striatofugal circuits. To do this we: (1) studied the effects of transection of corticostriatal fibers on amphetamine-evoked c-*fos* expression in striatofugal circuits; (2) characterized the effects of an ampakine, which preferentially enhances ongoing cortical activity, on amphetamine-evoked c-*fos* expression; and (3) determined the effect of a selective MEK inhibitor on amphetamine-evoked c-*fos* expression in striatofugal circuits.

Materials and methods

Animals

Male Sprague–Dawley rats (Harlan Sprague Dawley, Indianapolis, IN, USA) weighing 150–225 g upon arrival, were housed individually in clear square plastic cages containing shredded paper bedding, and were given a 1-week acclimatization period before any experimental manipulation. The rooms were temperature- and humidity-controlled, and maintained on a 14 : 10 h light : dark cycle, with food and water available *ad libitum*. All experimental procedures were approved by the University of Michigan Committee on the Use and Care of Animals.

Drugs

D-Amphetamine sulfate (Sigma, St Louis, MO, USA) and the ampakine CX516 (generously donated by Cortex Pharmaceuticals, Irvine, CA, USA) were dissolved in sterile 0.9% saline. The MEK inhibitor SL327 (generously donated by Bristol-Myers-Squibb, Wilmington, DE, USA) was dissolved in 25% dimethylsulfoxide (DMSO) (diluted 1 : 3 in sterile water). All drugs were administered by i.p. injection in a volume of 1 mL/kg. Drug weights refer to the weight of the salts.

Groups and test procedures

Effect of transection of corticostriatal fibers on amphetamine-evoked psychomotor activation and c-fos mRNA expression in identified striatal cell populations

Corticostriatal afferents originating from prefrontal and prelimbic cortices, and anterior portions of cingulate, primary sensorimotor and medial agranular cortices were transected using a modified version of the method described by Cenci and Bjorklund (1993). Rats were anesthetized with a mixture of ketamine hydrochloride (100 mg/mL i.p.; Fort Dodge Animal Health, Fort Dodge, IA, USA), xylazine hydrochloride (20 mg/mL i.p.; Ben Venue Laboratories, Bedford, OH, USA) and acepromazine maleate (10 mg/mL i.p.; Vedco, St Joseph, MO, USA). Using standard stereotaxic procedures, a series of coronal cuts (4 mm wide total) were made either unilaterally or bilaterally with an inverted T-shaped knife that had a 2-mm wide blade (coordinates from bregma: anterior/posterior (A/P) 2.7 mm; medial/lateral (M/L) ± 1.3; ± 2.3; ± 3.3 mm; dorsal/ventral (D/V) − 6.5 from the skull surface). Sham animals received a similar coronal cut, but the knife was only lowered 2 mm into the cortex (D/V − 3.0 mm from skull surface; i.e the white matter was not transected). A third group of animals had no surgery.

After a 1-week recovery period, rats were transported to a testing room where they received an injection of saline or amphetamine (2 mg/kg) and were placed into clear rectangular tubs (217 × 447 × 230 mm; i.e. a novel test cage) containing a clear plastic insert in the center of the cage (64 × 230 × 230 mm) that formed a corridor through which rats could locomote, and ground corncob bedding on the floor. Behavior was recorded for 50 min and the total number of crossovers (defined by two consecutive beambreaks of sets of infrared photocells spaced 230 mm apart across the length of the tub) was used as an index of locomotor activity. Fifty minutes after injection, animals were decapitated. Brains were removed, frozen in isopentane and stored at − 70°C.

Effect of an ampakine on amphetamine-induced psychomotor activation and c-fos mRNA expression in identified striatal cell populations

Rats were transferred from the main animal colony room to a testing room and placed individually into cages identical to those described above for 1 week before testing. On the day of testing rats received an injection of saline or the ampakine CX516 (35 mg/kg) immediately followed by an injection of saline or amphetamine (2 mg/kg). Ampakines are positive modulators of α-amino-3-hydroxy-5-methylisoxazole-4-proprionate (AMPA) glutamate receptors, which slow the deactivation and desensitization of AMPA receptors, and so increase cortical activity by enhancing the amplitude and duration of fast, excitatory postsynaptic currents (Arai *et al*. 1996a, 1996b; Hess *et al*. 2003). CX516 has some preference for GluR2 receptors (A. Arai, personal communication), which are most abundant in the cortex and hippocampus (Martin *et al*. 1993; Beneyto and Meador-Woodruff 2004). Behavior was recorded for 50 min and then the animals were decapitated. Brains were removed, frozen in isopentane and stored at − 70°C.

Effect of a MEK inhibitor on amphetamine-evoked c-fos mRNA expression in identified striatal cell populations

On the day of testing, rats received the highly selective MEK inhibitor SL327 (10, 20 or 40 mg/kg) dissolved in 25% DMSO

(diluted 1 : 3 in sterile water), in the animal colony room. Thirty minutes later animals were transported to a testing room where they received an injection of saline or amphetamine (2 mg/kg), and then were placed individually into orange circular buckets with ground corncob bedding on the floor. Fifty minutes after the second injection animals were decapitated. Brains were removed, frozen in isopentane and stored at − 70°C.

