The Journal of Neuroscience, July 1995, 15(7): 5379–5388

D_1 Receptors Modulate Glutamate Transmission in the Ventral Tegmental Area

Peter W. Kalivas and Patricia Duffy

Alcohol and Drug Abuse Program, Washington State University, Pullman, Washington 99164-6520

Perfusion of the D_1 agonist, SKF-82958, through a microdialysis probe implanted in the ventral tegmental area produced a dose-dependent increase in extracellular glutamate and GABA. The increase in extracellular glutamate occurred at approximately 30× lower dose than the elevation in GABA. The increase in extracellular glutamate by SKF-82958 was blocked by coperfusion of the D_1 antagonist, SCH-23390, and was not mimicked by perfusion of the $D_{2/3}$ agonist, quinpirole, into the ventral tegmental area. In contrast, the elevation in extracellular GABA was insensitive to blockade by SCH-23390. Systemic administration of cocaine (15 mg/kg, i.p.) produced a rapid elevation in extracellular glutamate lasting for 20 min that was prevented by pretreating the ventral tegmental area with SCH-23390. In contrast, acute cocaine produced a reduction in extracellular GABA content in the ventral tegmental area that was not affected by SCH-23390. These data indicate that the stimulation of D_1 receptors in the ventral tegmental area increases the release of glutamate and that increasing extracellular levels of somatodendritic dopamine by systemic cocaine can mimic this effect.

[Key words: dopamine, glutamate, GABA, ventral tegmental area, microdialysis, cocaine]

Somatodendritic dopamine release from neurons in the ventral mesencephalon is calcium dependent (Robertson et al., 1991; Grace, 1991; Kalivas and Duffy, 1991) and provides regulatory feedback on dopamine cellular activity (Bunney et al., 1973; Wang, 1981; Grace, 1987). Stimulation of somatodendritic dopamine autoreceptors hyperpolarizes dopamine cells via a G protein-dependent increase in potassium efflux (Innis and Aghajanian, 1987; Lacey et al., 1987). The ensuing membrane hyperpolarization inhibits dopamine cell firing (Bunney et al., 1973; Wang, 1981; Grace, 1987), as well as further release of somatodendritic dopamine (Kalivas and Duffy, 1991). The D_2 dopamine receptor subtype is a somatodendritic autoreceptor. Not only have pharmacological studies using receptor selective agonists identified D_2 receptor involvement (Lacey et al., 1987), but D_2 receptor binding and mRNA are eliminated in the ventral mesencephalon following selective dopamine lesions (Bouthenet et al., 1987; Meador-Woodruff et al., 1991)

In addition to D_2 receptors, somatodendritically released dopamine can stimulate other receptor subtypes. Both D_1 and D_3 receptor subtypes are present in the ventral mesencephalon (Bouthenet et al., 1991; Mansour et al., 1992). Similar to D_2 receptors, D_3 receptors may serve an autoreceptor function. While pharmacological verification of this possibility remains to be documented, dopamine neurons have been reported to contain D_3 receptor mRNA (Bouthenet et al., 1991). In contrast to D_2 and D_3 receptors, dopamine neurons do not synthesize D_1 receptors in measurable quantities (Mansour et al., 1992). In the substantia nigra the majority of D_1 receptor binding is in the pars reticulata and is eliminated by destruction of striatonigral afferents but remains unaffected by nigral dopamine lesions (Altar and Hauser, 1987; Beckstead, 1988). It is known that striatal efferents to the ventral mesencephalon are GABAergic (Smith and Bolam, 1990; Kalivas et al., 1993), and are capable of inhibiting somatodendritic dopamine release (Klitenick et al., 1992), as well as the firing frequency of dopamine neurons by a $GABA_B$-dependent increase in potassium conductance (Grace and Bunney, 1985; Lacey et al., 1987; Johnson and North, 1992). Because of the localization of D_1 receptors on GABAergic afferents, it has been proposed that activation of D_1 receptors may provide inhibitory regulation of dopamine cells by presynaptic stimulation of GABA release. Accordingly, using nigral tissue slices it was shown that D_1 agonists promote the release of preloaded ^{3}H-GABA (Starr, 1987). Furthermore, *in vivo* application of the D_1 agonist, CY-208-243, into the substantia nigra via a dialysis probe increases the extracellular content of GABA (Timmerman et al., 1991) and perfusion of ventral mesencephalic tissue slices with D_1 agonist promotes $GABA_B$-dependent hyperpolarizations in dopamine cells (Cameron and Williams, 1993).

In addition to GABAergic afferents, there exist excitatory amino acid (EAA) afferents to the ventral mesencephalon arising from the prefrontal cortex, amygdala, subthalamus and pedunculopontine region (see Kalivas, 1993, for review), and it is possible that D_1 receptor stimulation may also modulate EAA release. This possibility is of potential clinical significance because it could constitute a positive feedback mechanism onto dopamine cells and a number of psychopathologies have been associated with hyperactivity in mesocorticolimbic dopamine projections (Goldstein and Deutch, 1992; Sato, 1992; Koob et al., 1993).

The first experiments in the present study use microdialysis to examine the effect of *in vivo* D_1 receptor stimulation on extracellular glutamate content and GABA release in the medial dopamine cell group of the ventral mesencephalon, located in the ventral tegmental area (VTA). It was then determined if systemic administration of the psychostimulant, cocaine, alters the

Received Dec. 7, 1994; revised Mar. 13, 1995; accepted Mar. 16, 1995.

We thank Jenny Baylon for assistance in preparing the manuscript. This research was supported in part by USPHS Grants MH-40817 and DA-03906 and Research Career Development Award DA-00158 (P.W.K.).

Correspondence should be addressed to Peter Kalivas, Ph.D., Department of VCAPP, Washington State University, Pullman, WA 99164-6520.

Copyright © 1995 Society for Neuroscience 0270-6474/95/155379-10$05.00/0

extracellular content of glutamate and GABA in the VTA, and if this effect is mediated by D_1 receptors.

Materials and Methods

Surgery and animal housing. Male Sprague–Dawley rats (Simonsen Laboratories, Gilroy, CA) were individually housed with food and water available ad libitum. A 12 hr/12 hr light/dark cycle was used with the lights on at 6:30 a.m. Prior to surgery, rats weighing 250–350 gm were anesthetized with Equithesin (3.0 ml/kg) and mounted in a stereotaxic apparatus. Unilateral dialysis guide cannulae (14 mm, 20 gauge stainless steel) were implanted 3 mm dorsal to the VTA (2.5 mm A/P, 0.7 mm M/L, −2.5 mm D/V; relative to the interaural line according to Pellegrino et al., 1979) and cemented in place by affixing dental acrylic to three stainless steel screws tapped into the skull.

Microdialysis and behavior. The dialysis probes were constructed as described by Robinson and Whishaw (1988), with 1.0–1.5 mm of active dialysis membrane exposed at the tip. The probes were inserted through the guide cannula into the VTA the night prior to the experiment. The next day, dialysis buffer (5 mM KCl, 120 mM NaCl, 1.4 mM $CaCl_2$, 1.2 mM $MgCl_2$, 5.0 mM D-glucose, plus 0.2 mM phosphate-buffered saline to give a pH value of 7.4 and a final sodium concentration of 120.7 mM) was advanced through the probe at a rate of 1.9 ml/min via a syringe pump (Harvard Instruments, Boston, MA) for 2 hr. Twenty min baseline samples were collected for 100 min, followed by treatments involving passing various concentrations of the D_1 agonist, SKF-82958 (Research Biochemicals, Inc., Natick, MA), the D_1 antagonist, SCH-23390 (Research Biochemicals, Inc.) or the D_2/D_3 agonist, quinpirole (Research Biochemicals Inc.) through the dialysis probe and/or systemic injection of cocaine (15 mg/kg, i.p.; donated by the National Institute of Drug Abuse) or saline (1 ml/kg, i.p.). Intracranial drugs were dissolved in the dialysis perfusion buffer and cocaine was dissolved in saline. During both baseline and drug administration, samples were taken every 20 min. When cocaine was administered, motor activity was monitored using a photocell system (Omnitech Electronics, Columbus, OH). At the end of the experiment, the dialysis probe was removed and the animal was administered an overdose of pentobarbital (>100 mg/kg, i.p.).

