

Glen M. Sizemore · Conchita Co · Timothy R. Koves ·
Thomas J. Martin · James E. Smith

Time-dependent recovery from the effects of 6-hydroxydopamine lesions of the rat nucleus accumbens on cocaine self-administration and the levels of dopamine in microdialysates

Received: 11 March 2003 / Accepted: 16 July 2003 / Published online: 23 September 2003
© Springer-Verlag 2003

Abstract *Rationale:* Neurotoxin induced lesions of dopamine-releasing neurons that innervate the nucleus accumbens (NAcc) alter cocaine self-administration. In addition, elevated extracellular levels of NAcc dopamine (DA) are thought to be central to the biological mechanisms that underlie this behavior. *Objectives:* This study assessed the long-term effects of 6-hydroxydopamine (6-OHDA) induced lesions of the NAcc on cocaine self-administration and the dialysate levels of dopamine ([DA]_d) in this structure to determine if recovery of drug intake was correlated with the DA response. *Methods:* Rats implanted with jugular catheters and bilateral cannulas were trained to self-administer cocaine and subsequently received bilateral intracranial micro-injections of 6-OHDA or vehicle into the NAcc. The levels of DA and cocaine were determined in microdialysates of the NAcc collected during experimental sessions 6–7, 14–16, 29–30, and 44–46 days post-treatment. *Results:* The 6-OHDA induced lesions significantly reduced cocaine self-administration for 3 weeks while vehicle treatment had a moderate effect for the first several days. Cocaine-induced increases in NAcc [DA]_d did not return to sham/vehicle treated control levels for 6 weeks in the lesioned group and DA content in the NAcc was 46% of control at 44 days post-lesion. *Conclusions:* Although

dopaminergic lesions of the NAcc produced profound effects on cocaine self-administration, responding recovered to control levels before cocaine-induced increases in NAcc [DA]_d while content of DA in the NAcc did not recover. These data suggest that the plasticity of neuronal systems in the NAcc related to cocaine self-administration and their response following 6-OHDA lesions is more complex than restoration of DAergic tone.

Keywords Cocaine self-administration · Nucleus accumbens · 6-Hydroxydopamine lesions · Dopamine

Introduction

The search for the neurobiological mechanisms of cocaine reinforcement has focused on the role of dopamine (DA) releasing neurons for more than a decade. Pharmacological antagonism of dopamine receptors alters the rate of responding maintained by intravenous cocaine infusions under fixed-ratio schedules in a manner consistent with decreased reinforcement (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 decreases breakpoint of progressive-ratio (PR) schedules (Hubner and Morton 1991, Richardson et al. 1993). In contrast, serotonergic (Porrino et al. 1989; Peltier and Schenk 1991, Lane et al. 1992; Woolverton 1992; Lacosta and Roberts 1993) and noradrenergic antagonists (De Wit and Wise 1997; Woolverton 1987, 1992) do not alter cocaine self-administration.

The nucleus accumbens (NAcc) has been thought to be the locus of the DA innervations of the forebrain responsible for cocaine self-administration. 6-Hydroxydopamine (6-OHDA) induced lesions of these regions resulted in attenuation of rat intravenous cocaine self-administration (Roberts et al. 1977, 1980; Pettit et al. 1984; Caine and Koob 1994b). In addition, the injection of DA receptor antagonists directly into this region modified drug intake in a manner consistent with those seen following systemic administration of these antago-

G. M. Sizemore · C. Co · T. J. Martin · J. E. Smith
Center for the Neurobiological Investigation of Drug Abuse,
Department of Physiology and Pharmacology,
Wake Forest University School of Medicine,
Winston-Salem, NC 27157-1083, USA

T. R. Koves
Department of Physiology,
Eastern Carolina University,
East Fifth Street, Greenville, NC 27858-4353, USA

J. E. Smith (✉)
Department of Physiology and Pharmacology,
Wake Forest University School of Medicine,
Medical Center Blvd, Winston-Salem, NC 27157-1083, USA
e-mail: jamsmith@wfubmc.edu
Tel.: +1-336-7168506
Fax: +1-336-7168501

nists (Maldonado et al. 1993; McGregor and Roberts 1993; Phillips et al. 1994), further supporting a principal role. Furthermore, elevated levels of dopamine in dialysates ($[DA]_d$) taken from the NAcc have been found in rats during cocaine self-administration (Pettit and Justice 1989) that are significantly higher than those observed in yoked controls or with response-independent infusions to the self-administering animals (Hemby et al. 1997). Indeed, it has been suggested that all drug reinforcers increase NAcc $[DA]_d$ and that this action may constitute a direct measure of abuse liability (Di Chiara and Imperato 1988). This research project was initiated to further investigate the proposed central role of the DA innervations of the NAcc in the processes that underlie cocaine self-administration by assessing the time dependent recovery from the effects of bilateral 6-OHDA induced lesions of this region on self-administration, and on $[DA]_d$ assessed with in-vivo microdialysis.

Materials and methods

Subjects

Male, Fischer 344 ($n=16$) rats (90–150 days of age; 300–350 g) were individually housed with access to food and water ad libitum. The lighting in the room was on a reversed 12-h light/dark cycle (lights on: 5:00 pm) with self-administration sessions conducted during the dark cycle.

Surgical procedures

Rats were pretreated with atropine methyl nitrate (10.0 mg/kg; IP) and implanted with indwelling jugular catheters under pentobarbital anesthesia (50.0 mg/kg IP) using a previously described procedure (Weeks 1962, 1972) with penicillin G procaine (75,000 IU; 0.25 ml, IM) administered post-surgery. The catheter (Tygon tubing, S-54-HL) was anchored to tissue surrounding the external jugular vein and exited through a plastic backplate enclosed in Teflon mesh, which was implanted subcutaneously between the scapulae for attachment of a leash. The catheter was enclosed in a leash (stainless-steel 11-gauge tubing with steel spring on each end) that was anchored to the plastic backplate. The leash passed through the ceiling of the chamber and was attached to the single channel fluid swivel (Brown et al. 1976), which was secured to one end of a counter-balanced arm permitting uninhibited mobility. The swivel was connected to a syringe that was operated by a syringe pump (Med Associates).