Dual *in situ* hybridization

Brains were sectioned using a cryostat and 16-μm coronal sections were thaw-mounted on to Superfrost/Plus slides (Fisher Scientific, Pittsburgh, PA, USA) and stored at − 70°C until processing for dual *in situ* hybridization. The method was a modification of that described by Curran and Watson (1995). Slides containing four tissue sections were processed using a ^{35}S-UTP and ^{35}S-CTP-labeled riboprobe complementary to c-*fos* mRNA (680-mer; courtesy of Dr T. Curran, St Jude Children's Research Hospital, Memphis, TN, USA) and a digoxigenin-UTP-labeled riboprobe complementary to preproenkephalin mRNA (693-mer; courtesy of Dr J. Douglass, Amgen, Thousand Oaks, CA, USA). The radioactive riboprobe was generated by incubating linearized c-*fos* DNA (1 μg) at 37°C for 1.5 h in 1 × transcription buffer, 100 μCi α-^{35}S-UTP (100 Ci/mM, 20 mCi/mL; Amersham, Arlington Heights, IL, USA), 160 μCi α-^{35}S-CTP (800 Ci/mM, 40 mCi/mL; Amersham), 400 μM GTP, 400 μM ATP, 8 mM dithiothreitol, 10 U Rnase inhibitor and 50 U T7 RNA polymerase. The non-radioactive riboprobe was generated by incubating linearized preproenkephalin DNA (1 μg) at 37°C for 1.5 h in 1 × transcription buffer, 320 μM digoxigenin-UTP (Boehringer, Manheim, Germany), 80 μM UTP, 400 μM GTP, 400 μM ATP, 400 μM CTP, 10 mM dithiothreitol, 10 U Rnase inhibitor and 50 U T7 RNA polymerase. The resulting probes were incubated at room temperature (22°C) with 83 U Rnase-free Dnase for 15 min and then separated from free nucleotides on Micro Bio-Spin Chromatography columns (Bio-Rad, Hercules, CA, USA).

Before hybridization, tissue sections were fixed in 4% phosphate-buffered paraformaldehyde for 1 h at room temperature, rinsed three times in 2 × saline sodium citrate buffer (SSC), placed into a solution of 0.1 M triethanolamine/0.25% acetic acid for 10 min, rinsed in water and dehydrated in a series of graded alcohols (50–100%). The ^{35}S-labeled and digoxigenin-UTP-labeled probes were diluted in hybridization buffer (50% formamide, 10% dextran sulfate, 3 × SSC, 50 mM sodium phosphate, pH 7.4, 1 × Denhardt's solution and 10 mg/mL yeast tRNA) to give an approximate concentration of 3–4 × 10^6 d.p.m. per 80 μL and 2.5 μL per 80 μL respectively. Slides were coverslipped with diluted probe (80 μL) and placed in hybridization trays lined with filter paper dampened with 50% formamide/50% water. The trays were sealed and incubated at 55°C for 16 h. Coverslips were floated off in 2 × SSC and the slides were rinsed three times in 2 × SSC. The slides were then incubated in Rnase A (200 μg/mL) at 37°C for 1 h, rinsed in 2 ×, 1 × and 0.5 × SSC, incubated in 0.1 × SCC at 65°C for 1 h, and then cooled to room temperature. The slides were rinsed in 0.1 M sodium phosphate buffer (pH 7.4), incubated with shaking for 1 h at room temperature in a blocking solution (0.25% carageenan, 0.5% Triton X-100, 0.1 M sodium phosphate buffer), and then incubated overnight with shaking at room temperature with an antibody against digoxigenin conjugated to alkaline phosphatase (Fab fragments; Boehringer) that was diluted 1 : 10 000 in a blocking solution. Slides were then incubated twice each for 1 h at room temperature with shaking in 0.1 M sodium phosphate buffer, twice each for 30 min in Tris-buffered saline and rinsed in alkaline substrate buffer (ASB; 100 mM Tris base, 50 mM NaCl, 50 mM $MgCl_2$, pH 9.5). The color reaction was carried out in the dark at room temperature in ASB containing 5% polyvinyl alcohol, 0.025% levamisole and 2% nitro blue tetrazolium chloride/5-bromo-4-chloro-3-indoyl phosphate (NBT/BCIP) (Boehringer). After approximately 4 h the color reaction was stopped by washing the slides extensively in water, incubating in 0.1 M glycine containing 0.2% Triton X-100 (pH 2.2) for 10 min at room temperature and rinsing in water. Slides were then fixed in 2.5% glutaraldehyde for 2 h, rinsed in water and air-dried.

Slides were exposed to X-ray film for 5 days (Kodak Biomax MR; Kodak, Rochester, NY, USA) and then dipped in emulsion (Ilford KD-5; Polysciences, Warrington, PA, USA) and stored in light-tight boxes at 4°C for 18 days. Slides were developed (Kodak D-19) for 2.5 min at 17°C, rinsed in water and fixed (Kodak Rapid Fix) for 5 min at 17°C. They were then washed extensively in water, dehydrated in a series of graded alcohols (50–100%), washed extensively in xylene and coverslipped with Permount mounting medium.

Control experiments using sense probes or tissue pretreated with Rnase A (200 μg/mL at 37°C for 1 h) were performed to ensure probe specificity; no binding was observed with either control.

Quantification of gene expression and data analysis

Initial quantification of c-*fos* mRNA expression was conducted on autoradiographs across the rostrocaudal extent of the dorsomedial portion of the striatum (Paxinos and Watson 1998). This region was selected for analysis because it receives a dense glutamatergic projection from cortical regions, such as prefrontal, cingulate and parietal cortices (McGeorge and Faull 1989; Willuhn *et al*. 2003) and amphetamine-evoked c-*fos* expression is greatest in this region of the striatum (Badiani *et al*. 1998; Uslaner *et al*. 2001a). For some experiments, orbital, prefrontal, parietal and cingulate cortices were also quantified. Sections were quantified as described previously (Badiani *et al*. 1998).

