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Ventral pallidal extracellular fluid levels of dopamine, serotonin, gamma amino butyric acid, and glutamate during cocaine self-administration in rats

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Abstract *Rationale:* Dopamine innervation of the nucleus accumbens is thought to have a major role in the biological processes underlying cocaine self-administration. Recent data suggest that dopamine innervation of the ventral pallidum (VP) may also play an important role. *Objectives:* This experiment was initiated to assess extracellular fluid levels of dopamine (DA), serotonin (5-HT), gamma-aminobutyric acid (GABA), and glutamate (Glu) in the VP of rats self-administering cocaine using in vivo microdialysis. *Methods:* Rats were implanted with intravenous jugular catheters and a microdialysis probe guide cannula into the VP and trained to self-administer (SA) three different doses of cocaine during each daily session. Other rats (yoked rats) were surgically prepared in identical fashion and received vehicle infusions during microdialysis sessions when the SA rat to whom they were yoked produced cocaine infusions. When stable baselines of self-administration were obtained, microdialysates were collected during two consecutive daily self-administration sessions. Neurotransmitter levels were measured using HPLC with electrochemical (DA and 5-HT) or fluorescence detection (GABA and Glu). *Results:* In SA rats, extracellular fluid levels of DA [$[DA]_e$] and 5-HT [$[5-HT]_e$] were elevated throughout the session and levels of Glu [$[Glu]_e$] showed small increases at a few isolated time points during the session. The increases in $[DA]_e$ and $[5-HT]_e$ were dose-dependent. Extracellular fluid levels of GABA [$[GABA]_e$] were unchanged, as were levels of all four neurotransmitters in the yoked rats. *Conclusions:* These data support a potential role for DA and 5-HT innervations of the VP in intravenous cocaine self-administration.

Key words Ventral pallidum · Cocaine self-administration · Dopamine · Serotonin · GABA · Glutamate

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Introduction

During the last two decades, significant progress has been made toward understanding the biological mechanisms underlying cocaine self-administration. Recent research has focused on the role of dopamine (DA) in the actions of cocaine. Systemic administration of D_1 and D_2 receptor antagonists to animals self-administering cocaine has been reported to increase the rate of intake under fixed-ratio schedules (DeWit and Wise 1977; Roberts and Vickers 1984; Koob et al. 1987; Gerber and Wise 1989; Caine and Koob 1994a; Hemby et al. 1996) and to decrease breakpoint under progressive-ratio schedules of availability (Hubner and Morton 1991; Richardson et al. 1993). In contrast, cocaine self-administration in rodents appears not to be disrupted by systemic administration of serotonergic (Porrino et al. 1989; Peltier and Schenk 1991) or noradrenergic antagonists (DeWit and Wise 1977). Further support for the importance of DA is the demonstration that receptor agonists are self-administered (Yokel and Wise 1978; Woolverton et al. 1984; Self and Stein 1992; Weed et al. 1993; Nader and Mach 1996).

The brain site containing the DA innervations that mediate stimulant self-administration is thought to be the nucleus accumbens (Roberts et al. 1977). More recently, increased nucleus accumbens (NA) $[DA]_e$ has been demonstrated during cocaine self-administration (Pettit and Justice 1989; Hemby et al. 1997), that is significantly higher than that seen in yoked controls or with response independent infusions to the self-administering animals (Hemby et al. 1997). 6-Hydroxydopamine (6-OHDA) induced lesions of DA innervations of this area attenuate intravenous cocaine self-administration by rats (Roberts et al. 1977, 1980; Pettit et al. 1984; Caine and Koob 1994b). In addition, the direct injection of DA receptor antagonists into the NA produce the same effects on drug intake as those seen following systemic administration (Maldonado et al. 1993; McGregor and Roberts 1993; Phillips et al. 1994). These data collectively suggest a major role for DA innervation of the NA in cocaine self-administration.

