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Amphetamine-induced c-*fos* mRNA expression in the caudate-putamen and subthalamic nucleus: interactions between dose, environment, and neuronal phenotype

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Abstract

When administered in a novel environment relatively low doses of amphetamine induce c-*fos* mRNA in the subthalamic nucleus (STN) and in preproenkephalin mRNA-containing (ENK+) neurons in the caudate-putamen (CPu). When administered at home, however, low doses of amphetamine do not produce these effects. Environmental novelty also facilitates the behavioral effects of acute and repeated amphetamine, but this is dose-dependent. The purpose of the present experiment therefore was to determine if the effect of context on amphetamine-induced c-*fos* expression is also dose-dependent. It was found that: (i) No dose of amphetamine tested (1–10 mg/kg) induced c-*fos* in many ENK+ cells when given at home. (ii) When given in a novel environment low to moderate doses of amphetamine (1–5 mg/kg) induced c-*fos* in substantial numbers of ENK+ cells, but the highest dose examined (10 mg/kg) did not. (iii) Environmental novelty enhanced the ability of low to moderate doses of amphetamine to induce c-*fos* in the STN, but the highest dose of amphetamine induced robust c-*fos* mRNA expression in the STN regardless of context. The results do not support the idea that engaging ENK+ cells, at least as indicated by c-*fos* mRNA expression, is critical to produce robust behavioral sensitization, but do suggest a possible role for the STN. Furthermore, the results highlight the importance of drug–environment interactions on the neurobiological effects of drugs, and have implications for thinking about the circuits by which context modulates the acute and long-lasting consequences of amphetamine treatment.

Keywords: context, enkephalin, Fos, gene expression, striatum, substance P.

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The ability of amphetamine and cocaine to induce the expression of immediate early genes (IEGs) in the caudate-putamen (CPu) depends upon the context in which these drugs are administered (Badiani *et al*. 1998; Uslaner *et al*. 2001a; Klebaur *et al*. 2002). Amphetamine or cocaine given in the home cage induces IEGs preferentially in striatonigral neurons that coexpress mRNA for the dopamine D1 receptor, preprodynorphin, and preprotachykinin (substance P; SP+ cells) (Berretta *et al*. 1992; Cenci *et al*. 1992; Berretta *et al*. 1993; Johansson *et al*. 1994; Ruskin and Marshall 1994; Kosofsky *et al*. 1995; Berke *et al*. 1998). Under these conditions, psychostimulants fail to induce IEGs in many striatopallidal neurons that coexpress mRNA for the dopamine D2 receptor and preproenkephalin (ENK+ cells). However, when given in a distinct and novel test cage these drugs induce the IEG c-*fos* and Fos protein in both SP+ and ENK+ cells (Jaber *et al*. 1995; Badiani *et al*. 1999; Uslaner *et al*. 2001b). Furthermore, when given in a novel test cage, these drugs induce much more robust c-*fos* expression in the subthalamic nucleus (STN) (Ostrander *et al*. 2000; Uslaner *et al*. 2001b), a structure that receives input from ENK+ cells via the so-called ‘indirect pathway’ (Albin *et al*. 1989).

In addition to modulating psychostimulant-induced c-*fos* expression in the CPu and STN, environmental novelty also modulates the acute psychomotor activating effects of amphetamine, as well as its ability to produce a form of drug-experience dependent plasticity, behavioral sensitization.

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Abbreviations used: CPu, caudate-putamen; ENK+, preproenkephalin mRNA-containing; IEG, immediate early gene; LTP, long-term potentiation; SP, substance P; STN, subthalamic nucleus.

When given in a novel environment amphetamine produces more robust psychomotor activation, and more robust behavioral sensitization than when given in the home cage (Badiani *et al*. 1995a; Badiani *et al*. 1995b; Crombag *et al*. 1996; Badiani *et al*. 1997). We hypothesized therefore that the ability of environmental novelty to recruit ENK+ cells in the CPu and to engage the STN may be related to the ability of environmental novelty to enhance amphetamine-induced psychomotor activation and behavioral sensitization (Badiani *et al*. 1999; Uslaner *et al*. 2001b).

To explore this hypothesis we took advantage of the fact that the ability of environmental novelty to modulate amphetamine-induced psychomotor activation and behavioral sensitization is not absolute, but it is dose-dependent. As the dose of amphetamine is increased, the effect of environmental context is lost, such that relatively high doses promote robust psychomotor activation and robust behavioral sensitization, whether they are given in a novel environment or at home (Browman *et al*. 1998a,b). The current study was designed therefore to characterize interactions between the effects of dose and environmental context on amphetamine-evoked c-*fos* mRNA expression in the STN and in SP+ and ENK+ cells in the CPu. We hypothesized that if recruiting the STN or ENK+ cells in the CPu is important to facilitate acute psychomotor activation and/or behavioral sensitization, as dose is increased amphetamine should come to induce c-*fos* in the STN and in ENK+ cells, even when it is given at home.

