European Journal of Neuroscience, Vol. 13, pp. 1977–1983, 2001

Amphetamine and cocaine induce different patterns of c-*fos* mRNA expression in the striatum and subthalamic nucleus depending on environmental context

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Keywords: caudate, c-*fos*, environmental context, immediate early genes, novelty, rat, striatum, subthalamic nucleus

Abstract

In the dorsal striatum, there are two major populations of medium spiny projection neurons. One population is positive for dynorphin mRNA (DYN+), and these cells project preferentially to the substantia nigra, forming the so-called 'direct pathway'. A second population is positive for enkephalin mRNA (ENK+), and these cells influence the substantia nigra indirectly, via the globus pallidus and subthalamic nucleus. Psychostimulant drugs, such as amphetamine and cocaine, are reported to induce immediate early genes (IEGs) in only one subpopulation of dorsal striatal projection neurons, DYN+ cells. However, this apparent selectivity appears to be a function of environmental context. We found that when given in the animal's home cage, amphetamine and cocaine increased expression of the IEG, c-*fos*, almost exclusively in DYN+ cells. However, when given in a novel environment, amphetamine and cocaine increased c-*fos* mRNA in both DYN+ and ENK+ cells. Furthermore, amphetamine and cocaine increased c-*fos* mRNA expression in the subthalamic nucleus when administered in the novel environment, but not when given at home. We conclude that the neural circuitry engaged by psychostimulant drugs, and their ability to induce specific patterns of gene expression, are determined by the environmental context in which they are experienced. This may be related to the ability of environmental novelty to facilitate psychostimulant drug-induced neuroplasticity.

Introduction

Psychostimulant drugs, such as amphetamine and cocaine, induce the expression of immediate early genes (IEGs) in neurons throughout the striatal complex (Graybiel *et al*., 1990; Young *et al*., 1991; Berke *et al*., 1998). This may reflect their ability to engage striatal circuitry (Dragunow & Faull, 1989), and also may be indicative of an initial step in the development of cellular adaptations underlying drug experience-dependent plasticity (Morgan & Curran, 1991; Nestler *et al*., 1993). There are two neurochemically distinct populations of medium spiny projection neurons in the striatum (Albin *et al*., 1989; Alexander & Crutcher, 1990; Gerfen *et al*., 1990), and there have been many studies designed to delineate which population expresses IEGs following psychostimulant drug administration. There is almost complete consensus that amphetamine and cocaine induce IEG expression only in striatal neurons that are positive for prodynorphin mRNA (dynorphin+, DYN+, or enkephalin-negative, ENK–, neurons). These neurons project preferentially to the substantia nigra, forming the so-called 'direct' pathway. Amphetamine and cocaine reportedly fail to induce IEGs in striatal neurons that are positive for proenkephalin mRNA (ENK+ neurons), which project preferentially to the pallidum and subthalamic nucleus (STN), forming the so-called 'indirect' pathway (Berretta *et al*., 1992, 1993; Cenci *et al*., 1992a, b; Johansson *et al*., 1994; Ruskin & Marshall, 1994; Kosofsky *et al*., 1995; Moratalla *et al*., 1996; Berke *et al*., 1998; Harlan & Garcia, 1998).

We have found, however, that the ability of amphetamine to induce the IEG, c-*fos*, is modulated powerfully by the environmental context in which it is experienced. In the striatum, for example, amphetamine induces much higher levels of c-*fos* mRNA when it is given in a novel environment, relative to when it is given in the home cage (Badiani *et al*., 1998). Also, consistent with the literature, we found that when amphetamine was given at home it induced c-*fos* mRNA expression in cells positive for dopamine (DA) D1 receptor mRNA (the DYN+ cells), but not in cells positive for DA D2 receptor mRNA (the ENK+ cells). In contrast, when we gave amphetamine in a novel environment it induced c-*fos* mRNA expression in both D1 and D2 mRNA+ cells (Badiani *et al*., 1999). This suggests that the context in which amphetamine is experienced may not only modulate the magnitude of IEG expression, but may determine which populations of striatal cells are engaged.

To further address this issue we have characterized the ability of amphetamine to induce the IEG, c-*fos*, in neurochemically distinct striatal cell populations, as a function of environmental context, but using probes for either proenkephalin mRNA (to identify the ENK+ cell population) or prodynorphin mRNA (to identify the ENK– cell population). In addition, in our earlier experiments we quantified c-*fos* expression in the intact hemisphere of rats that had a unilateral 6-hydroxydopamine (6-OHDA) lesion (Badiani *et al*., 1998, 1999). This is potentially problematic because a unilateral 6-OHDA lesion

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Received 12 January 2001, revised 20 March 2001, accepted 26 March 2001

may alter neurotransmission even in the intact hemisphere (e.g. Robinson, 1991), and therefore, the present study was conducted using neurologically intact rats. Also, in our previous experiment we only studied amphetamine, and in the present experiment we sought to determine if environmental context has a similar effect on c-*fos* expression evoked by cocaine. Finally, to further explore the involvement of the 'indirect' pathway, we also examined c-*fos* expression in the STN, one component of this circuit.