Quantification of amino acid content. The concentrations of GABA and glutamate were determined using HPLC with electrochemical detection. Samples were typically split and separate isocratic HPLC systems were employed for quantifying GABA and glutamate. The dialysis samples were collected into 10 μl of mobile phase containing 2.0 pmol of aminovaleric acid and homoserine as the internal standards for GABA and glutamate, respectively. The mobile phases for glutamate and GABA are described by Donzanti and Yamamoto (1988) and Bourdelais and Kalivas (1992), respectively. A reversed-phase column (10 cm; ODS) was used to separate the amino acids and precolumn derivatization of amino acids with *o*-phthaldehyde was performed using an autosampler. For GABA the electrodes were set as follows: preinjection port guard = +0.7 V, preoxidation = +0.2 V, working = +0.45 V, and for glutamate the electrodes were set at; guard = +0.7 V, prereduction = −0.35 V, working = +0.65 V. Peak heights were measured and compared to an external standard curve for quantification.

Histology and statistics. Rats were perfused intracardially with phosphate-buffered saline (60 ml) followed by 10% formalin (60 ml). The brains were removed and stored in 10% formalin for at least 1 week. The brains then were blocked and coronal sections (100 μm thick) were taken at the level of the VTA with a vibratome. The sections were mounted on gelatin-coated slides and stained with cresyl violet. Probe placements were determined according to the atlas of Paxinos and Watson (1986) by an individual unaware of the rats' neurochemical response. The data were evaluated using a one- or two-way ANOVA with repeated measures over time. Comparisons between treatment groups or to predrug baseline were made using a least significant difference test described by Milliken and Johnson (1984) or a Dunnet's test for comparison to baseline.

Results

Effect of D_1 receptor stimulation. Figure 1 shows that perfusion of SKF-82958 through a dialysis probe in the VTA increased the extracellular content of both GABA and glutamate in the VTA. However, SKF-82958 was more potent in augmenting the levels of glutamate compared to GABA. The minimum effective dose for increasing glutamate was 3.0 μM while 300 μM of SKF-82958 was required to significantly elevate GABA. In contrast, at no dose of the D_2/D_3 agonist, quinpirole, were the levels of extracellular glutamate or GABA significantly altered (Fig. 2). Figure 2 also shows that blockade of D_1 receptors with SCH-23390 produced a significant increase in the extracellular concentration of glutamate at the lowest dose employed (0.3 μM)

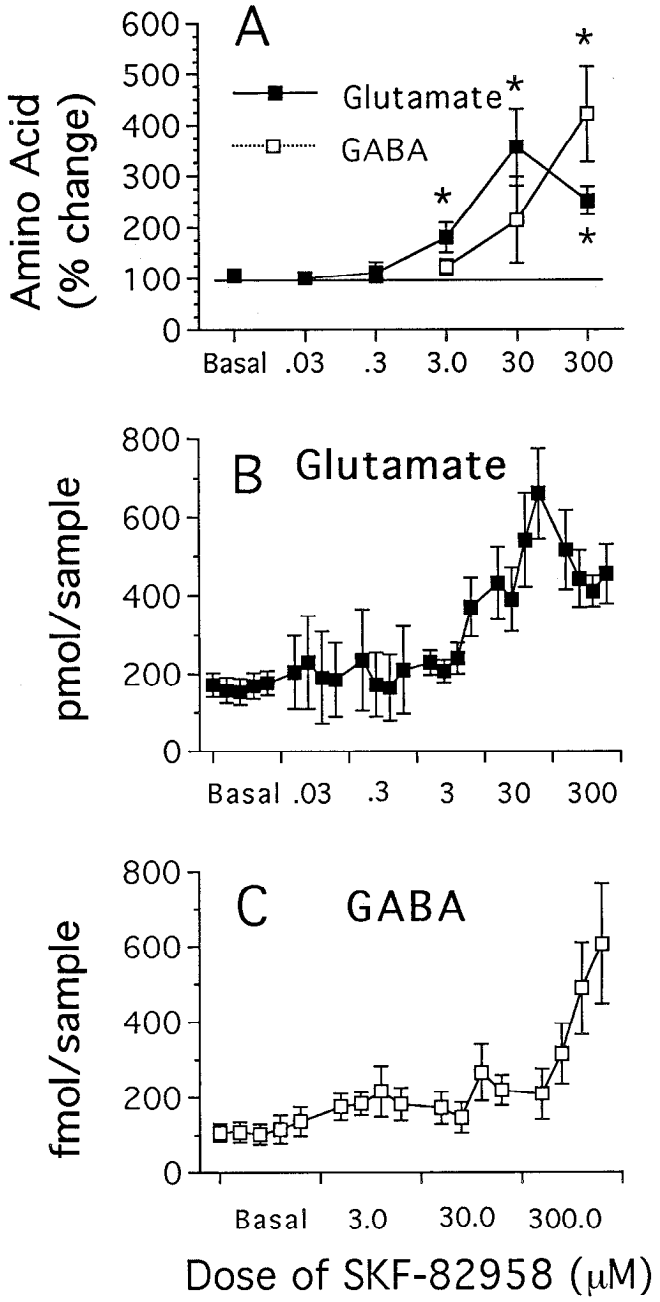


Figure 1. Effect of SKF-82958 on extracellular levels of glutamate and GABA in the VTA. Following the collection of five baseline samples (20 min each), increasing concentrations of SKF-82958 (0.03–300 μM) were passed through the dialysis probe for 100 min each (five samples). The *top panel* shows data normalized to percent change from baseline, while the *bottom panels* show the raw data. The data are shown as mean ± SEM. The data were evaluated using a one-way repeated measures ANOVA. For GABA, $N = 6$, $F_{(3,19)} = 7.17$, $p = 0.005$. For glutamate, $N = 10$, $F_{(3,27)} = 16.42$, $p < 0.001$. *, $p < 0.05$, compared to basal using a Dunnet's post hoc test.

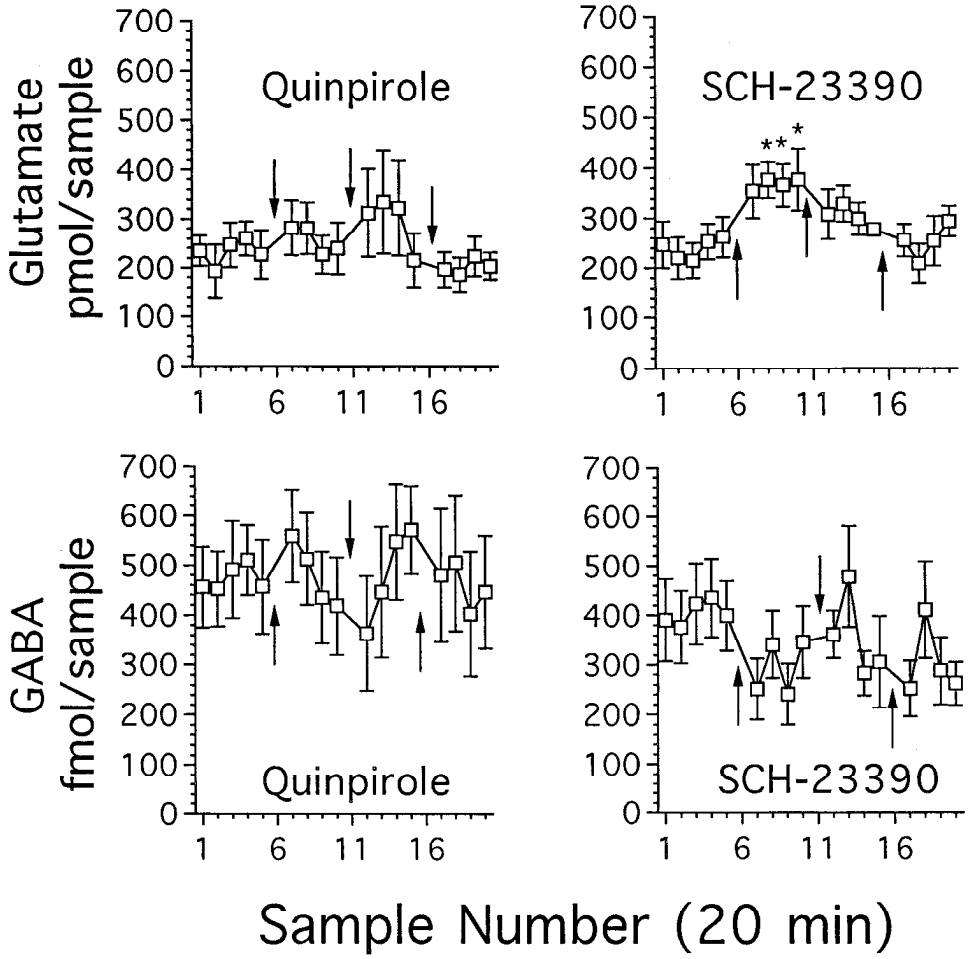


Figure 2. Effect of quinpirole (1.0, 10.0, and 100.0 μM) or SCH-23390 (0.3, 3.0, and 30.0 μM) on extracellular levels of glutamate and GABA in the VTA. Following collection of five baseline samples, *arrows* indicate sample where the concentration of quinpirole or SCH-23390 was changed in ascending order by dose. The data are shown as mean ± SEM and were evaluated using a one-way repeated measures ANOVA. For glutamate/quinpirole, $N = 4$, $F_{(12,51)} = 1.97$, $p = 0.058$. For glutamate/SCH-23390, $N = 4$, $F_{(12,51)} = 2.35$, $p = 0.024$. For GABA/quinpirole, $N = 5$, $F_{(12,64)} = 1.13$, $p = 0.356$. For GABA/SCH-23390, $N = 5$, $F_{(12,64)} = 11.04$, $p = 0.429$. *, $p < 0.05$, compared to the averaged basal value using a Dunnet's post hoc test.

and returned to baseline values following 3.0 and 30.0 μM SCH-23390. In contrast, no significant effect of SCH-23390 was measured on GABA levels.