In spite of the volumes of data demonstrating the importance of the NA, experimental evidence also suggests other anatomically related brain regions and neurotransmitter systems are involved in the biological processes underlying cocaine self-administration (Smith et al., unpublished data). Recent measures of neurotransmitter turnover rates during intravenous cocaine self-administration have demonstrated an increase in DA turnover with no changes in 5-HT, norepinephrine, Glu or GABA in the VP (Smith et al., unpublished data). The VP contains both D₁ and D₂ dopamine receptors (Napier and Maslowski-Cobuzzi 1994) and receives major GABA innervation from the NA. In addition, the NA and VP both receive DA innervation from the ventral tegmental area (VTA) and sites within the VP support electrical self-stimulation (Panagis et al. 1995; Murray and Shizgal 1996). Non-specific excitotoxic lesions of the VP alter rat intravenous cocaine self-administration (Robledo and Koob 1993) and microinjection of cocaine into the VP increases locomotion and produces a conditioned place preference (CPP; Gong et al. 1996). 6-OHDA-induced lesions of the VP block the CPP produced with systemic administration of cocaine (Gong et al. 1997). The purpose of the experiment reported here was to examine the role of the VP in cocaine self-administration using *in vivo* microdialysis to assess [DA]_e, [5-HT]_e, [GABA]_e, and [Glu]_e in the VP in rats self-administering three different doses of cocaine (0.17, 0.33, and 0.67 mg/infusion) in each daily session and in rats receiving response-independent infusions of saline on a schedule yoked to animals self-administering cocaine.

Materials and methods

Subjects

Eleven male, Fischer 344 rats (90–150 days; 275–350 g) were used. Subjects were individually housed and had access to food and water *ad libitum* except during experimental sessions. The lighting in the room in which they were housed was on a 12-h/12-h light/dark cycle (lights on: 5:00 p.m.) and sessions were conducted during the dark cycle.

Apparatus

Behavioral

Six standard operant conditioning chambers (Med Associates; 28.1×21.0×21.0 cm) enclosed in sound-attenuating, ventilated, enclosures (Med Associates) were used. Each chamber contained a lever (4.5×2.0 cm) approximately 7.2 cm from the top of the bars of the grid floor above which was located a stimulus lamp and each enclosure contained a houselight, Sonalert, and exhaust fan. Centered above the lever was a lens cap (2.7 cm diameter; center 5.7 cm above the lever) which could be transilluminated from behind by a light source (28 V, 100 mA). Experimental sessions were controlled by microcomputers using Med-PC (Med Associates) software.

Self-administration and guide cannula

The catheter, leash, swivel and syringe pump have been described elsewhere (Sizemore et al. 1997). Microdialysis probe guide

cannulas (22 g stainless steel) were implanted using a Kopf stereotaxic frame (Plastics One Inc., Roanoke, Va., USA). The guide cannula was secured with skull screws and dental acrylic cement and a dummy cannula (28 g) inserted inside the guide cannula to prevent blockage.

Microdialysis and HPLC

Microdialysis probes Microdialysis probes were constructed using a previously described procedure (Hemby et al. 1995a, 1995b). Other details of the microdialysis apparatus are the same as those described by Hemby et al. (1995a, 1995b).

HPLC for biogenic amines The HPLC system for the biogenic amine analysis has been described elsewhere (Hemby et al. 1995a, 1995b).

HPLC for amino acids The gradient HPLC system for the measurement of the amino acids consisted of two Gilson pumps (model 302), a manometric module (model 802B), a dynamic mixer (model 811), a Rheodyne injection valve (model 7520) with a 1.0 µl sample loop, a reverse phase C₁₈ microbore column (Vydac 201TP, 1 mm×150 mm, 5 µm) and a Gilson fluorometer (model 121). The *o*-phthalaldehyde (OPT) derivatives were assessed using fluorescent detection using a 430–470 nm excitation filter and a 305–395 nm emission filter.

HPLC for cocaine The HPLC system used for the measurement of cocaine was the same as that described by Hemby (1995a).

Procedure

Surgical

The catheterization procedure has been described elsewhere (Sizemore et al. 1997).

Subsequent to catheterization, subjects were implanted with a guide cannula into the ventral pallidum also under pentobarbital anesthesia as above. The guide cannula was implanted to terminate at the dorsal surface of the ventral pallidum (+7.5 mm from lambda, ±2.2 mm lateral from the midline, and –6.4 mm ventral from dura; König and Klippel 1974).

Behavioral

Self-administration (SA) Lever-pressing was engendered under a within-session dosing procedure in which three doses of cocaine (0.17, 0.33, and 0.67 mg/infusion) were available each session and every lever-press resulted in an infusion. Each dose was available for 1 h and the doses occurred in ascending order. These components were separated by 10-min blackouts during which the chamber was dark and responses had no programmed consequences. Each component began with the response-independent administration of the currently scheduled dose. When responding was established, the requirement was changed such that two responses (FR2) were required for each infusion. The dose of cocaine was determined by the duration of pump operation (2.8, 5.6, and 11.2 s). The lever-light was illuminated during the session except for 20 s following the initiation of infusions, during which time the houselight was illuminated and a Sonalert was activated, and during blackouts. When responding was stable, saline was substituted for cocaine for two or more sessions until responding occurred at a very low frequency (extinction). Several days after responding was subsequently re-established, subjects were implanted with a microdialysis guide cannula. Following cannulation and a 2- to 3-day recovery period, responding was maintained until stable intake was again observed (approximately ten sessions) when microdialysis was initiated.

