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Cocaine-induced psychomotor activity is associated with its ability to induce c-*fos* mRNA expression in the subthalamic nucleus: effects of dose and repeated treatment

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Abstract

Factors that modulate the psychomotor activating effects of amphetamine and cocaine, such as environmental novelty and dose, also regulate the ability of these drugs to induce c-*fos* mRNA expression in the subthalamic nucleus (STN). We hypothesized therefore that engagement of the STN may be important for stimulant-induced psychomotor activation. To further test this hypothesis we examined whether repeated treatment with cocaine, which enhances its psychomotor activating effects (i.e. produces behavioural sensitization), also enhances its ability to induce c-*fos* expression in the STN. In addition, given that STN activity is thought to be influenced by preproenkephalin mRNA-containing (ENK+) neurons in the caudate–putamen, we also examined whether repeated cocaine treatment alters c-*fos* expression in ENK+ cells. We report that: (i) cocaine pretreatment enhances the ability of a cocaine challenge to induce c-*fos* mRNA expression in the STN, and this effect is most robust at challenge doses where behavioural sensitization is observed; (ii) the ability of cocaine to induce c-*fos* in the STN is independent of the ability of cocaine to engage ENK+ cells. These results support the idea that the STN is involved in stimulant-induced psychomotor activation and sensitization, but suggest that stimulant-induced engagement of the STN is not dependent on ENK+ cells in the caudate–putamen. These findings may have implications concerning the neurobiological mechanisms underlying the behavioural effects of psychostimulant drugs.

Introduction

Over the past several decades much research has sought to identify the neural systems that mediate the behavioural effects of psychostimulant drugs, such as amphetamine and cocaine. It is now clear that many of their behavioural effects are due in large part to their ability to increase dopamine neurotransmission in the nucleus accumbens and caudate–putamen (CPu). On the other hand, very little is known about the importance of striatofugal targets. One such structure, the subthalamic nucleus (STN), is a principal component of the so-called 'indirect pathway', a pathway which arises from enkephalin-containing cells in the CPu (ENK+ cells) (Albin *et al*., 1989).

We have recently found that factors which influence the ability of psychostimulants to produce psychomotor activation, such as environmental context and dose, also modulate their ability to induce c-*fos* mRNA expression in the STN. Specifically, low to moderate doses of amphetamine or cocaine given in a novel test environment induce more robust psychomotor activation and c-*fos* expression in the STN than when these doses are given in the home cage (Uslaner *et al*., 2001b; Ostrander *et al*., 2003; Uslaner *et al*., 2003). Higher doses of amphetamine, which produce robust psychomotor activation when given in either the home cage or a novel environment (Browman *et al*., 1998), induce robust c-*fos* expression in the STN independent of environmental context (Uslaner *et al*., 2003). Therefore, we hypothesized that the ability of stimulant drugs to engage the STN may be important for their ability to produce psychomotor activation.

To further test this hypothesis we examined whether repeated treatment with cocaine (7 days with 15 mg/kg/day), which enhances its ability to produce psychomotor activation (i.e. behavioural sensitization), would also enhance its ability to induce c-*fos* expression in the STN. In addition, in order to examine whether ENK+ cells in the CPu (and presumably the indirect pathway) are involved in the ability of cocaine to engage the STN, we characterized the ability of cocaine pretreatment to modulate cocaine-induced c-*fos* expression in ENK+ cells in the CPu, using dual *in situ* hybridization histochemistry.

Materials and methods

Animals

Seventy-three male Sprague-Dawley rats (Harlan, Indianapolis, IN, USA) weighing 200–250 g were housed individually in square clear plastic cages with 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 07.00 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

Cocaine hydrochloride (7.5, 15 or 30 mg/mL) was dissolved in 0.9% saline and administered i.p. in a volume of 1 mL/kg. All drug weights refer to the weight of the salts.

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Testing environment

All rats were treated in clear rectangular tubs (22 × 45 × 23 cm), each of which contained a clear plastic insert in the centre of the cage (6.5 × 23 × 23 cm) forming a corridor through which rats could locomote. Three sets of infrared photocells were located along the length of the tub and were spaced ≈11.5 cm apart from one another. A break was counted each time a photo beam was interrupted.

