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D_1 Dopamine Receptor Activation Is Necessary for the Induction of Sensitization by Amphetamine in the Ventral Tegmental Area

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Repeated intermittent exposure to amphetamine produces long-term enhancements in the ability of this drug to produce locomotion and increase extracellular dopamine (DA) in the nucleus accumbens (NAcc). Three experiments were conducted to evaluate the role played by D_1 DA receptors in the production of these changes in response to amphetamine. Rats were preexposed to amphetamine, alone or with a DA receptor antagonist, and tested for sensitization 1–3 weeks after the last drug injection. On the test for sensitization, locomotor (experiments 1 and 2) and NAcc DA (experiment 3) responses of the animals to a systemic amphetamine injection were assessed. In the first experiment, systemic injections of the D_1 DA receptor antagonist SCH23390, but not other DA receptor antagonists with greater affinity for D_2 DA and 5-HT_2 receptors, blocked the development of locomotor sensitization produced by systemic injections of amphetamine. In the second experiment, locomotor sensitization induced by infusion of amphetamine into the ventral tegmental area (VTA) was blocked when these injections were preceded by systemic injections of SCH23390. Finally, in experiment three, co-injecting SCH23390, but not its inactive enantiomer, with amphetamine into the VTA during preexposure prevented sensitization of the NAcc DA response to this drug. These results indicate that while D_2 DA receptor activation is not necessary for the induction of locomotor sensitization to amphetamine, D_1 DA receptors located in the VTA play a critical role in the development of sensitized locomotor and NAcc DA response to this drug.

Key words: amphetamine; sensitization; ventral tegmental area; nucleus accumbens; locomotion; dopamine

Psychomotor-stimulant drugs such as amphetamine produce locomotor stimulant effects that become enhanced with repeated intermittent injection. This enhancement in behavioral response, termed behavioral sensitization, is enduring and has been demonstrated up to 1 year after drug exposure in the rat (Paulson et al., 1991). Studies of the neurobiological basis of behavioral sensitization to psychomotor-stimulant drugs have concentrated on the midbrain dopamine (DA) systems and in particular on the mesoaccumbens DA system because of the considerable evidence indicating that this system mediates the locomotor-activating effect of these drugs as well as their ability to elicit craving and lead to abuse (Kuczenski, 1983; Robinson and Becker, 1986; Kuczenski and Segal, 1989; Kalivas and Stewart, 1991; Nestler, 1992; Robinson and Berridge, 1993). A number of short-term changes in mesoaccumbens DA neurotransmission have been reported (Wolf et al., 1994). These are observed 1 hr to 3 d after the last drug injection, and they diminish with time. The change in mesoaccumbens DA neurotransmission associated most consistently with behavioral sensitization to psychomotor stimulant drugs, on the other hand, is not seen 3–4 d after the last drug injection but rather seems to increase with time. Enhanced drug-induced increases in levels of extracellular nucleus accumbens (NAcc) DA have been demonstrated 1 week to 2 months after the last drug injection, indicating that this change may be associated with the persistence of behavioral sensitization to these drugs (Robinson et al., 1988; Robinson, 1991; Segal and Kuczenski, 1992a,b; Kalivas and Duffy, 1993a; Wolf et al., 1993; Paulson and Robinson, 1995).

While amphetamine is known to increase acutely extracellular levels of DA in the NAcc as well as in the ventral tegmental area (VTA) (Kalivas and Duffy, 1993a,b), it is an action of amphetamine in the latter cell body region of mesoaccumbens DA neurons that is responsible for the induction of behavioral sensitization. Infusions of amphetamine into the VTA lead to sensitized behavioral response to subsequent systemic injections of amphetamine, cocaine, and morphine (Kalivas and Weber, 1988; Vezina and Stewart, 1990; Hooks et al., 1992) as well as to injections of amphetamine into the NAcc (Perugini and Vezina, 1994; Cador et al., 1995). The increase in NAcc DA produced by systemic amphetamine is also enhanced by previous exposure to intra-VTA amphetamine (Vezina, 1993).

Such findings suggest that the DA released somatodendritically by amphetamine may act at DA receptors in the VTA to produce sensitized behavioral and NAcc DA responses to subsequent drug challenge. The D_1, D_2, and D_3 DA receptor subtypes are expressed in the VTA. While D_2 and D_3 receptors are associated with DA neurons, and at least the D_2 receptor plays an autoreceptor role, the D_1 receptor does not seem to be synthesized by DA neurons (Mansour et al., 1990, 1992; Bouthenet et al., 1991). Recently, it was shown that the stimulation of D_1 receptors in the VTA produces a dose-dependent increase in extracellular levels of GABA and glutamate in this site (Cameron and Williams, 1993; Kalivas and Duffy, 1995). GABA and excitatory amino acid neuron terminals, as well as those of several other neurotransmit-

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ters, have been identified in the VTA, and both classes of neurotransmitter, as well as others, are known to modulate mesoaccumbens DA neuron activity (see Kalivas, 1993). D_1 receptors, perhaps by virtue of being expressed on these afferent terminals, seem to be positioned critically to exercise control on DA neurotransmission in the VTA. It is possible, therefore, that an action of the DA released somatodendritically by amphetamine at these D_1 receptors contributes importantly to the induction of sensitization by amphetamine in the VTA. Three experiments were conducted to evaluate this possibility.

MATERIALS AND METHODS

Subjects

Male Sprague–Dawley rats were used. The rats weighed 251–275 gm on arrival from Charles River Canada (St. Constant, Quebec, Canada) or Harlan Sprague–Dawley (Madison, WI). They were housed individually in a 12 hr light/dark reverse cycle room, with food and water available at all times, and were allowed to acclimate to these conditions for 3–4 d before the start of any procedures. In experiments requiring intracranial cannulation, rats were anesthetized with sodium pentobarbital (55 mg/kg, i.p.) and 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 [anterior-posterior (A/P), −3.6; lateral (L), ±0.6; dorsoventral (D/V), −8.9 from skull] and with an additional guide cannula (20 gauge, Kinetrods, Ottawa, Canada) aimed at the left NAcc (A/P, +3.6; L, −1.5; D/V, −6.5 to −8.5 from skull) in the microdialysis experiment. Cannulae aimed at the VTA were angled at 16° to the vertical and positioned 1 mm above the final injection site. The cannula aimed at the NAcc was implanted vertically and positioned 5 mm above the ventral-most aspect of this nucleus. All cannulae were secured with dental acrylic cement anchored to stainless steel screws fixed to the skull. After surgery, 28 gauge Plastics One obturators and 25 gauge dummy probes (cellulose fiber absent and stainless steel tubing sealed) were inserted into the VTA and NAcc guide cannulae, respectively, and the rats were returned to their home cages for a 10 d recovery period. At the completion of the experiments, rats were anesthetized and perfused via intracardiac infusion of saline and 10% formalin. Brains were removed and postfixed in 10% formalin and 30% sucrose, and 40 μm coronal sections were stained subsequently with cresyl violet for verification of cannulae tip and dialysis probe placements.