For the cortical transection experiment, level 1.2 mm from bregma was selected for further analysis because transection of corticostriatal afferents produced the greatest decrease in amphetamine-evoked c-*fos* expression at this level. Level − 0.8 mm from bregma was also selected for further analysis because there was no effect of cortical transection at this level and so it was used as a positive control. In unilaterally transected/sham animals, only the transected/sham hemisphere was quantified. To allow for comparisons with the unilaterally transected animals, only one hemisphere was quantified in the bilaterally transected/sham animals and the animals that did not receive surgery; side was determined randomly.

For the ampakine experiment, the dorsomedial striatum at level 0.0 mm from bregma was selected for further analysis because this region receives a dense projection from parietal cortex (McGeorge and Faull 1989), and ampakines have been reported to preferentially enhance psychostimulant-evoked c-*fos* expression in parietal cortex (Hess *et al*. 2003). The ventromedial striatum at level 0.0 mm from bregma was also selected for further analysis; it was used as a positive control because there was no effect of ampakine pretreat-

ment on amphetamine-evoked c-*fos* expression in orbital cortex, which sends a dense projection to this region (McGeorge and Faull 1989).

For the MEK inhibitor experiment, levels −0.4 and −0.8 mm from bregma were selected for further analysis because amphetamine evokes the greatest amount of c-*fos* mRNA expression at more caudal levels (Badiani *et al*. 1998; Ostrander *et al*. 2003; Willuhn *et al*. 2003).

Quantification was carried out by an experimenter blinded to the experimental conditions. Sections from three to 10 animals per group were examined at 20 × magnification using a microscope (Letiz DMR; Leica, Wetzler, Germany). For the cortical transection experiment, the number of single- and double-labeled cells in the dorsomedial striatum was counted in nine 250-μm^2 grids at level 1.2 mm from bregma and in four 250-μm^2 grids at level −0.8 mm from bregma in one hemisphere (see Fig. 3). For the other experiments, the number of cells in two 250-μm^2 grids in each hemisphere was counted (a total of four grids per region or level). ^{35}S-labeled cells (containing c-*fos* mRNA; c-*fos*+) appeared as silver grains under dark-field conditions and digoxigenin-labeled cells (containing preproenkephalin mRNA; Enk+) appeared as purple precipitate under bright-field conditions. Only ^{35}S-labeled cells that contained dense clusters of silver grains (at least 10 silver grains/cell) and digoxigenin-labeled cells that were uniformly darkly stained (at least 10 times above background staining) were considered to be positively labeled. The number of preproenkephalin mRNA-positive cells did not differ between groups (data not shown). In all experiments, an untreated group that received no experimental manipulation was used to assess basal numbers of c-*fos*+ cells, which was extremely low (i.e. fewer than five c-*fos*+ cells/mm^2).

The vast majority of cells in the striatum are medium spiny projection neurons that either co-express mRNA for dopamine D2 receptors and preproenkephalin and form part of the striatopallidal pathway (Enk+ or striatopallidal cells), or co-express mRNA for dopamine D1 receptors, preprodynorphin and preprotachykinin (but not preproenkephalin) and are part of the striatonigral pathway (Enk− or striatonigral cells). We have found that under our *in situ* conditions, preproenkephalin mRNA and preprotachykin mRNA co-localize in only 4% of cells in the striatum, confirming that Enk+ and Enk− cells represent two different and segregated cell populations (Gerfen 1992; Uslaner *et al*. 2003a). Amphetamine reportedly does not induce c-*fos* in interneurons in the striatum (Harlan and Garcia 1998) and, under our conditions, we have found that all amphetamine-evoked c-*fos*+ cells are either co-labeled with preproenkephalin mRNA or preprodynorphin/preprotachykinin mRNA (Ferguson *et al*. 2003). Therefore, in the present study the number of c-*fos*+ and Enk+ cells (c-*fos*/Enk+) was subtracted from the total number of c-*fos*+ cells in the striatum for each animal to give the number of c-*fos*+ and Enk− (c-*fos*/Enk−) cells. This number was then used as an indication of the number of cells in the striatonigral pathway that were activated following each treatment.

Statistical analysis

For the cortical transection experiment, there were no group differences in locomotor activity, c-*fos* mRNA expression, number of c-*fos*/Enk+ cells or number of c-*fos*/Enk− cells in animals that received unilateral sham, bilateral sham or no surgery (as assessed by one-way ANOVA); these three groups were therefore pooled to form a single control group. In addition, there were no group differences in these measurements between animals that received unilateral or bilateral transections (assessed by means of a *t*-test), so these groups were pooled to form a single experimental group. For both the cortical transection and ampakine experiments, group differences in locomotor activity, c-*fos* mRNA expression, the number of c-*fos*/Enk+ cells and the number of c-*fos*/Enk− cells were tested using two-way ANOVA followed by Bonferroni's post-hoc test. For the MEK inhibitor experiment, group differences in the number of c-*fos*/Enk+ cells and the number of c-*fos*/Enk− cells were examined using one-way ANOVA followed by Dunnett's post-hoc test. For all comparisons, $\alpha = 0.05$.