Testing procedures

Each pretreatment day, rats were transported from their home cages, placed into the testing environment and left to habituate for 30 min. The habituation period was used to attenuate the development of a conditioned response (Crombag *et al*., 2001). Following the habituation period, each rat received an i.p. injection of either saline or 15 mg/kg of cocaine. Sixty minutes following the injection, the rats were returned to their home cages in the animal colony. All rats received saline or cocaine treatment for 7 consecutive days, after which they were left undisturbed in the animal colony room for a 3-week withdrawal period. Following the withdrawal period, saline- and cocaine-pretreated rats were brought back to the test environment, habituated for 30 min and then given a challenge injection of saline or 7.5, 15, or 30 mg/kg of cocaine ($n = 8$–11/group).

Fifty minutes after the challenge injection each rat was decapitated and the brain was removed and immediately frozen in isopentane (−40 to −50 °C). The 50-min timepoint was chosen because it was used in previous studies and because it coincides with maximal c-*fos* mRNA expression (Badiani *et al*., 1998, 1999; Uslaner *et al*., 2001a; Uslaner *et al*., 2001b). The brains were stored in a −70 °C freezer until they were sectioned. A cryostat was used to cut 10-μm-thick coronal sections at the level of the CPu and STN (≈0.8 and 4 mm posterior to bregma, respectively). The sections were thaw-mounted onto 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 previously (Uslaner *et al*., 2001b) except that dual *in situ* hybridization was performed using ^{35}S-UTP- and ^{35}S-CTP-labelled riboprobes for c-*fos* mRNA (680-mer; courtesy of Dr T. Curran), and digoxigenin–UTP-labelled riboprobes complimentary for preprotachykinin mRNA [substance P (SP)] 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 & Watson (1995). Sections containing the STN were processed for single *in situ* hybridization using the riboprobe complementary to c-*fos* mRNA. Sections containing the CPu were processed for dual *in situ* hybridization using the riboprobe complementary to c-*fos* mRNA and preprotachykinin or preproenkephalin mRNA. Single-labelled sections were exposed to X-ray film (Kodak Biomax, MR) for ≈3 days. Double-labelled sections were dipped in Ilford KD-5 emulsion (Polysciences) and stored at 4 °C (≈7 days). After development (Kodak D-19), 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 Sony CCD camera from the X-ray film, and semiquantitative analysis was performed on the digitized autoradiograms using National Institute of Health Image Software (NIH Image). Pixels were counted when the optical density values were at least 3.5 SD 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 (Badiani *et al*., 1998; Day *et al*., 2001). Adjacent sections were stained with Cresyl Violet to help with anatomical localization.

Double-labelled tissue from the CPu was quantified using a Leica microscope (Leitz DMR, Wetzler, Germany) at a total magnification of 200×. Digoxigenin-labelled cells appeared as a purple precipitate in brightfield conditions, and a cell was considered positive only when it was a much darker hue of purple than background (background obtained from corpus callosum). ^{35}S-labelled cells appeared as densely packed silver grains in darkfield conditions, and a cell was considered positive only when the density of silver grains was clearly above background. The same criteria were used to analyse all slides, and the experimenter quantifying the tissue was blind to treatment conditions. We analysed 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. We examined these regions within the CPu because in previous studies we found that cocaine-induced c-*fos* expression was greatest in this area of the caudal CPu (Uslaner *et al*., 2001a).

Controls

To ensure that nonspecific 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 labelled separate cells within the CPu by performing dual *in situ* hybridization using ^{35}S-UTP- and ^{35}S-CTP-labelled preprotachykinin and digoxigenin-UTP-labelled preproenkephalin. We examined the same regions of the CPu as in the rest of the study. There were very few cells that were positive for both preproenkephalin and preprotachykinin mRNA in the CPu [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 *et al*., 1988).

Statistical analysis

To investigate whether there was a difference in locomotor behaviour (beam breaks) during the pretreatment phase, a mixed two-way ANOVA was conducted (Dose, two levels; Day, seven levels).