Design and procedure

All experiments consisted of a drug preexposure phase followed by a test for sensitization 1–3 weeks later. Animals were injected and tested only during their dark cycle.

Experiment 1. During the drug preexposure phase, different groups of rats were administered either saline (1 ml/kg, i.p.), the D_1 DA receptor antagonist SCH23390 (0.1 mg/kg, s.c.), or a D_2 DA receptor antagonist before each of five injections of saline or amphetamine (1.0 mg/kg, i.p.) given once every third day. The D_2 DA receptor antagonists tested were spiperone (2.0 mg/kg, i.p.), eticlopride (1.0 mg/kg, i.p.), and (±)sulpiride (100 or 200 mg/kg, i.p.). The first injection preceded the second by 0.25–1.0 hr, depending on the DA receptor antagonist that was injected. Immediately after the second injection, rats were placed in activity boxes, and their locomotor activity was measured for 2 hr. The doses of DA receptor antagonists used were determined on the basis of previous reports and their ability to block completely the locomotor-activating effects produced by 1.0 mg/kg (i.p.) amphetamine. On the test for sensitization, which was conducted 7–10 d after the last preexposure injection, all animals were injected with amphetamine (0.5 mg/kg, i.p.; no DA receptor antagonists were administered) and placed in the activity boxes, and their locomotor activity was measured again for 2 hr.

Experiment 2. During the drug preexposure phase, different groups of rats received three bilateral injections into the VTA of saline (0.5 μl/side), amphetamine (2.5 μg/0.5 μl/side), or amphetamine preceded 30 min earlier by an injection of SCH23390 (0.1 mg/kg, s.c.). Injections were given once every third day, and after each injection the rats were placed in activity boxes for 1 hr. On the test for sensitization, 2 weeks after the last preexposure injection, animals were injected with amphetamine (1.0 mg/kg, i.p.) and placed in the activity boxes, and their locomotor activity was measured for 2 hr.

Experiment 3. During the drug preexposure phase, different groups of rats received three injections into the VTA of saline (0.5 μl/side), amphetamine (2.5 μg/0.5 μl/side), amphetamine + SCH23390 (0.25 or 1.0 μg/0.5 μl/side), or amphetamine + SCH23388 (0.25 or 1.0 μg/0.5 μl/side). Injections were given once every third day while the rats were in their home cages. On the test for sensitization, 2–3 weeks after the last preexposure injection, *in vivo* microdialysis was used to assess extracellular levels of DA in the NAcc of all rats before and after a challenge injection of amphetamine (1.0 mg/kg, i.p.).

Locomotor activity

A bank of 12 activity boxes was used to measure locomotor activity in experiments 1 and 2. Each box (22 × 43 × 33 cm) was constructed of opaque plastic (rear and two side walls), a Plexiglas front-hinged door, and a tubular stainless steel ceiling and floor. Two photocells, positioned 3.5 cm above the floor and spaced evenly along the longitudinal axis of each box, estimated horizontal locomotion. Separate interruptions of photocell beams were detected and recorded via an electrical interface by a computer situated in an adjacent room. The activity boxes were kept in a room dimly lit with red light.

Intracranial microinjections

Bilateral intracranial microinjections into the VTA were made in the freely moving rat. Injection cannulae connected to 1 μl syringes (Hamilton, Reno, NV) via PE-20 tubing were inserted to a depth of 1 mm below the guide cannula tips. Injections were made in a volume of 0.5 μl/side during a period of 45 sec. The injection cannulae were removed, and the obturators were replaced 60 sec later.

In vivo *microdialysis*

On the day before microdialysis testing, rats were anesthetized briefly with halothane, and a microdialysis probe was lowered into the NAcc. The probes were constructed in the laboratory and modified slightly from those described by Robinson and Whishaw (1988) to the extent that they were removable and, once inserted, held in place by a plastic screw mounted to the side of the chronic 20 gauge guide cannula. They were of concentric design with an active surface length of 2 mm [Spectrum (Los Angeles, CA) regenerated cellulose hollow fiber with a 250 μm o.d. and a 5000 MW cut-off] attached to the end of a 25 gauge length of stainless steel tubing, and they were connected to a liquid swivel, located above the animal's chamber, by a length (~38 cm) of coiled steel tether protecting the inlet (20 gauge polyethylene tubing) and outlet (fused silica capillary with a 150 μm o.d. and a 75 μm i.d.; Polymicro Technologies, Phoenix, AZ) tubing leading to and from the probe. The rats were free to move within the 38 × 32 × 34 cm plastic dialysis test chambers, which were placed in opaque plastic shells. The swivels and collection vials were positioned outside to shield the animals from extraneous stimuli during the dialysis session. The probes were perfused with a modified Ringer's dialysate (145 mM Na^+, 1.2 mM Ca^{2+}, 2.7 mM K^+, 1.0 mM Mg^{++}, and 150 mM Cl^-, pH 7.4; 0.3 μl/min overnight and 1.5 μl/min during testing the following day, 18–20 hr after probe insertion). On the microdialysis test day, three baseline samples were collected. All rats were then injected with amphetamine after which nine more samples were collected. Samples were collected at 20 min intervals, and 20 μl aliquots were injected immediately onto a chromatography column for assessment by HPLC-electrochemical detection (HPLC-EC) of extracellular DA in the NAcc.

HPLC-EC

The HPLC-EC system consisted of a single-piston Gilson 302 pump (Gilson, Middleton, WI) set to 1.1 ml/min, a Gilson diaphragm-type pulse dampener, a 10 cm ODS-C18 3 μm column maintained at 35°C, an ESA model 5100 Coulochem detector with a conditioning cell (oxidizing at +300 mV) placed before a model 5011 high-sensitivity analytical cell (electrodes set to +50 and −350 mV) and a 0.04 M sodium acetate mobile phase containing 0.3 mM Na_2EDTA, 0.5 mM octyl sodium sulfate, and 3.3% acetonitrile (adjusted to pH 3.75 with glacial acetic acid). Extracellular concentrations of DA were estimated from peak areas by a Gilson 715 HPLC System Controller Computer System. To control for differences in active surface length between probes, DA concentrations were corrected for individual probe recoveries. These were determined *in vitro* at 20°C after each microdialysis testing session and ranged from 5 to 9%.