Figure 3, *A* and *B*, shows that the capacity of SKF-82958 (100 μM) to increase extracellular glutamate was prevented by coperfusion of the D_1 receptor antagonist SCH-23390 (30 μM). This dose of SKF-82958 also increased the extracellular content of GABA, but this effect was not susceptible to blockade by SCH-23390.

Effect of acute cocaine. Figure 4 shows that the systemic administration of cocaine (15 mg/kg, i.p.) produced a significant increase in the extracellular content of glutamate in the VTA which was associated with an increase in motor activity. The time course of the change in glutamate and behavior was similar, with both showing a peak response within the first 20 min after injection and a return to basal levels by 40–60 min after injection. Figure 4 also shows that the cocaine-induced increase in glutamate was abolished following the inhibition of D_1 receptors in the VTA by perfusing SCH-23390 (30 μM) through the dialysis probe. However, SCH-23390 did not significantly alter the behavioral stimulation produced by cocaine; although the peak behavioral response occurred at 40 min rather than 20 min after cocaine administration. In a control study ($N = 5$), after collecting five baseline samples rats were injected with saline (1.0 ml/kg, i.p.). Compared to basal levels, no alteration in extracellular glutamate content was measured during the 2 hr following saline injection (data not shown).

In contrast to the effect on extracellular glutamate, Figure 5 shows that an acute cocaine challenge (15 mg/kg, i.p.) produced an inconsistent, albeit statistically significant, reduction in the extracellular content of GABA in the VTA. This effect on extracellular GABA reached statistical significance at 60 and 100 min after administering cocaine. The reduction in extracellular GABA was not blocked by perfusing SCH-23390 into the VTA; although, the levels of GABA were significantly altered at different times after cocaine administration (40 and 120 min).

Histology. Figure 6 shows the location of the dialysis probes in the VTA. The probes were placed in the lateral VTA, corresponding to the nucleus parabrachialis pigmentosus and nucleus paranigralis, as well as along the midline in the nucleus interfascicularis and nucleus linearis. While the entire VTA was responsive to SKF-82958 or cocaine-induced elevation in extracellular glutamate, in the six rats (one rat from the experiment in Fig. 1, three rats from Fig. 3, and two rats from Fig. 4) having probes located in the substantia nigra the drugs were not effective. Figure 7, *A* and *B*, contains micrographs showing examples of dialysis probe placement in the VTA and substantia nigra. The effect of cocaine on extracellular glutamate corresponding to each rat is shown in Figure 7, *C* and *D*, respectively. While cocaine produced a marked elevation in glutamate in the VTA, it did not alter levels in the substantia nigra. Figure 7 also shows a micrograph of probe placement from an animal employed in the experiment shown in Figure 1. This micrograph at higher magnification verifies that the highest dose of SKF-82958 (300 μM) did not result in significant damage outside the mechanical disruption accompanying insertion of the probe.

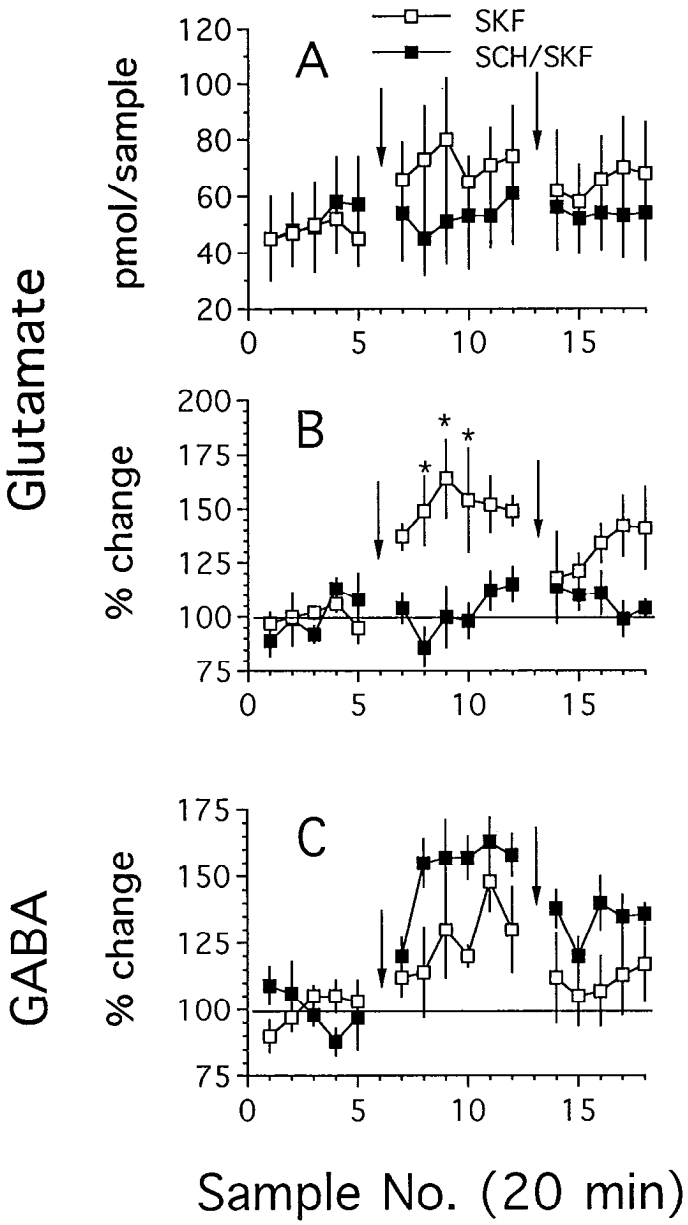


Figure 3. Capacity of SCH-23390 to block the elevation in glutamate, but not GABA, produced by SKF-82958. Following five baseline samples, either SKF-82958 (100 μM) alone or in combination with SCH-23390 (30 μM) was perfused through the dialysis probe for six samples, at which time control buffer was perfused until the end of the experiment. The period of drug perfusion is delineated by the *arrows*. *A* shows the raw glutamate levels, *B* shows the glutamate levels normalized to percent change from baseline, and *C* shows the levels of GABA normalized to percent change from baseline. The data are shown as mean ± SEM and were evaluated using a two-way ANOVA with repeated measures over time. For glutamate: $N = 5$ and 6; treatment $F_{(1,9)} = 13.28$, $p = 0.005$; time $F_{(15,135)} = 3.24$, $p < 0.001$; interaction $F_{(15,135)} = 2.59$; $p = 0.002$. For GABA: $N - 6$ and 7; treatment $F_{(1,11)} = 2.31$, $p = 0.096$; time $F_{(15,165)} = 3.48$, $p < 0.001$; interaction $F_{(15,165)} = 1.41$; $p = 0.148$. *, $p < 0.05$; comparing SKF/SCH with SKF alone at individual times using a least significant difference post hoc comparison (Milliken and Johnson, 1984).