Materials and methods

Animals

Eighty male Sprague–Dawley rats (Harlan, Indianapolis, IN, USA) weighing 200–250 g were initially housed individually in square plastic clear cages that had pine shavings on the floor and food and water freely available. The room was kept at a constant temperature and humidity and was on a 14 : 10 h light/dark cycle (lights on at 7 : 00 h). The animals were acclimated to the colony room for 7 days prior to any experimental manipulation. All experimental procedures were approved by the University of Michigan Committee on the Use and Care of Animals.

Drugs

D-Amphetamine sulfate (1, 5, or 10 mg/mL) was dissolved in 0.9% saline and administered in a volume of 1 mL/kg via intraperitoneal (i.p.) injection. All drug weights refer to the weight of the salts.

Testing procedures

Rats were randomly assigned to one of eight groups ($n = 10$ per group). Animals in four of the groups (home groups) were transferred from the main animal colony room to a room where they were housed in circular orange plastic buckets (25 cm diameter at base) with ground corncob bedding on the floor. They remained in these buckets with food and water available *ad libitum* for the rest of the experiment. Animals in the other four groups (novel groups) were left in the clear square cages in the animal colony room. Ten days later, rats in the home groups were administered saline or 1, 5, or 10 mg/kg of amphetamine in their home cages. Rats in the four novel groups were transferred from their cages in the main colony room to the circular orange buckets (for them a novel environment) identical to the ones in which the home groups lived, and were immediately administered saline or 1, 5, or 10 mg/kg of amphetamine. We chose this dose range because 1 mg/kg of amphetamine is just above the threshold dose necessary for inducing robust behavioral sensitization (Segal 1975), and doses between 7.5 and 10 mg/kg promote robust behavioral sensitization without producing marked dyskinetic effects (Segal and Mandell 1974; Taylor *et al*. 1974; Eichler *et al*. 1980).

Fifty minutes following treatment, each rat was decapitated. Its brain was removed, immediately frozen in isopentane (−40 to −50°C), and then stored in a −70°C freezer until it was sectioned. A cryostat was used to cut 10 μm thick coronal sections at the level of the CPu and the STN (approximately 0.8 and 4 mm posterior to bregma, respectively). The sections were thaw-mounted on slides coated with polylysine and stored at −70°C until processing for *in situ* hybridization.

In situ hybridization and quantification of mRNA

All *in situ* hybridization methods were as described in previous experiments (Uslaner *et al*. 2001b) except that the dual *in situ* hybridization was performed using [^{35}S]UTP and [^{35}S]CTP-labeled riboprobes for c-*fos* mRNA (680-mer; courtesy of Dr T. Curran), and digoxigenin-UTP-labeled riboprobes complimentary for preprotachykinin or preproproenkephalin mRNA (567-mer and 693-mer, respectively; courtesy of Dr J. Douglass, Amgen, Thousand Oaks, CA, USA). 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 by Curran and Watson (1995). Sections containing the STN were processed for single *in situ* hybridization using the riboprobe complementary to c-*fos*. Sections containing the CPu were processed for dual *in situ* hybridization using the riboprobe complimentary to c-*fos* and preprotachykinin or preproenkephalin. Single-labeled sections were exposed to X-ray film (Kodak Biomax, MR) for approximately 3 days. Double-labeled sections were dipped in Ilford KD-5 emulsion (Polysciences) and stored at 4°C (approximately 7 days). After development (Kodak D-19), the slides were dehydrated in graded alcohols and coverslipped with Permount.

Quantification

Single-labeled sections were quantified as described previously (Badiani *et al*. 1998). Briefly, brain images were captured with a Sony CCD camera from the X-ray film, and semiquantitative analysis was performed on the digitized autoradiograms using National Institute of Health Image Software. Pixels were counted when the optical density values were at least 3.5 standard deviations above background value (background obtained from corpus callosum; macro written by Dr S. Campeau, University of Colorado, Boulder). Thus, the data are represented as *relative integrated optical density* in arbitrary units, which reflects both signal intensity and the number of pixels above background, divided by total area (see Badiani *et al*. 1998; Day *et al*. 2001). Adjacent sections were stained with cresyl violet to help with anatomical location.

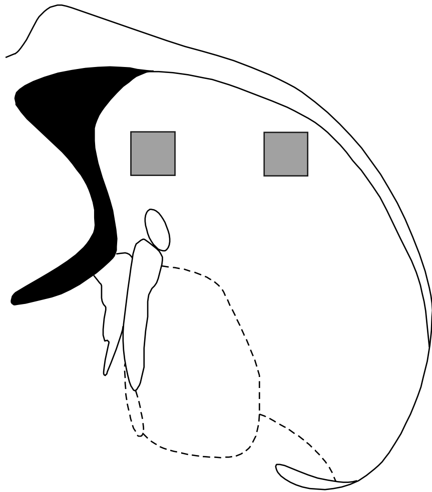

Fig. 1 The stippled area indicates the region of the CPu that was analyzed. Each stippled region is 500 × 500 μm in size.