Results

Transection of corticostriatal fibers selectively blocks amphetamine-evoked c-*fos* expression in striatopallidal (Enk+) neurons

Histology

The extent of the cortical transection was examined in cresyl violet-stained coronal brain sections. A representative example of a unilateral transection is shown in Fig. 1. At levels corresponding to the knife cut, there was a clear absence of tissue. One transected animal was excluded from the study because no tissue damage was found, and two sham animals were excluded because tissue damage was observed in the corpus callosum.

Locomotor behavior

Animals given amphetamine made significantly more crossovers during the first 50 min after drug treatment than animals given saline (main effect of drug, $F_{1,48} = 26.44$, $p < 0.0001$)(Fig. 2) . However, there was no effect of cortical transection on saline- or amphetamine-induced locomotor activity (main effect of surgery and interaction between surgery and drug factors not significant, $F_{1,48} = 0.01–3.28$, $p = 0.08–0.94$) (Fig. 2).

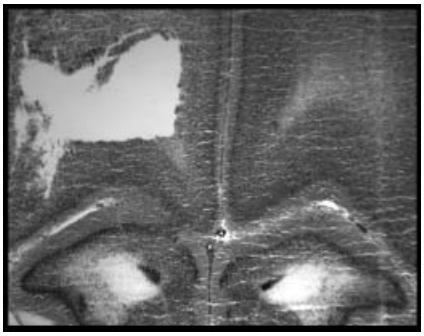

Fig. 1 Representative example of a cresyl violet-stained coronal brain section from a unilaterally transected animal. This level corresponds to the knife cut; there is a clear absence of tissue in and around the forceps minor of the corpus callosum.

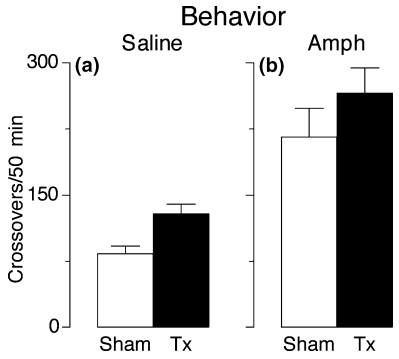

Fig. 2 Cortical transection does not alter the acute locomotor response to amphetamine (Amph). The mean ± SEM number of crossovers made during the first 50 min after (a) saline treatment and (b) amphetamine treatment is shown in sham operated and transected (Tx) animals.

c-fos *mRNA*

Amphetamine evoked significantly more c-*fos* mRNA expression in the dorsomedial striatum than saline (main effect of drug at each level quantified, $F_{1,45-48} = 9.86$–144.60, $p = 0.0001$–0.003) (Fig. 3a). The effect of the corticostriatal transection on amphetamine-evoked c-*fos* expression in the dorsomedial striatum varied as a function of rostral–caudal position (Fig. 3a). At more rostral levels (1.6, 1.2 and 0.8 mm from bregma), two-way ANOVA revealed significant interactions between surgery and drug factors ($F_{1,45-47} = 6.40$–16.01, $p = 0.0002$–0.01) (Fig. 3a). Post-hoc tests showed that there were no differences between sham and transected animals given saline ($t = 0.07$–1.05, $p > 0.05$) (Fig. 3a), but there were significant differences between sham and transected animals given amphetamine ($t = 2.7$–5.92; $p < 0.001$–0.05) (Fig. 3a), due to a decrease in amphetamine-evoked c-*fos* expression in transected animals. However, at more caudal levels (0.4 and −0.8 mm from bregma), there were no effects of cortical transection on saline- or amphetamine-evoked c-*fos* expression (main effect of surgery and interaction between surgery and drug factors not significant at each level, $F_{1,46-48} = 0.0001$–2.62, $p = 0.11$–0.99) (Fig. 3a).

Figure 3 also shows the effect of transection on the number of amphetamine-evoked c-*fos*+ cells in the striatum that were also Enk+ (striatopallidal cells) or Enk− (striatonigral cells) at levels 1.2 mm (Figs 3b–e) and −0.8 mm (Figs 3f–i) from bregma. Figure 3(j) shows representative examples of double-labeled c-*fos*/Enk+ cells. At both levels, amphetamine significantly increased the number of c-*fos*/Enk+ cells and the number of c-*fos*/Enk−cells compared with those in saline controls (main effect of drug at each level for each cell type, $F_{1,39-48} = 6.82$–108.50, $p = 0.001$–0.0001) (Figs 3b–i). At level 1.2 mm from bregma, there was a significant interaction in the number of c-*fos*/Enk+ cells between surgery and drug factors ($F_{1,48} = 4.44$, $p = 0.04$) (Figs 3d and e). Post-hoc tests revealed that there was no difference between sham and transected animals given saline ($t = 0.80$, $p > 0.05$) (Fig. 3d), but there was a significant difference between sham and transected animals given amphetamine ($t = 4.06$, $p < 0.001$) (Fig. 3e), due to a (mean ± SEM) 56 ± 7% decrease in the number of amphetamine-evoked c-*fos*/Enk+ cells in cortically transected animals. However, there was no effect of cortical transection on the number of saline- or amphetamine-evoked c-*fos*/Enk− cells (main effect of surgery and interaction between surgery and drug factors not significant, $F_{1,48} = 0.01$–0.28, $p = 0.60$–0.94) (Figs 3b and c). In contrast, at level −0.8 mm from bregma, there was no effect of cortical transection on the number of saline- or amphetamine-evoked c-*fos*/Enk− cells or c-*fos*/Enk+ cells (main effect of surgery and interaction between surgery and drug factors not significant for each cell type, $F_{1,39} = 0.01$–0.46, $p = 0.50$–0.93) (Figs 3f–i).