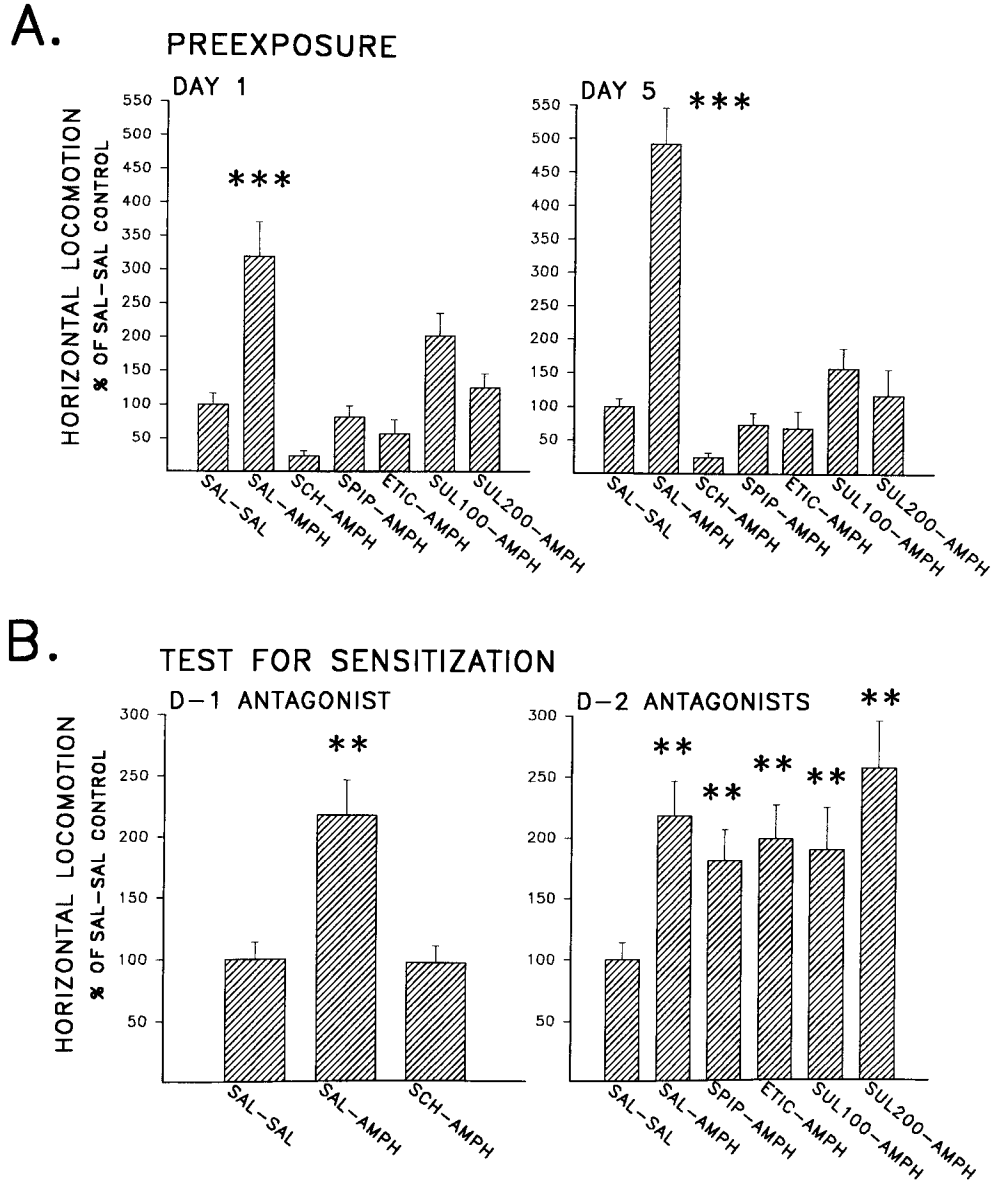

Figure 1. Effect of DA receptor blockade on the development of sensitization of the locomotor-activating effect of amphetamine. Group mean (±SEM) horizontal locomotor activity counts, shown for the first and last day of preexposure, represent 2 hr totals for each day and are expressed as percentage change of the counts displayed by the *SAL-SAL* control group (expressed as 100%; $n = 11$). Eticlopride (*ETIC*) ($n = 12$) was injected 15 min, saline (*SAL*) and SCH23390 (*SCH*) ($n = 6$) were injected 30 min, and spiperone (*SPIP*) ($n = 12$) and (±)sulpiride (*SUL*) ($n = 4$/dose) were injected 60 min before saline or amphetamine (*AMPH*); $n = 11$ for group *SAL-AMPH*. *A*, Both the D_1 and the D_2 DA receptor antagonists blocked the acute locomotor effect of amphetamine. *B*, Only the D_1 DA receptor antagonist blocked the induction of sensitization to amphetamine as revealed on a test conducted 7–10 d after the last preexposure injection. No antagonists were administered on this test, and all animals received amphetamine. Symbols indicate significant differences as revealed by post-hoc Scheffé comparisons after a one-way ANOVA. $^{**}p < 0.01$, SAL-SAL and SCH-AMPH compared with all other groups. $^{***}p < 0.001$, SAL-AMPH compared with all other groups.

Drugs

All DA receptor antagonists were purchased from Research Biomedicals International (Natick, MA). S(+)amphetamine sulfate was supplied by SmithKline Beecham Pharma (Oakville, Ontario, Canada). Spiperone HCl was dissolved in 0.1 M tartaric acid and diluted in water. (±)Sulpiride was dissolved in 5% acetic acid and diluted with water. The remaining drugs [amphetamine, S(−)eticlopride HCl, SCH23390, and 23388 HCl] were dissolved in water. All doses refer to weight of the respective salts.

Data analyses

The data were analyzed with one-way analyses of variance (ANOVA). Post-hoc Scheffé comparisons were made according to Kirk (1968).