Double-labeled tissue from the CPu was quantified using a Leica microscope (Leitz DMR, Wetzler, Germany) at a total magnification of 200 ×. Digoxigenin-labeled cells appeared as a purple precipitate in brightfield conditions and ^{35}S-labeled cells appeared as densely packed silver grains in darkfield conditions. We analyzed a 500 × 500 μm portion of the dorsomedial and dorsolateral CPu in each hemisphere. There were no differences in cell counts between these areas, so they were combined. Figure 1 shows the portion of the CPu that was analyzed. We examined this portion of the CPu because in previous studies we found the drug–environment interaction is greatest in the caudal CPu (Uslaner *et al*. 2001a).

Controls

In order to ensure that non-specific binding was minimal we performed control experiments on striatal tissue using sense strands for c-*fos*, preprotachykinin, and preproenkephalin mRNA. There was no binding of the sense probes. Furthermore, we examined whether the preprotachykinin and preproenkephalin antisense probes labeled separate cells within the CPu by performing dual *in situ* hybridization using [^{35}S]UTP and [^{35}S]CTP labeled preprotachykinin and digoxigenin-UTP labeled preproenkephalin. We examined the same region of the CPu as in the rest of the study.

Table 1 Mean (± SEM) number of ENK+ and SP+ cells in the CPu as a function of environment and dose

Treatment	ENK+ cells	SP+ cells
Home 0	264 ± 10	237 ± 7
Home 1	269 ± 19	240 ± 7
Home 5	253 ± 12	242 ± 10
Home 10	253 ± 16	229 ± 8
Novel 0	242 ± 21	228 ± 7
Novel 1	254 ± 18	232 ± 5
Novel 5	253 ± 16	240 ± 11
Novel 10	250 ± 16	229 ± 8

There were very few cells that were positive for both preproenkephalin and preprotachykinin mRNA [101 of 2154 ENK+ cells were also SP+ (4.7%); 101 out of 2284 SP+ cells were also ENK+ (4.4%)], confirming previous reports that, based on mRNA expression, these two cell populations in the CPu are almost completely segregated (Gerfen and Young 1988; Albin *et al*. 1989).

We also determined if there was any effect of treatment condition on the total number ENK+ or SP+ cells because this could bias quantification of double-labeled cells. Table 1 shows that there was no effect of treatment condition on the total number of ENK+ or SP+ cells in the region of the CPu we sampled.

Statistical analysis

Three sets of comparisons were made: (i) To determine if a particular treatment induced c-*fos* mRNA expression above baseline, data were normalized by subtracting the mean of the home saline group from the value for each individual subject, and then a planned one-sample *t*-test was used to determine whether group means were significantly greater than zero. (ii) To determine if amphetamine-induced c-*fos* mRNA expression varied as a function of context and/or dose, planned two-way ANOVAs (context, two levels; dose, three levels) were conducted, followed by Fisher's PLSD tests for pairwise comparisons. (iii) To determine if amphetamine administered in the novel environment induced levels of c-*fos* mRNA expression greater than novelty alone, Fisher's PLSD tests for pairwise comparisons were used. For all comparisons $\alpha < 0.05$.

Results

Expression of c-*fos* mRNA in the Cpu

Figure 2(a) shows the mean number of c-*fos* mRNA positive cells (c-*fos*+) in the CPu as a function of treatment condition. All doses of amphetamine given at home or in the novel environment significantly increased the number of c-*fos*+ cells above baseline ($p < 0.0005$), and the effect of amphetamine given in the novel environment was greater than the effect of novelty alone ($p < 0.0002$). However, there was also a significant dose by context interaction ($F_{2,53} = 5.78$; $p < 0.006$). This was because environmental context influenced the ability of the two lower doses of amphetamine (1 and 5 mg/kg) to increase the number of c-*fos*+ cells, whereas environment had no effect on the highest dose (10 mg/kg).

Figure 2(b) shows the mean number of c-*fos*+ cells that were also positive for SP mRNA (SP/c-*fos*+) as a function of treatment condition. All treatments increased the number of SP/c-*fos*+ cells above baseline ($p < 0.0008$) and the effect of amphetamine given in the novel environment was greater than the effect of novelty alone ($p < 0.01$). Higher doses of amphetamine produced a greater effect than lower doses (dose, $F_{2,53} = 19.97$; $p < 0.0001$), but there was no effect of environmental context (context, $F_{1,53} = 0.93$; $p = 0.3$), and no interaction between context and dose ($F_{2,53} = 2.06$; $p = 0.13$).