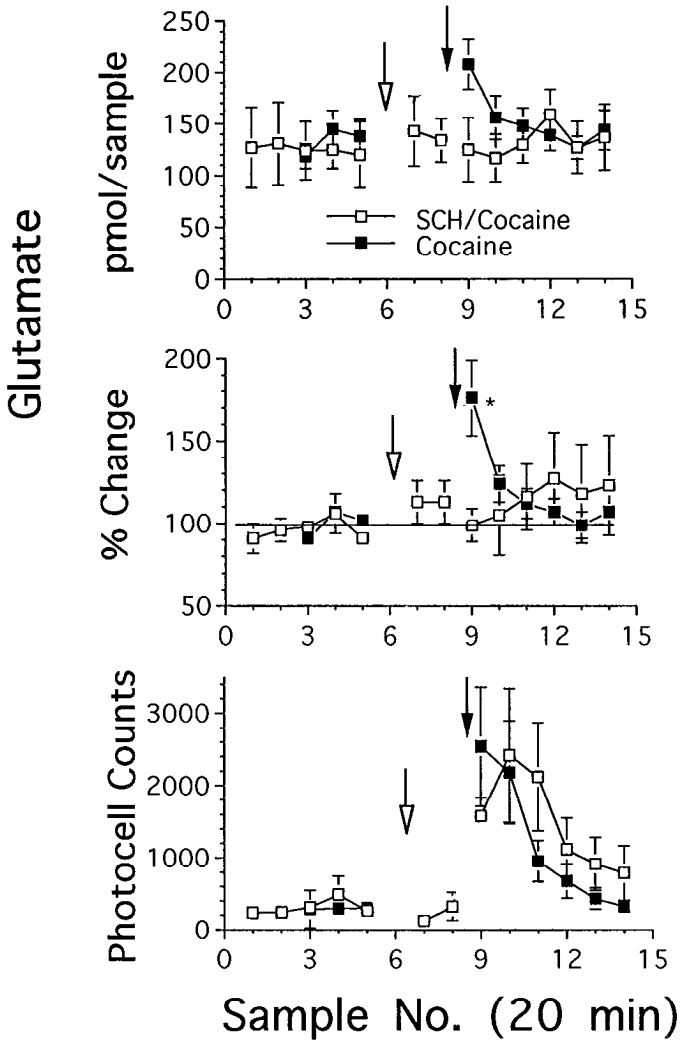


Figure 4. Effect of cocaine on extracellular glutamate and blockade by SCH-23390. The *top panel* shows raw data, the *middle panel* shows the levels of glutamate normalized to percent change from baseline, and the *bottom panel* shows the behavioral response. Two experiments were conducted. In the first experiment (cocaine; $N = 9$), following collection of three baseline dialysis samples (samples 3–5) rats were given a systemic injection of cocaine (15 mg/kg, i.p.) and six subsequent samples obtained (samples 9–15). In the second experiment (SCH/cocaine; $N = 5$), after five baseline samples (samples 1–5), SCH-23390 (30 μM) was added to the dialysis buffer (samples 6–14), and 60 min later the rats were injected with cocaine (15 mg/kg, i.p.), corresponding to samples 9–15. Thus, the sample number corresponds to time for the SCH/cocaine group (experiment 2). For the cocaine group (experiment 1), sample 3 corresponds to the beginning of the experiment and samples 5 and 9 correspond to adjacent collections. The *solid arrow* indicates the injection of cocaine, and the *open arrow* indicates the time when SCH-23390 was added to the dialysis buffer of the SCH/cocaine group. Data are shown as mean ± SEM and samples were statistically compared using a two-way ANOVA with repeated measures over time. *Middle*, treatment $F_{(1,12)} = 0.01$, $p = 0.925$; time $F_{(8,96)} = 3.27$, $p = 0.002$; interaction $F_{(8,96)} = 3.04$, $p = 0.004$. *Bottom*, treatment $F_{(1,12)} = 0.25$, $p = 0.629$; time $F_{(8,96)} = 9.04$, $p < 0.001$; interaction $F_{(8,96)} = 1.25$, $p = 0.278$. *, $p < 0.05$; comparing cocaine to cocaine/SCH-23390 at individual times using a least significant difference post hoc analysis (Milliken and Johnson, 1984).

Discussion

These data demonstrate that stimulation of D_1 receptors in the VTA increases the extracellular content of glutamate. The involvement of D_1 receptors is indicated by the fact that the effect of SKF-82958 on glutamate was prevented by coperfusion of a D_1 antagonist and was not elicited by the D_2/D_3 agonist, quinpirole. Furthermore, systemic administration of cocaine, which is known to elevate extracellular somatodendritic dopamine (Bradberry et al., 1989; Kalivas and Duffy, 1993; Parsons and

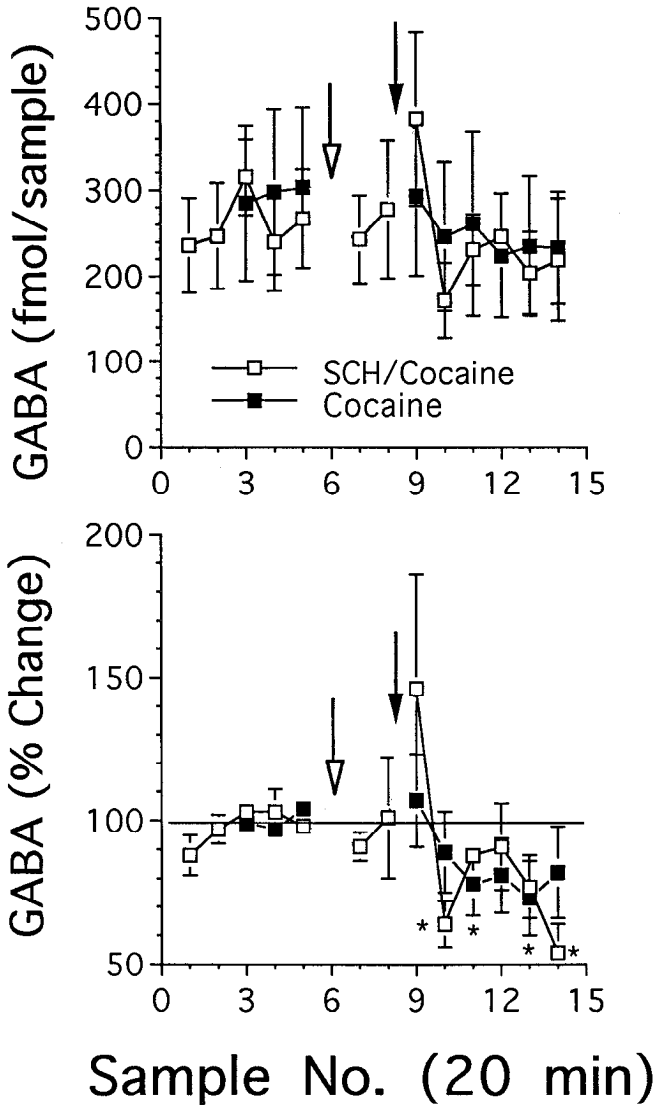


Figure 5. Effect of cocaine on extracellular GABA and lack of effect by SCH-23390. The experiments were conducted as described in the Figure 4 caption. The *top panel* shows raw data, and the *bottom panel* shows the levels of GABA normalized to percent change from baseline. The *solid arrow* indicates the injection of cocaine, and the *open arrow* indicates the time when SCH-23390 was introduced into the dialysis buffer of the SCH/cocaine group. Data are shown as mean ± SEM and were statistically evaluated using a two-way ANOVA with repeated measures over time. *Bottom*, treatment $F_{(1,8)} = 0.14$, $p = 0.719$; time $F_{(8,80)} = 2.69$, $p = 0.010$; interaction $F_{(8,80)} = 1.55$, $p = 0.132$. *, $p < 0.05$; comparing all times to the last baseline value within each treatment group, using a least significant difference post hoc analysis. The SCH/Cocaine group revealed a significant reduction from baseline at 40 and 100 min after cocaine administration while the cocaine group demonstrated a reduction at 60 and 100 min following cocaine.

Justice, 1993), increased extracellular glutamate and this effect was blocked by local perfusion of SCH-23390. In contrast, the stimulation of extracellular GABA levels by SKF-82958 perfusion into the VTA was not blocked by SCH-23390. Moreover, systemic cocaine administration produced inconsistent reductions in extracellular GABA in the VTA, an effect also not prevented by SCH-23390 perfusion into the VTA.

Synaptic organization of the VTA. The location of D_1 receptors on afferent terminals in the ventral mesencephalon supports the presynaptic regulation of GABA and glutamate release by D_1 receptor stimulation. This localization has been characterized in the substantia nigra, where it was shown that destruction of the GABAergic striatonigral projection eliminates the majority of D_1 receptors, and that mRNA for D_1 receptors is not present in ventral mesencephalic neurons (Beckstead, 1988; Mansour et al., 1992). By analogy, D_1 receptors in the VTA may reside primarily on GABAergic afferents arising from the nucleus accumbens and ventral pallidum (Kalivas et al., 1993). However, in addition to GABA, there exists excitatory amino acid afferents to the VTA and substantia nigra which could also express presynaptic D_1 receptors. Indeed, the relatively poor potency of SKF-82958 to increase extracellular GABA and the lack of sensitivity to blockade by SCH-23390 argues that D_1 receptors may preferentially modulate EAA release. To some extent the EAA afferents to the ventral mesencephalon are topographically organized. For example, EAA projections emanating from the prefrontal cortex and amygdala preferentially innervate the VTA, while EAA afferents from the subthalamic nucleus and pedunculopontine region project preferentially to the substantia nigra (Kita and Kitai, 1986; Scarnati et al., 1986; Christie et al., 1987). Considering the relative insensitivity of the substantia nigra to SKF-82958 or cocaine-induced elevation in glutamate, it may be that only EAA afferents projecting to the VTA express D_1 receptors. Supporting this, a portion of the cells in the prefrontal cortex that are retrogradely labeled from the VTA express mRNA for D_1 receptors (unpublished observation).

