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Previous Exposure to VTA Amphetamine Enhances Cocaine Self-administration under a Progressive Ratio Schedule in a D_1 Dopamine Receptor Dependent Manner

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The effect of previous exposure to amphetamine (AMPH) in the ventral tegmental area (VTA) on the subsequent self-administration of cocaine was assessed. Rats in different groups were pre-exposed to three injections into the VTA of either saline (0.5μl/side) or AMPH (2.5μg/0.5μl/side). Injections were given once every third day. Starting 7–10 days after the last pre-exposure injection, rats were trained to self-administer cocaine (0.3 mg/kg/infusion) under fixed ratio 1 and 2 (FR1 and FR2) schedules and then tested under a progressive ratio (PR) schedule of reinforcement for six consecutive days. No differences between groups were observed during self-administration training under the FR schedules of reinforcement. However, when tested under the PR schedule, VTA AMPH pre-exposed rats worked more and, as a result, obtained more infusions of cocaine than saline pre-exposed rats. Rats in a separate group pre-exposed to VTA AMPH but co-infused with the D_1-like dopamine (DA) receptor antagonist SCH23390 (0.25 μg/0.5μl/side) did not show enhanced cocaine self-administration. These rats, as well as others pre-exposed to VTA SCH23390 alone showed levels of cocaine self-administration similar to saline pre-exposed rats. Thus, in a manner paralleling the sensitization of AMPH-induced locomotion and nucleus accumbens DA overflow, previous exposure to AMPH in the VTA leads to enhanced intravenous self-administration of cocaine and activation of D_1 DA receptors in this site during pre-exposure is necessary for the production of this effect.
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KEY WORDS: *Amphetamine; Cocaine; Ventral tegmental area; D_1 Dopamine receptor; Self-administration; Progressive ratio schedule of reinforcement; Sensitization; Cross-sensitization; SCH23390; Drug pre-exposure*

Repeated exposure to psychostimulants is known to result in long-lasting augmentations of the behavioral and neurochemical responses to these drugs. These enhanced responses, manifestations of psychostimulant sensitization (for review, see Kalivas and Stewart 1991) have been proposed by some to model psychostimulant addiction (e.g., Robinson and Berridge 1993; Di Chiara 1995). Consistent with this view, previous exposure to systemic amphetamine (AMPH) has been shown to en-

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hance the self-administration of AMPH (Piazza et al. 1989; Pierre and Vezina 1997, 1998) and cocaine (Horger et al. 1992; Valadez and Schenk 1994) when rats were tested under fixed ratio (FR) schedules of reinforcement. More recently, similar AMPH pre-exposure regimens have also been shown to promote the self-administration of higher doses of this drug in rats tested under a progressive ratio (PR) schedule of reinforcement (Mendrek et al. 1998; Lorrain et al. 2000; Vezina et al. 2002).

Amphetamine has been shown to act in the ventral tegmental area (VTA), but not other sites such as the nucleus accumbens (NAcc) or the prefrontal cortex (PFC), to initiate neuronal events leading to psychostimulant sensitization (for review, see Vanderschuren and Kalivas 2000). For example, repeated application of AMPH into the VTA is known to lead to sensitized locomotor (Kalivas and Weber 1988; Vezina and Stewart 1990; Hooks et al. 1992; Perugini and Vezina 1994; Cador et al. 1995; Vezina 1996) and NAcc dopamine (DA: Vezina 1993, 1996) responding to subsequent infusion of this and other drugs. Furthermore, rats previously exposed to AMPH in the VTA, but not in the NAcc, subsequently exhibit enhanced pursuit and self-administration of the drug (Vezina et al. 2002). Thus, AMPH actions in the VTA appear to be critical in inducing not only locomotor and neurochemical sensitization but also in promoting its self-administration.

It is likely that AMPH produces its effects in the VTA via actions at D_1 DA receptors in this site. AMPH, whether administered systemically (Kalivas and Duffy 1995; Wolf and Xue 1999) or locally into the VTA (Wolf and Xue 1998), is known to increase extracellular levels of DA in this site. This DA would then be available to activate DA receptors in this region. Indeed, it is known that induction of locomotor sensitization by AMPH requires the activation of D_1, but not D_2, DA receptors in the VTA (Stewart and Vezina 1989; Bjijou et al. 1996; see also Pierce et al. 1996). Similarly, it has been shown that sensitization of the ability of AMPH to increase extracellular levels of DA in the NAcc is prevented when the D_1-like DA receptor antagonist SCH23390 is co-administered with AMPH into the VTA during pre-exposure (Vezina 1996). Consistent with these findings, evidence also exists suggesting that the induction of enhanced self-administration by repeated AMPH pre-exposure also requires activation of D_1 DA receptors. Pierre and Vezina (1998) reported, for example, that systemically administering SCH23390 with AMPH during pre-exposure prevented the subsequent facilitation of self-administration of a low dose of the drug that is normally observed.