Figure 2(c) shows the mean number of c-*fos*+ cells that were also positive for ENK mRNA (ENK/c-*fos*+), and Fig. 3

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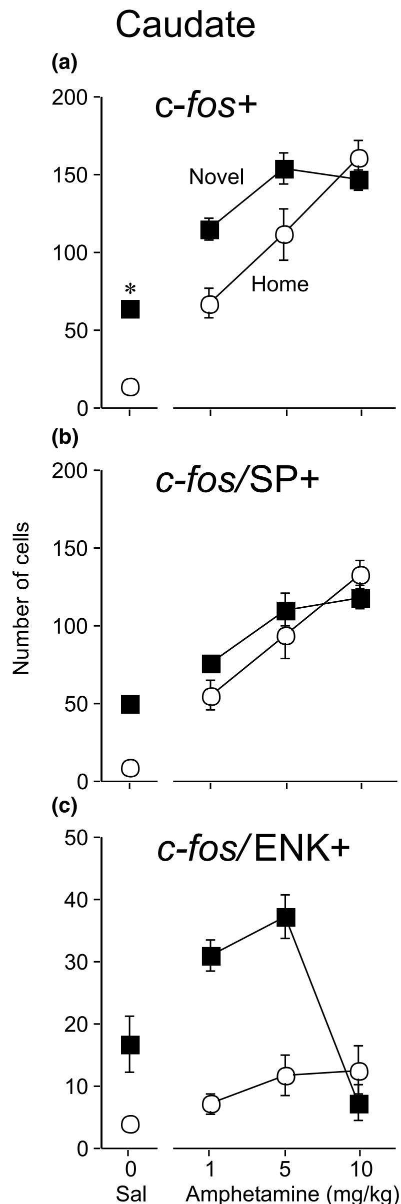

Fig. 2 The expression of c-*fos* mRNA in the CPu of rats given saline or 1, 5 or 10 mg/kg of amphetamine at home or in a novel environment. (a) The mean (± SEM) number of cells positive for c-*fos* mRNA as a function of dose and environmental context. (b) The mean (± SEM) number of cells positive for c-*fos* mRNA that were also positive for preprotachykinin mRNA (SP+) as a function of dose and environmental context. (c) The mean (± SEM) number of cells positive for c-*fos* mRNA that were also positive for preproenkephalin mRNA (ENK+) as a function of dose and environmental context.

provides representative examples of cells double-labeled for c-*fos* and preproenkephalin. When given at home, amphetamine induced c-*fos* in very few ENK+ cells (only the effect of 5 mg/kg was statistically significant). When given in the novel environment the effect of amphetamine on the number of ENK/c-*fos*+ cells differed markedly as a function of dose. Doses of 1 and 5 mg/kg increased the number of ENK/c-*fos*+ cells above baseline ($p < 0.0001$), above that produced by novelty alone ($p < 0.005$), and well above that produced by these doses of amphetamine given at home ($p < 0.0001$). In striking contrast, 10 mg/kg did not increase the number of ENK/c-*fos*+ cells above baseline or above novelty alone.

Expression of c-*fos* mRNA in the subthalamic nucleus

Figure 4 shows the dose-effect curve for amphetamine-induced c-*fos* mRNA expression (integrated density) in the STN as a function of environmental condition. When given at home, 1 mg/kg of amphetamine failed to induce c-*fos* mRNA above baseline, whereas 5 and 10 mg/kg of amphetamine did. When given in the novel environment, though, all doses of amphetamine significantly increased c-*fos* mRNA expression, both relative to baseline and to saline given in the novel environment ($p < 0.03$). In addition, there was a significant dose by context interaction ($F_{2,52} = 3.18$, $p < 0.05$). When given in the novel context, 1 and 5 mg/kg of amphetamine induced greater c-*fos* mRNA expression relative to when these doses were given at home ($p < 0.03$). However, there was no effect of environment on the highest dose tested (10 mg/kg; $p = 0.44$). Figure 5 provides representative sections illustrating c-*fos* mRNA expression at the level of the STN.

Discussion

We report three main findings: (i) The number of SP+ cells in the CPu that expressed c-*fos* mRNA increased progressively over the dose range studied, whether amphetamine was given at home or in a novel environment. (ii) The ability of amphetamine to engage ENK+ cells in the CPu was critically dependent on an interaction between dose and environmental context. When given at home, all doses of amphetamine were relatively ineffective in inducing c-*fos* mRNA in ENK+ cells. When given in a novel environment, however, low to

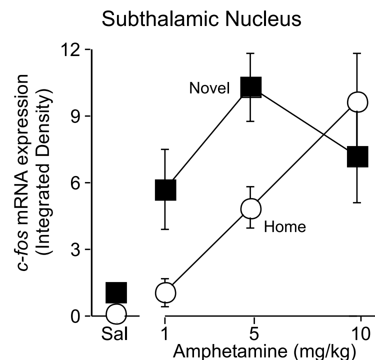

Fig. 4 The expression of c-*fos* mRNA in the STN of rats given saline or 1, 5 or 10 mg/kg of amphetamine at home or in a novel environment, as indicated by analysis of relative integrated optical density values (arbitrary units).