Materials and methods

Subjects

Fifty-six male Sprague–Dawley rats (Harlan, Indianapolis, IN, USA) weighing 200–250 g were housed initially in stainless steel hanging cages in a temperature- and humidity-controlled colony room. The rats were kept on a 14 : 10 h light : dark cycle (lights on at 07.00 h) and were given food and water *ad libitum*. The animals were kept in the colony room for 7 days before any experimental manipulation. The experimental procedures were approved by the University of Michigan Committee on the Use and Care of Animals.

Testing procedures

Rats were assigned to one of seven groups. Animals in three groups were housed singly in 40.6 × 25.4 × 20.3 cm high white plastic tubs with ground corncob bedding on the floor. Animals in three other groups were housed singly in stainless steel hanging cages. After 10 days of adaptation to these housing conditions, animals in these six groups received an intraperitoneal (i.p.) injection of saline or drug in the white plastic tubs. Thus, three groups were administered saline (home/saline group, $n = 5$), 1.5 mg/kg of amphetamine (home/amphetamine group, $n = 9$) or 15 mg/kg of cocaine (home/cocaine group, $n = 9$) in their home cages, whereas animals in the other three groups were transferred from their home cages to novel test cages (white plastic tubs), where they immediately received saline (novelty group, $n = 9$), 1.5 mg/kg of amphetamine (novel/amphetamine group, $n = 11$) or 15 mg/kg of cocaine (novel/cocaine group, $n = 9$). Note that all animals received their injections in physically identical cages, but for some animals this was home and for others it was a novel environment. In addition, a seventh group was housed in the white plastic tubs but received no injections (untreated group, $n = 4$).

Drugs

D-amphetamine sulphate (1.5 mg/mL) and cocaine HCl (15 mg/mL) were dissolved in 0.9% saline. All drug weights refer to the weight of the salts. Drug solutions were administered by i.p. injection (1 mL/kg) and control treatments consisted of saline.

In situ *hybridization methods*

Fifty minutes following drug treatments, the rats were decapitated, their brains were removed and immediately frozen in isopentane (–40 to –50 °C), and then stored in a –70 °C freezer. Coronal brain sections (10 μm) were cut using a cryostat. Brain sections were taken at the level of the dorsal striatum and the STN (at approximately 0.8 mm posterior and 4 mm posterior to bregma, respectively), thaw-mounted on slides coated with polylysine and stored at –70 °C until processing for *in situ* hybridization. This level of the dorsal striatum was chosen because it is where we have previously found the largest effect of environmental context on amphetamine-induced c-*fos* expression (Badiani *et al*., 1998).

The single *in situ* hybridization method was adapted from that described by Cullinan *et al*. (1995) and the double *in situ* hybridization method from that described by Curran & Watson (1995). Briefly, brain sections were fixed in 4% paraformaldehyde. Sections containing the STN were processed for single *in situ* hybridization using ^{35}S-UTP and CTP-labelled riboprobes complementary to c-*fos* (680-mer; courtesy of Dr T. Curran, St Jude Children's Research Hospital, Memphis, TN, USA). Sections