RESULTS

Experiment 1. D_1 DA receptor blockade prevents the induction of sensitization by systemic injections of amphetamine: locomotor activity

All DA receptor antagonists blocked the acute locomotor-activating effect of 1.0 mg/kg (i.p.) amphetamine at the doses tested. This is shown for the first and last day of preexposure in Figure 1*A*. The ANOVAs conducted on the day 1 and day 5 data each revealed a significant effect of groups [$F(6,53) = 13.24$ and 26.25, respectively; $p < 0.0001$]. Rats that received amphetamine

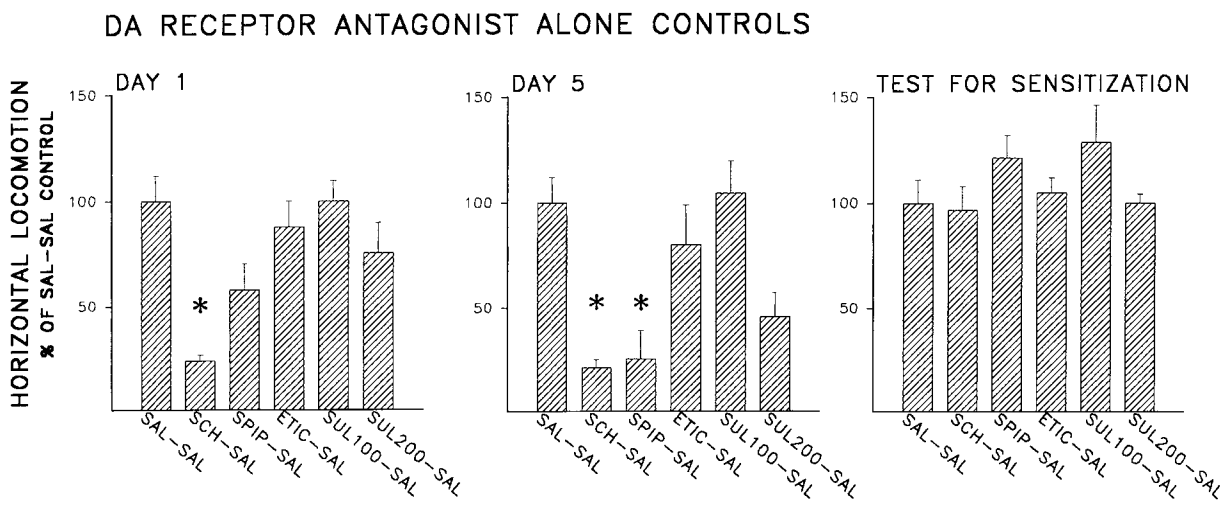

Figure 2. Preexposure to DA receptor antagonists alone did not affect the locomotor response to amphetamine on the subsequent test for sensitization. Data are shown as described in Figure 1. $^*p < 0.05$, *SCH-SAL* and *SPIP-SAL* each decreased locomotion significantly compared with saline and the other antagonists during preexposure. No significant group differences were found on the test for sensitization; $n = 4–8$/group.

without an antagonist showed significantly higher levels of locomotion than all other animals did ($p < 0.001$). The remaining groups did not differ significantly from one another.

On the test for sensitization, conducted 7–10 d after the last preexposure injection, no receptor antagonists were administered, and all animals were injected with amphetamine. Figure 1*B* shows that only the D_1 DA receptor antagonist SCH23390 blocked the induction of sensitization to the locomotor-activating effect of amphetamine. The ANOVA conducted on the test data revealed a significant effect of groups [$F(6,53) = 3.94$, $p < 0.0025$]. A sensitized locomotor response was shown by all groups treated previously with saline or a D_2 DA receptor antagonist before each of the five injections of amphetamine during preexposure. All of these groups showed significantly greater locomotion than the saline control group that received amphetamine for the first time on this test or animals treated previously with SCH23390 before amphetamine during preexposure ($p < 0.01$). These latter two groups did not differ significantly from each other.

Figure 2 shows that repeated exposure to DA receptor antagonists alone at doses sufficient to block the acute locomotor effect of amphetamine does not lead to an enhanced locomotor response on the test for sensitization with amphetamine. The ANOVAs conducted on the day 1 and day 5 data each revealed a significant effect of groups [$F(5,30) = 5.91$ and 6.70, respectively; $p < 0.002$]. SCH23390 and spiperone, at the doses tested, produced significant decreases in locomotion during preexposure compared with saline and the other antagonists ($p < 0.05$). The ANOVA conducted on the test day data revealed no significant effects.

Experiment 2. D_1 DA receptor blockade prevents the induction of sensitization by injections of amphetamine into the VTA: locomotor activity

Consistent with previous reports (Kalivas and Weber, 1988; Vezina and Stewart, 1990; Hooks et al., 1992), exposure to injections of amphetamine into the VTA 2 weeks earlier produced a sensitized locomotor response to a systemic amphetamine challenge injection when compared with that of animals preexposed to VTA saline (Fig. 3). Treating animals with SCH23390 (subcutaneously) before each of the intra-VTA amphetamine injections during preexposure blocked the induction of sensitization [$F(2,14) = 6.92$; $p < 0.01$]. Sensitized animals showed significantly greater locomotion than the other two groups did ($p < 0.01$). These did not differ significantly from one another.

Experiment 3. D_1 DA receptor blockade prevents the induction of sensitization by injections of amphetamine into the VTA: NAcc DA

As reported previously (Vezina, 1993), animals preexposed 2–3 weeks earlier to injections of amphetamine into the VTA showed enhanced levels of DA in the NAcc in response to a systemic amphetamine challenge injection on the test for sensitization compared with those produced in animals exposed previously to

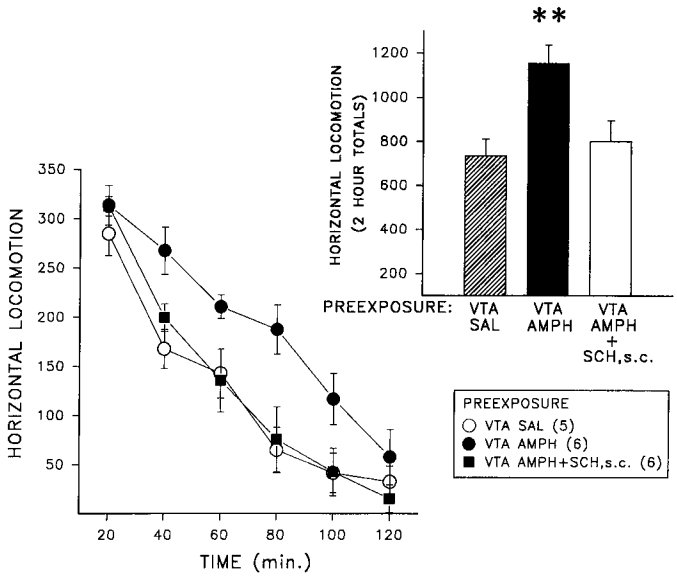

Figure 3. Group mean (± SEM) horizontal locomotor activity counts after a systemic injection of amphetamine on a test for sensitization conducted 2 weeks after preexposure to intra-VTA amphetamine. D_1 DA receptor blockade prevented the induction of locomotor sensitization by injections of amphetamine into the VTA. SCH23390 was injected subcutaneously 30 min before the intra-VTA amphetamine injections during preexposure. *Inset* shows the results as 2 hr session totals. $^{**}p < 0.01$, significantly higher locomotor counts compared with the other two groups. Numbers in parentheses indicate number per group.