An ampakine selectively enhances amphetamine-evoked c-*fos* expression in striatopallidal (Enk+) neurons

Locomotor behavior

Animals given amphetamine made significantly more crossovers during the first 50 min following treatment than those given saline (main effect of drug, $F_{1,34} = 39.72$, $p < 0.0001$) (Fig. 4). However, ampakine pretreatment had no effect on saline- or amphetamine-induced locomotor activity (main effect of pretreatment and interaction between pretreatment and drug factors not significant, $F_{1,34} = 0.03$–0.55, $p = 0.46$–0.86) (Fig. 4).

c-fos *mRNA*

In all regions examined, there was no effect of ampakine pretreatment on a subsequent injection of saline. To simplify data presentation therefore, Fig. 5 shows data from amphetamine-treated groups only. The effect of ampakine pretreatment on amphetamine-evoked c-*fos* expression was examined in prefrontal (i.e. Cg1, prelimbic and infralimbic regions), orbital, cingulate and parietal cortices. Amphetamine evoked significantly more c-*fos* expression than saline in all cortical regions examined (main effect of drug for each region, $F_{1,34} = 16.76$–94.66, $p < 0.0001$; data not shown). In prefrontal, orbital and cingulate cortices, ampakine pretreatment had no effect on saline- or amphetamine-evoked c-*fos* expression main effect of pretreatment and interaction between pretreatment and drug factors not significant ($F_{1,34} = 0.01$–3.63, $p = 0.07$–0.94) (Fig. 5d shows data for orbital cortex; data not shown for other regions). However, in parietal cortex there was a significant interaction in the amount of c-*fos* expression between pretreatment and drug factors ($F_{1,34} = 10.09$, $p = 0.003$). Post-hoc tests revealed that there was no difference between saline- and

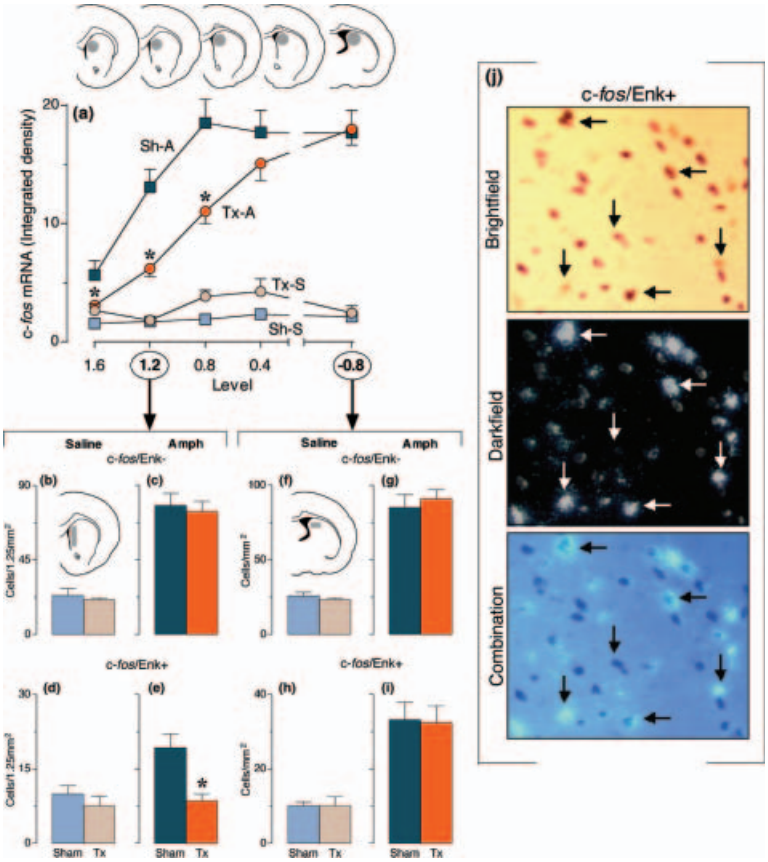

Fig. 3 Cortical transection selectively blocks the number of amphetamine-evoked c-*fos*/Enk+ cells in the dorsomedial striatum. (a) Mean ± SEM c-*fos* expression (integrated density) across the rostral–caudal extent of the dorsomedial striatum. Sh-A, sham operated and amphetamine treated; Tx-A, transected and amphetamine treated; Sh-S, sham operated and saline treated; Tx-S, transected and saline treated. (b, c) Mean ± SEM number of c-*fos*/Enk− cells at level 1.2 after saline treatment (b) and amphetamine (Amph) treatment (c). Blue bars represent sham groups and orange bars represent transected (Tx) groups. (d, e) Mean ± SEM number of c-*fos*/Enk+ cells at level 1.2 after saline treatment (d) and amphetamine treatment (e). (f, g) Mean ± SEM number of c-*fos*/Enk− cells at level −0.8 after saline treatment (f) and amphetamine treatment (g). (h, i) Mean ± SEM number of c-*fos*/Enk+ cells at level −0.8 after saline treatment (h) and amphetamine treatment (i). (j) Representative histological plates showing sections from the dorsomedial striatum that were double-labeled for c-*fos* mRNA and preproenkephalin mRNA. Sections were taken from an animal that received amphetamine treatment (2 mg/kg i.p.) in a novel test environment. (Top) Brightfield image in which Enk+ cells are indicated by orange precipitate. (Middle) Darkfield image in which c-*fos* cells are indicated by clusters of silver grains. (Bottom) Overlay of brightfield and darkfield images. Downward facing arrows indicate single-labeled cells (c-*fos*+ or Enk+). Left facing arrows indicate double-labeled cells (c-*fos*/Enk+). *$p < 0.05$ versus sham-operated amphetamine-treated group (two-way ANOVA with Bonferroni's post-hoc test).