Seemingly inconsistent with the synaptic organization of the VTA outlined above, the low dose of SCH-23390 (0.03 μM) significantly elevated the concentration of extracellular glutamate. This poses the possibility that the effect of D_1 receptor stimulation on glutamate release is biphasic. At basal levels of stimulation D_1 receptors may be inhibiting the release of glutamate, while at higher levels of stimulation release is promoted. If true, the release of glutamate by D_1 receptors may occur only following pharmacological stimulation or under pathological conditions (see below).

Functional significance of D_1 receptor regulated release. Based upon both neurochemical and electrophysiological studies (Starr, 1987; Timmerman et al., 1991; Cameron and Williams, 1993), the prevailing view is that D_1 receptor-mediated stimulation of GABA release provides a form of negative feedback, which, in combination with D_2 autoreceptor stimulation, regulates dopamine cell firing frequency and somatodendritic dopamine release. However, the observation that SKF-82958 increased extracellular glutamate levels at lower, presumably more physiological levels of stimulation, indicates that D_1 receptors may provide positive feedback onto dopamine neurons. Stimulation of either NMDA or non-NMDA receptors increases the firing frequency of dopamine neurons (Grace, 1987; Mereu et al., 1991; Johnson and North, 1992), and NMDA receptor activation appears to selectively increase the occurrence of burst firing patterns (Johnson et al., 1992; Overton and Clark, 1992). Thus, enhanced glutamate release by D_1 receptor stimulation may increase the firing frequency of dopamine neurons. In apparent contrast to this conclusion, Cameron and Williams (1993) show in tissue slices that stimulation of D_1 receptors hyperpolarizes dopamine cells via increasing GABA stimulation of $GABA_B$ receptors. However, these studies were conducted in the presence of the NMDA and non-NMDA blockers, AP5 and CNQX, respectively, which would eliminate any polarization arising from enhanced glutamate release.

In addition to dopamine cells, there exist nondopaminergic

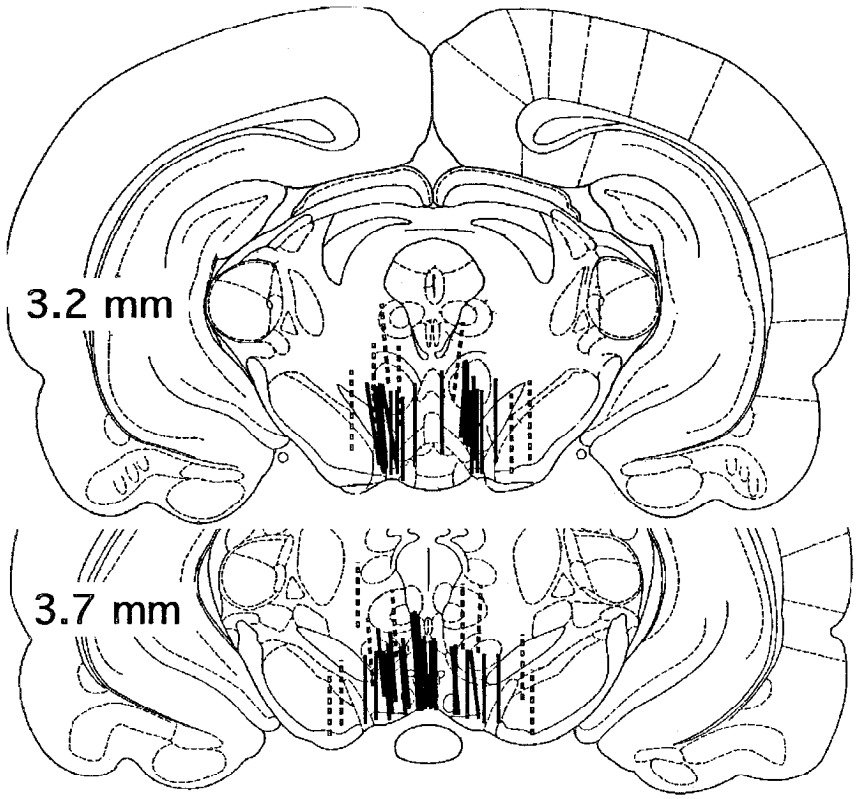


Figure 6. Illustration of the location of the dialysis probes in the VTA and adjacent nuclei. *Solid lines* indicate probe tracks within the VTA that were used for data analysis, while the *dashed line* indicates placement outside the VTA from animals that were excluded from data analysis. In addition to the probe tracks shown in the drawing, seven rats were excluded from the study with placements caudal to the diagrams. The drawings were derived from the atlas of Paxinos and Watson (1986).

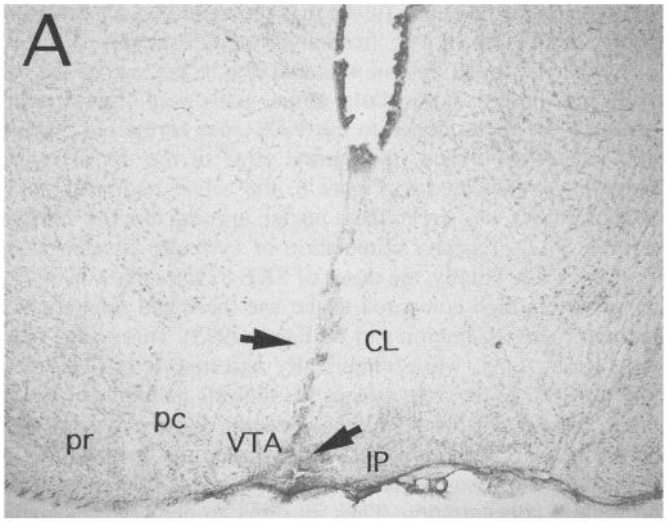
neurons in the VTA which are, at least partly, GABAergic (Oertel et al., 1982; Nagai et al., 1983; Smith and Bolam, 1990), and the firing frequency of these neurons is elevated by glutamate analogues (Mereu et al., 1991). It is possible that D_1 receptor stimulation may selectively release glutamate from terminals synapsing on GABAergic interneurons, which are known to inhibit dopamine cells (Grace and Bunny, 1979; Johnson and North, 1992). Supporting this possibility, D_1 receptor agonists stimulate the firing frequency of cells in the substantia nigra, pars reticulata, which are predominantly GABAergic (Waszczak, 1990; Martin and Waszczak, 1994). In contrast to the excitatory effect of glutamate directly on dopamine cells, increased EAA transmission at GABAergic interneurons would inhibit dopamine cell firing frequency by promoting GABA release (Grace and Bunney, 1979, 1985; Mereu et al., 1991; Johnson and North, 1992).

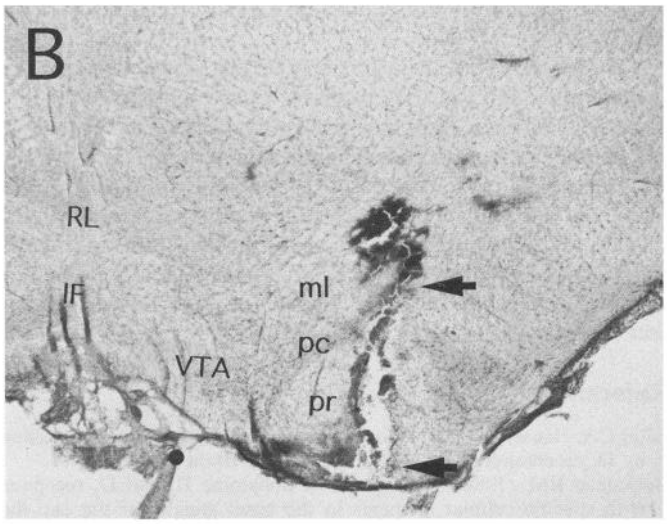
Effects of cocaine. Cocaine increases extracellular somatodendritic dopamine levels in the VTA (Bradberry and Roth, 1989; Kalivas and Duffy, 1993; Parsons and Justice, 1993) A number of neuropsychiatric pathologies have been associated with hyperactivity of dopamine transmission in the mesoaccumbens projection (Grace, 1991; Goldstein and Deutch, 1992; Sato, 1992). Indeed, chronic abuse of amphetamine or cocaine can elicit paranoid psychosis and panic attacks which are indistinguishable from idiopathic psychopathologies (Post and Weiss, 1988; Sato, 1992). This drug induced psychopathology is termed behavioral sensitization and its neural mechanisms have been studied in some detail using an animal model wherein repeated administration of psychostimulants to rodents results in a progressive augmentation in motor activity (Segal and Schuckitt, 1983; Robinson and Becker, 1986; Kalivas and Stewart, 1991). The fact that systemic cocaine administration increased extracellular glutamate by indirectly stimulating D_1 receptors in the VTA indicates a potential role for this mechanism in sensitization-based psychopathologies. Moreover, behavioral sensitization to repeated psychostimulant administration in rats is prevented by pretreating the VTA with either a D_1 (Stewart and Vezina, 1989; Bjijou et al., 1994) or NMDA receptor antagonist (Kalivas and Alesdatter, 1993). These findings indicate that D_1 receptor-mediated activation of glutamate transmission in the VTA is an important sequence of synaptic events in the development of behavioral sensitization to psychostimulants.