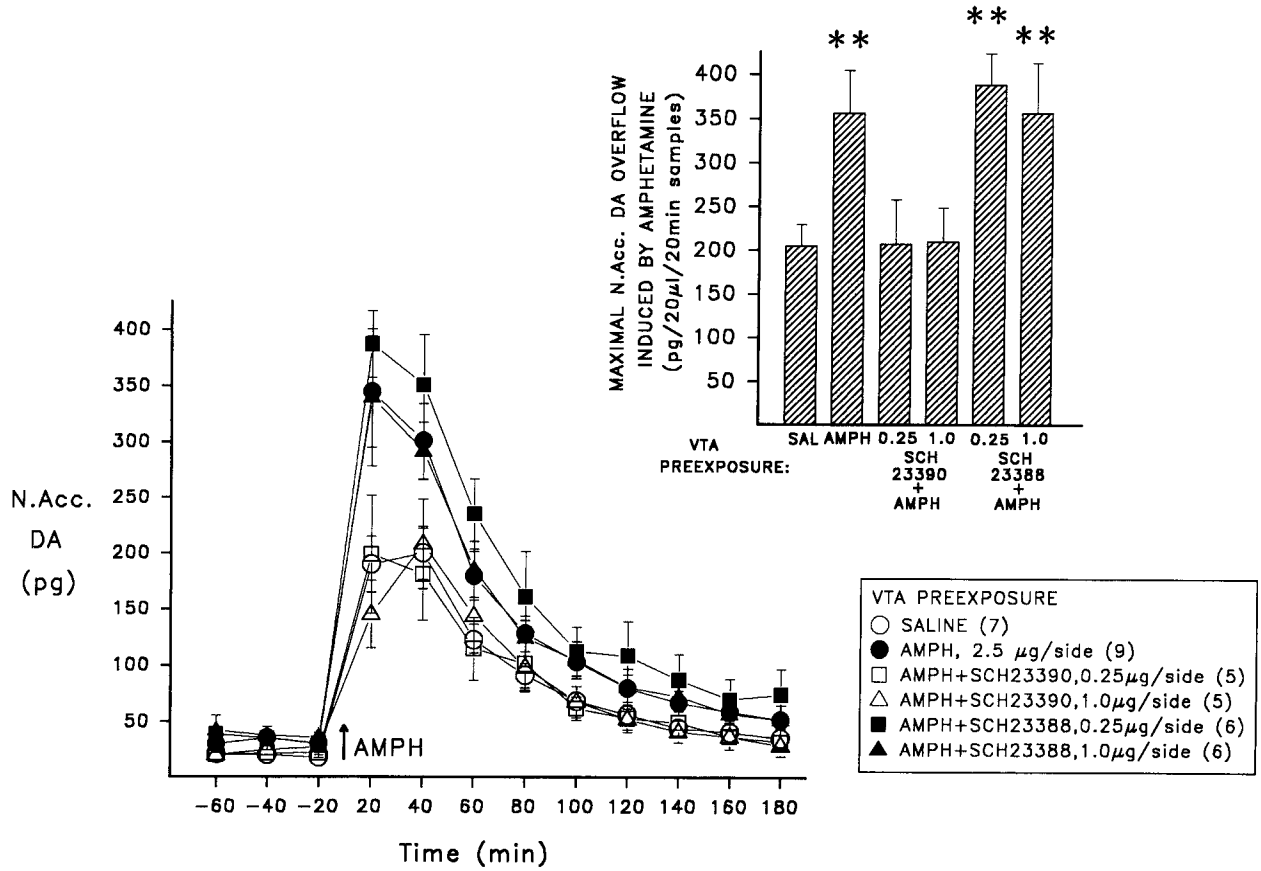

Figure 4. Extracellular concentrations of DA in the NAcc before and after a systemic challenge injection of amphetamine on a test for sensitization conducted 2–3 weeks after preexposure. Induction of the sensitized NAcc DA response was blocked by co-injections of SCH23390, but not of its inactive enantiomer SCH23388, during preexposure to intra-VTA amphetamine. Data are shown as absolute values (group mean ± SEM) corrected for probe recovery. Results are summarized in the *inset* as group mean maximal NAcc DA overflow induced by amphetamine (+ SEM). Maximal overflow for each animal was taken as the highest DA peak obtained in a test session and always occurred in the first or second sample after the amphetamine challenge. $^{**}p < 0.02$, a significantly higher DA response to amphetamine was shown by animals preexposed to VTA amphetamine or amphetamine plus either dose of SCH23388 compared with animals preexposed to VTA saline or amphetamine plus either dose of SCH23390. Numbers in parentheses indicate number per group.

VTA saline. Induction of this sensitized NAcc DA response to amphetamine was blocked by co-injecting SCH23390, but not its inactive enantiomer SCH23388, with amphetamine into the VTA during preexposure (Fig. 4). Basal levels of DA did not differ significantly between groups, and maximal NAcc DA overflow, taken as the highest DA peak obtained in a test session, occurred for all animals in the first or second sample (20–40 min) after the amphetamine challenge. The ANOVA conducted on these data revealed a significant effect of groups [$F(5,32) = 3.30$; $p < 0.02$]. Animals preexposed to VTA amphetamine or VTA amphetamine + SCH23388 showed significantly greater NAcc DA responses to the intraperitoneal amphetamine challenge than did animals preexposed to VTA saline or VTA amphetamine + SCH23390 ($p < 0.02$). These latter three groups did not differ significantly from one another.

Histology

Only data from animals with injection cannula tips located bilaterally in the VTA (experiments 2 and 3) and the active portion of the microdialysis probe located in the NAcc (experiment 3) were considered. In experiment 2, four animals [one from the saline (SAL), two from the amphetamine (AMPH), and one from the AMPH+SCH groups] were excluded because at least one of their injection cannula tips was located either dorsal or caudal to the VTA. Figure 5 shows the location of the active portion of the microdialysis probes in the NAcc for all animals included in experiment 3. One animal with a probe positioned lateral to the NAcc was excluded. Also shown is a representative photomicrograph showing the steel and active portion of a microdialysis probe in the NAcc of one of the animals tested. The region of the NAcc sampled corresponds to the rostral pole of this nucleus (Deutch et al., 1993).