Rats were implanted with injection guide cannulae (CMA11, CMA, North Chelmsford, Mass., USA) to terminate at the dorsal surface of the nucleus accumbens [9.4 mm from lambda, ± 1.7 mm lateral from the midline, and 5.0 mm ventral from the dura (König and Klippel 1974)] using a stereotaxic (Stoelting, Wood Dale, Ill., USA). The guide cannulae were secured to the skull with stainless steel screws and dental acrylic cement and obturators (28 g) inserted to prevent blockage. These cannulae served as guides for both micro-injection and microdialysis probes. When stable baselines of self-administration were obtained (18–23 sessions), the rats received two treatments of either the bilateral injection of 6-OHDA ($n=8$) or sham/vehicle treatment ($n=8$) 2 days apart (Wednesday and Friday). On the first lesion day, rats were pretreated with desipramine (20 mg/kg) IP and, 15 min later, with pargyline (20 mg/kg, IP) and anesthetized with methohexital (2 mg/ml, IV). Fifteen minutes later, 6-OHDA (4.0 μ g in 2.0 μ l per side) or vehicle (9.0 mg sodium chloride and 0.2 mg ascorbic acid dissolved in 1.0 ml sterile water) was administered through the

guide cannulae (over 10.0 min at a rate of 0.2 μ l/min). On the second lesion day, the treatment was identical to the first except that there was no desipramine or pargyline pretreatment.

Self-administration

Self-administration sessions were conducted in operant conditioning chambers (Med Associates) enclosed in sound-attenuating, ventilated enclosures (Med Associates) with each chamber containing a retractable lever, a stimulus light above the lever. Each enclosure contained a house light, Sonalert and exhaust fan and experimental sessions were controlled by microcomputers using Med-PC (Med Associates) software. Lever-pressing was established 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 which was raised to a fixed ratio 2 over the first several sessions. The dose of cocaine was determined by the duration of infusion pump operation (3.1, 6.2, and 12.4 s of 1.647 mg/ml cocaine) and each dose was available for 1 h and presented in an ascending order. Each component began with the response-independent administration of the currently scheduled dose and was separated from the preceding component by a 30-min timeout during which the chamber was dark. The lever-light was illuminated during the session except for the 2 min following the initiation of infusions, during which the house light was illuminated, a Sonalert was activated, and the lever was retracted. The lever was re-introduced into the chamber upon the termination of this 2-min stimulus complex. When drug intake was stable (five consecutive sessions during which the number of infusions delivered of each dose of cocaine did not vary by more than 10% of the mean), saline was substituted for cocaine for two or more sessions until five or fewer infusions were delivered (extinction). Stable responding as defined above was subsequently re-established prior to any further experimental manipulations.

Microdialysis

Microdialysis sessions were conducted at four separate time periods: days 6–7, 14–16, 29–30, and 44–46 following the first lesion or sham/vehicle treatment. Probes were inserted through the previously implanted guide cannula approximately 18 h prior to the self-administration session during which microdialysis was scheduled to occur. The inlet tubing to the microdialysis probe was connected to a syringe containing artificial cerebrospinal fluid and one channel of a dual channel fluid swivel (Instech Laboratories, Plymouth Meeting, Pa., USA), while the other channel was used for intravenous cocaine infusions. Artificial cerebrospinal fluid (aCSF; 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) was perfused at a rate of 0.5 μ l/min. Microdialysis samples were collected into microcentrifuge tubes from the free end of the outlet tubing at 10-min intervals 30 min pre-session, during the sessions and 90 min post-session and immediately frozen on dry ice and stored at $-70^\circ C$ until analysis. Microdialysis was performed at both sites alternately at each consecutive time period for a total of two sessions per side. Thus, the same side was used for microdialysis sessions on days 6–7 and 29–30 and the other side for days 14–16 and 44–46.

Analysis of DA and cocaine in microdialysates by HPLC

DA was measured in a 1- μ l aliquot of each dialysate using high pressure liquid chromatography with electrochemical detection (HPLC-EC). DA was separated by HPLC consisting of a syringe pump (model LC-260D; ISCO, Lincoln, Neb., USA) with an air-actuated injection valve (model AC14UW; Valco) and a 1.0 μ l sample loop, a microbore column (Spherisorb, ODS2, 5.0 μ m, 0.5 mm i.d. \times 100 mm), a dual glassy carbon working electrode (model PM; EG&G Princeton Applied Research, Princeton, N.J.,

USA), a reference electrode (RE-1; Bioanalytical Systems Inc., W. Lafayette, Ind., USA) and an EC detector (model 400; EG&G Princeton Applied Research) with the applied potential set at +700 mV as referenced to Ag/AgCl. The mobile phase consisted of 20 mM citric acid, 46 mM NaH₂PO₄, 0.25 mM EDTA, 0.7 mM 1-decanesulfonic acid, 10 mM triethylamine and 21% methanol (v/v), pH 5.4 (Parsons et al. 1995) and the flow rate of 15 µl/min resulted in a retention time of 5 min for DA. DA was quantified by comparing samples with standards of known concentration and the limit of detection was 0.5 fmol, which corresponded to a concentration of 0.5 nM.

Cocaine was measured in a 0.5 µl aliquot of each dialysate sample using HPLC and UV detection. The HPLC consisted of an SSI pump (model 222D; Scientific Systems, Inc., State College, Pa., USA), a Rheodyne injection valve (model 7520) with a 0.5 µl sample loop, a Spherisorb microbore column (0.5 mm×100 mm, 5 µm C₁₈) and an LDC analytical variable wavelength detector (model 3200). The concentration of cocaine in the microdialysates was measured using UV absorbance at a wavelength of 235 nm. The mobile phase consisted of 50 mM NaH₂PO₄, 10 mM triethylamine, 0.1 mM EDTA, 22% acetonitrile and 15% methanol with pH adjusted to 5.6 and flow rate of 25 µl/min that resulted in a retention time of 8 min. The detection limit for cocaine was 100 fmol, which corresponded to 0.2 µM. Absolute concentrations of cocaine in the dialysate were determined by comparing with known concentrations of standards.