The results from the challenge test were analysed by making two sets of comparisons. (i) To determine whether a particular treatment enhanced locomotor behaviour (number of beam breaks) or c-*fos* mRNA expression above baseline, data were normalized by subtracting the mean of the saline challenged groups from the value for each individual subject, and then a one-sample *t*-test was used to determine whether group means were significantly different from zero. The cocaine- and saline-pretreated groups that received a saline challenge injection did not differ on any of the measurements examined, so they were combined. (ii) To determine whether cocaine-induced locomotor behaviour or c-*fos* mRNA expression varied as a function of pretreatment and/or dose, two-way ANOVAs (Pretreatment, two levels; Dose, four levels) were conducted, followed by Fisher's PLSD tests for pairwise comparisons. For all comparisons $\alpha < 0.05$.

In addition, we examined the relationship between c-*fos* expression in the STN and locomotor behaviour on the day of the challenge by calculating correlation coefficients, followed by Fisher's exact tests.

Results

Cocaine-induced psychomotor activity

The insert in the top panel of Fig. 1 shows the behavioural results from the pretreatment phase of the experiment. ANOVA revealed a significant interaction between Day and Dose ($F_{6,483} = 5.64$, $P < 0.0001$), which resulted from the increasing behavioural response produced by cocaine across pretreatment days (i.e. sensitization).

The top panel of Fig. 1 shows the behavioural results from the challenge injection administered 3 weeks after the final (7th) pretreatment injection. Saline-pretreated rats showed a significantly greater locomotor response to an injection of 30 mg/kg of cocaine, relative to baseline ($P < 0.04$), but none of the other doses produced a significant effect in saline-pretreated rats. In contrast, cocaine-pretreated rats that received either a 15 or 30 mg/kg cocaine challenge injection showed a significantly greater locomotor response than baseline ($P < 0.012$). Furthermore, there was an interaction between Dose and Pretreatment ($F_{3,64} = 3.73$, $P < 0.017$), which was because cocaine pretreatment produced a shift to the left in the dose–effect function for psychomotor activity. The effect of cocaine pretreatment was most pronounced and reached statistical significance when a challenge dose of 15 mg/kg was given ($P < 0.02$). Figure 2 shows the time course of the behavioural response to the challenge injection.