Considering the above findings, together with others showing cross-sensitization between AMPH and the locomotor effects of cocaine (Schenk et al. 1991; Hooks et al. 1992; Bonate et al. 1997) as well as the ability of cocaine to produce conditioned place preference (Shippenberg and Heidbreder 1995), it was hypothesized that infusions of AMPH into the VTA would lead to enhanced self-administration of cocaine. Furthermore, it was hypothesized that this enhancement would depend on the stimulation of D_1 DA receptors in the VTA during pre-exposure to AMPH in this site. To test these hypotheses, AMPH was infused into the VTA alone or in combination with SCH23390. Starting 9 to 15 days later, rats were tested for their self-administration of cocaine under a PR schedule of reinforcement.

METHODS

Subjects

Male Long-Evans rats (Harlan Sprague Dawley, Madison, WI; Toconic, Germantown, NY) weighing 250–275 g on arrival were used. They were individually housed with food and water freely available in a reverse cycle room (12/12 h light/dark; lights-on 7:30 p.m.) for the duration of the experiment. Rats were always tested during the dark period of the light cycle.

Apparatus

Fifteen chambers, each measuring 25 × 31 × 33 cm, were used for cocaine self-administration. Each chamber was made of stainless steel (rear and two side walls), a Plexiglas front hinged door and a tubular stainless steel ceiling and floor. These chambers were placed in a plastic box that shielded rats from extraneous disturbances. White noise was supplied in each box by a ventilating fan. A lever (5 cm above the floor) and a stimulus light (13.5 cm above the lever) were positioned on the right side wall. Each chamber was equipped with a liquid swivel system comprising of a steel-spring tether, a liquid swivel, and an infusion pump (Model A.E., Razel Scientific Inc., Stamford, CT) that allowed free movement of the rat in the chamber and delivery of drug upon depression of the lever. The tether was connected to the rat by screwing its captive collar onto the threaded portion of a custom-designed L-shaped Plastics One cannula (20 gauge) secured to the rat's skull (see Pierre and Vezina 1997). Lever presses and drug infusions were recorded and controlled via an electrical interface by a computer using locally developed software.

Drugs

S(+)-amphetamine sulfate (AMPH), (−)-cocaine hydrochloride (cocaine) and R(+)-SCH23390 hydrochloride (SCH23390) were obtained from Sigma, Inc. (Saint Louis, MO). Drugs were dissolved in sterile saline (0.9% w/v) for both i.v. and i.c. routes of administration. Doses refer to the weight of the salt.

Surgery

For all surgical procedures described below, rats were anesthetized with a mix of ketamine (100 mg/kg, i.p.) and xylazine (6 mg/kg, i.p.). For intra-cranial cannula implantation, rats were placed in a stereotaxic instrument with the incisor bar positioned 5.0 mm above the interaural line (Pellegrino et al. 1979). They were, then, implanted with chronic bilateral guide cannulae (22 gauge, Plastics One, Roanoke, VA) aimed at the VTA (A/P, −3.6; L, ±0.6; DV, −8.9 from bregma and skull). Cannulae were angled at 16° to the vertical and positioned 1 mm above the final injection site. After surgery, 28 gauge obturators were placed in the guide cannulae and rats were returned to their home cages for a 7–10 day recovery period.

For cocaine self-administration, rats were surgically implanted with an i.v. catheter into their right external jugular vein as described by Pierre and Vezina (1997). The intravenous catheter used was made of silastic tubing (Dow Corning, Inc., Midland, MI). Catheters were flushed daily with a 0.9% sterile saline solution containing 30 IU/ml heparin and 250 mg/ml ampicillin in order to promote patency. The catheters of nine rats nonetheless developed leaks or became blocked during the course of the experiment. These rats (VTA AMPH: 3; VTA saline: 4; VTA AMPH+SCH23390: 1; VTA SCH23390: 1) were excluded from further consideration.

All surgical procedures were conducted using aseptic techniques according to an approved IACUC protocol.

Intra-cranial Microinjections

Bilateral intra-cranial microinjections into the VTA were made in the freely moving rat. Injection cannulae (28 gauge) connected to 1 μl syringes (Hamilton, Reno, NV) via PE-20 tubing were inserted to a depth 1 mm below the guide cannula tips. Injections were made in a volume of 0.5 μl/side over 30 s. Sixty seconds later, the injection cannulae were withdrawn and the obturators were replaced.

Experimental Design

The experiment consisted of three phases: pre-exposure, cocaine self-administration training under FR schedules of reinforcements and cocaine self-administration testing under a PR schedule of reinforcement. Rats were randomly assigned to four experimental groups: VTA AMPH, VTA saline, VTA AMPH+SCH23390, VTA SCH23390.