DA content and histology

Animals were killed at the end of the last microdialysis session and the brains were removed and frozen at -80°C. Frozen brains were warmed to -20°C and 1.0 mm coronal sections were made for NAcc dissection. Sections (20 µm) were taken at the cannula tracks and placement verified by microscope following fixation and staining (Klüver and Barrera 1953).

Dopamine was extracted from the remaining tissue using a previously reported procedure (Co et al. 1982). Briefly, the NAcc was homogenized in 1 N formic acid: acetone (15:85) and 3,4-dihydroxybenzylamine was added to each sample to correct for recovery. Lipids were removed by a heptane:chloroform (8:1) wash and the aqueous layer dried under a stream of dry N₂. The dried samples were reconstituted in the mobile phase (0.05 M citrate-phosphate, 0.1 mM EDTA at pH 3.7, 0.4 mM sodium octyl sulfate and 10% methanol) and the levels of DA determined by HPLC-EC [(Gilson 302 pump, BAS (West Lafayette, Ind., USA) LC-4B detector, a Rheodyne sample injector, a BAS biophase ODS analytical column, 4.6×250 mm, 5 µm and Hewlett Packard 3390A integrator) with the oxidation potential set at +0.7 V against the reference electrode and the flow rate of 1.0 ml/min.

Data analysis

All data were analyzed using a three-way ANOVA with intracranial treatment (6-OHDA or sham lesion), cocaine dose and day following treatment serving as the independent variables and either number of cocaine infusions or percent baseline of [DA]_d serving as the dependent measures. Data were further analyzed by two-way ANOVA with cocaine dose and day following intracranial treatment serving as the independent variables and either number of cocaine infusions or percent baseline of [DA]_d serving as the dependent measures, comparing prelesion baseline data to post-lesion data separately for animals treated with 6-OHDA or sham lesion. Post-hoc analyses were performed using the Bonferroni/Dunn method for multiple comparisons to a control, with prelesion data serving as the control for each treatment group. The effects of treatments on NAcc, DA, norepinephrine and serotonin tissue content were analyzed using *t*-tests of significance between means.

Results

Cocaine self-administration

6-OHDA induced lesions of the NAcc decreased responding maintained by cocaine infusions for up to 20 days following treatment while sham treatment produced a lesser effect that persisted for up to 8 days after treatment. Analysis of the data by three-way ANOVA demonstrated a significant main effect of 6-OHDA treatment [$F(1,897)=42.77$, $P\leq 0.0001$], cocaine dose [$F(2,897)=460.8$, $P\leq 0.0001$] and day following intracranial treatment [$F(27,897)=6.4$, $P\leq 0.0001$]. The effect of 6-OHDA was dependent upon both the dose of cocaine available for self-administration and the number of days elapsed since 6-OHDA or sham lesion, as there were significant interactions between intracranial treatment and cocaine dose [$F(2,897)=26.3$, $P\leq 0.0001$] and between intracranial treatment and day after treatment [$F(27,897)=2.8$, $P\leq 0.0001$]. The effects of 6-OHDA treatment resolved with a similar time course with respect to cocaine dose, in that there were no significant interactions between cocaine dose and day after treatment and no significant three-way interaction between intracranial treatment, cocaine dose and day after 6-OHDA or sham lesion ($P>0.05$ for both analyses). Post-hoc analysis of the number of cocaine infusions with respect to time after treatment revealed a significant effect of sham treatment on days 6, 7 and 8 for all three doses of cocaine ($P\leq 0.005$) compared to pretreatment baseline values. In contrast, the effect of 6-OHDA treatment on number of infusions of cocaine delivered was significantly lower than prelesion values at all time points up to 20 days post-treatment ($P\leq 0.05$), but was not significantly different thereafter. The number of infusions of cocaine delivered was dose-responsive at all time points for both groups, and the number of infusions delivered was significantly different for all pairwise comparisons with respect to cocaine dose ($P\leq 0.05$).

Dopamine concentrations in microdialysates of the NAcc

Analysis of the microdialysis data yielded similar findings as the number of infusions of cocaine, however the time course of recovery from 6-OHDA treatment was different than that found with the behavioral data. 6-OHDA treatment decreased the percent of baseline values for [DA]_d for up to 30 days, while sham treatment had no significant effect (Figs 1, 2, 3, 4). There was a significant main effect of 6-OHDA compared to sham treatment [$F(1,116)=55.8$, $P\leq 0.0001$] and the level of DA in the microdialysates was dose-dependent [$F(2,116)=14.4$, $P\leq 0.0001$]. There was a significant interaction between treatment and cocaine dose [$F(2,116)=5.3$, $P\leq 0.006$] and between treatment and time after treatment [$F(3,116)=5.6$, $P\leq 0.001$]. The effect of sham or 6-OHDA treatment on percent baseline of [DA]_d over time was consistent across all three doses of cocaine, since there