Yoked infusions In addition to the seven SA rats, four naive rats were also exposed to microdialysis. These control subjects were

seen as essential to demonstrate that the administration of cocaine was related to changes observed and not incidental aspects of the experimental conditions (stimulus lights, tones, or fluid infusion) given the paucity of information concerning the neurochemical changes in the VP during cocaine self-administration. The microdialysis sessions for each of these subjects were conducted simultaneously with those of an SA rat. Thus, there were four pairs of rats, each pair consisting of an SA rat and a yoked vehicle-infused rat. The chambers of each of these pairs were functionally connected such that SA and yoked rats were exposed simultaneously to the same stimuli except that the yoked rats received saline infusions whenever the SA rat produced a cocaine infusion. For DA, GABA, and Glu, samples could only be collected and/or analyzed on three of the four yoked rats.

Microdialysis

A microdialysis probe was inserted through the previously implanted guide cannula approximately 18 h prior to microdialysis sessions. Artificial cerebrospinal fluid was perfused at a flow rate of 0.5 $\mu\text{l}/\text{min}$. Artificial cerebrospinal fluid (aCSF) consisted of 145 mM NaCl, 1.2 mM CaCl_2 , 2.8 mM KCl, 1.2 mM MgCl_2 , 5.4 mM D-glucose, and 1.25 mM NaH_2PO_4 at a pH of 7.2. Microdialysis samples were collected every 10 min during the sessions, 30 min pre-session and 90 min post-session and immediately frozen on dry ice and stored at -70°C until analysis. Most subjects were dialyzed on 2 successive days to provide enough extracellular fluid for examination of all four neurotransmitters. Altogether there were samples sufficient to provide data on DA and 5-HT for all seven SA rats and to provide data on GABA and Glu for four SA rats.

HPLC

Biogenic amines Aliquots of 1 μl of the dialysate sample were injected into the biogenic amine HPLC system for the analysis of DA and 5-HT. The mobile phase was the same as that described by Parsons et al., (1995). The flow rate was 15 $\mu\text{l}/\text{min}$ and the retention time for DA was 5 min and 10 min for 5-HT. DA and 5-HT were quantified by comparing samples with standards of known concentrations. Limits of detection for DA and 5-HT were 0.5 fmol and 0.2 fmol, respectively, which corresponded to concentrations of 0.5 nM and 0.2 nM.

Cocaine The procedure for measuring cocaine concentration in the dialysates was the same as that reported by Hemby et al. (1995a). The detection limit for cocaine was 100 fmol which corresponded to 0.2 nM.

Amino acids The gradient mobile phase consisted of (A) 50 mM sodium acetate and 0.1 mM EDTA at pH 6.2 and (B) methanol 15–50%. A 2 μl aliquot of the dialysate was derivatized with 1 μl OPT reagent for 4 min. A 1 μl aliquot of the OPT-amino acids was injected into the HPLC system. The stock OPT reagent consisted of 4 mg OPT dissolved in 100 μl methanol and 8 μl 2-mercaptoethanol in 1 ml of 0.4 M sodium borate pH 9.5. The working reagent was prepared fresh daily by diluting the above stock solution with 0.4 M sodium borate, pH 9.5 (1/20:v/v). Flow rate was 80 $\mu\text{l}/\text{min}$ and retention for glutamate and GABA were 3.5 min and 18.5 min, respectively. The detection limits for glutamate and GABA was 50.0 fmol or 75.0 nM.

Histology

The placements of the guide cannula tracts were verified by histological procedures. Brains were removed, frozen and 20 μm coronal sections near the cannula tracts were taken at -20°C . Sections were stained by the Klüver-Barrera (1953) method and the probe placements verified by light microscope.

Results

Baseline behavior

Performance was consistent with that previously reported for cocaine self-administration under ratio schedules (Pickens and Thompson 1968) and similar to that reported for this strain of rats when these doses were made available either within-session (random order), as substitutions for a standard daily dose (0.33 mg/infusion), or when these doses were the only ones available for several sessions (Sizemore et al. 1997). The number of infusions per component was a monotonic, decreasing function of dose and infusions were evenly spaced. The lowest dose (0.17 mg/infusion) was self-administered at the highest rate, the highest dose (0.67 mg/infusion) was self-administered at the lowest rate, and the dose intermediate to these (0.33 mg/infusion) was self-administered at an intermediate rate.