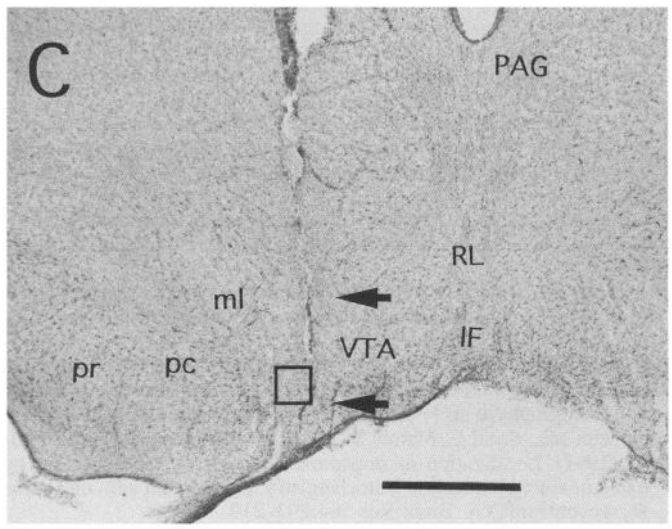
In contrast to glutamate, cocaine treatment reduced extracellular GABA levels in the VTA, which is the opposite of what occurs with local application of D_1 agonist into the VTA. Further, this reduction was not blocked by intra-VTA perfusion of a D_1 antagonist. This lack of D_1 receptor involvement combined with the fact that administration of the $D_{2/3}$ agonist, quinpirole, into the VTA did not alter extracellular GABA argues that the reduction in GABA transmission in the VTA does not arise from cocaine-induced elevation of somatodendritic dopamine. In addition to the dopamine transporter, cocaine binds to the serotonin (5-HT) transporter (Ritz et al., 1990), producing an elevation in extracellular 5-HT in the VTA (Parsons and Justice, 1993). Cameron and Williams (1994) recently demonstrated that cocaine perfusion of VTA tissue slices reduces GABA mediated hyper-

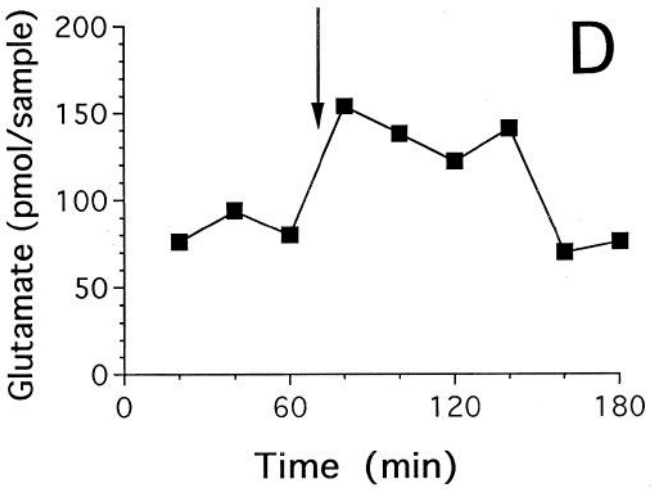
→

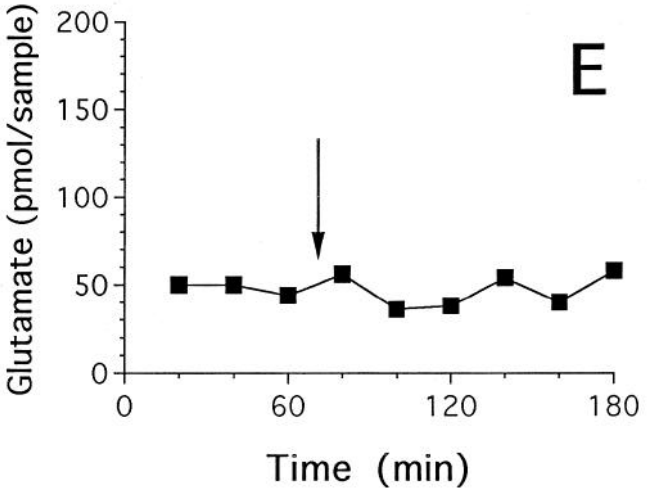
Figure 7. Micrographs showing the location of probe tracks in the VTA (*A* and *C*) and in the substantia nigra (*B*). *D and E* show the level of extracellular glutamate following an injection of cocaine (15 mg/kg, i.p.) obtained from the rats in *A* and *B*, respectively. Note the lack of increase in extracellular glutamate by cocaine in the animal implanted in the substantia nigra. The micrograph in *C* was obtained from an animal used in

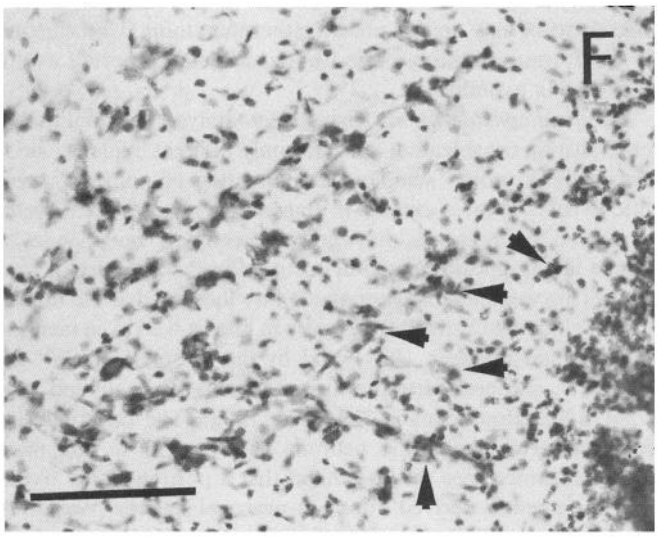












the experiment depicted in Figure 1 that received the full dose–response curve of SKF-82958 (i.e., including 300 μM). *F*, The higher magnification micrograph (delineated by the *box* in *C*) shows that this treatment did not produce significant neurotoxicity beyond the mechanical damage resulting from the probe itself. The *arrowheads* identify fusiform shaped neurons adjacent to the glial scar produced by the probe. *CL*, Nucleus linearis caudalis; *IF*, nucleus interfascicularis; *IP*, nucleus interpeduncularis; *ml*, lemniscus medialis; *PAG*, periaqueductal gray; *pc*, substantia nigra, pars compacta; *pr*, substantia nigra, pars reticulata; *RL*, nucleus linearis rostralis; *VTA*, ventral tegmental area. Scale bars: *C*, 1 mm; *F*, 0.1 mm. *Arrows* delineate the approximate length of the active dialysis membrane (*A–C*) or the time of cocaine injection (*D, E*).

polarizations of dopamine neurons. Further, this effect of cocaine resulted from the stimulation of 5-HT_{1B} receptors. Thus, cocaine may indirectly elicit two presynaptic effects in the VTA by D_1 and 5-HT_{1B} presynaptic receptors, and both of these actions will increase excitatory input to VTA neurons by enhancing EAA or inhibiting GABA release, respectively. The fact cocaine reduces the extracellular content of GABA in the VTA appear to contradict the electrophysiological observations that cocaine inhibits the firing frequency of dopamine neurons. Cocaine-induced inhibition of dopamine cell firing is thought to arise via stimulation of D_2 receptors (Brodie and Dunwiddie, 1990; Lacey et al., 1990) and/or activation of descending GABAergic afferents (Einhorn et al., 1988). Two explanations for this discrepancy can be offered. (1) As discussed above, the reduction in extracellular GABA may be dominated by effects on descending GABAergic afferents to inhibitory GABAergic interneurons which would result in increase GABA release from the interneurons and inhibition of dopamine cells. However, Cameron and Williams (1994) recorded directly from dopamine neurons to demonstrate a reduction in stimulated GABA transmission. (2) The electrophysiological studies were conducted in anesthetized rats or tissue slices which alter functional status of EAA and GABAergic afferents to the VTA. In tissue slices the afferents have been lesioned and anesthesia decreases the spontaneous activity of accumbal medium spiny neurons which contribute to the descending GABAergic innervation of the VTA (Henriksen and Giachino, 1993; Kalivas et al., 1993).