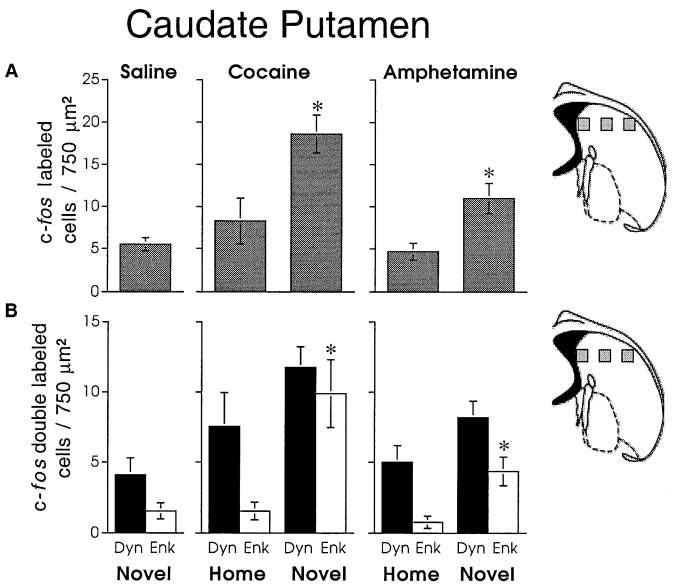

FIG. 1. The expression of c-*fos* mRNA in the dorsal striatum of rats given cocaine or amphetamine at home, or in a novel environment, or given saline in a novel environment. The illustrations to the right of each panel contain stippled regions that indicate the area of the dorsal striatum that was quantified. (A) The mean (± SEM) number of cells positive for c-*fos* mRNA as a function of drug and environment. The mean (± SEM) for the control group was 0.222 ± 0.15, and this number was subtracted from the value for each animal in all experimental groups. Thus, the mean of the control group is represented by a value of zero, and all values greater than zero indicate significant expression. All groups showed greater expression than the control group (one-sample *t*-tests; novel/saline, $t = 7.19$, $P < 0.0001$; home/cocaine, $t = 2.79$, $P < 0.02$; novel/cocaine, $t = 8.34$, $P < 0.0001$; home/amphetamine, $t = 4.84$, $P < 0.002$; novel/amphetamine, $t = 6.14$, $P < 0.0003$). Animals given cocaine or amphetamine in the novel environment showed higher levels of expression than animals treated at home (novel/cocaine vs. home/cocaine, $t = 4.86$, $P < 0.0001$; novel/amphetamine vs. home/amphetamine, $t = 2.97$, $P < 0.009$). Both the novel/cocaine and novel/amphetamine groups differed from the saline group ($t = 2.68$–5.52, $P < 0.02$). (B) The mean (± SEM) number of cells positive for c-*fos* mRNA that were also positive for either dynorphin (DYN) mRNA or enkephalin (ENK) mRNA, as a function of drug and environment. In the control group, no ENK+ cells were positive for c-*fos* mRNA, and only 0.222 ± 0.15 DYN+ cells were positive for c-*fos* mRNA. In all groups, there was an increase in the number DYN+ cells that expressed c-*fos* mRNA, relative to the control group (novel/saline, $t = 3.5$, $P < 0.009$; home/cocaine, $t = 3.1$, $P < 0.02$; novel/cocaine, $t = 7.95$, $P < 0.0001$; home/amphetamine, $t = 4.25$, $P < 0.003$; novel/amphetamine, $t = 7.01$, $P < 0.0001$). Also, in all groups except the home/amphetamine group, there was an increase in the number of ENK+ cells that expressed c-*fos*, relative to the control group (novel/saline, $t = 3.1$, $P < 0.015$; home/cocaine, $t = 2.5$, $P < 0.038$; novel/cocaine, $t = 4.05$, $P < 0.004$; home/amphetamine, $t = 1.79$, $P = 0.11$; novel/amphetamine, $t = 4.31$, $P < 0.002$). There was no effect of environment on the number of DYN+ cells that expressed c-*fos* mRNA (novel/cocaine vs. home/cocaine, $t = 1.91$, $P = 0.073$; novel/amphetamine vs. home/amphetamine, $t = 1.53$, $P = 0.147$). In contrast, there was a strong effect of environment on the number of ENK+ cells that expressed c-*fos* mRNA (novel/cocaine vs. home/cocaine, $t = 3.01$, $P < 0.008$; novel/amphetamine vs. home/amphetamine, $t = 3.31$, $P < 0.005$). The novel/cocaine and novel/amphetamine groups also had more c-*fos*/ENK+ cells ($t = 2.32$–3.34, $P < 0.033$) and c-*fos*/DYN+ cells ($t = 2.45$–4.05, $P < 0.026$) than the novel/saline group. Asterisks indicate that novel is greater than home.

containing the dorsal striatum were processed for dual *in situ* hybridization using ^{35}S-UTP and CTP-labelled riboprobes complementary to prodynorphin or proenkephalin (733-mer and 693-mer, respectively; courtesy of Dr J. Douglass, Amgen, Thousand Oaks, CA, USA) and a digoxigenin-UTP-labelled riboprobe complimentary to c-*fos* (680-mer; courtesy of Dr T. Curran). The only alterations to the published protocol were (i) 1.25 μg of linearized plasmid was used to generate the digoxigenin-UTP-labelled probe, and (ii) sections were incubated in 0.1 M glycine and 0.5% Triton-X 100, pH 2.2, for 10 min at room temperature and later fixed in 0.5% glutaraldehyde. These latter steps were included because they help to decrease the colour background after development of emulsion-dipped slides (Day *et al*., 1999).