The dark-field photomicrographs in Figure 6*A,B* show many tyrosine hydroxylase (TH)-positive fluorescent cell bodies and processes in the VTA immediately adjacent to the area damaged by the injection procedure 1 mm beyond the tip of a guide cannula in one of the animals tested in experiment 3. Thus, although some destruction from the three microinjections into this site was unavoidable, many DA neurons adjacent to the injection cannulae in the VTA remained. Six animals were excluded from this experiment because at least one of their injection cannula tips was located either dorsal or lateral to the VTA (two animals from the SAL and two from each of the AMPH+SCH23388 groups). The latter four AMPH+SCH23388 animals did not show a sensitized NAcc DA response to the amphetamine challenge injection on the test for sensitization, nor did four additional animals injected previously with amphetamine into sites dorsal to the VTA. The

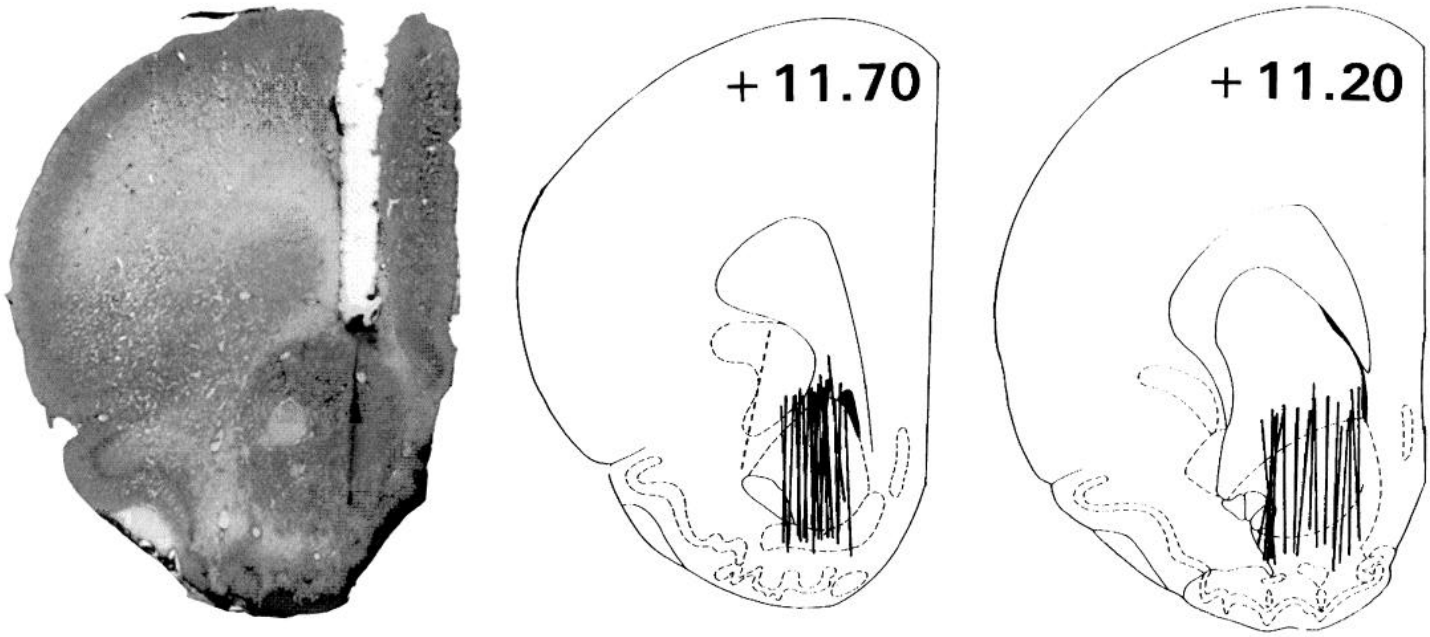

Figure 5. Location of the active portion of the microdialysis probes in the NAcc. *Solid lines* indicate probe placements for all animals included in experiment 3. *Dashed line* indicates the probe placement of an animal that was excluded. The photomicrograph to the left shows the steel and active portion of a microdialysis probe in the rostral pole of the NAcc of one of the animals tested. Numbers indicating millimeters rostral to the interaural line and line drawings are from Paxinos and Watson (1986).

injection cannula tip placements for these eight animals are shown in Figure 6*C* (*open circles*) together with placements in the VTA (*filled circles*) from the nine AMPH animals that showed enhanced NAcc DA response on the test for sensitization in experiment 3. *Open squares* indicate injection cannula tip placements for the 10 AMPH+SCH23390 rats. Animals preexposed to amphetamine but in sites outside the VTA showed levels of NAcc DA in response to amphetamine similar to those of animals exposed previously to VTA saline (Fig. 6*D*). These were significantly lower than those shown by animals exposed previously to VTA amphetamine [$F(2,21) = 10.42$; $p < 0.001$].

DISCUSSION

These results indicate that activation of D_1 DA receptors is necessary for the induction of sensitization by amphetamine in the VTA. First, D_1 but not D_2 DA receptor blockade prevented the development of sensitization of the locomotor-activating effect of systemically administered amphetamine. Second, D_1 DA receptor blockade prevented the induction of locomotor sensitization produced by infusions of amphetamine into the VTA. Third, coinjecting a D_1 DA receptor antagonist, but not its inactive enantiomer, with amphetamine into the VTA blocked the development of the sensitized NAcc DA response to a subsequent systemic amphetamine challenge injection.

The present findings are consistent with and extend those of previous studies to show that while SCH23390 (>1000 times more potent at the D_1 than at the D_2 DA receptor subtype; Seeman and Grigoriadis, 1987) blocks the development of behavioral sensitization to amphetamine when injected systemically (Vezina and Stewart, 1989; Drew and Glick, 1990; present report), several D_2 DA receptor antagonists (>2500–10^6 times more potent at the D_2 than the D_1 subtype) do not. The benzamide YM-09151–2 is a notable exception to this pattern; it blocks the development of the sensitized locomotor and striatal DA responses to methamphetamine (Ujike et al., 1989; Hamamura et al., 1991). Although this receptor antagonist selectively binds to the D_2 DA receptor subtype (Niznik et al., 1985), it has also been shown more recently, unlike the other benzamide sulpiride, to activate rapidly and transiently nigrostriatal DA neurons in a Ca^{2+}-, TTX-, reuptake site-, and D_2 receptor-independent manner (Tomiyama et al., 1993), suggesting the possibility that actions other than its propensity to bind to D_2 receptors may contribute to its effects on methamphetamine sensitization. Taken together, the above evidence does not support a role for the D_2 DA receptor subtype in sensitization to amphetamine. Rather, it is D_1 DA receptor activation that seems critical. It is unlikely that SCH23390 blocked the development of sensitization to amphetamine by acting at 5-HT_2 receptors where it has been shown to bind competitively (Bischoff et al., 1986; McQuade et al., 1988). The dose of SCH23390 used in the present experiments (0.1 mg/kg) is well below its ED_{50} for binding to 5-HT_2 receptors *in vivo*, and as indicated above, spiperone, which exhibits similar potencies for D_2 and 5-HT_2 receptors and slightly higher potency for the latter receptor compared with SCH23390 (Seeman and Grigoriadis, 1987), did not block the development of sensitization to amphetamine at a dose that completely blocked the acute locomotor effect of this drug.