Although the levels of extracellular GABA were significantly reduced by cocaine or cocaine plus SCH-23390, the effect in both groups was inconsistent over time. No clear explanation for the variable time course of GABA reduction is revealed by the present experiments. However, similar inconsistent reductions in GABA in the ventral pallidum have been observed following dopamine agonist administration (Bourdelais and Kalivas, 1990). It is possible that GABA transmission is under strong homeostatic regulation and presynaptic modifications of membrane potential may only bias transmitter release and not provide obligatory control. Thus, the changes in extracellular GABA concentration may appear phasic rather than tonic. Unfortunately, the 20 min sampling period utilized does not permit evaluation of this hypothesis.

Technical considerations. Using microdialysis as a tool to discern synaptic organization provides only indirect evidence since microdialysis probes sample the summation of numerous synaptic events. Thus, a change in transmitter release from a subpopulation of synapses may go undetected if no change or change in an opposite direction occurred in the majority of synapses. This weakness is compounded by the fact that the uptake of both glutamate and GABA occurs in glia as well as in neurons (McGeer and McGeer, 1989), and by the presence of Ca^{2+}-independent, carrier-mediated efflux of GABA (Bernath and Zigmond, 1988; Bourdelais and Kalivas, 1992). The metabolic interaction between glia and neurons with regards to glutamate and GABA increases the likelihood that a portion of the extracellular content is derived from non-neuronal sources (Westerink and de Vries, 1989). In spite of difficulties in identifying the source of amino acids, the responsiveness of extracellular levels to the stimulation or inhibition of neurotransmitter receptors (e.g., D_1) indicates that the levels measured by dialysis can reflect synaptic events. However, especially for glutamate where the basal levels in the VTA are in the range of 0.5–3.0 μM (present report; Li et al., 1994), it is probable that unstimulated levels are derived, in part, from nonsynaptic sources.

Another technical limitation is that due to the size of the microdialysis probes, extracellular amino acids were often derived, in part, from nuclei dorsal to the VTA, such as the red nucleus and, to a lesser extent, the ventral edge of the dorsal raphe. However, as indicated in Figure 6, the active region of many dialysis probes was not in these nuclei, arguing that the changes induced by D_1 receptor stimulation or systemic cocaine arose from the VTA. Finally, the doses of SKF-82958 and SCH-23390 are relatively high compared to the use these and related compounds *in vitro* (Cameron and Williams, 1993). This results from two factors. First, while empirically untested, it is likely that only 10–20% of the drug crosses the dialysis membrane into the brain (Parsons and Justice, 1994). Second, *in vitro* systems permit perfusion of the entire tissue slice. In contrast, with a dialysis probe only the tissue adjacent to the probe will be exposed to the highest concentration. Thus, in order to elicit a measurable response it may require perfusion of relatively large concentrations for pharmacologically relevant doses of drug to access the majority of the VTA.

Conclusions. These data demonstrate that somatodendritic dopamine release in the VTA increases extracellular levels of glutamate. This results from the stimulation of D_1 receptors that are presumably located on glutamatergic axon terminals in the VTA. This synaptic arrangement poses the possibility of positive feedback onto dopamine cells which may be important in dopamine related psychiatric disorders, notably psychostimulant-induced psychosis. Supporting this postulate, systemic cocaine administration elevates extracellular glutamate in the VTA and this effect is prevented by blocking D_1 receptors.

References

Altar CA, Hauser K (1987) Topography of substantia nigra innervation by D_1 receptor-containing striatal neurons. Brain Res 410:1–11.

Beckstead RM (1988) Association of dopamine D_1 and D_2 receptors with specific cellular elements in the basal ganglia of the cat: the uneven topography of dopamine receptors in the striatum is determined by intrinsic striatal cells, not nigrostriatal axons. Neuroscience 27:851–863.

Bernath S, Zigmond MJ (1988) Characterization of [^{3}H]-GABA release from striatal slices: evidence for a calcium-independent process via the GABA uptake system. Neuroscience 27:677–682.

Bjijou Y, Stinus L, LeMoal M, Cador M (1994) Selective involvement of dopamine D1 receptors in the VTA in the behavioral sensitization induced by intra-VTA amphetamine injections. Soc Neurosci Abstr 20:1623.

Bourdelais AJ, Kalivas PW (1990) Amphetamine lowers extracellular GABA concentration in the ventral pallidum. Brain Res 516:132–136.

Bourdelais AJ, Kalivas PW (1992) Modulation of extracellular GABA in the ventral pallidum using *in vivo* microdialysis. J Neurochem 58: 2311–2320.

Bouthenet M-L, Martres M-P, Sales N, Schwartz J-C (1987) A detailed mapping of dopamine D-2 receptors in rat central nervous system by autoradiography with [^{125}I]iodosulpride. Neuroscience 20:117–155.

Bouthenet ML, Souil E, Matres M-P, Sokoloff P, Giros B, Schwartz, J-C (1991) Localization of dopamine D_3 receptor mRNA in rat by using *in situ* hybridization histochemistry: comparison with dopamine D_2 receptor mRNA. Brain Res 564:203–219.

Bradberry CW, Roth RH (1989) Cocaine increases extracellular dopamine in rat nucleus accumbens and ventral tegmental area as shown by *in vivo* microdialysis. Neurosci Lett 103:97–102.

Brodie MS, Dunwiddie TV (1990) Cocaine effects in the ventral tegmental area: evidence for an indirect dopaminergic mechanism of action. Naunyn Schmiedeberg's Arch Pharmacol 342:660–665.