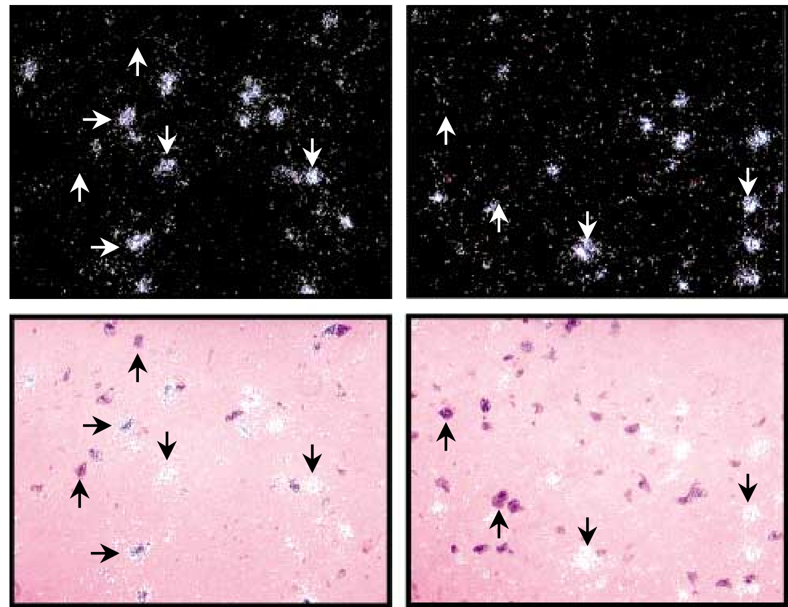

Fig. 3 Representative histological plates from the CPu illustrating cells double-labeled for c-*fos* mRNA and preproenkephalin mRNA in animals given either 5 or 10 mg/kg of amphetamine in the novel environment. (Top) Darkfield images in which cells positive for c-*fos* mRNA are identified by clusters of silver grains. (Bottom) Overlay of darkfield and brightfield images. Cells positive for preproenkephalin mRNA are identified with purple precipitate and cells positive for c-*fos* mRNA are identified by silver grains. Arrows pointing down indicate cells positive for c-*fos* but not preproenkephalin mRNA. Arrows pointing up indicate cells labeled with preproenkephalin mRNA but not c-*fos* mRNA. Arrows pointing to the right indicate double-labeled cells (c-*fos*/ENK+).

moderate doses of amphetamine (1 and 5 mg/kg) increased c-*fos* mRNA in a substantial number of ENK+ cells. In striking contrast, a higher dose of amphetamine (10 mg/kg) failed to induce c-*fos* in ENK+ cells. (iii) The ability of amphetamine to engage the STN was modulated by both dose and environmental context. Environmental novelty enhanced the ability of low to moderate doses of amphetamine to induce c-*fos* in the STN, but at the highest dose tested amphetamine induced equally robust c-*fos* mRNA expression in either context.

The ability of amphetamine to induce c-*fos* in ENK+ cells is determined by an interaction between dose and environmental context

Most previous studies report that amphetamine or cocaine induces c-*fos* or Fos-IR almost exclusively in SP+ cells in the