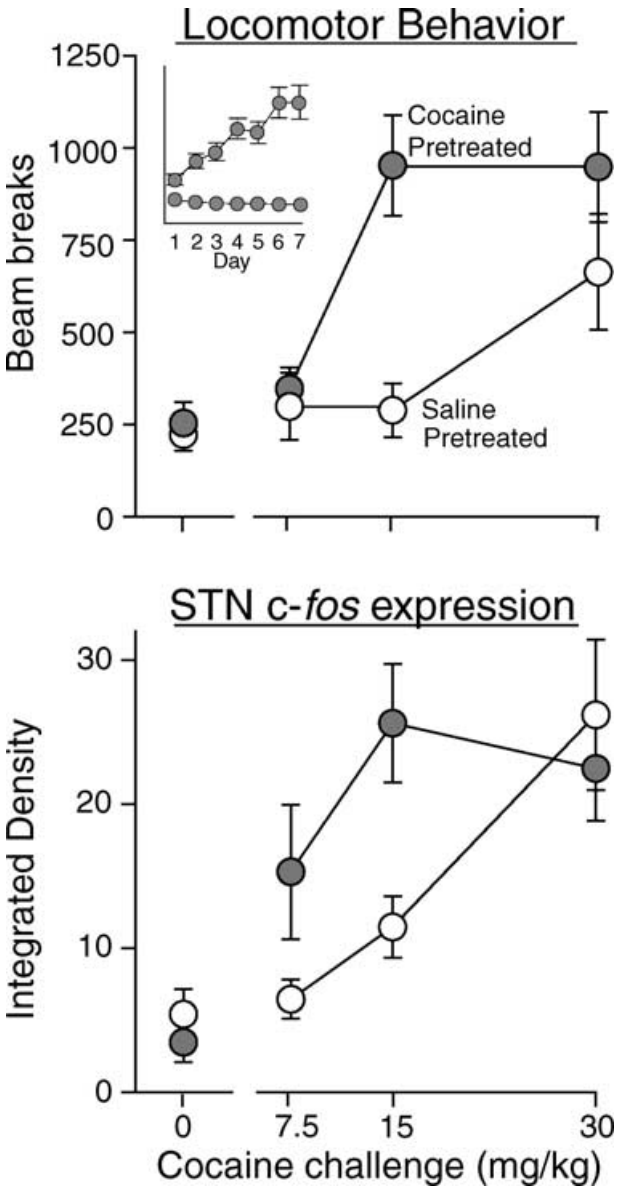

FIG. 1. Cocaine-induced psychomotor activation (beam breaks) and c-*fos* mRNA expression in the STN. Beam breaks were summed across the 45 min following saline or cocaine treatment. (Insert, top panel) The mean (± SEM) number beam breaks produced by saline or cocaine (15 mg/kg) during the 7 days of pretreatment. The open circles represent the group that was pretreated with saline, and the closed circles represent the group that was pretreated with cocaine. (Top panel) The mean (± SEM) number of beam breaks produced by a challenge injection of saline or of 7.5, 15 or 30 mg/kg of cocaine in rats pretreated with saline or cocaine. (Bottom panel) The expression of c-*fos* mRNA in the STN produced by a challenge injection of saline or of 7.5, 15 or 30 mg/kg of cocaine in rats pretreated with saline or cocaine, as indicated by analysis of relative integrated optical density values (arbitrary units).

*Cocaine-induced c-*fos *expression in the STN*

The bottom panel of Fig. 1 shows the dose–effect curve for cocaine-induced c-*fos* mRNA expression (integrated density) in the STN as a function of pretreatment condition. Animals pretreated with saline and given a 15 or 30 mg/kg cocaine challenge injection showed significantly enhanced levels of c-*fos* expression in the STN, compared to baseline ($P < 0.026$), but the effect of 7.5 mg/kg was not statistically significant. In contrast, animals pretreated with cocaine and challenged with either 7.5, 15 or 30 mg/kg of cocaine all showed a significant increase in c-*fos* expression in the STN, relative to baseline ($P < 0.032$). ANOVA revealed a significant Dose × Pretreatment interaction ($F_{3,64} = 2.94$, $P < 0.04$). Thus, similar to the psychomotor activating response, cocaine pretreatment produced a shift to the left in the dose–effect function for cocaine-induced c-*fos* expression in the STN. This effect of cocaine pretreatment was most pronounced and reached statistical significance when a challenge dose of 15 mg/kg of

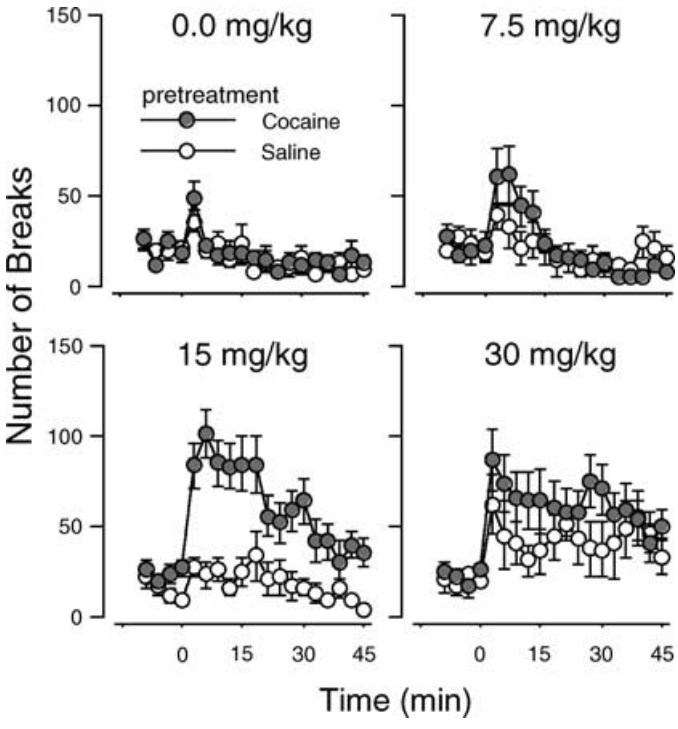

FIG. 2. The mean (± SEM) number of beam breaks over time (3-min intervals) produced by a challenge injection of saline or of 7.5, 15 or 30 mg/kg of cocaine in rats pretreated with saline or cocaine. Injections were given at time point 0. The open circles represent the group that had been pretreated with saline and the closed circles represent the group that had been pretreated with cocaine.

cocaine was given ($P < 0.02$), again similar to the effect of cocaine pretreatment on psychomotor activity (see above).