Consistent with previous reports, preexposure to injections of amphetamine into the VTA produced a sensitized locomotor response to a subsequent systemic challenge injection of amphetamine. Injecting animals systemically with SCH23390 before each of the intra-VTA amphetamine injections blocked the induction of this sensitization, again indicating that D_1 DA receptor activation is necessary. Given that amphetamine produces sensitization when injected into the VTA but not into sites 1–3 mm dorsolateral to this area (Perugini and Vezina, 1994) or into the NAcc or the medial prefrontal cortex (Dougherty and Ellinwood, 1981; Kalivas and Weber, 1988; Vezina and Stewart, 1990; Hooks et al., 1992), the major subcortical and cortical terminal regions of VTA DA neurons, respectively these results indicate a critical role for D_1

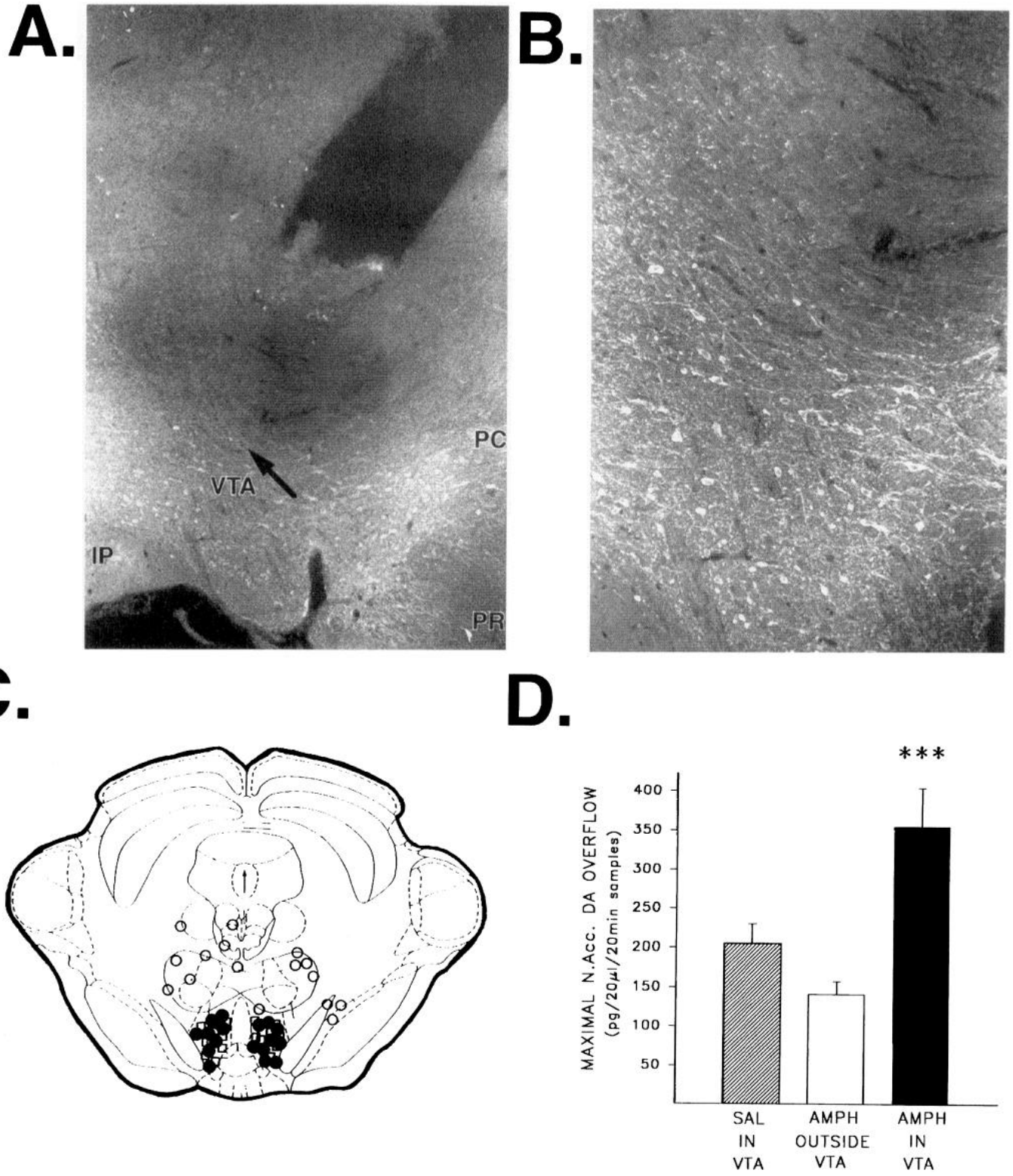

Figure 6. Placement of cannulae aimed at the VTA in experiment 3. Dark-field photomicrograph in *A* shows a guide cannula aimed at the VTA. Note the many TH-positive fluorescent cell bodies and processes in the VTA immediately adjacent to the area damaged by the injection procedure extending ~1 mm beyond the tip of the guide cannula (*arrow*). These are shown at higher magnification in *B*. *C*, Depiction of injection cannula tip placements in the mesencephalon of animals that subsequently showed a sensitized NAcc. DA response to amphetamine on the test for sensitization (nine pairs of *filled circles* representing animals that received amphetamine) and that of animals that did not (eight pairs of *open circles* located dorsal and lateral to the VTA representing four animals that received amphetamine and four that received AMPH+SCH23388). Also shown (*open squares*) are the injection cannula tip placements for the 10 AMPH+SCH23390 animals. The line drawing is from Paxinos and Watson (1986) and depicts the caudal surface of a coronal section extending +3.0 to +3.8 mm from the interaural line. The greater maximal NAcc DA response to amphetamine on the test for sensitization shown by animals preexposed to VTA amphetamine is represented graphically in *D* and compared with that of animals preexposed to VTA saline or to amphetamine in sites outside the VTA. Data for the *SAL IN VTA* and *AMPH IN VTA* groups are from Figure 4. ***$p < 0.001$, significantly greater DA response compared with the other two groups. *IP*, interpeduncular nuclei; *PC*, pars compacta, substantia nigra; *PR*, pars reticulata, substantia nigra; *VTA*, ventral tegmental area.