Microdialysis

DA and 5-HT: baseline levels

Baseline $[\text{DA}]_e$ were 2.51 ± 0.76 and 2.4 ± 1.59 fmol for, respectively, the self-administering and yoked rats. Baseline $[\text{5-HT}]_e$ were 0.68 ± 0.08 and 0.59 ± 0.17 fmol.

DA and 5-HT: levels during self-administration

The extracellular fluid concentrations of DA and 5-HT increased rapidly in the cocaine self-administering rats and remained 250–300% above baseline for the duration of the session. In contrast, there was no change in $[\text{DA}]_e$ and $[\text{5-HT}]_e$ for the yoked-saline animals. This is apparent from Fig. 1, which shows $[\text{DA}]_e$ (top panel) and $[\text{5-HT}]_e$ (middle panel) for each 10-min period beginning 30 min prior to the start of the session, during the session, and for 90 min post-session. Comparisons among all six 10-min periods within a component and the three 10-min periods preceding the start of the session (one-way repeated-measures ANOVA) revealed statistically significant differences in $[\text{DA}]_e$ and $[\text{5-HT}]_e$ at all three doses of cocaine [DA: 0.17 mg/infusion; $F(8,48)=26.3$, $P<0.001$; 0.33 mg/infusion; $F(8,48)=33.8$, $P<0.001$; 0.67 mg/infusion; $F(8,48)=35.5$, $P<0.001$. 5-HT: 0.17 mg/infusion; $F(8,48)=12.2$, $P<0.001$; 0.33 mg/infusion; $F(8,48)=8.3$, $P<0.001$; 0.67 mg/infusion; $F(8,48)=14.5$, $P<0.001$]. More detailed analyses (Neuman-Keuls pairwise comparisons) revealed that $[\text{DA}]_e$ and $[\text{5-HT}]_e$ at each 10-min period of each component was different than baseline levels for the SA rats. In addition, for DA, there were significant within-component differences at 0.17 and 0.33 mg/infusion. Specifically, at 0.17 mg/infusion, the $[\text{DA}]_e$ during the first 10-min period was different than any other subsequent period. At 0.33 mg/infusion, $[\text{DA}]_e$ during the first 10-min period was different than during

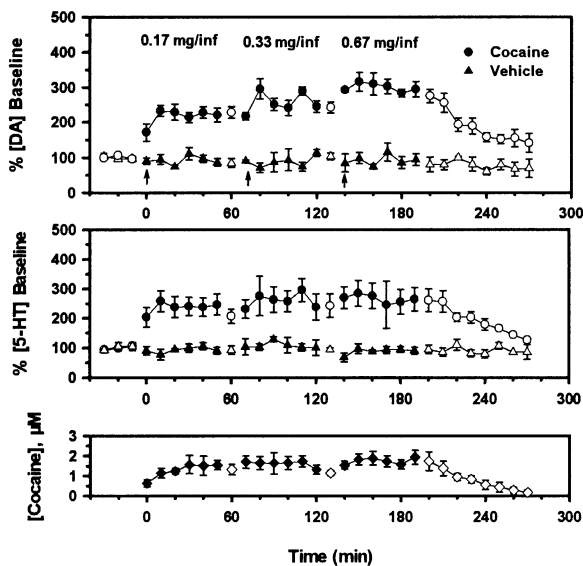


Fig. 1 Extracellular concentration of DA, 5-HT, and cocaine (*top, middle, and bottom panels, respectively*). Levels of DA and 5-HT are expressed as a percentage of baseline. Shown are data from 30 min prior, during, and 90 min after experimental sessions for both self-administration and yoked-vehicle animals. *Filled symbols* show data from during self-administration components. *Open symbols* show data from before or after the session as well as from the 10-min between component blackouts

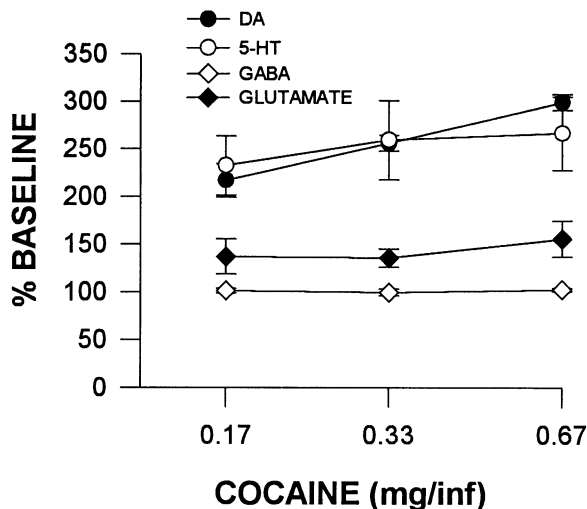


Fig. 2 Extracellular fluid concentrations (% baseline) of DA, 5-HT, GABA, and Glu averaged over each component as a function of dose of cocaine

the second and fifth 10-min periods, and $[\text{DA}]_e$ during the second and fourth 10-min periods differed.