Given the similarity of the dose–effect functions for cocaine-induced c-*fos* expression in the STN and locomotor activity, correlations between these variables were examined. When all animals were included in the analysis and plotted individually, there was a significant correlation between c-*fos* expression in the STN and the number of beam breaks ($r = 0.66$, $P < 0.0001$; top panel of Fig. 3). Importantly, the correlation was greatest for those treatments that produced robust cocaine-induced behavioural effects. Thus, correlations between beam breaks and c-*fos* induction in the STN was greatest for animals receiving 15 or 30 mg/kg of cocaine following cocaine pretreatment ($r = 0.592$ and 0.627, respectively) or 30 mg/kg of cocaine following saline pretreatment ($r = 0.555$; data not shown). When the correlation analysis was conducted on the group means for c-*fos* expression in the STN and psychomotor activity, the correlation between these two measures was even greater ($r = 0.89$, $P < 0.02$; bottom panel of Fig. 3).

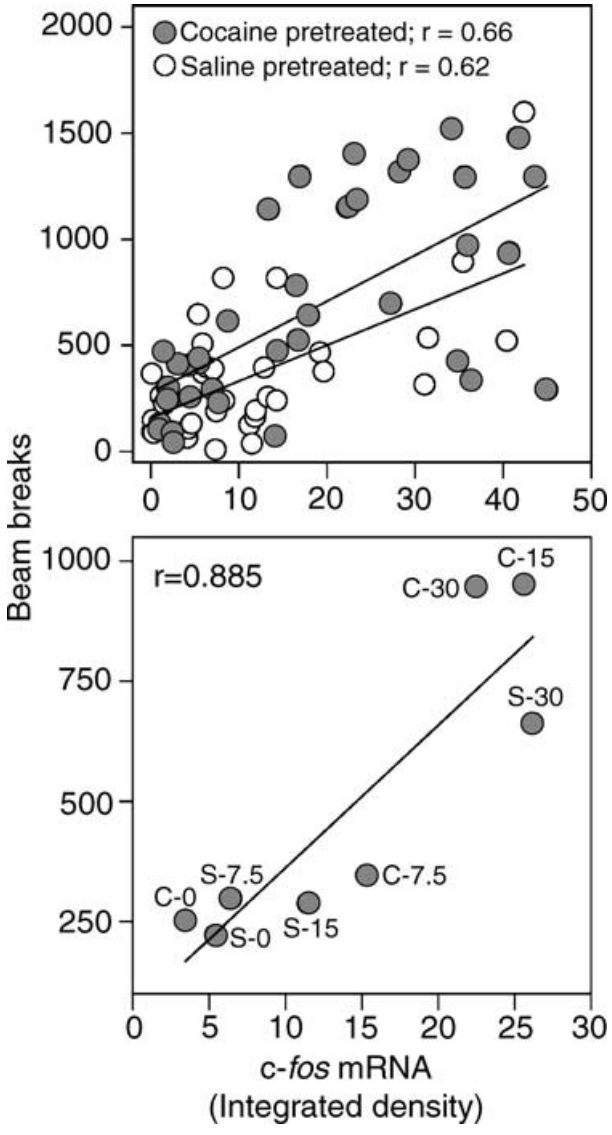

FIG. 3. Association between cocaine-induced psychomotor activation (*y*-axis; beam breaks) and c-*fos* expression in the STN (*x*-axis) when individual animals are plotted separately (top panel), and when group means are plotted separately (bottom panel). Abbreviations: r, correlation coefficient; S, saline pretreated; C, cocaine pretreated; 0, 7.5, 15 or 30, challenge dose of cocaine (mg/kg).