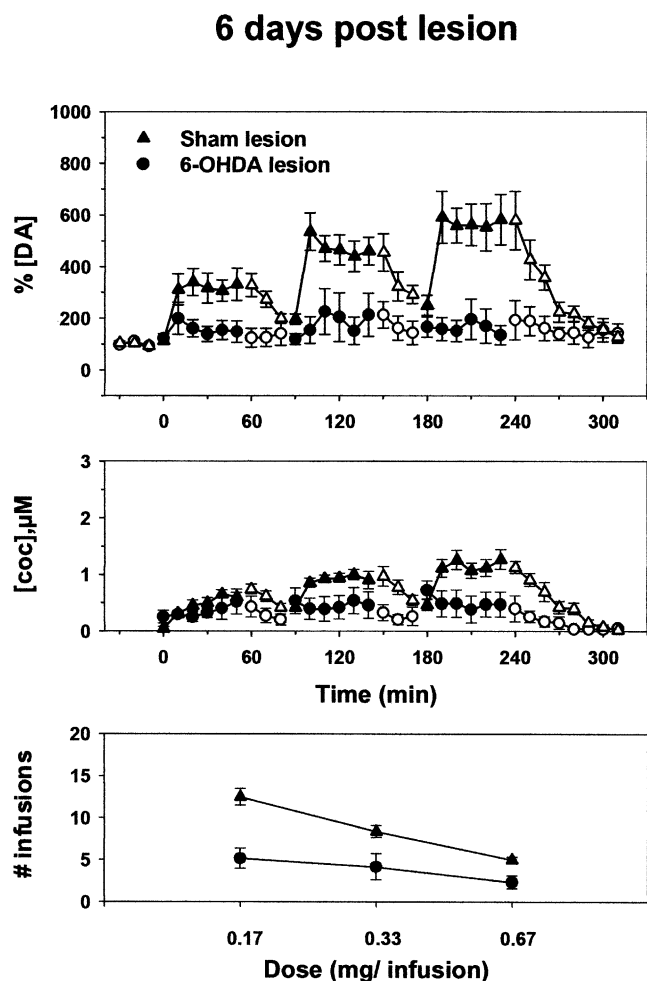


Fig. 1 The effects of sham-vehicle or 6-OHDA lesions of the nucleus accumbens on cocaine self-administration and the levels of DA and cocaine in microdialysates on day 6 or 7 post-treatment. *Top panel* shows dopamine levels and the *middle panel* cocaine levels in microdialysates of the nucleus accumbens. The *bottom panel* shows the drug intake during the microdialysis session. DA levels are expressed as a percentage of baseline in samples collected at 10-min intervals (error bars represent SEMs) and *open symbols* represent data for pre- and post-session and time-out periods between doses. 6-OHDA treatment had a significant effect on the percent baseline values for $[DA]_d$, demonstrating a significant decrease on day 6. The baseline intake for the two groups were not different (sham treated 16.5 ± 0.4 , 12.2 ± 0.3 and 6.8 ± 0.3 ; 6-OHDA treated 15.9 ± 0.7 , 12.5 ± 0.5 and 4.5 ± 0.5 for the 0.17, 0.33 and 0.67 mg/kg doses, respectively) (mean \pm SEM)

was no significant three-way interaction between treatment, cocaine dose and time after treatment [$F(6,116)=0.44$, $P \leq 0.85$]. In contrast, the sham lesion procedure did not influence the percent baseline values of $[DA]_d$ with respect to day after treatment [$F(2,58)=2.5$, $P \leq 0.07$]. Similar to the data obtained for number of infusions of cocaine, 6-OHDA treatment produced a significant effect on percent baseline values for $[DA]_d$ that was dependent upon the time following treatment [$F(3,58)=5.0$, $P \leq 0.004$]. Post-hoc analysis of these data demonstrated a significant decrease in percent baseline $[DA]_d$ on days 6, 16 and 30 but not on day 44 after the

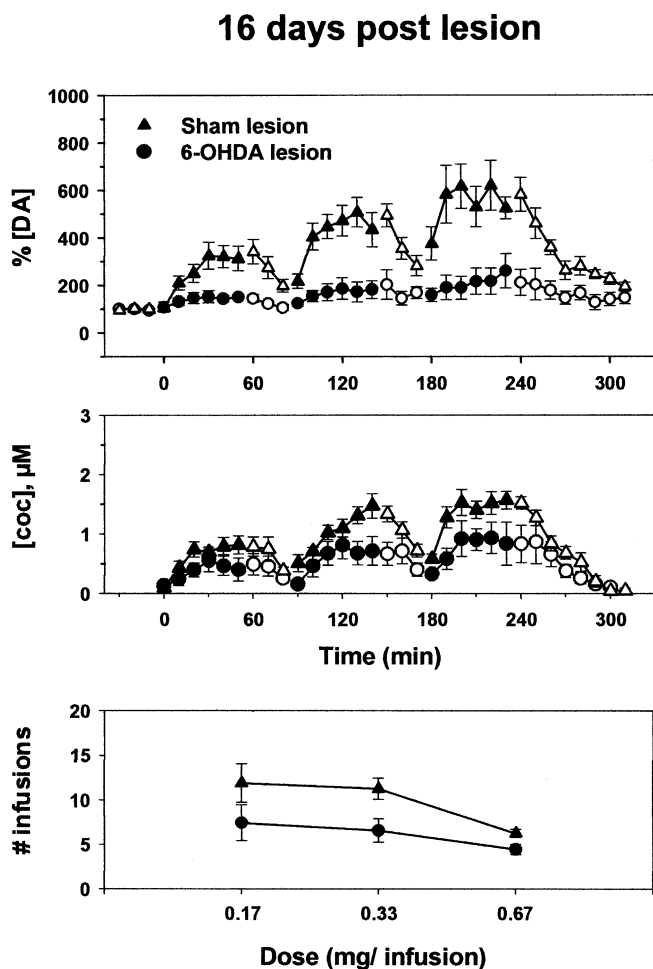


Fig. 2 The effects of sham-vehicle or 6-OHDA lesions of the nucleus accumbens on cocaine self-administration and the levels of DA and cocaine in microdialysates on day 16 or 17 post-treatment. 6-OHDA treatment had a significant effect on the percent baseline values for $[DA]_d$, demonstrating a significant decrease on day 16

lesion ($P \leq 0.05$) in contrast to the effect on cocaine infusions which persisted for only 20 days after the lesion (see above). Therefore, there is a significant effect of 6-OHDA treatment on the neurochemical response to cocaine self-administration that persists longer than the effect on responding for infusions of cocaine.

Baseline levels of NAcc $[DA]_d$ were not different between the sham/vehicle treated and 6-OHDA treated groups at the 6-day (6-OHDA: 1.68 ± 0.32 nM; sham/vehicle: 1.33 ± 0.49 nM) (mean \pm SEM), 16-day (6-OHDA: 1.06 ± 0.29 nM; sham/vehicle: 1.68 ± 0.37 nM), 30-day (6-OHDA: 1.73 ± 0.44 nM; sham/vehicle: 1.36 ± 0.31 nM) and 44-day (6-OHDA: 1.71 ± 0.59 nM and sham/vehicle: 1.49 ± 0.68 nM) time points.