As can be seen in Fig. 2, which shows levels of all four neurotransmitters averaged over each component for the SA rats, average $[\text{DA}]_e$ and $[\text{5-HT}]_e$ were increasing functions of dose of cocaine. A statistical comparison (one-way repeated-measures ANOVA) of $[\text{DA}]_e$ and $[\text{5-HT}]_e$, averaged over each component, revealed significant differences [DA : $F(2,12)=14.5$, $P<0.001$; 5-HT:

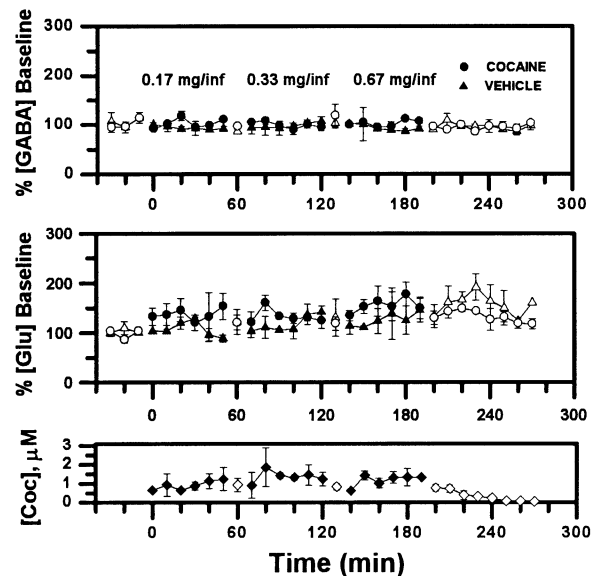


Fig. 3 Extracellular concentration of GABA, Glu, and cocaine (*top, middle, and bottom panels, respectively*). Levels of GABA and Glu are expressed as a percentage of baseline. Shown are data from 30 min prior, during, and 90 min after experimental sessions for both self-administration and yoked-vehicle animals. *Filled symbols* show data from during self-administration components. *Open symbols* show data from before or after the session as well as from the 10-min between-component blackouts

$F(2,12)=4.0$, $P<0.05$). Pair-wise comparisons (Neuman-Keuls) revealed that average $[\text{DA}]_e$ at each dose differed and that average $[\text{5-HT}]_e$ during the low and high doses differed from each other.

GABA and Glu: baseline levels

Baseline $[\text{GABA}]_e$ were 97.2 ± 21.4 and 96.3 ± 25.6 fmoles for, respectively, self-administering rats and yoked rats, and $[\text{Glu}]_e$ were 474.0 ± 118.0 and 389.0 ± 68.0 fmol.

GABA and Glu: levels during self-administration

As can be seen from Fig. 3, $[\text{GABA}]_e$ did not change and closely resembled levels in the yoked rats while $[\text{Glu}]_e$ was slightly elevated. Statistical comparison of all 10-min periods within each component to each other and to the three 10-min periods preceding the session (one-way repeated-measures ANOVA) yielded no significant differences for GABA at any dose. For Glu, there were significant differences at every dose [0.17 mg/infusion: $F(8,24)=2.8$, $P<0.05$; 0.33 mg/infusion: $F(8,24)=4.0$, $P<0.01$; 0.67 mg/infusion: $F(8,24)=3.8$, $P<0.01$] but pairwise comparisons (Neuman-Keuls) revealed that no differences could be detected among individual 10-min periods at 0.17 mg/infusion when compared to each other or to the three 10-min periods preceding the session. At 0.33 mg/infusion, the second 10-min period differed from the three 10-min baseline periods and at