Pre-exposure. Seven to ten days after implantation of bilateral guide cannulae aimed at their VTA, rats received a total of three microinjections corresponding to their pre-exposure condition: AMPH (2.5μg/0.5μl/side), saline (0.5μl/side), SCH23390 (0.25μg/0.5μl/side) or AMPH+SCH23390. The doses of AMPH and SCH23390 were selected based on the findings of Vezina (1996) and Vezina et al. (2002). In these studies, this dose of AMPH was sufficient to induce sensitization of its locomotor activating effects, its ability to increase extracellular levels of DA in the NAcc as well as its ability to support self-administration when microinjected into the VTA but not into sites adjacent to it. Co-administration of SCH23390 with AMPH at the above dose completely prevented the induction of sensitized NAcc DA responding and was without effect when administered alone (Vezina 1996). Although SCH23390 exhibits some affinity for $5HT_2$ receptors, it is unlikely that this characteristic contributes to its ability to block the induction of sensitization by AMPH because receptor antagonists with similar or greater affinity for this receptor but without action at the D_1 DA receptor have been found to be without effect (Vezina 1996). Injections were made once every third day.

Cocaine Self-administration Training under FR Schedules of Reinforcements. Training for cocaine self-administration began 7–10 days after the final drug pre-exposure injection and 3–5 days after rats received their i.v. catheters. Cocaine self-administration sessions were held daily and lasted for a maximum of 3 h. In all cases, reinforced presses on the lever delivered an infusion of cocaine through the i.v. catheter (0.3 mg/kg/infusion). This dose has been shown to lie at the lower end of the ascending limb of the dose-effect curve for cocaine on the PR schedule of reinforcement (see Richardson and Roberts 1996) and was selected to maximize detection of higher final ratios in the PR testing phase of the present experiment (see below). The cocaine solution was injected in volumes of 0.10–0.13 ml/infusion at a rate of 1.6 ml/min. For 15 s immediately following reinforced depressions of the lever, a stimulus light above the lever was lit and lever presses were recorded but did not lead to further infusions.

An experimenter-delivered priming infusion of cocaine (0.3 mg/kg, i.v.) was given at the beginning of each session. The initial schedule used was an FR1 and it was increased to an FR2 once rats successfully administered an additional nine infusions within the 3-h session. Rats were then again required to self-administer an additional nine infusions within a 3-h session under an FR2 schedule. Because the purpose of the present experiment was to study the effect of previous exposure to AMPH on the subsequent self-administration of cocaine, it was thought preferable to keep exposure to cocaine during self-administration training to a minimum. By using these procedures, previously described by Mendrek et al. (1998), Lorrain et al. (2000), and Vezina et al. (2002), it was possible to do this and establish lever press responding reinforced by cocaine (see saline

self-administration controls below). Rats that did not satisfy each of the FR1 and the FR2 criteria (i.e., nine infusions in a 3-h session) within five days were excluded from the study (VTA AMPH: 4; VTA saline: 3; VTA AMPH+SCH23390: 2; VTA SCH23390: 4). Each training session lasted until rats self-administered nine infusions or until a maximum of 3 h elapsed. Number of infusions obtained and days to satisfaction of the training criterion under each FR schedule were recorded.

Cocaine Self-administration Testing under a PR Schedule of Reinforcement. Upon satisfactory completion of self-administration training under the FR schedules, rats were tested on six consecutive days under a PR schedule of reinforcement. Under this schedule, the number of responses required to obtain each successive infusion of cocaine was determined by the expression $[5e^{(0.25 \times \text{infusion number})}] - 5$, rounded to produce the following sequence of required lever presses: 1, 3, 6, 9, 12, 17, 24, 32, 42, 56, 73, 95, 124, 161, 208 etc. (Richardson and Roberts 1996). The daily PR sessions lasted a maximum of 3 h or until 1 h elapsed without a drug infusion. The group mean time to final ratio attained (break point) observed throughout PR testing in cocaine self-administering rats ranged from 32.1 min in those rats pre-exposed to VTA saline to 117.7 min in rats previously exposed to VTA AMPH. Time to final ratio attained in the saline self-administering rats described below ranged from 4 min at the end of testing to 28.9 min at the beginning of testing. Priming infusions were not given during these sessions. The number of infusions obtained in each PR session was recorded.

Saline Self-administration Controls. In order to confirm that the self-administration behavior observed in the above rats was in fact reinforced by the cocaine infusions, eight additional rats were tested. These were surgically prepared with bilateral guide cannulae aimed at the VTA. Four were subsequently pre-exposed to VTA saline and the remaining four to VTA AMPH as described above. They were then fitted with i.v. catheters and beginning four days later (7–10 days after the final pre-exposure injection) were given the opportunity to self-administer saline (0.10–0.13 ml/infusion at a rate of 1.6 ml/min). Using procedures identical to those described for cocaine, these rats were first subjected to two days of saline self-administration training under an FR1 schedule of reinforcement followed by two days of training under an FR2 schedule. Two days were allotted to each schedule because the group mean number of days to criterion observed in the groups of rats self-administering cocaine fell between 1 and 2 (see Figure 1). An experimenter-delivered priming infusion of saline was given at the beginning of each training session. Control rats were then tested on six consecutive days for their self-administration of saline under the PR schedule of reinforcement. Priming infusions of saline were not given during these sessions. The number of infusions obtained under each schedule of reinforcement was recorded. In a manner paralleling the findings of experiments measuring locomotion following a saline challenge injection (e.g., Perugini and Vezina 1994), no statistically significant differences in the self-administration of saline were observed at any time between control rats pre-exposed to VTA saline and control rats pre-exposed to VTA AMPH. The data obtained for these two groups were therefore combined and compared with those obtained for cocaine self-administering rats previously exposed to VTA saline.