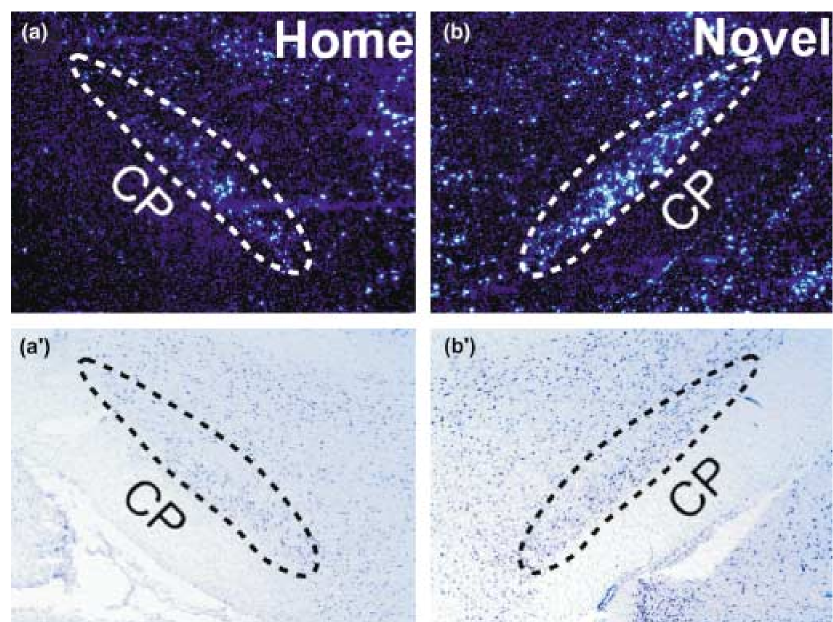

Fig. 5 Representative sections at the level of the subthalamic nucleus (STN) from animals given 5 mg/kg of amphetamine at home or in the novel context. (a and b) Emulsion dipped sections illustrating expression of c-*fos* mRNA (silver grains). (a′ and b′) Adjacent cresyl violet stained sections. Stippled region indicates the area of the STN that was analyzed. The cerebral peduncle (CP) is labeled to aid with anatomical location.

CPu (Berretta *et al*. 1992; Cenci *et al*. 1992; Berretta *et al*. 1993; Johansson *et al*. 1994; Ruskin and Marshall 1994; Kosofsky *et al*. 1995; Moratalla *et al*. 1996; Berke *et al*. 1998; Harlan and Garcia 1998). As best as we can tell, however, in these studies amphetamine was administered in the home cage. Consistent with this we found that when amphetamine was given at home about 90% of c-*fos*+ cells were SP+. We are aware of only one report, other than studies from our laboratory (Badiani *et al*. 1998; Uslaner *et al*. 2001b), showing that amphetamine induces c-*fos* or Fos-IR in significant numbers of ENK+ cells. Jaber *et al*. (1995) found that although amphetamine increased Fos-IR predominantly in SP+ cells (77% of Fos + cells were SP+), Fos-IR was also found in many ENK+ cells (33% of Fos + cells were ENK+). Importantly, Jaber *et al*. (1995) treated animals in a distinct test cage, different from the animal's home cage. We conclude therefore that the apparent selectivity of amphetamine-induced c-*fos* or Fos-IR for SP+ cells is likely a function of the context in which the drug is administered.

However, the ability of environmental novelty to modulate amphetamine-induced c-*fos* expression in ENK+ cells was strongly dose-dependent. When given in the novel environment 1 and 5 mg/kg of amphetamine significantly increased the number of c-*fos*/ENK+ cells, but 10 mg/kg of amphetamine did not. This inverted U-shaped dose-effect function for amphetamine-induced c-*fos* expression in ENK+ cells has not been previously reported, presumably because in all the previous studies where amphetamine was given in a novel environment doses ≤ 5 mg/kg were used (Jaber *et al*. 1995; Badiani *et al*. 1999; Uslaner *et al*. 2001b).

It is generally thought that amphetamine- and cocaine-induced IEG expression in the CPu is dependent on both dopamine and glutamate NMDA receptors because dopamine D1 receptor antagonists (Graybiel *et al*. 1990; Nguyen *et al*. 1992; Steiner and Gerfen 1995; Yoshida *et al*. 1995; Ishida *et al*. 1998) and NMDA antagonists (Snyder-Keller 1991; Ohno *et al*. 1994; Wang *et al*. 1994; Wang and McGinty 1996; Ishida *et al*. 1998) block psychostimulant drug-induced IEG expression. But these findings may only apply to IEG expression in SP+ cells, because the pharmacology of amphetamine-induced IEG expression in ENK+ cells has never been studied. However, there is reason to believe that glutamate may be important for the ability of amphetamine to induce c-*fos* in ENK+ cells. Disinhibition of corticostriatal glutamate projections (Arnauld *et al*. 1996; Berretta *et al*. 1997; Parthasarathy and Graybiel 1997; Gerfen *et al*. 2002) or local infusion of glutamate agonists into the CPu (Berretta *et al*. 1992) enhances c-*fos* mRNA or Fos-IR in ENK+ cells, while having little effect on SP+ cells. Furthermore, mere exposure to a novel environment induces c-*fos* expression in a significant number of ENK+ cells in the CPu. Indeed, the percentage of c-*fos*/ENK+ cells in animals exposed to novelty alone (approximately 30% of c-*fos*+ cells were ENK+) is very similar to the percentage for animals given 1 or 5 mg/kg of amphetamine in the novel environment, even though the absolute number of c-*fos*/ENK+ cells is much less. Exposure to a novel environment also induces marked c-*fos* expression throughout the neocortex (Badiani *et al*. 1998; Uslaner *et al*. 2001a). Therefore, induction of c-*fos* in ENK+ cells produced by mere exposure to novelty may reflect activation of corticostriatal glutamate projections, and amphetamine-induced dopamine release may amplify this effect. Indeed, it is well known that dopamine can enhance the ability of glutamate to engage striatal cells (see below). In support of this hypothesis we recently found that amphetamine-induced c-*fos* expression in ENK+ cells is preferentially attenuated by low doses of a dopamine D1 or D2 receptor antagonist, a non-selective NMDA receptor antagonist, and an NMDA antagonist specific for the NR2B subunit (Ferguson *et al*. 2002).