ampakine-pretreated animals given saline ($t = 0.91$, $p > 0.05$) (data not shown) but there was a significant difference between saline- and ampakine-pretreated animals given amphetamine ($t = 3.66$, $p < 0.01$) (Fig. 5a), due to an increase in amphetamine-evoked c-*fos* expression in ampakine-pretreated animals. These results are consistent with a previous finding that ampakine pretreatment enhanced methamphetamine-induced c-*fos* expression in parietal but not frontal cortex (Hess *et al*. 2003), and suggest that the effect of the ampakine is region specific.

The effect of ampakine pretreatment on the number of amphetamine-evoked c-*fos*/Enk+ and c-*fos*/Enk− cells in the dorsomedial (Figs 5b and c) and the ventromedial (Figs 6e and f) striatum at level 0.0 was also examined. In both regions, amphetamine significantly increased the number of c-*fos*/Enk+ cells and the number of c-*fos*/Enk− cells compared with saline (main effect of drug in each region for each cell type, $F_{1,34} = 43.06–139.6$, $p < 0.0001$) (data not shown). In the dorsomedial striatum, there was a significant interaction in the number of c-*fos*/Enk+ cells between pretreatment and drug factors ($F_{1,34} = 9.72$, $p = 0.004$). Post-hoc tests showed that there was no difference between saline- and ampakine-pretreated animals given saline ($t = 0.56$, $p > 0.05$) (data not shown), but there was

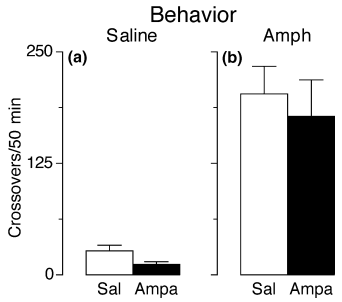

Fig. 4 Ampakine pretreatment does not alter the acute locomotor response to amphetamine. The mean ± SEM number of crossovers made during the first 50 min after treatment with (a) saline and (b) amphetamine (Amph) in animals pretreated with saline (Sal) or ampakine (Ampa) is shown.

a significant difference between saline- and ampakine-pretreated animals given amphetamine ($t = 3.94$, $p < 0.01$) (Fig. 5c), due to a 120 ± 37% increase in the number of amphetamine-evoked c-*fos*/Enk+ cells in ampakine-pretreated animals. However, there was no effect of ampakine pretreatment on the number of saline- or amphetamine-evoked c-*fos*/Enk− cells (main effect of pretreatment and interaction between pretreatment and drug factors not significant, $F_{1,34} = 0.03–0.68$, $p = 0.42–0.86$) (Fig. 5b for amphetamine groups; data not shown for saline groups). Furthermore, in animals given amphetamine, there was a significant correlation between c-*fos* expression in the parietal cortex and the number of c-*fos*/Enk+ cells ($r = 0.49$, $p = 0.03$); the correlation with c-*fos*/Enk− cells was not significant. In contrast, in the ventromedial striatum, there was no effect of ampakine pretreatment on the number of saline- or amphetamine-evoked c-*fos*/Enk− or c-*fos*/Enk+ cells (main effect of pretreatment and interaction between pretreatment and drug factors not significant for each cell type, $F_{1,34} = 0.002–1.14$, $p = 0.29–0.97$) (Figs 5e and f for amphetamine groups; data not shown for saline groups).

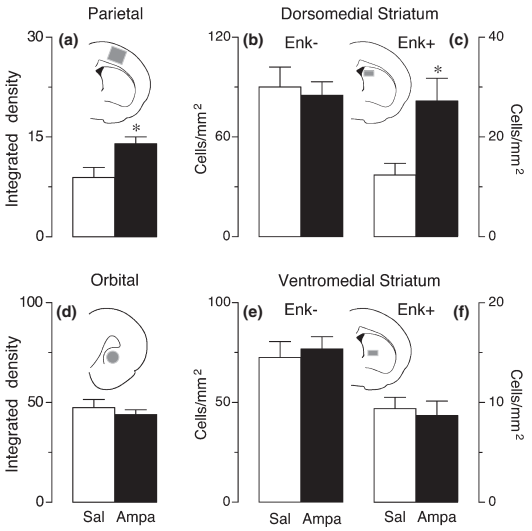

Fig. 5 Ampakine (Ampa) pretreatment selectively augments the number of amphetamine-evoked c-*fos*/Enk+ cells in the dorsomedial striatum. White bars represent the saline-pretreated group and black bars the ampakine-pretreated group. (a) Mean ± SEM c-*fos* expression (integrated density) in parietal cortex at level 0.0 after amphetamine treatment. (b, c) Mean ± SEM number of amphetamine-evoked c-*fos*/Enk− cells (b) and c-*fos*/Enk+ cells (c) in dorsomedial striatum. (d) Mean ± SEM c-*fos* expression (integrated density) in orbital cortex at level 2.6 after amphetamine treatment. (e, f) Mean ± SEM number of amphetamine-evoked c-*fos*/Enk− cells (e) and c-*fos*/Enk+ cells (f) in ventromedial striatum. *$p < 0.05$ versus saline-pretreated amphetamine group (two-way ANOVA with Bonferroni's post-hoc test).