Histology

After completion of the experiments, rats were anesthetized with sodium pentobarbital and perfused via intracardiac infusion of saline and 10% formalin. Brains were removed and post-fixed in 10% formalin. Coronal sections (40 μm) were mounted onto gelatin-coated slides and subsequently stained with cresyl violet for verification of cannula tip placements. The brains of three additional rats were post-fixed in saline solution containing 10% formalin and 30% sucrose and prepared for tyrosine hydroxylase (TH) immunohistochemistry

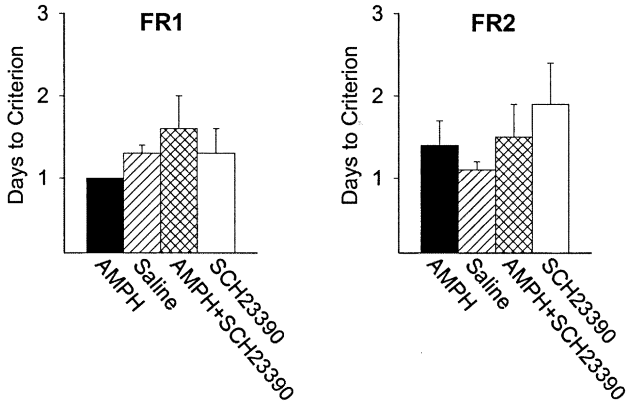

Figure 1. Cocaine self-administration during training under the FR1 (left) and the FR2 (right) schedules of reinforcement. Data are shown as the mean (+ SEM) number of days rats took to reach criterion (nine self-administered infusions within a 3-h session) for each schedule. Rats were initially exposed to an FR1 schedule of reinforcement and, following a single priming infusion of cocaine (0.3 mg/kg), they were allowed to self-administer nine additional infusions of the drug. Once rats self-administered the required nine infusions, they were switched to an FR2 schedule, once again given a single priming infusion and then allowed to self-administer nine additional infusions. Pre-exposure condition, indicated by group names at abscissae (VTA AMPH (n = 9), VTA saline (n = 10), VTA AMPH+SCH23390 (n = 11), VTA SCH23390 (n = 9)), did not significantly influence the acquisition of self-administration of this dose of cocaine under either schedule.

using procedures adapted from those described by Bencsics et al. (1996).

Data Analyses

The data obtained during self-administration training (days to criterion) were analyzed with 2-way between ANOVA with pre-exposure (two levels: AMPH and saline) and D_1 DA receptor blockade (two levels: SCH23390 and saline) as the between factors. The data obtained during self-administration testing (number of infusions obtained under the PR schedule of reinforcement) were analyzed with 2-way between 1-way within ANOVA with the above between factors and days of testing (6) as the within factor.

The data obtained for the saline self-administration controls were compared with those obtained for cocaine self-administering rats previously exposed to VTA saline using *t*-tests for independent samples (FR1 and FR2 schedules) and 1-way between 1-way within ANOVA with self-administered drug as the between factor (two levels: AMPH and saline) and days of testing (6) as the within factor. For all schedules, the data analyzed were number of infusions obtained. For each of the FR schedules, the number of infusions obtained on the two days of training was averaged for each of the saline self-administering rats as well as for the few cocaine self-administering rats that required more than one day to satisfy the FR criteria.

The number of infusions obtained in a PR session was used for statistical analysis rather than the number of presses emitted or the final ratios obtained, since the latter were, by definition, generated from an exponential function (Richardson and Roberts 1996). Post-hoc Scheffé tests were made according to Kirk (1968).

RESULTS

Cocaine Self-administration Training under the FR Schedules of Reinforcement

In agreement with previous reports (Mendrek et al. 1998; Lorrain et al. 2000; Vezina et al. 2002), rats in all four pre-exposure groups readily satisfied the self-administration training criteria and did so in similar fashion. On average, rats in the different groups satisfied each of the FR1 and FR2 criteria in one to two days (Figure 1). The ANOVA conducted on these data revealed no significant effects. Days to achieve the FR1 criterion: pre-exposure ($F_{1,35} = 0.26$, ns), D_1 DA receptor blockade ($F_{1,35} = 1.14$, ns), interaction ($F_{1,35} = 0.89$, ns). Days to achieve the FR2 criterion: pre-exposure ($F_{1,35} = 0.08$, ns), D_1 DA receptor blockade ($F_{1,35} = 1.65$, ns), interaction ($F_{1,35} = 0.89$, ns).