Assuming that a dopamine/glutamate interaction is important for the ability of 1 and 5 mg/kg of amphetamine to induce c-*fos* in ENK+ cells, why would 10 mg/kg fail to induce c-*fos* in these same cells? First, it is important to note that this inverted U-shaped dose-effect curve is not specific to amphetamine-induced c-*fos* mRNA expression in ENK+ cells. The ability of amphetamine to induce *arc* mRNA expression in ENK+ cells is also characterized by an inverted-U shaped dose-effect curve (unpublished observations). In addition, low doses of methamphetamine increase neuropeptide immunoreactivity and release in the caudate-putamen but high doses have either no effect or the opposite effect (Wagstaff *et al*. 1996; Alburges *et al*. 2001; Hanson *et al*. 2002). Finally, in situations where low doses of amphetamine increase the firing rate of cells in the CPu, higher doses can decrease firing rate (Rebec and Curtis 1983; Gardiner *et al*. 1988).

Given these differences between high and low doses of amphetamine, it is tempting to conclude that amphetamine recruits different neurotransmitter systems and/or intracellular signaling pathways as a function of dose, which could be true. It is also possible, however, that an interaction between dopamine and glutamate underlies both the increased response of ENK+ cells to moderate doses of amphetamine given in the novel environment, and the lack of response to higher doses. Although the interaction between dopamine and glutamate is yet to be clearly elucidated, and there are conflicting results (see Nicola *et al*. 2000 for review), many electrophysiological studies suggest that the interaction between dopamine and glutamate is not linear. Using single-unit recording techniques it has been shown that local application of low levels of DA enhance the response to iontophoretically applied glutamate in most striatal cells in anesthetized (Chiodo and Berger 1986; Hu and Wang 1988; Hu *et al*. 1990; Kiyatkin and Rebec 1996; Hu and White 1997) and freely moving rats (Nisenbaum *et al*. 1988), as well as in striatal cells administered a depolarizing pulse

(Akaike *et al*. 1987). In contrast, this effect is reversed at higher DA levels, which inhibit glutamatergic-induced neuronal firing. This occurs not only in the striatum, but also in the medial prefrontal cortex, where low levels of dopamine enhance the inward current produced by NMDA, but higher concentrations suppress the inward current (Zheng *et al*. 1999). Importantly, the ability of amphetamine to enhance extracellular dopamine in the CPu increases progressively between 1 and 10 mg/kg (Sharp *et al*. 1987; Butcher *et al*. 1988). Therefore, low and moderate doses of amphetamine may increase dopamine to levels that enhance glutamatergic activity, whereas the even greater levels of synaptic dopamine produced by very high doses of amphetamine may suppress glutamatergic activity. Given the role of glutamate in mediating IEG expression in ENK+ cells (discussed above), we speculate that the differential ability of high and low levels of dopamine to modulate glutamate activity in the CPu may be responsible for the inverted U-shaped dose-effect curve for amphetamine-induced c-*fos* in ENK+ cells.

The effect of amphetamine on the STN

The ability of amphetamine to increase c-*fos* expression in the STN has been reported previously (Wirtshafter and Asin 1999), and we recently found that environmental novelty enhances amphetamine- and cocaine-induced c-*fos* expression in the STN, relative to drug given at home (Ostrander *et al*. 2000; Uslaner *et al*. 2001b). Here we extend this finding to other doses of amphetamine (1 and 5 mg/kg), and show that the ability of novelty to enhance amphetamine-induced c-*fos* expression in the STN is dose-dependent. Specifically, at the highest dose examined (10 mg/kg) amphetamine enhanced c-*fos* expression in the STN to the same extent when it was given at home or in the novel environment.