Cocaine-induced c-fos expression in the CPu

The top panel of Fig. 4 shows the number of c-*fos*+ cells in the CPu as a function of pretreatment condition and challenge dose. All doses of cocaine, irrespective of pretreatment, increased the number of c-*fos*+ cells in the CPu relative to baseline ($P < 0.008$). Higher doses of

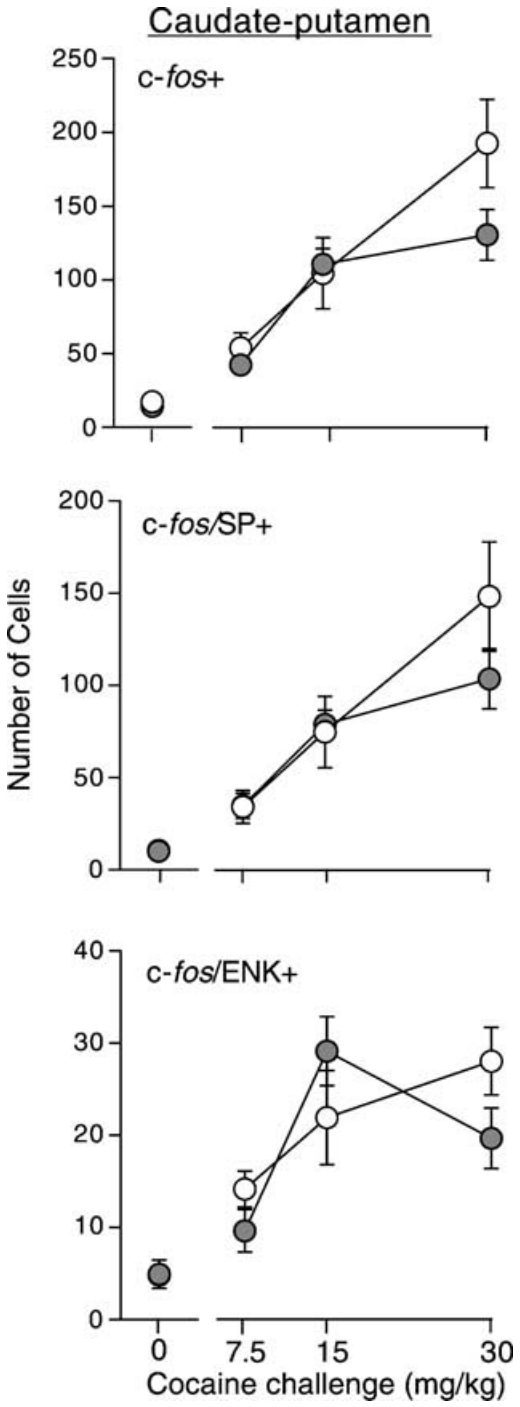

FIG. 4. Expression of c-*fos* mRNA in the CPu of rats given saline or 7.5, 15 or 30 mg/kg of cocaine following pretreatment with saline or cocaine. (A) The mean (± SEM) number of cells positive for c-*fos* mRNA as a function of dose and pretreatment. (B) The mean (± SEM) number of cells positive for c-*fos* mRNA that were also positive for preprotachykinin mRNA (SP+) as a function of dose and pretreatment. (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 pretreatment.

cocaine increased the number of c-*fos*+ cells in the CPu to a greater extent than lower doses (effect of Dose, $F_{3,65} = 36.452$, $P < 0.0001$), but there was no effect of Pretreatment ($F_{1,65} = 2.74$, $P = 0.1$) and no Dose × Pretreatment interaction ($F_{3,65} = 2.04$, $P = 0.117$).

The middle panel of Fig. 4 shows the number of SP+ cells that were also positive for c-*fos* (SP/c-*fos*+) in the CPu as a function of treatment condition and challenge dose. Irrespective of pretreatment condition, all doses of cocaine increased the number of SP/c-*fos*+ cells ($P < 0.03$). Higher doses increased the number of SP/c-*fos*+ cells to a greater extent than lower doses ($F_{3,64} = 27.094$, $P < 0.0001$), but there was no effect of Pretreatment ($F_{1,64} = 1.058$, $P = 0.308$) and no Dose × Pretreatment interaction ($F_{3,64} = 1.39$, $P = 0.253$).