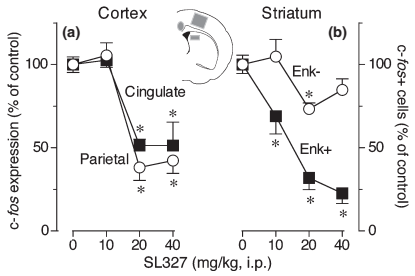

Fig. 6 Blockade of the ERK/MAPK signaling cascade preferentially attenuates the number of amphetamine-evoked c-*fos*/Enk+ cells in the dorsomedial striatum. Data represent an average of cell counts from levels −0.4 and −0.8. (a) Mean ± SEM percentage reduction in amphetamine-evoked c-*fos* expression in parietal (○) and cingulate (■) cortices. (b) Mean ± SEM percentage reduction in the number of amphetamine-evoked c-*fos*/Enk− cells (○) and c-*fos*/Enk+ cells (■). *$p < 0.05$ versus saline-pretreated amphetamine group (one-way ANOVA with Dunnett's post-hoc test).

A MEK inhibitor attenuates amphetamine-evoked c-*fos* expression in the cortex and striatopallidal (Enk+) neurons in the striatum

c-fos *mRNA*

The effects of SL327, a selective MEK inhibitor, on amphetamine-evoked c-*fos* expression in the cortex and striatum are shown in Fig. 6. Pretreatment with SL327 produced a significant dose-dependent decrease in amphetamine-evoked c-*fos* expression in parietal and cingulate cortices ($F_{3,19} = 12.23–26.77$, $p = 0.0001$) (Fig. 6a). At the highest dose of SL327 tested, amphetamine-evoked c-*fos* expression in parietal cortex was decreased by 58 ± 8% and amphetamine-evoked c-*fos* expression in cingulate cortex was decreased by 53 ± 10%. Only pretreatment with 20 mg/kg SL327 produced a significant decrease in the number of amphetamine-evoked c-*fos*/Enk− cells ($F_{3,19} = 5.35$, $p = 0.008$) (Fig. 6b). However, SL327 pretreatment produced a dose-dependent

decrease in the number of amphetamine-evoked c-*fos*/Enk+ cells, which was statistically significant at all doses tested ($F_{3,19} = 23.27$, $p < 0.0001$) (Fig. 6b). At the highest dose of SL327 tested, the number of amphetamine-evoked c-*fos*/Enk− cells was decreased by 15 ± 7%, whereas the number of amphetamine-evoked c-*fos*/Enk+ cells was decreased by 77 ± 6%.

Discussion

Transection of corticostriatal projections reduced amphetamine-evoked c-*fos* expression in denervated regions of the striatum, consistent with previous reports (Cenci and Bjorklund 1993, 1994; Vargo and Marshall 1995, 1996). The present study extends these findings by demonstrating that the transection specifically altered the ability of amphetamine to engage striatopallidal (Enk+) neurons, leaving the number of amphetamine-evoked c-*fos*+ striatonigral (Enk−) neurons unchanged. If the ability of amphetamine to engage striatopallidal neurons is dependent on a corticostriatal input to the striatum, then activation of the cortex should facilitate amphetamine-evoked IEG expression selectively in striatopallidal neurons. Consistent with Hess *et al*. (2003), we found that administration of a positive modulator of AMPA glutamate receptors (i.e. an ampakine) selectively enhanced the ability of amphetamine to induce one marker of neuronal activity, c-*fos*, in the parietal cortex. In association with this increase in parietal activity, there was a two-fold increase in the number of c-*fos* + striatopallidal neurons specifically in the striatal region that receives parietal input. There was, however, no effect on the number of c-*fos*+ striatonigral neurons. Furthermore, in striatal regions innervated by cortical areas unaffected by the ampakine, there was no effect on the number of amphetamine-evoked c-*fos*+ striatopallidal neurons. Collectively, these results suggest that corticostriatal (presumably glutamatergic) activity modulates the ability of amphetamine to engage striatopallidal, but not striatonigral, neurons.

Stimulation of corticostriatal inputs, as well as administration of glutamate or glutamate receptor agonists, can increase extracellular dopamine concentrations in the striatum (Nieoullon *et al*. 1978; Glowinski *et al*. 1988). It is possible therefore, that activation of corticostriatal glutamatergic afferents facilitates amphetamine-evoked IEG expression in striatopallidal neurons indirectly through a presynaptic mechanism, such as stimulation of dopamine release from the terminals of nigrostriatal dopaminergic neurons. Amphetamine is thought to induce IEG expression in striatonigral neurons primarily through dopamine-mediated mechanisms (Graybiel *et al*. 1990; Cole *et al*. 1994; Konradi *et al*. 1994), but we saw no effect of either corticostriatal transection or ampakine treatment on this cell population. These data therefore, suggest that glutamate does not modulate amphetamine-evoked IEG expression simply through alterations in striatal dopaminergic neurotransmission.