Unfortunately, the neural mechanisms by which amphetamine engages the STN are largely unknown. We had previously hypothesized that the recruitment of ENK+ cells in the CPu may enhance c-*fos* mRNA expression in the STN by engaging the ‘indirect’ pathway. Standard descriptions of this circuit predict that engaging ENK+ cells should influence the STN via the striato-pallidal-STN pathway (Albin *et al*. 1989; Crossman 1990). However, our current results suggest that engaging the ‘indirect’ pathway is not the only means by which amphetamine induces c-*fos* in the STN, because high doses given at home or in the novel environment did not appear to engage ENK+ cells in the CPu, but did engage the STN. Consistent with this, others have reported that STN activity is not solely dependent upon the ‘indirect’ pathway. For example, a lesion of the globus pallidus, which prevents ENK+ cells from interacting with the STN through the ‘indirect’ pathway, has little effect on the ability of amphetamine to increase neuronal firing in the STN (Olds *et al*. 1998). Of course, the STN receives numerous projections, many of which could mediate the effects described here (Parent and Hazrati 1995). For example, the STN receives a direct DA input from the substantia nigra pars compacta (SNc) and ventral tegmental area (VTA) (Campbell *et al*. 1985; Canteras *et al*. 1990; Hassani *et al*. 1997) and DA agonists enhance neural activity (Mintz *et al*. 1986; Kreiss *et al*. 1996; Kreiss *et al*. 1997; Ni *et al*. 2001) and c-*fos* expression in the STN (Ruskin and Marshall 1995; Svenningsson and Le Moine 2002). In addition, the STN receives efferents from the prefrontal cortex (Canteras *et al*. 1990) and cortical stimulation enhances neuronal firing (Maurice *et al*. 1998) and c-*fos* expression in the STN (Sgambato *et al*. 1997). The possible influence of these circuits is particularly intriguing, given that the VTA, SNc, and the prefrontal cortex have been strongly implicated in the long-lasting behavioral and neurobiological effects of amphetamine (Eichler and Antelman 1979; Robinson *et al*. 1985; Vezina and Stewart 1990; Banks and Gratton 1995; Pierce and Kalivas 1997; Vanderschuren and Kalivas 2000).

The relationship between the ability of amphetamine to engage the STN, ENK+ cells and behavioral sensitization

Amphetamine produces more robust behavioral sensitization when it is administered repeatedly in a distinct and relatively novel environment, compared with when it is given in the home cage (Badiani *et al*. 1995a; Badiani *et al*. 1995b; Crombag *et al*. 1996; Badiani *et al*. 1997), but the ability of environmental context to modulate behavioral sensitization is dose-dependent. As dose is increased amphetamine induces robust behavioral sensitization at home and in a novel environment (Browman *et al*. 1998b). Therefore, if engaging ENK+ cells in the CPu were necessary to induce the robust behavioral sensitization produced by high doses, as dose is increased amphetamine should come to induce c-*fos* in ENK+ cells even when given at home. This did not happen. When given at home not only did increasing doses of amphetamine fail to recruit ENK+ cells, but so did 10 mg/kg given in the novel environment. This does not support the idea that engaging ENK+ cells, at least as indicated by c-*fos* mRNA expression, is critical to produce robust behavioral sensitization under all conditions.

Nevertheless, it is still possible that engaging the ‘indirect pathway’ (as indicated by c-*fos* expression in ENK+ cells) is responsible for the modulatory effect of context on sensitization. At low to moderate doses the induction of sensitization is facilitated by giving amphetamine in a distinct context, and this may be due to the recruitment of corticostriatal glutamate inputs, which is reflected by c-*fos* mRNA induction in ENK+ cells. At higher doses, however, the modulatory effect of environment on sensitization is lost, as is the ability to engage ENK+ cells. Thus, the neural mechanisms underlying the induction of sensitization may be qualitatively different depending upon the context surrounding drug

treatment and the dose of drug given. Obviously, this possibility carries significant implications for understanding the neurobiological mechanisms underlying sensitization.

Finally, the data reported here suggest that the role of the STN in sensitization may be under-appreciated. Low doses of amphetamine, which only produce sensitization when given in a novel environment, also induce c-*fos* in the STN only when given in a novel environment. As dose is increased, however, and the influence of context weakens, amphetamine begins to induce c-*fos* in the STN regardless of environmental context. Thus, there is a similar interaction between dose and context in producing sensitization and engaging the STN (as indicated by c-*fos* mRNA expression). The role of the STN in sensitization is unknown, but it is worth noting that activation of the STN enhances dopamine efflux in the striatum by increasing activity in the SNc (Smith and Grace 1992). Furthermore, stimulation of the STN can induce long-term potentiation (LTP) in the SNc (Overton *et al*. 1999). Given the hypothesized relationship between LTP, dopamine release, and behavioral sensitization (Wolf 1998; Hyman and Malenka 2001), further investigation of the role of the STN in sensitization is warranted.

In conclusion, this study clearly shows that the ability of amphetamine to engage neural circuits involved in the psychomotor activating and incentive motivational effects of drugs of abuse is powerfully modulated by the context in which drugs are administered, and that the effects of context interact with the effects of dose. This may be one reason why 'set and setting' are so important in modulating both the acute behavioral and subjective effects of drugs (Falk and Feingold 1987), as well as their ability to induce forms of drug experience-dependent plasticity, such as behavioral sensitization (Robinson *et al*. 1998).

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