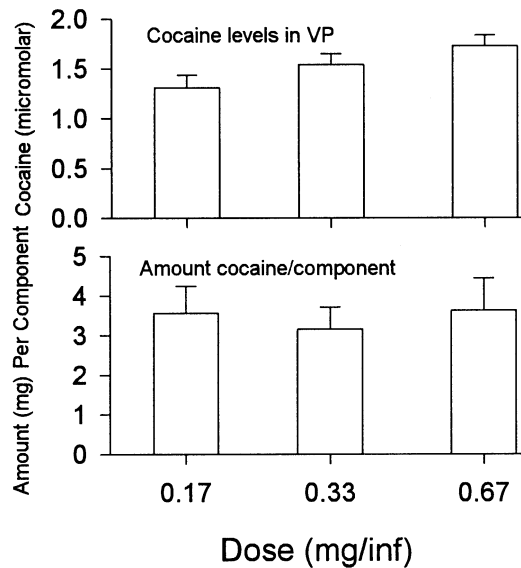


Fig. 4 Levels of cocaine in the VP averaged over each component (*top panel*) as well as the average amount of cocaine self-administered during each component (*bottom panel*)

0.67 mg/infusion, the fifth 10-min period differed from the last two 10-min baseline periods.

A comparison of levels averaged over each component (one-way repeated-measures ANOVA) showed that there was no difference in average levels of these neurochemicals as a function of dose (Fig. 2).

Cocaine levels

As can be seen in the bottom panel of Fig. 1, which shows data for each 10-min period from the start of the session through 90 min post-session, cocaine levels in the VP increased rapidly following the start of the session and remained relatively constant at about 1.5 μM for the duration of it. A comparison of cocaine levels in the VP at each 10-min period within a component (one-way repeated-measures ANOVA) revealed no statistically significant differences at any dose.

An analysis (one-way repeated-measures ANOVA) of average cocaine levels in the VP at each dose, although approaching significance, failed to reveal a significant difference between doses [$F(2,12)=3.1$, $P=0.08$]. Figure 4 shows levels of cocaine in the VP averaged over each component (*top panel*) as well as the average amount of cocaine self-administered during each component (*bottom panel*). As can be seen, the SA rats self-administered about the same amount of cocaine per component. There was no significant difference between the amounts self-administered at the three doses (one-way repeated-measures ANOVA).

The location of the microdialysis probe for each rat is shown in Fig. 5. The top panel shows the location for the seven self-administering rats and the bottom panel shows

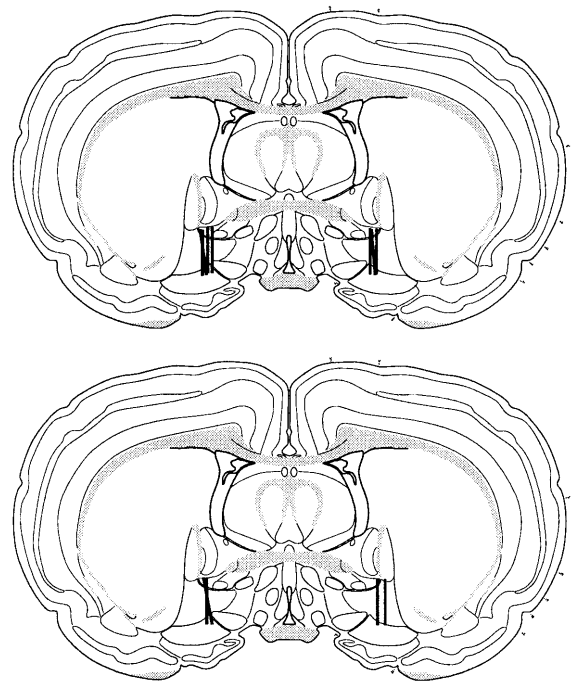


Fig. 5 Location of microdialysis probes for the seven self-administration rats (*top panel*) and the four yoked-vehicle rats (*bottom panel*). Drawings correspond to coronal levels -0.30 and -0.40 mm from Bregma according to the atlas of Paxinos and Watson (1986). Drawings were generated using Brain Maps® software (Biosoft Elsevier, Amsterdam, The Netherlands)

the location for the four yoked rats. For all 11 rats, the probe surface was almost entirely within the VP.

Discussion

The extracellular fluid levels of DA, 5-HT and, to a much lesser extent, Glu were increased in the VP during cocaine self-administration with the average levels of DA and 5-HT during a component related to the dose of cocaine self-administered. Increases in $[\text{Glu}]_e$ were small and statistical significance isolated to a few 10-min periods during the session. In contrast, VP $[\text{GABA}]_e$ was not increased relative to baseline during cocaine self-administration. $[\text{DA}]_e$, $[\text{5-HT}]_e$, $[\text{GABA}]_e$, or $[\text{Glu}]_e$ were not different from pre-session baseline in the yoked saline infused rats during the experimental session. These data suggest that the large increases in extracellular DA and 5-HT observed were dependent upon the self-administration of cocaine and not on exposure to the experimental procedure. However, whether these increased levels are characteristic of only self-administered cocaine remains to be determined; NA $[\text{DA}]_e$ has been shown to be significantly higher in self-administering rats than in yoked cocaine-infused controls (Hemby et al. 1997).