Neurochemical content of the NAcc

The content of DA, 5-HT and NE was assessed in the NAcc following the last microdialysis session (day 44

30 days post lesion

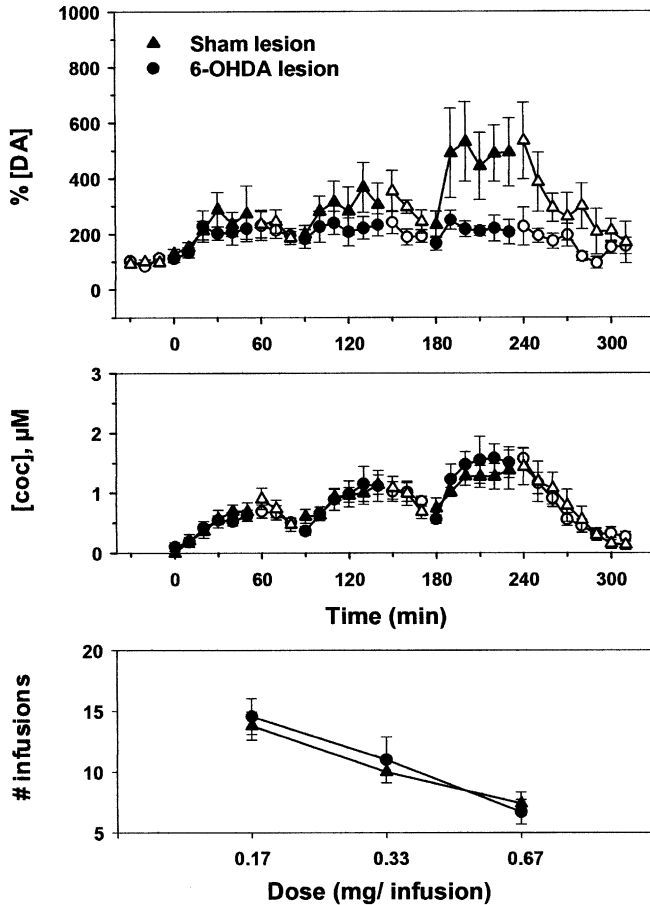


Fig. 3 The effects of sham-vehicle or 6-OHDA lesions of the nucleus accumbens on cocaine self-administration and the levels of DA and cocaine in microdialysates on day 30 or 31 post-treatment. 6-OHDA treatment had a significant effect on the percent baseline values for $[DA]_d$, demonstrating a significant decrease on day 30

post-lesion). There was a significant main effect of 6-OHDA treatment [$F(1,24)=21.7$, $P\leq 0.0001$] and a significant interaction between treatment and the neurotransmitter being analyzed [$F(2,24)=18.9$, $P\leq 0.0001$]. Only DA levels were significantly affected at this time point, being decreased from 502.3 ± 51.7 pmol/mg protein in sham-treated animals to 227.4 ± 28.8 pmol/mg protein in 6-OHDA-treated subjects (54.7% decrease, $P\leq 0.002$). The content of both 5-HT and NE was not significantly different between sham- and 6-OHDA-treated subjects.

Placement

The guide cannula for the 6-OHDA of sham treatments and microdialysis probes terminated at the dorsal surface of the NAcc and the microinjections were administered at the border of the shell and core. Half of the active portion of each microdialysis probe was within the core and the

44 days post lesion

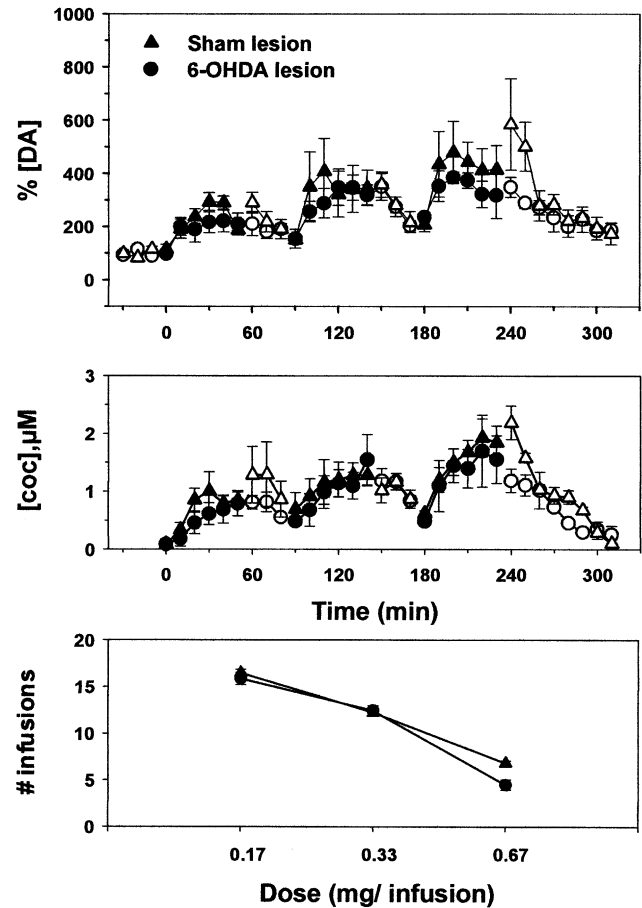


Fig. 4 The effects of sham-vehicle or 6-OHDA lesions of the nucleus accumbens on cocaine self-administration and the levels of DA and cocaine in microdialysates on day 44 or 45 post-treatment. 6-OHDA treatment had no significant effect on the percent baseline values for $[DA]_d$

other half in the shell region of the NAcc for both 6-OHDA- and sham-treated rats.