receptors located in the VTA. Consistent with these findings, injecting SCH23390 into the VTA before each systemic preexposure injection of amphetamine also blocks the development of locomotor sensitization to this drug (Stewart and Vezina, 1989). This latter finding has been criticized, however, as reflecting either non-D_1 receptor actions of SCH23390 in the VTA or an action of the diffused antagonist at D_1 receptors in forebrain DA terminal regions (Di Chiara, 1993; Jeziorski and White, 1995). Several considerations make these possibilities unlikely. As indicated above, sensitization produced by amphetamine in the VTA is blocked by systemically injected SCH23390. This finding, together with those showing that amphetamine does not produce behavioral sensitization when injected into sites outside the VTA, and other findings obtained in this laboratory (data not shown) showing that intra-VTA infusions of amphetamine at doses used to produce sensitization and of SCH23390 (in agreement with White and Wang, 1984) at doses used to block sensitization do not acutely affect levels of extracelluar DA in the NAcc, makes it unlikely that SCH23390 is blocking the induction of sensitization by amphetamine in the VTA via nonspecific non-D_1 receptor actions in this site or via D_1 receptor actions in forebrain sites.

Similarly (also see Vezina, 1993), exposure to injections of amphetamine into the VTA 2–3 weeks earlier produced a sensitized NAcc DA response to a systemic challenge injection of amphetamine. As with the sensitization of locomotion by VTA amphetamine (Perugini and Vezina, 1994), infusions of this drug into sites dorsal and lateral to the VTA were ineffective. Furthermore, and again consistent with what has been found for the sensitization of locomotion by VTA amphetamine, the induction of the sensitized NAcc DA response to amphetamine was blocked by SCH23390 but spared by its inactive enantiomer SCH23388 when these were co-injected with amphetamine into the VTA during preexposure. Again, it is unlikely that SCH23390 (~250 times more potent at the D_1 receptor than SCH23388; Andersen et al., 1992) blocked the development of sensitization via nonspecific effects. Rather, these latter findings argue strongly for a critical role of D_1 receptors in the VTA in the sensitization by amphetamine of mesoaccumbens DA neurons. The resulting enhancement of the ability of amphetamine to increase extracellular levels of DA in the NAcc may be associated with the persistence of locomotor sensitization to this drug. It is true that these two responses can be dissociated in the period soon after drug preexposure (see Paulson and Robinson, 1995, for a discussion and references). As already suggested, sensitized locomotor responses conceivably could be supported during this time in the absence of enhanced NAcc DA levels by an upregulation of postsynaptic D_1 receptors in this site (Henry and White, 1991; Wolf et al., 1994). Such changes in mesoaccumbens neurotransmission, however, have been shown to diminish with time.

The present findings are consistent with the view that the induction by amphetamine in the VTA of enduring enhancements in locomotor and NAcc DA responses to this drug involve the somatodendritic release of DA and its action at D_1 DA receptors in this site. The neural consequences of this activation of D_1 DA receptors and the sequence of events recruited to produce them remain unknown. Preexposure to systemic injections of amphetamine enhances the neuronal response of DA neurons to glutamate in the VTA (White et al., 1995). Such findings may reflect a change in the afferent regulation of DA neurons resulting from, for example, a decrease in the sensitivity of GABA interneurons and terminals for excitatory amino acids or a change in the reactivity of DA neurons themselves to excitatory amino acids. Such changes conceivably could enhance the locomotor and NAcc DA responses to amphetamine in sensitized animals. Although an upregulation of D_1 DA receptors in different areas of the substantia nigra reticulata occurs at different times after amphetamine and methamphetamine preexposure (Ujike et al., 1991; Bonhomme et al., 1995), it is not clear that such a change could contribute to an enhanced D_1 receptor-mediated increase in glutamate release in the VTA. Reports that animals sensitized to psychomotor stimulants show enhanced *in vitro* amphetamine- and K^+-stimulated DA release from mesolimbic DA neuron terminal field tissue, not including the DA cell bodies of origin or their various afferent inputs, point more to a change in the reactivity than in the afferent regulation of DA neurons (Kolta et al., 1985; Castaneda et al., 1988; Peris et al., 1990). Thus, although mesoaccumbens DA cell bodies and those regulatory afferent terminals having access to them in the VTA may be critical for the initiation of those long-term changes underlying sensitization to amphetamine, once these changes have become established in DA neuron terminals, DA cell bodies and their afferent inputs are no longer necessary for the expression of sensitization. Protein kinase (Steketee, 1994) and synthesis (Sorg and Ulibarri, 1995) inhibition in the VTA blocks the induction of behavioral sensitization to psychomotor stimulants. Such findings are consistent with the view that such long-term changes require the synthesis of new proteins in the cell bodies of mesoaccumbens DA neurons and their transport to the terminals of these neurons in the NAcc. The expression of the enhanced NAcc DA response to amphetamine thus may be delayed in the initial period immediately after drug preexposure by the slow nature of axonal transport or by changes in the levels and phosphorylation state of specific neurofilament proteins produced by the sensitizing drug regimen (Beitner-Johnson et al., 1992).

D_1 DA receptors in the VTA seem to be positioned critically to influence the initiation of such enduring changes in the reactivity of mesoaccumbens DA neurons that lead to long-term sensitized locomotor and NAcc DA responses to amphetamine. Given the rich and varied innervation of the VTA (see Kalivas, 1993), there are several possible neurotransmitter candidates or combinations of candidates available to interact with DA to produce sensitization. Two of these, GABA and glutamate, have been implicated because their release into the VTA is modulated by D_1 receptors (Cameron and Williams, 1993; Kalivas and Duffy, 1995), and both influence the induction of behavioral sensitization to psychomotor stimulants (Kalivas and Stewart, 1991; Kalivas and Alesdatter, 1993; Wolf et al., 1994). Furthermore, NMDA receptor blockade prevents the development of sensitization to the D_1 DA receptor agonist SKF38393 (Criswell et al., 1990). The presence of GABA and excitatory amino acid receptors on mesolimbic DA neuron cell bodies thus may be critical for the induction of long-term changes in these neurons. Their contribution, and possibly that of other neurotransmitters, could be recruited by the DA somatodendritically released by amphetamine.

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