$[\text{DA}]_e$ in the VP was dose-responsive, consistent with what has been postulated for the NA (Pettit and Justice 1991). However, the increase in $[\text{DA}]_e$ in the VP was less

than that previously observed in the NA (Pettit and Justice 1991; Hemby et al. 1997). This discrepancy between the VP and the NA is consistent with the content of DA in the VP and NA as measured in this laboratory in rats with a history of cocaine self-administration (Smith et al., unpublished data; NA: 849.0 ± 42.4 pmol/mg protein; VP: 164.0 ± 17.8 pmol/mg protein) and presumably reflects the density of nerve endings in which DA is stored. An additional difference between the VP and NA is that SA and yoked rats had nearly identical baseline levels of DA whereas Parsons et al. (1995) report that baseline levels of DA were higher in self-administering rats versus cocaine-naïve rats. It is possible that this difference reflects neuroadaptations that occur in the NA but not the VP but other procedural differences may account for the discrepancy. $[5\text{-HT}]_e$ was elevated during cocaine self-administration in the VP similar to what has been reported for the NA (Parsons et al. 1995), but the dose-responsiveness of this effect has not been investigated.

Although $[DA]_e$ and $[5\text{-HT}]_e$ in the microdialysates were dependent on the cocaine dose over the range investigated, the cocaine levels, though showing a trend, were not statistically different. However, levels of cocaine in discrete brain areas do not necessarily correlate with the levels of observed neurotransmitters. For example, $[DA]_e$ in the NA differs between rats that self-administer cocaine versus those that receive yoked response-independent cocaine infusions even though cocaine levels in the microdialysates are the same (Hemby et al. 1997). In addition, response-independent presentation of the identical pattern of cocaine self-administered the previous day to the self-administering rats resulted in significantly lower levels of $[DA]_e$ even though the levels of cocaine were identical in the microdialysates (Hemby et al. 1997).

Although both $[DA]_e$ and $[5\text{-HT}]_e$ both showed a dose-dependency, $[DA]_e$ was more sensitive to changes in dose (i.e., all three doses differed significantly whereas, for $[5\text{-HT}]_e$, only the high and low doses differed). This may be related to the proposed role of DA in the brain processes underlying the self-administration of cocaine, at least as demonstrated with antagonist-pretreatment studies (DeWit and Wise 1977; Roberts and Vickers 1984; Koob et al. 1987; Gerber and Wise 1989; Caine and Koob 1994a; Hemby et al. 1996) and may be related to the fact that turnover rate of DA is increased in self-administering animals while 5-HT turnover rates are not (Smith et al., unpublished data). The fact that both $[DA]_e$ and $[5\text{-HT}]_e$ are increased and both dose-dependent, however, suggests that 5-HT may be more important than was once thought and in recent years the investigation of 5-HT receptors has been consistent with this. When administered systemically, specific 5-HT_{1B} agonists (RU 24969 and CP 94,253) decrease the rate of cocaine self-administration under an FR5 schedule and increase breakpoint when responding is maintained under a PR schedule of cocaine infusion (Parsons et al. 1998). Similarly, the specific 5-HT indirect agonist, fenfluramine, decreases the rate of cocaine self-administra-

tion (Glowa et al. 1997). Parsons et al. (1998) interpreted their findings in terms of an enhancement of the reinforcing efficacy of cocaine but the role of 5-HT may be complicated. Rocha et al. (1997), for example, found that 5-HT_{1B} knockout mice acquired cocaine self-administration more rapidly than wild-type mice – a finding which appears inconsistent with Parsons et al. (1998). Similarly, Roberts et al. (1999) report that, when various tropane analogs were substituted for cocaine in rats trained to self-administer cocaine, breakpoints were more closely correlated with the DA/5-HT transporter selectivity ratio than the affinity at the DA transporter alone. Parsons et al. (1996) reported that the indirect 5-HT_{1B} agonist, CGS 12066B, reduced the rate of self-administration of GBR 12909, a selective DA uptake inhibitor, but did not reduce cocaine self-administration. Taken together these data, along with the earlier findings suggesting little or no role for direct 5-HT antagonists (see Introduction) point to a complicated, but important, role for 5-HT.

