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Heterogeneous Subregional Binding Patterns of ^{3}H-WIN 35,428 and ^{3}H-GBR 12,935 Are Differentially Regulated by Chronic Cocaine Self-Administration

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We examined the influence of chronic cocaine exposure, in an unlimited access self-administration paradigm, on density of the dopamine transporter (^{3}H-WIN 35,428 and ^{3}H-GBR 12,935 binding) and concentration of monoamine (dopamine, serotonin, noradrenaline and metabolites) neurotransmitters in rat brain. In normal rodent striatum ^{3}H-WIN 35,428 and ^{3}H-GBR 12,935 binding to the dopamine transporter, although generally similar, showed different subregional rostrocaudal and mediolateral gradients, suggesting that the two ligands might bind to different subtypes or states of the dopamine transporter. Following chronic, unlimited access cocaine self-administration, binding of ^{3}H-WIN 35,428 was significantly elevated in whole nucleus accumbens (+69%, $p < 0.001$) and striatum (+65%, $p < 0.001$) on the last day of cocaine exposure ("on-cocaine group"); whereas in the 3 week withdrawn animals ("cocaine-withdrawn group"), levels were either normal (striatum) or reduced (−30%, $p < 0.05$, nucleus accumbens). Although similar changes in ^{3}H-GBR 12,935 binding were observed, this dopamine transporter ligand showed a smaller and highly subregionally dependent increase in binding in striatal subdivisions of the on-cocaine group, but a more marked binding reduction in the cocaine-withdrawn animals. As compared with the controls, mean dopamine levels were reduced in striatum (−15%, $p < 0.05$) of the on-cocaine group and in nucleus accumbens (−40%, $p < 0.05$) of the cocaine-withdrawn group. These data provide additional support to the hypothesis that some of the long-term effects of cocaine exposure (drug craving, depression) could be consequent to reduced nucleus accumbens dopamine function. Our data also suggest that dopamine transporter concentration, and perhaps function, might undergo up- or downregulation, either as a direct effect of cocaine, or indirectly as part of a homeostatic response to altered synaptic dopamine levels, and therefore might participate in the neuronal events underlying cocaine-induced behavioral changes.

[Key words: cocaine, self-administration, dopamine transporter, quantitative autoradiography, nucleus accumbens]

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The psychostimulant cocaine binds to presynaptic dopamine, noradrenaline, and serotonin reuptake complexes, preventing removal of these monoamines from their sites of action and prolonging neurotransmission (Hadfield and Nugent, 1983; Reith et al., 1986). Substantial evidence links the reinforcing properties of cocaine and other psychomotor stimulants, such as amphetamine, with enhanced dopaminergic neurotransmission in the mesocorticolimbic system, which includes cells that project from the ventral tegmental area to the nucleus accumbens and medial frontal cortex (for reviews, see Koob and Goeders, 1989; Wise, 1990). Thus, treatment of rodents with dopamine receptor antagonists will increase response rates for intravenous injection of amphetamine (Yokel and Wise, 1975; Risner and Jones, 1976) and cocaine (Koob et al., 1987), whereas destruction of dopaminergic pathways will reduce cocaine self-administration (Roberts et al., 1980; Pettit et al., 1984). However, not all behavioral effects of psychostimulants are prevented by dopamine neuron lesions (Spyraki et al., 1982) or by dopamine receptor antagonists (Colpaert et al., 1976; Cunningham and Appel, 1982), suggesting the possible involvement of other monoaminergic neurotransmitter systems, especially serotonin (Scheel-Kruger et al., 1976; Pradhan et al., 1978).