Single-labelled sections were exposed to X-ray film (Kodak Biomax, MR, USA) for approximately 3 days and then dipped in emulsion (NTB2, Kodak) and stored at 4 °C. Dipped slides were developed (D-19, Kodak), dehydrated in graded alcohols, cresyl-violet stained, and coverslipped with Permount. Double-labelled sections were dipped in Ilford KD-5 emulsion (Polysciences) and stored at 4 °C (≈ 3 days for enkephalin and 2 weeks for dynorphin). After development (D-19, Kodak), the slides were dehydrated in graded alcohols and coverslipped with Permount.

Quantification

Single-labelled sections were quantified as described previously (Badiani *et al*., 1998). Briefly, brain images were captured with a CCD camera (TM-745, Pulnix, USA) from X-ray film. Semiquantitative analysis was performed on each brain image using National Institute of Health image software. Pixels were counted when the optical density values were greater than 3.5 SD above background value (background obtained from corpus callosum; macro written by Dr S. Campeau, University of Colorado, Boulder, CO, USA). Data are represented as relative integrated optical density in arbitrary units, which reflect both signal intensity and the number of pixels above background, divided by total area (Badiani *et al*., 1998; Day *et al*., 2001).

Double-labelled tissue from the dorsal striatum was quantified using a Leica microscope (Leitz DMR, Wetzler, Germany). Digoxigenin-labelled cells appeared as a purple precipitate in brightfield conditions and ^{35}S-labelled cells appeared as silver grains in darkfield conditions. A 250-mm^2 eyepiece grid was used to quantify the number of single- and double-labelled cells in three locations in the dorsal striatum (see Fig. 1). These data were combined because the pattern of expression did not differ across the three locations.

Statistics

Group differences in c-*fos* mRNA expression were analysed using planned *t*-tests. For all data, an initial comparison was made between the untreated group and the home/saline group to determine the effect of being picked up and given an i.p. injection of saline. For all measures there were no differences between these groups, so they were pooled to form one control group. For all comparisons $\alpha < 0.05$.

Results

*Expression of c-*fos *mRNA in the dorsal striatum*

Figure 1 shows the results from analysis of the dorsal striatum. In the control group the levels of expression were extremely low, and for all graphs the data were normalized by subtracting the mean level of expression in the control group. Therefore, values that are significantly greater than zero represent an increase in c-*fos* mRNA expression above control. The stippled areas on the illustrations to the right of each graph indicate the portion of the dorsal striatum that was analysed. We focused on this region of the dorsal striatum because it is where we previously found the largest drug–environment interaction (Badiani *et al*., 1998).

Figure 1A shows the mean number of cells expressing c-*fos* mRNA in the dorsal striatum for each group. The number of c-*fos*+ cells was increased significantly above control in all groups (all $P < 0.05$), but there were also significant group differences. The greatest number of c-*fos* mRNA+ cells was found in animals given amphetamine or cocaine in a novel environment, and these groups differed significantly from all other groups ($P < 0.02$).

Figure 1B shows the mean number of c-*fos* mRNA+ cells in the dorsal striatum that were also positive for either DYN mRNA or ENK mRNA, for each group. Figure 2 provides illustrations of representative sections processed for dual *in situ* hybridization histochemistry. When given at home or in a novel environment, both amphetamine and cocaine induced c-*fos* mRNA expression in DYN+ cells, and there was no significant effect of environment. In contrast, there was a large effect of environment on c-*fos* mRNA expression in ENK+ cells. When given at home, both amphetamine and cocaine increased c-*fos* mRNA expression in very few ENK+ cells, and the home/amphetamine group did not even differ from control. When given in a novel environment, however, both amphetamine and cocaine increased c-*fos* mRNA expression in many ENK+ cells, and in significantly more ENK+ cells than in the home/drug condition ($P < 0.008$). Like psychostimulant drug treatment at home, saline administered in a novel environment increased c-*fos* mRNA expression in very few ENK+ cells, although significantly more than in the control condition.

In summary, when given at home, amphetamine and cocaine induced c-*fos* mRNA expression in many DYN+ cells, but only in very few ENK+ cells. When given in a novel environment, however, amphetamine and cocaine induced c-*fos* mRNA expression in approximately equal numbers of DYN+ and ENK+ cells. Indeed, the increase in the total number of cells positive for c-*fos* mRNA seen when psychostimulants were given in a novel environment, relative to when they were given at home (see Fig. 1A), was largely accounted for by the increase in the number of ENK+ cells expressing c-*fos* mRNA (compare Fig. 1A and B).

Subthalamic nucleus

Figure 3 shows the effect of environment on amphetamine- and cocaine-induced c-*fos* mRNA levels in the STN. In the control group, levels of c-*fos* mRNA were very low and the data were normalized by subtraction of the average level of expression in control group (i.e. control expression equals zero). The doses of amphetamine and cocaine used here did not significantly increase c-*fos* mRNA expression in the STN when these drugs were administered at home, nor did saline administration in a novel environment. In contrast, when administered in a novel environment, both amphetamine and cocaine significantly increased c-*fos* mRNA expression in the STN ($P < 0.01$), and the levels of expression were significantly greater than in the home condition ($P < 0.02$). Figure 4 provides representative densitograms.