Cocaine Self-administration Testing Under the PR Schedule of Reinforcement

Unlike what was found during self-administration training under the FR schedules, pre-exposure condition significantly impacted performance under the PR schedule of reinforcement (Figure 2). As predicted, rats previously exposed to VTA AMPH worked more and obtained significantly more infusions than rats in the remaining groups. Importantly, rats previously exposed to AMPH but co-infused with SCH23390 did not

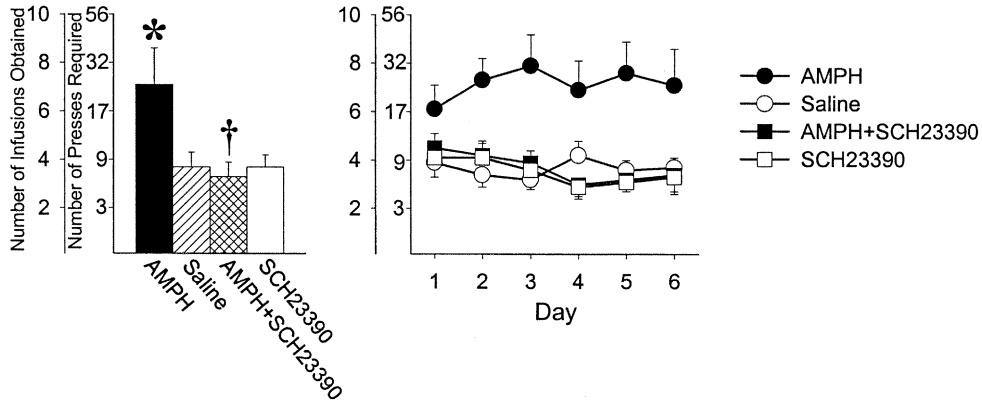

Figure 2. Previous exposure to VTA AMPH enhanced the self-administration of cocaine under the PR schedule of reinforcement in a D_1 DA receptor-dependent manner. Data are shown as mean (± SEM) number of infusions obtained. The number of presses required to obtain each successive infusion is also shown. Rats in the different pre-exposure groups, indicated by the names at the abscissa on the left and in the legend on the right (VTA AMPH (n = 9), VTA saline (n = 10), VTA AMPH+SCH23390 (n = 11), VTA SCH23390(n = 9)), were tested for their self-administration of cocaine (0.3 mg/kg/infusion) under the PR schedule. The bar graph to the left was derived from means of the values obtained for each subject on each of the six PR test days. These are shown to the right as group means. * $p < .05$, vs saline pre-exposed rats; † $p < .05$, vs AMPH pre-exposed rats; as revealed by post-hoc Scheffé comparisons following ANOVA.

show significantly enhanced cocaine self-administration on the PR test days. The ANOVA conducted on these data revealed significant effects of pre-exposure ($F_{1,35} = 5.65$, $p < .025$) and D_1 DA receptor blockade ($F_{1,35} = 6.98$, $p < .025$) but not day ($F_{5,175} = 0.77$, ns). The ANOVA also revealed significant Pre-exposure X D_1 DA receptor blockade ($F_{1,35} = 7.42$, $p < .01$) and D_1 DA receptor blockade X Day ($F_{5,175} = 2.28$, $p < .05$) interactions. The remaining interactions did not achieve statistical significance.

When the number of infusions obtained was averaged over the six PR test days (see Figure 2, left), the mean (±SEM) number of infusions obtained by the different groups was: VTA AMPH (7.15 ± 1.03), VTA saline (3.67 ± 0.58), VTA SCH23390 (3.71 ± 0.46), VTA AMPH+SCH23390 (3.28 ± 0.58). Post-hoc Scheffé comparisons showed that only VTA AMPH pre-exposed rats obtained significantly more cocaine infusions compared with saline pre-exposed rats ($p < .01$). Co-administration of the D_1-like DA receptor antagonist SCH23390 with AMPH during pre-exposure blocked this effect ($p < .01$). Previous exposure to VTA SCH23390 alone did not have any significant effect on cocaine self-administration when compared with saline pre-exposure.

Saline Self-administering Controls

When the behavior observed in cocaine self-administering rats previously exposed to VTA saline was compared with that of rats self-administering saline, it was clear that cocaine served as a reinforcer. Rats self-administering cocaine obtained significantly more infusions than rats self-administering saline on the FR1 ($t(16) = 5.17$, $p < .001$; Figure 3, panel A), the FR2 ($t(16) = 6.86$, $p < .001$; Figure 3, panel B) and the PR (Figure 3, panel C) schedules. The ANOVA conducted on the data obtained under the latter schedule revealed a significant effect of self-administered drug ($F_{1,16} = 9.74$, $p < .01$) and a significant Drug X Day interaction ($F_{5,80} = 3.64$, $p < .01$). It can be seen in Figure 3, panel C that the number of lever presses exhibited by saline self-administering rats diminished progressively with days of testing on the PR schedule so that by day 4, most rats exhibited little or no lever pressing and obtained few or no infusions.