A variety of biochemical approaches have been employed in the experimental animal to assess the consequences of long-term cocaine exposure on the nucleus accumbens presynaptic dopamine system, including determination of whole-tissue and extracellular dopamine levels and measurement of dopamine transporter concentration. The results of microdialysis studies, reporting reduced extracellular dopamine levels in the nucleus accumbens during the abstinence period following repeated cocaine administration, have generally been consistent (Parsons et al., 1991; Robertson et al., 1991; Rosetti et al., 1992; Weiss et al., 1992; but see Kalivas and Duffy, 1993), and have provided evidence that some of the long-term effects of cocaine exposure could be due to diminished nucleus accumbens dopamine function. However, the results of investigations examining the effects of long-term cocaine exposure on two other presynaptic dopaminergic indices, namely, whole-tissue dopamine levels and dopamine transporter concentration, have not yet been consistent. Thus, whereas some groups have reported no change in whole striatal dopamine levels within 24 hr of withdrawal from repeated cocaine administration (Kleven et al., 1988; Peris et al., 1990; Yeh and DeSouza, 1991; Yang et al., 1992), others have shown terminal field dopamine levels to be transiently increased (striatum, Roy et al., 1978; Hurd et al., 1989) or reduced (stria-

tum, Taylor and Ho, 1977; frontal cortex, Karoum et al., 1990). Following a short (3 d) withdrawal from limited access to cocaine self-administration, reduced nucleus accumbens dopamine levels have been reported (Bozarth, 1989). Two weeks or longer after cocaine withdrawal, dopamine levels have been reported to be normal (striatum, Kleven et al., 1988; striatum, frontal cortex, Yeh and DeSouza, 1991; striatum and nucleus accumbens, Kleven and Seiden, 1991) or reduced (frontal cortex, Wyatt et al., 1988), with dopamine metabolites being either normal (striatum, Kleven et al., 1988; striatum and nucleus accumbens, Kleven and Seiden, 1991) or reduced (striatum, Trulson and Ulissey, 1987; frontal cortex, Wyatt et al., 1988). With respect to dopamine transporter concentration, while some groups have reported unaltered binding of ^{3}H-GBR 12,935 and ^{3}H-mazindol to striatal and nucleus accumbens dopamine transporter 3–7 d after withdrawal from repeated cocaine (Allard et al., 1990; Yi and Johnson, 1990; Kula and Baldessarini, 1991; Benmansour et al., 1992), others have shown reduced ^{3}H-GBR 12,935 binding in striatum (Farfel et al., 1992) and medial prefrontal cortex (Hitri et al., 1989) and reduced ^{3}H-mazindol binding in nucleus accumbens (Sharpe et al., 1991) after 10–14 d of cocaine abstinence.

The conflicting results in the literature appear to arise from differences in administration schedules, doses, and withdrawal times. Indeed, the same dose of cocaine self-administered or passively received might be associated with a different affective value, for example, reinforcement or aversion, with different neuroadaptive changes underlying these behavioral responses. In this regard, rats that self-administer cocaine show a diminished susceptibility to the development of tolerance to the anorectic effects of cocaine and lower morbidity and mortality compared with yoked controls that receive the same dose and time course of drug noncontingent upon their responding (Dworkin et al., 1992). Furthermore, different cocaine administration schedules (such as intermittent vs continuous exposure) result in altered behavioral sensitivity to the drug (Reith et al., 1987; King et al., 1992). Similarly, the administration schedule used for dopamine receptor agonists has been shown to have different effects on dopamine receptor regulation (Gerfen, 1990). Thus, different paradigms of cocaine administration, resulting in perturbed dopaminergic neurotransmission, could have markedly different behavioral and neurochemical effects, which might explain some of the discrepant findings in the literature.