Bunney BS, Walters JR, Roth RH, Aghajanian GK (1973) Dopami-

nergic neurons: effect of antipsychotic drugs and amphetamine on single cell activity. J Pharmacol Exp Ther 185:560–571.
Cameron DL, Williams JT (1993) Dopamine D_1 receptors facilitate transmitter release. Nature 366:344–347.
Cameron DL, Williams JT (1994) Cocaine inhibits GABA release in the VTA through endogenous 5-HT. J Neurosci 14:6763–6767.
Christie MJ, Summers RJ, Stephenson JA, Cook CJ, Beart PM (1987) Excitatory amino acid projections to the nucleus accumbens septi in the rat: a retrograde transport study utilizing D[^{3}H]aspartate and [^{3}H]GABA. Neuroscience 22:425–439.
Donzanti BA, Yamamoto BK (1988) An improved and rapid HPLC-EC method for the isocratic separation of amino acid neurotransmitters from brain tissue and microdialysis perfusates. Life Sci 43:913–914.
Einhorn LC, Johansen PA, White FJ (1988) Electrophysiological effects of cocaine in the mesoaccumbens dopamine system: studies in the ventral tegmental area. J Neurosci 8:100–112.
Goldstein M, Deutch AY (1992) Dopaminergic mechanisms in the pathogenesis of schizophrenia. FASEB J 6:2413–2421.
Grace AA (1987) The regulation of dopamine neurons activity as determined by *in vivo* and *in vitro* intracellular recordings. In: The neurophysiology of dopamine systems (Chiodo LA, Freeman AS, eds), pp 1–66. Detroit: Lake Shore.
Grace AA (1991) Phasic versus tonic dopamine release and the modulation of dopamine system responsivity: a hypothesis for the etiology of schizophrenia. Neuroscience 41:1–24.
Grace AA, Bunney BS (1979) Paradoxical GABA excitation of nigral dopaminergic cells: indirect mediation through reticulata inhibitory neurons. Eur J Pharmacol 59:211–218.
Grace AA, Bunney BS (1985) Opposing effects of striatonigral feedback pathways on midbrain dopamine cell activity. Brain Res 333: 271–284.
Henriksen SJ, Giachino J (1993) Functional characteristics of nucleus accumbens neurons: evidence obtained from *in vivo* electrophysiological recordings. In: Limbic motor circuits and neuropsychiatry (Kalivas PW, Barnes CD, eds), pp 101–124. Boca Raton, FL: CRC.
Innis RB, Aghajanian GK (1987) Pertussis toxin blocks autoreceptor-mediated inhibition of dopaminergic neurons in rat substantia nigra. Brain Res 411:139–143.
Johnson SW, North RA (1992) Two types of neurone in the rat ventral tegmental area and their synaptic inputs. J Physiol (Lond) 450:491–502.
Johnson SW, Seutin V, North RA (1992) Burst firing in dopamine neurons induced by *N*-methyl-D-aspartate: role of electrogenic sodium pump. Science 258:665–667.
Kalivas PW (1993) Neurotransmitter regulation of dopamine neurons in the ventral tegmental area. Brain Res Rev 18:75–113.
Kalivas PW, Alesdatter JE (1993) Involvement of NMDA receptor stimulation in the VTA and amygdala in behavioral sensitization to cocaine. J Pharmacol Exp Ther 267:486–495.
Kalivas PW, Duffy P (1991) A comparison of axonal and somatodendritic dopamine release using *in vivo* dialysis. J Neurochem 56:961–967.
Kalivas PW, Duffy P (1993) Time course of extracellular dopamine and behavioral sensitization to cocaine. II. Dopamine perikarya. J Neurosci 13:276–284.
Kalivas PW, Stewart J (1991) Dopamine transmission in the initiation and expression of drug- and stress-induced sensitization of motor activity. Brain Res Rev 16:223–244.
Kalivas PW, Churchill L, Klitenick MA (1993) GABA and enkephalin projection from the nucleus accumbens and ventral pallidum to VTA. Neuroscience 57:1047–1060.
Kita H, Kitai ST (1986) Efferent projections of the subthalamic nucleus in the rat. Light and electron microscopic analysis with the PHA-L method. J Comp Neurol 372:21–30.
Klitenick MA, DeWitte P, Kalivas PW (1992) Regulation of somatodendritic dopamine release in the ventral tegmental area by opioids and GABA. J Neurosci 12:2623–2632.
Koob GF, Robledo P, Markou A, Caine SB (1993) The mesocorticolimbic circuit in drug dependence and reward—a role for the extended amygdala? In: Limbic motor circuits and neuropsychiatry (Kalivas PW, Barnes CD, eds), pp 289–310. Boca Raton, FL: CRC.
Lacey MG, Mercuri NB, North RA (1987) Dopamine acts at D_2 receptors to increase potassium conductance in neurons of the rat substantia nigra. J Physiol (Lond) 392:397–416.
Lacey MG, Mercuri NB, North RA (1990) Actions of cocaine on rat dopaminergic neurones *in vitro*. Br J Pharmacol 99:731–735.
Li Y, Xue C-J, Wolf ME (1994) Effects of acute and repeated amphetamine administration on extracellular glutamate and aspartate levels in the rat ventral tegmental area and nucleus accumbens. Soc Neurosci Abstr 20:1621.
Mansour A, Meador-Woodruff JH, Zhou Q, Civelli O, Akil H, Watson SJ Jr (1992) A comparison of D1 receptor binding and mRNA in rat brain using receptor autoradiographic and *in situ* hybridization techniques. Neuroscience 46:959–971.
Martin LP, Waszczak BL (1994) D_1 agonist-induced excitation of substantia nigra, pars reticulata neurons: mediation by D_1 receptors on striatonigral terminals via pertussis toxin-sensitive coupling pathway. J Neurosci 14:4494–4506.
McGeer PL, McGeer EG (1989) Amino acid neurotransmitters. In: Basic neurochemistry: molecular, cellular and medical aspects, 4th ed (Siegel G, Agranoff B, Albers RW, Molinoff P, eds), pp 311–332, New York: Raven.
Meador-Woodruff JH, Mansour A, Healy DJ, Kuehn R, Zhou Q-Y, Bunzow JR, Akil H, Civelli O, Watson SJ Jr (1991) Comparison of the distributions of D1 and D2 dopamine receptor mRNA's in rat brain. Neuropsychopharmacology 5:231–242.
Mereu G, Costa E, Armstrong DM, Vicini S (1991) Glutamate receptor subtypes mediate excitatory synaptic currents of dopamine neurons in midbrain slices. J Neurosci 11:1359–1366.
Milliken GA, Johnson DE (1984) Analysis of messy data. I. Designed experiments. Belmont, CA: Lifetime Learning.
Nagai T, McGeer PL, McGeer EG (1983) Distribution of GABA-T-intensive neurons in the rat forebrain and midbrain. J Comp Neurol 218:220–238.
Oertel WH, Tappaz ML, Berod A, Mugnaini E (1982) Two-color immunohistochemistry for dopamine and GABA neurons in rat substantia nigra and zona incerta. Brain Res Bull 9:463–474.
Overton P, Clark D (1992) Iontophoretically administered drugs acting at the *N*-methyl-D-aspartate receptor modulate burst firing in A9 dopamine neurons in the rat. Synapse 10:131–140.
Parsons LH, Justice JB Jr (1993) Serotonin and dopamine sensitization in the nucleus accumbens, ventral tegmental area and dorsal raphe nucleus following repeated cocaine administration. J Neurochem 61: 1611–1619.
Parsons LH, Justice JB Jr (1994) Quantitative approaches to *in vivo* brain microdialysis. Crit Rev Neurobiol 8:189–220.
Paxinos G, Watson C (1986) The rat brain in stereotaxic coordinates. New York: Academic.
Pellegrino LK, Pellegrino AS, Cushman AJ (1979) A stereotaxic atlas of the rat brain. New York: Plenum.
Post RM, Weiss SRB (1988) Sensitization and kindling: implications for the evolution of psychiatric symptomatology. In: Sensitization in the nervous system (Kalivas PW, Barnes CD, eds), pp 257–292. Caldwell, NJ: Telford.
Ritz MC, Cone EJ, Kuhar MJ (1990) Cocaine inhibition of ligand binding at dopamine, norepinephrine and serotonin transporters: a structure–activity study. Life Sci 46:635–645.
Robertson GS, Damsma G, Fibiger HC (1991) Characterization of dopamine release in the substantia nigra by *in vivo* microdialysis in the freely moving rat. J Neurosci 11:2209–2216.
Robinson TE, Becker JB (1986) Enduring changes in brain and behavior produced by chronic amphetamine administration: a review and evaluation of animal models of amphetamine psychosis. Brain Res Rev 11:157–198.
Robinson TE, Wishaw IQ (1988) Normalization of extracellular dopamine in the striatum following recovery from a partial unilateral 6-OHDA lesion of the substantia nigra: a microdialysis study in freely moving rats. Brain Res 450:209–224.
Sato M (1992) A lasting vulnerability to psychosis in patients with previous methamphetamine psychosis. Ann NY Acad Sci 654:160–170.
Scarnati E, Proia A, Campana E, Pacitti C (1986) A microiontophoretic study on the nature of the putative synaptic neurotransmitter involved in the pedunculopontine-substantia nigra pars compacta excitatory pathway of the rat. Exp Brain Res 62:470–478.
Segal DS, Schuckit MA (1983) Animal models of stimulant-induced psychosis. In: Stimulants: neurochemical, behavioral, and clinical perspectives (Creese I, ed), pp 131–167. New York: Raven.
Smith Y, Bolam JP (1990) The output neurones and the dopaminergic

neurones of the substantia nigra receive a GABA-containing input from the globus pallidus in the rat. J Comp Neurol 296:47–64.

Starr M (1987) Opposing roles of dopamine D_1 and D_2 receptors in nigral [^{3}H]aminobutyric acid release? J Neurochem 49:1042–1049.

Stewart J, Vezina P (1989) Microinjections of SCH-23390 into the ventral tegmental area and substantia nigra pars reticulata attenuate the development of sensitization to the locomotor activating effects of systemic amphetamine. Brain Res 495:401–406.

Timmerman W, Zwaveling J, Westerink BHC (1991) Dopaminergic modulation of the GABA release in the substantia nigra reticulata. In: Monitoring molecules in neuroscience (Rollema H, Westerink BHC, Drijfhout WJ, eds), pp 105–107. The Netherlands: Krips Repro.

Wang RY (1981) Dopaminergic neurons in the rat ventral tegmental area. II. Evidence for autoregulation. Brain Res Rev 3:141–152.

Waszczak BL (1990) Differential effects of D_1 and D_2 dopamine receptor agonists on substantia nigra pars reticulata neurons. Brain Res 513:125–135.

Westerink BHC, de Vries JB (1989) On the origin of extracellular GABA collected by brain microdialysis and assayed by a simplified on-line method. Naunyn-Schmiedeberg's Arch Pharmacol 336:502–507.