Discussion

We report four major findings. (i) In neurologically intact rats (our previous studies were with rats that had a unilateral 6-OHDA lesion),

both cocaine and amphetamine induced c-*fos* mRNA expression in significantly more cells in the dorsal striatum when the drugs were administered in a novel environment than when they were administered in the home cage. (ii) When given at home, both amphetamine and cocaine induced c-*fos* mRNA almost exclusively in striatal cells that were positive for dynorphin mRNA (i.e. the ENK– cell population). (iii) When given in a novel environment, however, amphetamine and cocaine induced c-*fos* mRNA not only in DYN+ cells, but in approximately equal numbers of ENK+ cells. (iv) Amphetamine and cocaine enhanced c-*fos* mRNA levels in the STN when they were administered in a novel environment, but not when they were administered at home.

Most researchers have reported that amphetamine and cocaine increase c-*fos* mRNA or Fos-like immunoreactivity (Fos-IR) almost exclusively in ENK– striatal neurons (Berretta *et al*., 1992; Cenci *et al*., 1992a, b; Johansson *et al*., 1994; Ruskin & Marshall, 1994; Kosofsky *et al*., 1995; Berke *et al*., 1998). For example, using immunohistochemistry, Berretta *et al*. (1992, p.769) reported that in rats given either amphetamine or cocaine 'Fos-positive nuclei were almost never found in enkephalin-positive neurons. Out of 2500 Fos-positive nuclei plotted, only two ... were colocalized with enkephalin'. Similarly, using *in situ* hybridization, Johansson *et al*. (1994, p. 845) reported that 'amphetamine and cocaine raised c-*fos* mainly in substance P-positive cells, but rarely in enkephalin-positive cells'. Therefore, our finding that amphetamine and cocaine increased c-*fos* mRNA in many ENK+ cells appears, at first glance, to be inconsistent with the literature. However, to the best of our knowledge, in all previous studies but one, psychostimulants were given in the home cage. For example, in all the studies from the Graybiel laboratory, amphetamine or cocaine was given in the home cage (A. Graybiel, personal communication, 18 November 2000). The one exception is a study by Jaber *et al*. (1995), who reported that although amphetamine induced Fos-IR predominantly in ENK– cells (77% of Fos+ cells were also substance P+), Fos was also expressed in ENK+ cells (33% of Fos+ cells were ENK+). Indeed, these authors report the highest level of psychostimulant-evoked IEG expression in ENK+ cells in the literature, other than the present study and our earlier study in rats with a unilateral 6-OHDA lesion (Badiani *et al*., 1999). It is especially important to note, therefore, that Jaber *et al*. (1995) treated rats in a test cage, not in their home cage.

It thus seems that the apparent selectivity of psychostimulant-induced IEG expression in the striatum may be a function of where drugs were administered. When given in a novel environment, the effect of amphetamine and cocaine on c-*fos* was not restricted to one population of projection neurons. This raises a number of questions regarding the neurotransmitter systems thought to govern psychostimulant-induced IEG expression in the striatum. The ability of amphetamine and cocaine to induce c-*fos* in the striatum is widely thought to be DA-dependent. The ability of amphetamine or cocaine to induce c-*fos* mRNA and Fos-IR in the striatum is decreased by a 6-OHDA lesion (Cenci *et al*., 1992b; Bhat & Baraban, 1993; Cenci & Bjorklund, 1994; Paul *et al*., 1995; Ishida *et al*., 1998), or by the coadministration of D1 antagonists (Graybiel *et al*., 1990; Berretta *et al*., 1992; Nguyen *et al*., 1992; Steiner & Gerfen, 1995; Yoshida *et al*., 1995; Ishida *et al*., 1998), as is the expression of other IEGs, including zif/268 and arc (Moratalla *et al*., 1992; Steiner & Gerfen, 1995; Daunais & McGinty, 1996; Kodama *et al*., 1998). The effect of D2 antagonists is more mixed, and more difficult to interpret, because D2 antagonists can themselves induce expression of c-*fos* (Graybiel *et al*., 1990; Young *et al*., 1991; Ruskin & Marshall, 1994). Nevertheless, LaHoste *et al*. (2000) reported recently that the D2 antagonist, L-741,626, but not the D3 antagonist, U99194A, or the D4 antagonist, L-745,870, attenuated amphetamine-stimulated Fos-IR in the striatum. However, all of these studies presumably characterized c-*fos* expression primarily in ENK– cells, because expression in ENK+ cells has not been studied.