Discussion

It is currently widely accepted that NAcc DA has a primary role in the reinforcing effects of cocaine. 6-OHDA induced lesions of the NAcc, specific to DA, produced significant decreases in cocaine self-administration consistent with previous reports (Roberts et al. 1977, 1980; Pettit et al. 1984; Caine and Koob 1994b). Although dopaminergic (DAergic) lesions of the NAcc decrease responding maintained by cocaine infusions, this effect is not permanent (Roberts et al. 1980). The response of forebrain systems to dopamine depletion by 6-OHDA has been studied for some time, and several changes in these systems are known to occur over time. DAergic terminals that remain following incomplete lesions with 6-OHDA sprout functional collateral endings

that increase DAergic tone (Zigmond and Hastings 1998). The DAergic terminals that are spared following 6-OHDA lesions also become hyperactive, releasing DA at greater rates and a greater percentage of the stored DA in these terminals appears to be in newly-synthesized readily releasable pools compared to non-lesioned tissue (Nisenbaum et al. 1986, 1988; Snyder et al. 1990). These mechanisms are thought to at least partially restore the DAergic tone in the forebrain following DA depletion, as measured by *in vivo* microdialysis (Robinson et al. 1990). Such mechanisms probably contribute to the restoration of cocaine self-administration in animals treated with 6-OHDA in the NAcc. However, the major finding of this study is that the reinforcing effects of cocaine return prior to full recovery of the neurochemical effects of cocaine self-administration after 6-OHDA induced lesion of this structure. The changes that occur following depletion of DAergic nerve endings in this area appear to be more complex than just restoration of DAergic tone. The neurochemical response to self-administered cocaine returned to prelesion levels at a time when DA content was significantly decreased in the NAcc following 6-OHDA lesions, suggesting that the restoration of DAergic tone indeed does occur at later time points and is probably relevant to the biological mechanisms underlying cocaine self-administration.

The ability of cocaine to maintain self-administration at prelesion levels prior to a return in the effects on dialysate levels of DA in the NAcc suggests that additional mechanisms may be involved in addition to those listed above. One mechanism could be an upregulation in DA receptor subtypes within the NAcc that are involved in the reinforcing effects of cocaine, thereby rendering lower concentrations of DA capable of producing a greater reinforcing stimulus than in sham treated animals. However, studies on the effects of 6-OHDA lesions of the basal forebrain on DA receptors has yielded equivocal findings. 6-OHDA lesions that reduce the DAergic neurons by 50% do not result in increases in DA receptors (Suzuki et al. 1995; Chritin et al. 1996). Modest alterations in D₁ and D₂ receptor density have been reported following more extensive lesions (Angulo et al. 1991; Jongen-Relo et al. 1994). Supersensitivity to both D₁ and D₂ agonists occurs following 6-OHDA lesions of the forebrain even in the absence of changes in receptor density (Breese et al. 1987) or to an extent that cannot be accounted for by modest increases in receptor density (Mandel et al. 1993). Increases in D₂ receptor-G-protein coupling have also been seen following 6-OHDA lesions, suggesting a possible mechanism for DA agonist supersensitivity in the absence of large changes in receptor number (Newman-Tancredi 2001).

Although DAergic levels in the NAcc are correlated with cocaine self-administration, recent studies have focused on the involvement of other brain regions and neurotransmitters in the reinforcing effects of cocaine. The ventral tegmental area (VTA) has been implicated in the self-administration of drugs and reinforcement processes in general (Wise and Bozarth 1985), and interest in

the involvement of the ventral pallidum (VP) in these processes as well has increased in the last decade (Robledo and Koob 1993; Gong et al. 1996; Sizemore et al. 2000). DA turnover rates are increased during intravenous cocaine self-administration in the VP and decreased in the NAcc (Smith et al. 2003) and [DA]_d levels in the VP increase during cocaine self-administration (Sizemore et al. 2000), similar to what has been reported in the NAcc (Hemby et al. 1997). The VP contains D₁ and D₂ receptors and receives major GABA innervations from the NAcc (Napier and Maslowski-Cobuzzi 1994). The VTA sends DA innervations to both the NAcc and VP, and several sites within the VP support electrical self-stimulation (Panagis et al. 1995; Murray and Shizgal 1996). Non-specific lesions of the VP dramatically alter intravenous cocaine self-administration in rats (Robledo and Koob 1993), and micro-injection of cocaine into the VP produces increases in locomotion and supports a conditioned place preference (CPP; Gong et al. 1996). 6-OHDA injections into the VP block the CPP produced by systemic administration of cocaine, and it is possible that this structure may assume a greater role after damage to dopaminergic innervation of the NAcc (Gong et al. 1997). The ability of dopaminergic agonists to elicit motor behavior and reduce GABA levels in the VP is enhanced following 6-OHDA lesions of the NAcc (Bourdelaïs and Kalivas 1992). The effects of cocaine in the VP could therefore possibly be enhanced following DAergic depletion in the NAcc. Since the medium spiny projection neurons from the NAcc to VP contain postsynaptic D₂ receptors, supersensitivity of these D₂ receptors as outlined above could increase the modulation of these neurons by accumbens DA. The interaction between the NAcc, VTA and VP following 6-OHDA lesions of the NAcc may be altered and, thus support cocaine self-administration in the absence of measurable increases in DA in the NAcc.

In conclusion, these data support the concept that extensive interconnections between the VTA, NAcc, VP and perhaps other forebrain structures are important for the self-administration of psychomotor stimulants. Exploring the neurochemical and pharmacological changes that occur in these structures following DAergic depletion in the NAcc related to cocaine self-administration may provide new models for the functional neuroanatomy of these systems. These studies could yield insights regarding the ability of cocaine to alter neuronal responses following DAergic depletion, as well as help to define the neuroplasticity that occurs within the limbic forebrain. Some potential therapies for cocaine abuse focus on diminishing the dopaminergic effects of cocaine. The present data suggest that the plasticity of the forebrain is sufficient to provide mechanisms that support high rates of self-administration despite dramatic reductions in the dopaminergic effects of cocaine.

Acknowledgements The conduct of this experiment was consistent with the ethical standards outlined in "Principles of laboratory animal care" (NIH publication No. 80-23, revised 1996). This

research was supported by grants from the National Institute on Drug Abuse: DA 03628, DA 06634 and DA00114.