The extracellular fluid levels of neurotransmitters other than DA and 5-HT in specific brain areas during drug self-administration have not been widely studied. The decrease in VP GABA and Glu turnover rates during self-administration (Smith et al., unpublished data) suggest that decreases in extracellular fluid levels might be observed; however, no decreases were observed. The fact that no decreases were observed in $[GABA]_e$ is difficult to understand both in terms of its apparent opposition to the turnover data, and in terms of theories concerning the role of DA in the NA. According to that hypothesis, the potential importance of the VP in cocaine self-administration is discussed in terms of its anatomical relationship with the NA; that is, as the terminus for GABAergic projections of the NA (Amalric and Koob 1993; Hooks and Kalivas 1995). Since D₂ DA receptors are the predominant receptor subtype on the GABAergic projections from the NA to the VP, DA stimulation might be expected to inhibit GABA tone in the VP (Johnson and Napier 1996). Indeed, systemically administered quinpirole (a D₂ agonist) can result in decreased firing rates in VP neurons (Maslowski and Napier 1991), and administration of amphetamine results in decreases in $[GABA]_e$ (Bourdelaïs and Kalivas 1990).

Even though no change in $[GABA]_e$ was observed here, GABA appears to have an important role in psychomotor stimulant self-administration. Baclofen (a GABA_B agonist) pretreatment reduced breakpoint on a progressive-ratio schedule of cocaine infusion, but had little effect on food-reinforced responding or on responding maintained under an FR1 schedule of cocaine infusion (Roberts et al. 1996). In addition, baclofen could postpone the onset of circadian rhythm-dependent cocaine-reinforced responding (Roberts and Andrews 1997). Similarly, baclofen administered systemically reduced the rate of responding in one component of a multiple schedule in which responding was maintained by cocaine infusions, but had only small effects on behavior maintained by food in the other component (Shoaib et al. 1998). These researchers also found that baclofen admini-

istered directly into the NA or VTA also suppressed responding maintained under an FR5 schedule of cocaine infusion. Further, the indirect agonist gamma-vinyl GABA attenuated the increases in extracellular DA in the NA produced by IP injection of cocaine, and this effect was reversed by co-administration of SCH 50911, a GABA_B antagonist (Ashby et al. 1999). Taken together, these data suggest that the activity of GABAergic innervation of the NA and VTA functions to inhibit DA release in these areas, thereby possibly attenuating the reinforcing efficacy of cocaine.

Although [Glu]_e was elevated during the self-administration session, only during a few isolated 10-min periods were levels elevated sufficiently to be statistically significant. It is possible that the elevations in [Glu]_e observed at other points in the session would have been significant if samples would have been obtained for more than four rats. There are few studies relevant to the role of Glu in cocaine self-administration and none directly related to the issue of changes in [Glu]_e in the VP. Most papers concerning Glu and cocaine self-administration involve the effects of Glu agonists and antagonists infused into the NA. Cornish et al. (1999) found that glutamatergic agonists infused into the NA decreased the rate of cocaine-maintained responding and reinstated extinguished responding, while antagonists had no selective effect upon cocaine self-administration. In contrast, Pulverenti et al. (1992) found that APV, an NMDA blocker, infused into the nucleus accumbens affected cocaine self-administration but not heroin administration. Other studies have suggested that cocaine self-administration produces long-term alteration in glutamatergic function (White et al. 1995; Keys et al. 1998). The findings of the present study and those mentioned above suggest a role for glutamate in cocaine self-administration. However, it should be noted that the status of microdialysis measurements of GABA and Glu has been examined critically (Timmerman and Westerink 1997) and the extent to which such measurements can be meaningfully interpreted has been called into question. These authors conclude that it is unlikely that levels of these transmitters meaningfully reflect changes in neurotransmission. Instead, it is more likely that basal levels reflect glial metabolism.

The data reported here, in conjunction with other data described (see Introduction) suggest that DA and 5-HT innervations of the VP may have an important role in the biological processes underlying psychomotor stimulant self-administration. In addition, the role of DA innervation of the NA may *not* be as absolute as was once thought. 6-OHDA lesions of the NA do not usually eliminate responding maintained by cocaine infusion (Roberts et al. 1977, 1980; Pettit et al. 1984) and micro-injections of cocaine directly into the NA do not maintain responding (but see McKinzie et al. 1999) although infusions into the medial prefrontal cortex will (Goeders and Smith 1983). Taken together, these data support the concept that extensive interconnections between the VTA, NA, and VP may be critically important for the self-administration of psychomotor stimulants.

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