The bottom panel of Fig. 4 shows the number of ENK+ cells that were also c-*fos*+ (ENK/c-*fos*+) in the CPu as a function of pretreatment condition and challenge dose. Other than the group pretreated with cocaine and given a 7.5-mg/kg challenge, all groups showed an increase in the number of ENK/c-*fos*+ cells relative to baseline. Higher doses increased the number of ENK/c-*fos*+ cells compared to lower doses ($F_{3,64} = 19.312$, $P < 0.0001$), but there was no effect of Pretreatment ($F_{1,64} = 0.392$, $P = 0.5337$) and no Dose × Pretreatment interaction ($F_{3,64} = 2.313$, $P = 0.0844$).

Discussion

We reported previously that conditions which enhance the ability of amphetamine and cocaine to produce psychomotor activation also increase their ability to induce c-*fos* expression in the STN (Uslaner *et al*., 2001b; Ostrander *et al*., 2003; Uslaner *et al*., 2003). We hypothesized therefore that the ability of these drugs to engage the STN may be important for their ability to produce robust psychomotor activation. This hypothesis was tested here by employing a cocaine pretreatment regimen that enhances the ability of a cocaine challenge to produce psychomotor activation (i.e. behavioural sensitization). We predicted that pretreatment with cocaine would also enhance its ability to induce c-*fos* expression in the STN. It did. Furthermore, given that ENK+ cells in the CPu influence the STN via the indirect pathway, we predicted that repeated treatment with cocaine would enhance its ability to induce c-*fos* expression in ENK+ cells. It did not. Each of these findings will be discussed in turn.

The relationship between psychomotor activation and engagement of the STN

The present findings clearly support the idea that engaging the STN may be important for the psychomotor activating effects of stimulant drugs. First, similar to our findings with a single injection of amphetamine (Uslaner *et al*., 2003), cocaine produced a dose-dependent increase in c-*fos* mRNA expression in the STN that was accompanied by a dose-dependent increase in psychomotor activation. Second, cocaine pretreatment produced a shift to the left in the dose–effect function for cocaine-induced c-*fos* mRNA expression in the STN that was very similar to the shift in the dose–effect function for psychomotor activation. Pretreatment with cocaine enhanced the ability of the 15 mg/kg cocaine challenge to induce c-*fos* mRNA expression in the STN and to produce psychomotor activation, relative to pretreatment with saline. The 30 mg/kg challenge of cocaine induced robust c-*fos* expression in the STN and robust psychomotor activation, regardless of the animal's drug history. These results extend previous findings from our laboratory demonstrating enhanced amphetamine-induced c-*fos* mRNA expression in the STN as a function of prior drug experience (Ostrander *et al*., 2003). Third, there was a positive correlation between cocaine-induced c-*fos* mRNA expression in the STN and psychomotor activation.

The role of the STN in stimulant-induced psychomotor activation has not received much attention until quite recently. There is, however, increasing evidence that it is involved. For example, it has been reported that acute injections of either methamphetamine or cocaine increases glucose utilization in the STN (Porrino *et al*., 1988; Pontieri *et al*., 1990), and acute amphetamine administration increases c-*fos* expression and cell firing in the STN (Ishida *et al*., 1998; Olds *et al*., 1998, 1999). In addition, bilateral STN lesions attenuate the ability of a single acute administration of cocaine to induce psychomotor activation (Baunez *et al*., 2002). Thus, the available literature, along with the results reported here, suggests that the STN plays an important role in mediating the psychomotor activating effects of stimulant drugs.

The mechanism(s) by which the STN influences the ability of stimulant drugs to produce psychomotor activation is unknown. However, glutamatergic projections from the STN to the ventral tegmental area (VTA) and the substantia nigra pars compacta (SNc) may be involved (Kita & Kitai, 1987; Groenewegen & Berendse, 1990). Stimulation of the STN enhances cell firing in midbrain dopamine neurons (Hammond *et al*., 1978; Smith & Grace, 1992), presumably engaging mesolimbic and nigrostriatal circuitry and inducing dopamine release in the striatum (Bruet *et al*., 2001). Indeed, glutamatergic agonists infused onto the STN induce Fos-immunoreactivity in the striatum and produce motor activation (Kearney & Albin, 2000). Importantly then, glutamate and dopamine are known to be critical for stimulant-induced psychomotor activation. Systemically administered glutamate or dopamine antagonists (Maj *et al*., 1972; Schlechter & Butcher, 1972; Karler *et al*., 1989; Karler *et al*., 1990), or lesions of dopamine projections to the striatum (Creese & Iversen, 1974; Kelly *et al*., 1975; Kelly & Iversen, 1976), block the psychomotor activating effects of both amphetamine and cocaine. Thus, by engaging mesolimbic and nigrostriatal circuitry, and thereby enhancing dopamine release in the striatum, engagement of the STN may potentiate stimulant-induced psychomotor activation. Of course, STN projections to other brain regions thought to be involved in stimulant-induced psychomotor activation, such as the globus pallidus (Kita & Kitai, 1987; Groenewegen & Berendse, 1990) and the striatum (Kita & Kitai, 1987; Parent & Smith, 1987), may also be important.