It is more likely that glutamate released from the cortex acts through a postsynaptic mechanism in the striatum to modulate amphetamine-evoked IEG expression in striatopallidal neurons. NMDA receptors in the striatum are mainly composed of NR1 subunits (which are necessary for formation of a functional receptor) in complex with either NR2A or NR2B subunits, which determine the pharmacological and physiological properties of the receptor (Buller *et al*. 1994; Laurie and Seeburg 1994; Standaert *et al*. 1994, 1999; Landwehrmeyer *et al*. 1995; Zukin and Bennett 1995). One promising target for the actions of glutamate is the NR2B-containing NMDA receptor located on medium spiny projection neurons. These receptors are regulated by corticostriatal afferents (Wullner *et al*. 1994; Kayadjanian *et al*. 1996), mediate a more sustained calcium influx than NR2A-containing NMDA receptors (Vicini *et al*. 1998) and are associated with enhanced behavioral and synaptic plasticity (Quinlan *et al*. 1999; Tang *et al*. 1999). In support of this hypothesis, we have shown that blockade of NR2B-containing NMDA receptors selectively attenuates the number of amphetamine-evoked c-*fos*+ striatopallidal neurons, whereas blockade of both NR2B- and NR2A-containing NMDA receptors reduces the number of amphetamine-evoked c-*fos*+ striatonigral and striatopallidal neurons (Ferguson *et al*. 2003). It should be noted, however, that dopamine modulates the ability of glutamate to engage striatopallidal neurons, as dopamine D1 and D2 receptor antagonists attenuate both amphetamine- and cortical stimulation-evoked IEG expression in these neurons (Liste *et al*. 1995; Ferguson *et al*. 2003).

It is widely accepted that psychostimulants induce IEGs through D1 receptor activation of the cyclic AMP/protein kinase A signaling cascade (Graybiel *et al*. 1990; Cole *et al*. 1994; Konradi *et al*. 1994). However, more recent work suggests that the ERK/MAPK signaling cascade is also an important pathway underlying psychostimulant-evoked IEG expression, because MEK inhibitors attenuate cocaine- and methamphetamine-evoked IEG expression in the striatum (Valjent *et al*. 2000; Salzmann *et al*. 2003). In addition, dopamine agonists evoke a greater c-*fos* response in the striatum of ERK1 knockout mice (which show enhanced stimulus-dependent phosphorylation of ERK2 protein) than in wild-type mice (Mazzucchelli *et al*. 2002). Importantly, the ERK/MAPK signaling cascade is activated primarily via increases in calcium, such as occurs following glutamatergic stimulation of NMDA receptors (Fiore *et al*. 1993; Xia *et al*. 1996; Sgambato *et al*. 1998; Vanhoutte *et al*. 1999; Gerfen *et al*. 2002). In the present study, we found that a MEK inhibitor produced only a small decrease in the number of amphetamine-evoked c-*fos*+ striatonigral neurons, but nearly

an 80% decrease in the number of c-*fos*+ striatopallidal neurons. The MEK inhibitor also produced a partial blockade (approximately 50–60% decrease from control) of amphetamine-evoked IEG expression in relevant cortical regions. We cannot ascertain whether the number of c-*fos*+ striatopallidal neurons was decreased through local actions of the MEK inhibitor within the striatum, or through actions at sites outside of the striatum (for example via decreases in corticostriatal afferent activity). Nonetheless, we can conclude that the ability of amphetamine to engage striatopallidal neurons is strongly dependent on activation of the ERK/MAPK signaling cascade, whereas this signaling cascade has only a minor influence on the ability of amphetamine to activate striatonigral neurons.

Thus, we propose the following process for amphetamine-evoked IEG expression in striatopallidal neurons. (1) Amphetamine administration in a novel test environment activates the neocortex. (2) This stimulates glutamate release in the striatum via corticostriatal afferents, which engages NR2B-containing NMDA receptors on striatopallidal medium spiny neurons. (3) In the presence of dopamine, these events initiate gene transcription through the ERK/MAPK signaling cascade. The nature of this dopamine–glutamate interaction is not yet understood. Furthermore, the functional significance of activation of striatopallidal neurons in mediating the actions of amphetamine is not yet known. However, conditions that promote cortical activation and engage striatopallidal neurons (amphetamine administration in a novel test environment) also promote amphetamine-induced behavioral sensitization (Badiani *et al*. 1995a, 1995b, 1998, 1999; Crombag *et al*. 1996; Uslaner *et al*. 2001b), and cortical lesions prevent the induction of locomotor sensitization to amphetamine (Wolf *et al*. 1995; Li and Wolf 1997; Cador *et al*. 1999).

Repeated psychostimulant treatment not only produces behavioral plasticity, but induces other forms of plasticity, such as NMDA receptor-dependent striatal long-term depression (Thomas *et al*. 2000; Thomas *et al*. 2001), and increased dendritic spine density in medium spiny striatal neurons (Robinson and Kolb 1997, 1999; Li *et al*. 2003). Importantly, glutamate and the ERK/MAPK signaling cascade have been implicated in these other forms of plasticity. For example, synaptic plasticity in the hippocampus is both NMDA receptor dependent (Brown *et al*. 1988) and ERK/MAPK dependent (English and Sweatt 1996, 1997), and MEK inhibitors prevent one form of NMDA receptor-mediated structural plasticity, increased spine formation (Wu *et al*. 2001; Goldin and Segal 2003).

In conclusion, we suggest that drug–environment interactions lead to recruitment of the ERK/MAPK signaling cascade in striatopallidal neurons via activation of glutamatergic corticostriatal afferents. This may promote behavioral sensitization, as well as the neurobiological adaptations associated with this form of drug experience-dependent plasticity.

Acknowledgements

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