References

- Angulo JA, Coirini H, Ledoux M, Schumacher M (1991) Regulation by dopaminergic neurotransmission of dopamine D₂ mRNA and receptor levels in the striatum and nucleus accumbens of the rat. *Mol Brain Res* 11:161–166
- Bourdelaís A, Kalivas PW (1992) Apomorphine decreases extracellular GABA in the ventral pallidum of rats with 6-OHDA lesions in the nucleus accumbens. *Brain Res* 577:306–311
- Breese GR, Duncan GE, Napier TC, Bondy SC, Iorio LC, Mueller RA (1987) 6-Hydroxydopamine treatments enhance behavioral response to intracerebral microinjection of D₁- and D₂-dopamine agonists into nucleus accumbens and striatum without changing dopamine antagonist binding. *J Pharmacol Exp Ther* 240:167–170
- Brown ZW, Amit Z, Weeks JR (1976) Simple flow-thru swivel for infusions into unrestrained animals. *Pharmacol Biochem Behav* 5:363–365
- Caine SB, Koob GF (1994a) Effects of dopamine D-1 and D-2 antagonists on cocaine administration under different schedules of reinforcement in the rat. *J Pharmacol Exp Ther* 270:209–218
- Caine SB, Koob GF (1994b) Effects of mesolimbic dopamine depletion on responding maintained by cocaine and food. *J Exp Anal Behav* 61:213–221
- Chritin M, Blanchard V, Raisman-Vozari R, Feuerstein C, Agid Y, Javoy-Agid F, Savasta M (1996) DA uptake sites, D₁ and D₂ receptors, D₂ and proenkephalin mRNAs and Fos immunoreactivity in rat striatal subregions after partial dopaminergic degeneration. *Eur J Neurosci* 8:2511–2520
- Co C, Smith JE, Lane JD (1982) Use of a single compartment LCEC cell in the determinations of biogenic amine content and turnover. *Pharmacol Biochem Behav* 39:799–802
- DeWit H, Wise RA (1977) Blockade of cocaine reinforcement in rats with dopamine receptor blocker pimoziide, but not with the noradrenergic blockers phentolamine or phenoxybenzamine. *Can J Psychol* 31:195–203
- Di Chiara G, Imperato A (1988). Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. *Proc Natl Acad Sci USA* 85:5274–5278
- Gerber GJ, Wise RA (1989) Pharmacological regulation of intravenous cocaine and heroin self-administration in rats: a variable dose paradigm. *Pharmacol Biochem Behav* 32:527–531
- Gong W, Neill D, Justice JB (1996) Conditioned place preference and locomotor activation produced by injection of psychostimulants into ventral pallidum. *Brain Res* 707:64–74
- Gong W, Neill D, Justice JB (1997) 6-Hydroxydopamine lesion of ventral pallidum blocks acquisition of place preference conditioning to cocaine. *Brain Res* 754:103–112
- Hemby SE, Smith JE, Dworkin SI (1996) The effects of eticlopride and naltrexone on cocaine, heroin, and cocaine/heroin combination self-administration. *J Pharmacol Exp Ther* 277:247–258
- Hemby SE, Co C, Koves TR, Smith JE, Dworkin SI (1997) Differences in extracellular dopamine concentrations in the nucleus accumbens during response-dependent and response-independent cocaine self-administration in the rat. *Psychopharmacology* 133:7–16
- Hubner CB, Morton JE (1991) Effects of selective D₁ and D₂ selective antagonists on cocaine self-administration in the rat. *Psychopharmacology* 105:151–156
- Jongen-Relo AL, Docter GJ, Jonker AJ, Vreugdenhil E, Groenewegen HJ, Voorn P (1994) Differential effects of dopamine depletion on the binding and mRNA levels of dopamine receptors in the shell and core of the rat nucleus accumbens. *Mol Brain Res* 25:333–343
- Klüver H, Barrera EA (1953) A method for the combined staining of cell and fibers in the nervous system. *J Neuropathol Exp Neurol* 12:400–403
- König JFR, Klippel RA (1974). The rat brain: a stereotaxic atlas of the forebrain and lower parts of the brainstem. Kreiger Publishing, Huntington, New York
- Koob GF, Vaccarino FJ, Almaric M, Bloom FE (1987) Positive reinforcement properties of drugs: search for neural substrates. In: Engel J, Oreland L (eds) *Brain reward systems and abuse*. Raven Press, New York, pp 35–50
- Lacosta S, Roberts DC (1993) MDL 72222, ketanserin, and methysergide pretreatments fail to alter breaking points on a progressive ratio schedule reinforced by intravenous cocaine. *Pharmacol Biochem Behav* 44:161–165
- Lane JD, Pickering CL, Hooper ML, Fagan K, Tyers MB, Emmett-Oglesby MW (1992) Failure of ondansetron to block discriminative or reinforcing stimulus effects of cocaine in the rat. *Drug Alcohol Depend* 30:151–162
- Maldonado R, Robledo P, Chover, AJ, Caine SB, Koob GF (1993) D-1 dopamine receptors in the nucleus accumbens modulate cocaine self-administration in the rat. *Pharmacol Biochem Behav* 45:239–242
- Mandel RJ, Hartgraves SL, Severson JA, Woodward JJ, Wilcox RE, Randall PK (1993) A quantitative estimate of the role of striatal D-2 receptor proliferation in dopaminergic behavioral supersensitivity: the contribution of mesolimbic dopamine to the magnitude of 6-OHDA lesion-induced agonist sensitivity in the rat. *Behav Brain Res* 59:53–64
- McGregor A, Roberts DCS (1993). Dopaminergic antagonism within the nucleus accumbens or the amygdala produces differential effects on intravenous cocaine self-administration under fixed and progressive ratio schedules of reinforcement. *Brain Res* 624:245–252
- Murray B, Shizgal P (1996). Physiological measures of conduction velocity and refractory period for putative reward-relevant MFB axons arising in the rostral MFB. *Physiol Behav* 59:427–437
- Napier TC, Maslowski-Cobuzzi RJ (1994) Electrophysiological verification of the presence of D₁ and D₂ dopamine receptors within the ventral pallidum. *Synapse* 17:160–166
- Newman-Tancredi A, Cussac D, Brocco M, Rivet J-M, Chaput C, Touzard M, Pasteau V, Millan MJ (2001) Dopamine D₂ receptor-mediated G-protein activation in rat striatum: functional autoradiography and influence of unilateral 6-hydroxydopamine lesions of the substantia nigra. *Brain Res* 920:41–54
- Nisenbaum ES, Stricker EM, Zigmond MJ, Berger TW (1986) Long-term effects of dopamine-depleting brain lesions on spontaneous activity of type II striatal neurons: relation to behavioral recovery. *Brain Res* 398:221–230
- Nisenbaum ES, Stricker EM, Zigmond MJ, Berger TW (1988) Spontaneous activity of type II but not type I striatal neurons is correlated with recovery of behavioral function after dopamine-depleting brain lesions. *Brain Res* 473:389–393
- Panagis G, Miliaressis E, Anagnostakis, Spyraiki C (1995). Ventral pallidum self-stimulation: a movable electrode mapping study. *Behav Brain Res* 68:165–172
- Parsons LH, Koob GF, Weiss F (1995) Serotonin dysfunction in the nucleus accumbens of rats during withdrawal after unlimited access to intravenous cocaine. *J Pharmacol Exp Ther* 274:1182–1191
- Peltier P, Schenk S (1991) GR38032F, a serotonin 5-HT antagonist, fails to alter cocaine self-administration in rats. *Pharmacol Biochem Behav* 39:133–136
- Pettit HO, Justice JB (1989) Dopamine in the nucleus accumbens during cocaine self-administration as studied by in vivo microdialysis. *Pharmacol Biochem Behav* 34:899–904
- Pettit HO, Ettenberg A, Bloom FE, Koob GF (1984) Destruction of dopamine in the nucleus accumbens selectively attenuates cocaine but not heroin self-administration in rats. *Psychopharmacology* 84:167–173
- Phillips GD, Howes SR, Whitelaw RB, Robbins TW, Everitt BJ (1994) Isolation rearing impairs the reinforcing efficacy of