The relationship between stimulant-induced engagement of the STN and ENK+ cells in the CPu

Given that ENK+ cells are thought to influence the STN via the indirect pathway (Albin *et al*., 1989), we had hypothesized that engaging ENK+ cells in the CPu would be important for the ability of stimulant drugs to engage the STN. However, our current findings suggest that the ability of psychostimulant drugs to engage the indirect pathway (at least as indicated by c-*fos* mRNA expression in ENK+ cells) is not necessary to induce c-*fos* in the STN. Pretreatment with cocaine enhanced the ability of a 15-mg/kg challenge of cocaine to induce c-*fos* in the STN, but had no effect on the ability of cocaine to engage ENK+ cells. Others have also reported that repeated treatment with stimulant drugs does not enhance their ability to induce c-*fos* or Fos-immunoreactivity in ENK+ cells (Jaber *et al*., 1995; Moratalla *et al*., 1996; Canales & Graybiel, 2000). In addition, we have previously found that a high dose of amphetamine (10 mg/kg) induces robust c-*fos* mRNA expression in the STN but does not induce c-*fos* in very many ENK+ cells (Uslaner *et al*., 2003). Moreover, lesions of the globus pallidus, which prevent ENK+ cells from influencing the STN via the indirect pathway, do not dramatically alter the ability of amphetamine to increase neuronal firing in the STN (Olds *et al*., 1998).

Of course, there are a variety of other inputs that could be important for the ability of cocaine to engage the STN. The SNc and VTA send dopaminergic projections to the STN (Campbell *et al*., 1985; Canteras

et al., 1990; Hassani *et al*., 1997), and dopamine agonists enhance neural activity (Mintz *et al*., 1986; Kreiss *et al*., 1996, 1997; Ni *et al*., 2001) and c-*fos* expression in the STN (Ruskin & Marshall, 1995; Svenningsson & 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 involvement of these projections seems especially interesting, given the hypothesized involvement of these structures in the psychomotor activating effects of stimulant drugs (Eichler & Antelman, 1979; Robinson *et al*., 1985; Vezina & Stewart, 1990; Banks & Gratton, 1995; Pierce & Kalivas, 1997).

Conclusions

The current findings suggest that the STN contributes to the psychomotor activating effects of cocaine. The ability of cocaine to induce c-*fos* in the STN was enhanced by doses and treatment regimens that produced robust psychomotor activation. Furthermore, there was a significant correlation between drug-induced c-*fos* expression in the STN and psychomotor activation. In addition, given that the expression of behavioural sensitization was observed at challenge doses where there was a significant effect of pretreatment on cocaine-induced c-*fos* in the STN, the role of the STN in sensitization also merits consideration. In this regard, it is worth noting that stimulation of the STN produces LTP in dopamine-containing neurons in the SNc (Overton *et al*., 1999), which may contribute to the sensitization process (Wolf, 1998). Finally, given the possible relationship between sensitization and addiction (Robinson & Berridge, 1993), future consideration of the role of the STN in the reinforcing effects of psychostimulants seems warranted. Indeed, the STN has also been implicated recently in the incentive motivational effects of cocaine (Baunez *et al*., 2002).

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Abbreviations

CPu, caudate–putamen; ENK/c-*fos*+, ENK+ cells that were also c-*fos*+; ENK+ cells, enkephalin-containing cells in the CPu; SP, substance P; SP/c-*fos*+, SP+ cells that were also positive for c-*fos*; STN, subthalamic nucleus.

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