Consistent with the notion that amphetamine-evoked c-*fos* mRNA expression in ENK– cells is DA-dependent, we found that a 6-OHDA lesion abolishes the ability of amphetamine to increase c-*fos* mRNA in cells also positive for D1 receptor mRNA (ENK– cells), regardless of environmental context. However, we also found that a 6-OHDA lesion does not reduce c-*fos* mRNA in striatal neurons positive for D2 receptor mRNA (ENK+ cells) when amphetamine is administered in a novel environment (Badiani *et al*., 1999). This is an important finding because it is the first to suggest that different neurotransmitter systems are responsible for psychostimulant-evoked increases in c-*fos* mRNA expression in different striatal projection neurons, and that the induction of c-*fos* in the ENK+ cell population requires the actions of a neurotransmitter(s) other than DA.

At this point it is pure speculation as to what neurotransmitters are involved in the induction of c-*fos* in ENK+ cells. Nevertheless, there are reasons to suspect that glutamate may be involved. First, NMDA receptor antagonists decrease amphetamine- and cocaine-evoked expression of a number of IEGs in the striatum (Snyder-Keller, 1991; Ohno *et al*., 1994; Wang *et al*., 1994; Wang & McGinty, 1996; Ishida *et al*., 1998). Second, damage to corticostriatal afferents, which are mostly glutamatergic, reduces IEG expression evoked by amphetamine (Cenci & Bjorklund, 1993; Cenci & Bjorklund, 1994). Third, activation of corticostriatal fibres by electrical stimulation induces c-*fos* mRNA or Fos-IR in the striatum and STN (Fu & Beckstead, 1992;

FIG. 2. Representative histological plates illustrating sections from the dorsal striatum double-labelled for c-*fos* mRNA and enkephalin (ENK) mRNA. Cells positive for c-*fos* mRNA are visualized by the purple precipitate and cells positive for enkephalin mRNA by silver grains. White arrows indicate single-labelled cells (c-*fos*+/ENK–). Black arrows indicate double-labelled cells (c-*fos*+/ENK+).

FIG. 3. The expression of c-*fos* mRNA in the subthalamic nucleus of rats given cocaine or amphetamine (AMPH) at home, or in a novel environment, or given saline in a novel environment, as indicated by analysis of optical density values (arbitrary units). The level of expression in the control group was very low (mean ± SEM, 2.8 ± 1.33 units), and this value was subtracted from the value for each animal in all experimental groups. Thus, the mean of the control group is represented by a value of zero, and all values greater than zero indicate significant expression. Statistics: only animals administered amphetamine or cocaine in a novel environment differed from the control group (one-sample *t*-tests: novel/saline, $t = 1.48$, $P = 0.178$; home/cocaine, $t = 1.51$, $P = 0.17$; novel/cocaine, $t = 3.6$, $P < 0.008$; home/amphetamine, $t = 1.67$, $P = 0.139$; novel/amphetamine, $t = 4.51$, $P < 0.002$). Animals given cocaine or amphetamine in a novel environment showed higher levels of c-*fos* mRNA expression than animals treated at home (novel/cocaine vs. home/cocaine, $t = 3.07$, $P < 0.008$; novel/amphetamine vs. home/amphetamine, $t = 2.57$, $P < 0.02$). Both the novel/cocaine and novel/amphetamine groups differed from the novel/saline group

FIG. 4. Representative densitograms illustrating the expression of c-*fos* mRNA (S35-labelled) in the subthalamic nucleus (STN) of rats administered cocaine (COC) or amphetamine (AMPH). Increasing intensity of the signal is indicated by the transition from white to yellow to red. The cerebral peduncle (CP) is labelled to assist with orientation.

Wan *et al*., 1992; Liste *et al*., 1995; Parthasarathy & Graybiel, 1997; Sgambato *et al*., 1997), and this effect is attenuated by glutamate antagonists (Liste *et al*., 1995; Berretta *et al*., 1997). Fourth, local cortical disinhibition by application of the GABA-A antagonist, picrotoxin (Arnauld *et al*., 1996; Berretta *et al*., 1997; Berretta *et al*., 1999) or local infusion of glutamate agonists into the striatum, induces Fos-IR in ENK+, but not ENK–, neurons (Berretta *et al*., 1997). Fifth, when amphetamine or cocaine are given in a novel environment there is a massive increase in c-*fos* mRNA expression throughout the neocortex, including the prefrontal cortex and the amygdala, which in part may reflect activation of glutamatergic corticostriatal systems. This cortical activation is probably due the action of a novel environment as a stressor (Cullinan *et al*., 1995), because mere exposure to a novel environment (without drug) increases corticosterone secretion and induces c-*fos* mRNA in the neocortex and portions of the amygdala (Badiani *et al*., 1998; Uslaner