Histology

Only data obtained from rats with both cannula tips placed in the VTA were retained for statistical analyses. Fifteen rats were excluded because they did not satisfy this criterion (VTA AMPH: 2; VTA saline: 4; VTA AMPH+SCH23390: 5; VTA SCH23390: 4). Figure 4, panel A, illustrates the location of the injection cannula tips in the VTA for the remaining rats in the different pre-exposure groups that were tested for cocaine self-administration. Also shown are photomicrographs illustrating a representative injection cannula tip in the VTA (arrow in Figure 4, panel B) and, at higher power magnification, TH positive cells in close proximity to the injection cannula tip (Figure 4, panel C). Little evidence for neurotoxicity was detected beyond the mechanical damage produced by penetration of the cannulae.

DISCUSSION

In the present experiment, it was found that previous exposure to AMPH in the VTA enhanced the subsequent self-administration of cocaine. This effect required the local activation of D_1 DA receptors because blockade of these receptors in the VTA during pre-exposure to AMPH prevented the facilitation of cocaine self-administration produced by this drug. Previous exposure to SCH23390 alone had no effect on rats' subsequent self-administration of cocaine. These findings parallel those obtained for sensitization of locomotion and NAcc DA overflow by VTA AMPH. Previous exposure to the regimen of VTA AMPH infusions used in the present experiment is known to lead to sensitization of its locomotor (Kalivas and Weber 1988; Stewart and Vezina 1989; Hooks et al. 1992; Perugini and Vezina 1994; Cador et al. 1995; Vezina 1996) and NAcc DA (Vezina 1993, 1996) activating effects. Moreover, both incidences of sensitization are prevented when SCH23390 is co-infused with AMPH into the VTA during pre-exposure (Stewart and Vezina 1989; Bjijou et al. 1995; Vezina 1996). Together, these findings suggest that exposure to AMPH in the VTA initiates common neuroadaptational processes involving D_1 DA receptors in this site that may contribute to the sensitization of a number of responses associated with psychostimulant drugs, including locomotion, NAcc DA overflow and drug self-administration.

The present results are consistent with and extend previous findings showing locomotor cross-sensitization between systemic AMPH pre-exposure and cocaine (Schenk et al. 1991; Hooks et al. 1992; Bonate et al. 1997), facilitating effects of systemic AMPH pre-exposure on cocaine (Horger et al. 1992; Valadez and Schenk 1994) and AMPH (Piazza et al. 1989; Pierre and Vezina 1997; Mendrek et al. 1998; Lorrain et al. 2000) self-administration as well as facilitation of AMPH self-administration by VTA, but not NAcc, AMPH pre-exposure (Vezina et al. 2002). The enhancement of cocaine self-administration by VTA AMPH pre-exposure was observed, in the present experiment, not when rats were trained under the FR1 and FR2 schedules but rather when they were required to work progressively more for each successive cocaine infusion under the PR schedule of reinforcement. This finding, again consis-