- intravenous cocaine on intra-accumbens *d*-amphetamine: impaired response in intra-accumbens D₁ and D₂/D₃ dopamine receptor antagonists. *Psychopharmacology* 1115:419–429
- Porrino MC, Ritz MC, Goodman NL, Sharpe LG, Kuhar M.J, Goldberg SR (1989) Differential effects of the pharmacological manipulations of serotonin systems on cocaine and amphetamine self-administration in rats. *Life Sci* 45:1529–1535
- Richardson NR, Piercy MF, Svennson K, Collins RJ, Myers JE, Roberts DCS (1993) Antagonism of cocaine self-administration by the preferential autoreceptor antagonist, (+)-AJ76. *Brain Res* 619:15–21
- Roberts DCS, Vickers G (1984) Atypical neuroleptics increase self-administration of cocaine: an evaluation of a behavioral screen for antipsychotic activity. *Psychopharmacology* 82:135–139
- Roberts DCS, Corcoran ME, Fibiger HC (1977) On the role of ascending catecholaminergic systems in intravenous self-administration of cocaine. *Pharmacol Biochem Behav* 6:615–620
- Roberts DCS, Koob GF, Klonoff P, Fibiger HC (1980) Extinction and recovery of cocaine self-administration following 6-hydroxydopamine lesions of the nucleus accumbens. *Pharmacol Biochem Behav* 12:781–787
- Robinson TE, Castaneda E, Whishaw IQ (1990) Compensatory changes in striatal dopamine neurons following recovery from injury induced by 6-OHDA or methamphetamine: a review of evidence from microdialysis studies. *Can J Psychol* 44:253–275
- Robledo P, Koob GF (1993) Two discrete nucleus accumbens projection areas differentially mediate cocaine self-administration in the rat. *Behav Brain Res* 55:159–166
- Sizemore GM, Co C, Smith JE (2000) Ventral pallidal extracellular fluid levels of dopamine, serotonin, gamma amino butyric acid, and glutamate during cocaine self-administration in rats. *Psychopharmacology* 150:391–398
- Smith JE, Koves TR, Co C (2003) Neurotransmitter turnover rates in brain regions of rats during intravenous cocaine self-administration. *Neuroscience* 117:461–475
- Snyder GL, Keller RW Jr, Zigmond MJ (1990) Dopamine efflux from striatal slices after intracerebral 6-hydroxydopamine: evidence for compensatory hyperactivity of residual terminals. *J Pharmacol Exp Ther* 253:867–876
- Suzuki M, Kawasaki Y, Murata M, Shibata R, Kurachi M, Mori H (1995) Effects of 6-hydroxydopamine lesions of the medial prefrontal cortex on local cerebral blood flow and D₁ and D₂ dopamine receptor binding in rats: a quantitative autoradiographic study. *Eur Neuropsychopharmacol* 5:95–101
- Weeks JR (1962) Experimental morphine addiction: methods for automatic intravenous injections in unrestrained rats. *Science* 138:143–144
- Weeks JR (1972) Long term intravenous infusions. In: Meyers RD (ed) *Methods in psychobiology*. Academic Press, New York, vol 2, pp 155–168
- Wise RA, Bozarth MA (1985) Brain mechanisms of drug reward and euphoria. *Psychiatr Med* 3:445–460
- Woolverton WL (1987) Evaluation of the role of norepinephrine in the reinforcing effects of psychomotor stimulants in rhesus monkeys. *Pharmacol Biochem Behav* 26:835–839
- Woolverton WL (1992) Cocaine self-administration: pharmacology and behavior. *NIDA Res Monogr* 124:189–202
- Zigmond MJ, Hastings TG (1998) Neurochemical responses to lesions of dopaminergic neurons: implications for compensation and neuropathology. *Adv Pharmacol* 42:788–792