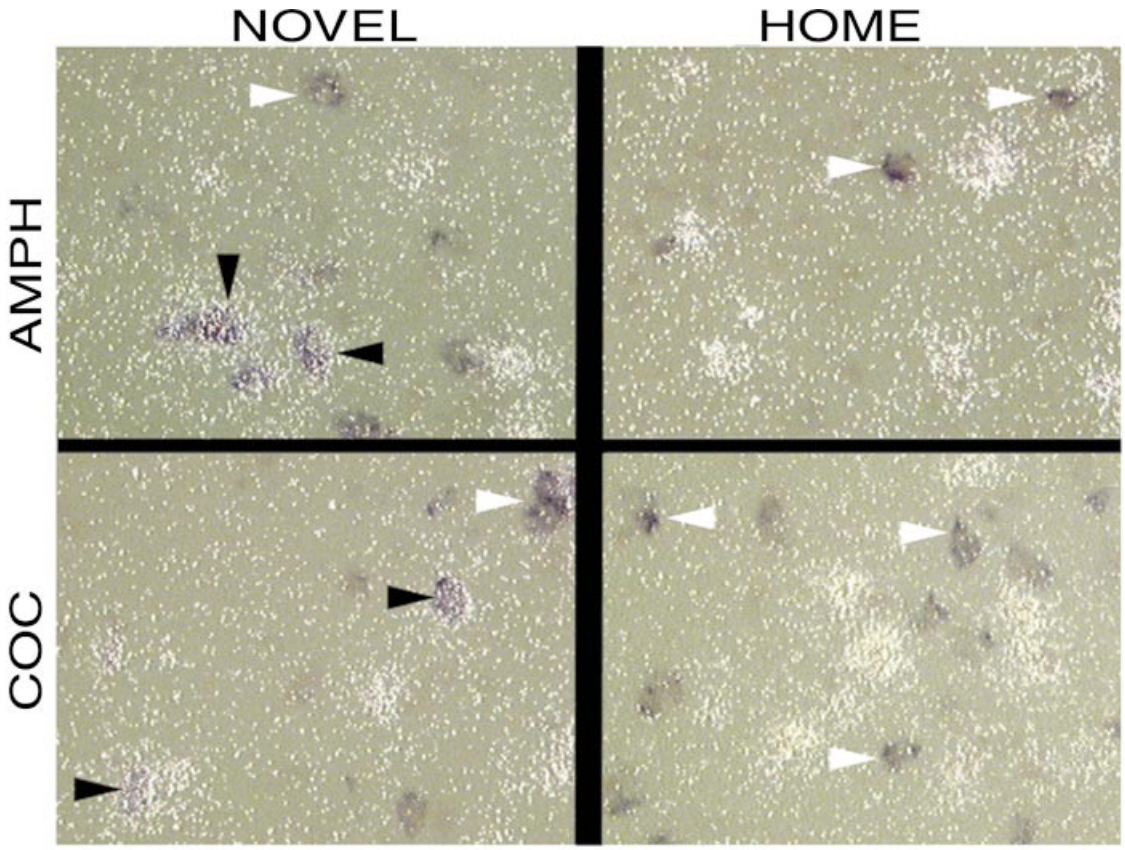

FIG. 2.

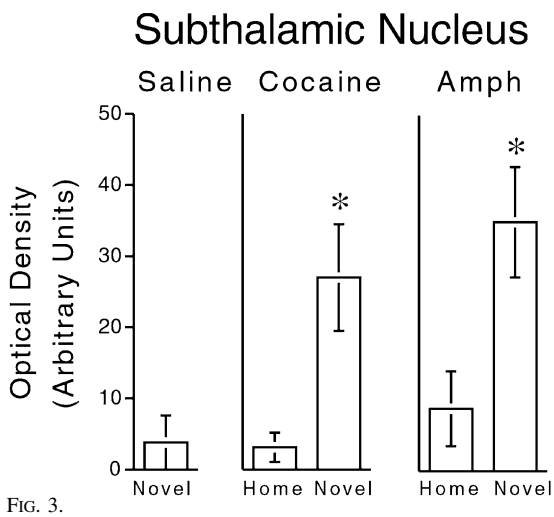

FIG. 3.