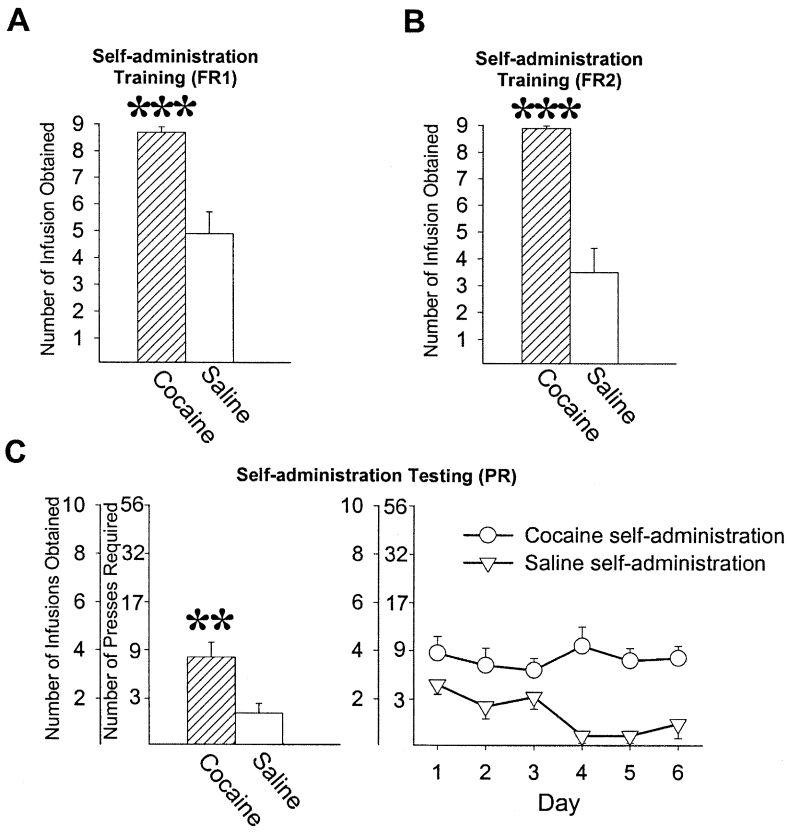

Figure 3. Comparison of self-administration behavior supported by cocaine and saline. Rats previously exposed to VTA saline and self-administering cocaine (0.3 mg/kg/infusion) obtained significantly more infusions than rats self-administering saline on each of the FR1 **(A)**, the FR2 **(B)** and the PR **(C)** schedules of reinforcement. Data are shown as mean (± SEM) number of infusions obtained. PR data for cocaine self-administering rats are from Figure 2. Group names at the abscissae indicate solution self-administered. ** $p < .01$, *** $p < .001$, vs saline self-administering rats.

tent with previous reports (Mendrek et al. 1998; Lorrain et al. 2000; Vezina et al. 2002), suggests that the effects of previous drug exposure are linked not so much to the self-administration of cocaine per se but rather to enhanced motivation to engage in this behavior as revealed under conditions of progressively increasing workload.

The present results are also consistent with, and extend, earlier findings showing that the systemic administration of SCH 23390 with AMPH during pre-exposure prevents the facilitation of AMPH self-administration normally produced by this drug (Pierre and Vezina 1998). These findings, together with others showing that systemic (Vezina and Stewart 1989; Drew and Glick 1990; Vezina 1996) and intra-VTA (Stewart and Vezina 1989; Bjijou et al. 1996; Vezina 1996) infusions of SCH23390 equally prevent the induction of sensitization of the locomotor and NAcc DA activating effects of AMPH, point to a critical role for D_1 DA receptors in the VTA in the initiation of long-term neuronal and behavioral adaptations by AMPH in this site. Indeed, selective activation of D_1 DA receptors in the VTA is known to produce sensitization of cocaine's locomotor and NAcc DA activating effects (Pierce et al. 1996). In addition, evidence indicates that D_1 DA receptor-initiated second messenger cascades in the VTA, such as those involving components of the cAMP signal transduction pathway, may be critical for the development of behavioral and neurochemical sensitization by AMPH. For example, locomotor sensitization by psychostimulant drugs is prevented by inhibition of adenylate cyclase and cAMP-dependent protein kinase in this site (Steketee 1994; Tolliver et al. 1999). Conversely, repeated infusion of the activator of adenylate cyclase, cholera toxin, into the VTA has been reported to produce long-lasting sensitization of the locomotor response to AMPH (Tolliver et al. 1999).

Because D_1 DA receptors do not appear to be synthesized by DA neurons in the VTA (Mansour et al. 1992), it is likely that they contribute to the induction of sensitization by AMPH by virtue of their expression on afferent terminals in this site. Of the varied neurotransmitter systems known to innervate the VTA (Kalivas 1993), neurons containing γ-aminobutyric acid (GABA) and glutamate have attracted attention because their release into this site is known to be modulated by D_1 DA receptors (Cameron and Williams 1993; Kalivas and Duffy 1995; Wolf and Xue 1998) and to influence the induction of psychostimulant sensitization (Kalivas and Stewart 1991; Kalivas and Alesdatter 1993; Kim and Vezina 1998; Cador et al. 1999; Vezina and Queen 2000). One possible result of the sensitization process may thus be an alternation in the ability of D_1 DA receptors to influence the afferent regulation of VTA DA neurons

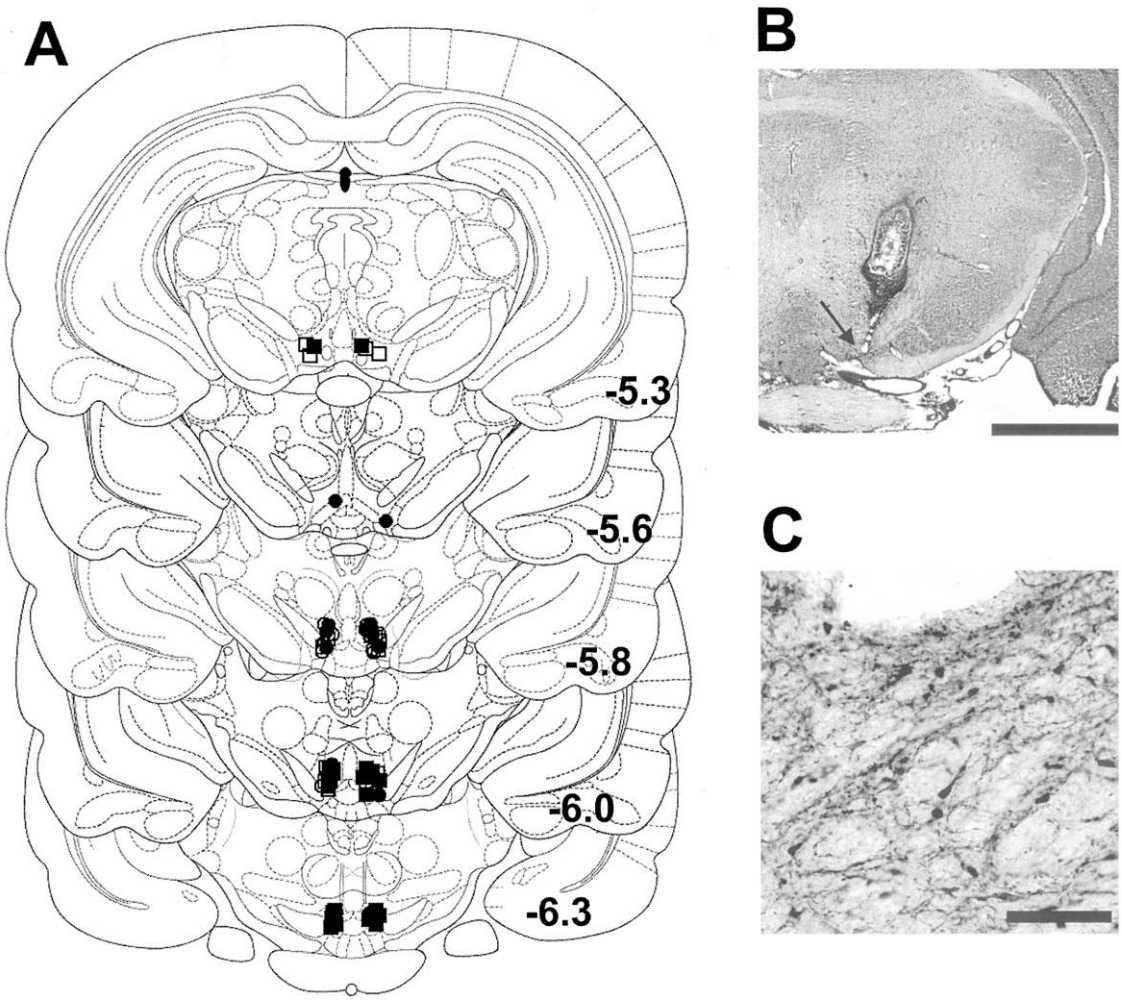

Figure 4. Injection cannula tip placements in the VTA. Location of the injection cannula tips of the rats in the different pre-exposure groups tested for cocaine self-administration and included in the data analyses is illustrated in **A**. Line drawings are from Paxinos and Watson (1997). Numbers to the right indicate mm from bregma. Symbols indicate group affiliation: ● VTA AMPH; ○ VTA saline; ■ VTA AMPH+SCH23390, □ VTA SCH23390. The photomicrographs illustrate a representative injection cannula tip in the VTA (arrow in **B**) and TH positive cells in close proximity to it **(C)**. Scale bars, 2 mm in **B** and 100 μm in **C**.

by GABA and glutamate. For example, previous exposure to systemic cocaine has been reported to inhibit the $GABA_B$ receptor-mediated inhibitory postsynaptic potentials observed in VTA DA cells (Bonci and Williams 1996) and to augment the increase in the extracellular glutamate levels (Kalivas and Duffy 1998) normally produced by D_1 DA receptor activation in this site. Alternatively, the activation of D_1 DA receptors by somatodendritically released DA during the sensitization process may recruit glutamate and its activation of glutamate receptors expressed by DA perikarya in the VTA (Wang and French 1993) to initiate long-term intracellular adaptations in these neurons. The inhibition of protein synthesis in the VTA, for example, has been reported to block the induction of locomotor sensitization by cocaine (Sorg and Ulibarri 1995), possibly by preventing the accumulation of altered levels of candidate proteins for transport to the NAcc where psychostimulants are known to produce enhanced responding in sensitized rats (Perugini and Vezina 1994; Cador et al. 1995).

The descending excitatory amino acid projection from the PFC to the VTA has been specifically implicated in the induction of psychostimulant sensitization. These neurons express D_1 DA receptors on their terminals (Lu et al. 1997) and lesions of the PFC that remove their input to the VTA prevent the development of sensitization by systemic cocaine (Tzschentke and Schmidt 1998) as well as systemic and intra-VTA AMPH (Cador et al. 1999). Interestingly, recent neuroanatomical (Carr and Sesack 2000), neurochemical (Takahata and Moghaddam 2000) and electrophysiological (Lokwan et

al. 1999) data suggest that these PFC-VTA neurons may not exert their effects via direct actions on VTA-NAcc DA neurons. Rather, their impact on these DA neurons appears to be polysynaptically mediated and may involve local midbrain circuits using GABA, glutamate, acetylcholine, and perhaps other neurotransmitters originating in the VTA and sites (e.g., pedunculopontine and laterodorsal tegmentum; see Lokwan et al. 1999; Forster and Blaha 2000) projecting to it. The contribution of these local circuits to the enhancement of psychostimulant self-administration produced by previous exposure to VTA AMPH remains to be elucidated.

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