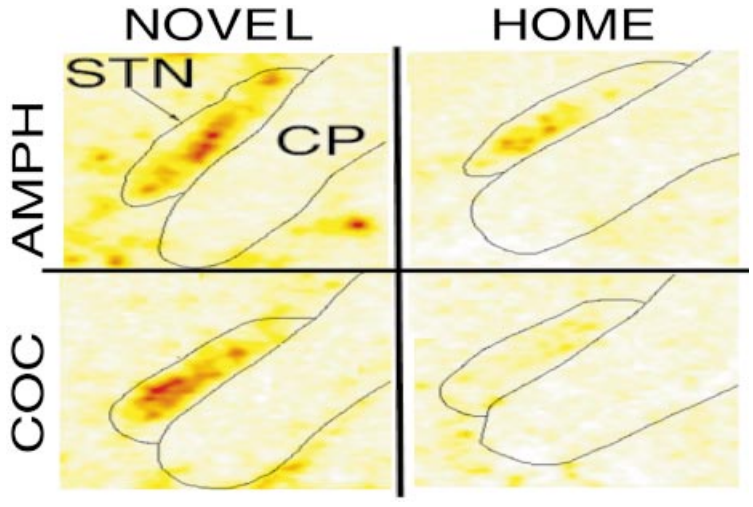

FIG. 4.

et al., 1999; Day *et al*., 2001). Importantly, these same cortical systems are not engaged when amphetamine or cocaine are given at home (Badiani *et al*., 1998; Uslaner *et al*., 1999; Day *et al*., 2001).

We hypothesize therefore that when amphetamine and cocaine are given in a novel environment, the primary neuropharmacological effects of these drugs on monoamine neurotransmission (which predominate in the home condition) interact with the effects of environmental novelty on corticostriatal glutamate systems, and this interaction leads to the recruitment of the ENK+ cell population in the striatum. The identity of the neurotransmitter systems that engage corticostriatal cells is unknown, but neurotransmitter systems activated by stressors, such as monoamines, are prime suspects. Note, however, that the cortical activation produced by exposure to a novel environment alone (in the absence of drugs) is not sufficient to engage ENK+ cells or the STN (see Figs 1B and 3). This appears to require a drug–environment interaction at the level of the striatum, and we hypothesize this involves a monoamine–glutamate interaction.

Finally, it is tempting to speculate that engaging the ENK+ cell population in the striatum leads to increased activity in the 'indirect' pathway, and that this accounts for the increase in c-*fos* mRNA expression in the STN seen when amphetamine or cocaine are given in a novel environment. It has been reported previously that amphetamine increases Fos-IR (Wirtshafter & Asin, 1999), glucose utilization (Trugman & James, 1993; Pontieri *et al*., 1995) and unit activity (Olds *et al*., 1999) in the STN, although in these studies the effect of environmental context was not examined. Of course, it is also possible that the effect of environmental novelty on psychostimulant-evoked c-*fos* mRNA expression in the STN is not related directly to the recruitment of ENK+ cells in the striatum, but to activation of one of many other afferents to the STN, including those arising in the neocortex (for review seeParent & Hazrati, 1995).

It is worth noting that the monoamine–glutamate interaction hypothesized here may not only facilitate striatal and STN IEG expression, but because psychostimulant administration in a novel environment facilitates behavioural sensitization (Badiani *et al*., 1995a, b; Crombag *et al*., 1996; Browman *et al*., 1998), it may also promote drug experience-dependent neuroplasticity. Indeed, stimulation of the STN can produce long-term potentiation (LTP)-like changes in the substantia nigra (Overton *et al*., 1999). This is important because of the proposed relationship between LTP, sensitization and glutamate neurotransmission (Wolf, 1998).

Conclusions

In conclusion, the pattern of gene expression induced by psychostimulant drugs is determined, to an amazing extent, by the setting in which these drugs are experienced. This may account for some of the effects of environmental novelty on both the acute psychomotor actions of these agents, as well as their ability to induce drug experience-dependent neuroplasticity. Most importantly, these data indicate that to understand the neurobiological actions of drugs of abuse that are critical in the development of addiction, such as their ability to promote neuroplasticity, we must pay close attention not only to their direct pharmacological actions, but also, as put by Falk & Feingold (1987, p. 1503), to the 'cluster of interactions among the pharmacological substance, the individual set of the organism, and the environmental setting'.

Acknowledgements

This research was supported by a NIDA grant to T.E.R. (DA04294) and a NIMH grant to H.A. and S.J.W. (MH42251). T.E.R. is also supported by a Senior Research Scientist Award from NIDA (KO5 DA00473). We thank Sharon Burke and Michelle Ostrander for their assistance in conducting these experiments.

Abbreviations

6-OHDA, 6-hydroxydopamine; DA, dopamine; DYN, dynorphin; ENK, enkephalin; IEGs, immediate early genes; i.p., intraperitoneal; IR, immunoreactivity; LTP, long-term potentiation; neg, negative; pos, positive; STN, subthalamic nucleus.

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