We suggest that experimental animal studies of reinforcing drugs should mimic, as closely as possible, the human situation. With the low cost of crack cocaine, and the high income from drug dealing, many inner city residents have unlimited access to cocaine. Therefore, we used an unlimited access cocaine self-administration paradigm to identify the brain biochemical effects of chronic cocaine exposure as it might relate to such use in humans. Using the unlimited access paradigm, rats self-administer the drug in repetitive episodes or "binges" that resemble the episodic drug-taking behavior observed in humans (Gawin and Ellinwood, 1988). As in the human situation, an unlimited access schedule will occasionally result in mortality (20–30%) due to cocaine overdose (M. E. Carroll and S. T. Lac, unpublished observation). The self-administration paradigm allows the investigator to determine only the dose of drug that is administered for each lever press, while the animal has control over the number of injections and total quantity of drug administered. Therefore, the amount of drug self-administered reflects a reward-relevant intake chosen by the animal and provides a good model for investigating the neuropharmacology of cocaine dependence and withdrawal. In this investigation we used an unlimited access cocaine self-administration paradigm to measure the concentration of two presynaptic indices of monoaminergic neurotransmission, namely, whole-tissue levels and binding to the presynaptic dopamine transporter. Our data provide additional support for the hypothesis that some of the behavioral consequences of cocaine exposure and withdrawal could be related to reduced nucleus accumbens dopamine function.

Materials and Methods

Subjects. Two groups of male Wistar rats (350–400 gm) were implanted with chronic indwelling jugular vein catheters, as described previously (Weeks, 1972; Carroll et al., 1981a). The rats were given free access to food and water and allowed to recover from surgery for 24–48 hr. The rats lived in their operant chambers for the duration of the experiment; chambers were located in a temperature- (24°C) and humidity-controlled room with lights on between 7 A.M. and 7 P.M. The cocaine self-administration experiment was approved by the University Institutional Animal Care Committee under protocol number 9303025.

Apparatus. The experimental chambers and infusion system have previously been described in detail (Carroll and Boe, 1982). Briefly, each octagonally shaped chamber consisted of alternating stainless steel and Plexiglas walls and contained two response levers, a food receptacle, and a standard water bottle with the spout protruding into the chamber. A stimulus light was mounted above each lever and was illuminated for the duration of an infusion (3–4 sec) after a response was made. Infusion pumps were located outside wooden enclosures that contained the experimental chambers. Experimental events and data recording were controlled by microcomputers (Carroll et al., 1981b) located in an adjacent room.

Cocaine HCl was provided by the National Institute of Drug Abuse (Research Triangle Institute, Research Triangle Park, NC). Infusion solutions were mixed in sterile saline and contained in 500 ml reservoirs above the experimental chamber.

Cocaine self-administration. One to two days following surgery the drug-naive rats, with no prior experience in the operant chamber, were given unlimited access to cocaine self-administration. Daily sessions began at 10:00 A.M. and lasted 24 hr/d for at least 3 weeks (mean self-administration period = 7 weeks ± 5 d). At 9:30 A.M. cages were cleaned, intake measurements were made, and food and liquids were replenished. Cocaine (0.1 mg per infusion) was delivered following a response on the left lever and the dose was controlled by the duration of infusion (1 sec per 100 gm body weight).

One group of rats (n = 10; "on-cocaine group") was killed on the last day of cocaine access with no withdrawal from cocaine. The maximal interval between the last cocaine infusion and death was 4 hr. A second group (n = 8; "cocaine-withdrawn group") was killed after a 3 week withdrawal period. Food intake was limited to 20 gm/d during the self-administration period so that all rats would consume similar amounts of food. Previous work in the laboratory indicates that rats given continuous access to cocaine consume approximately 20 gm of food per day. When unlimited access to food is allowed, there is greater variability in cocaine self-administration (Carroll and Lac, 1993). Since the experimental chamber acts as the home environment for the self-administering animals, control animals (n = 15; age and food matched) remained in their home cages throughout.

Rats were killed by decapitation, and the brain rapidly removed and divided longitudinally into two halves. One half was immediately frozen over dry ice and stored at −80°C until cryostat sectioning. Serial coronal sections (20 μm) were cut at −20°C, thaw mounted onto gelatin-coated slides, and stored at −80°C until assayed. The second half was dissected over a cold plate into discrete brain areas, including striatum, nucleus accumbens, hypothalamus, thalamus, septum, medial prefrontal cortex, lateral prefrontal cortex, occipital cortex, hippocampus, cerebellum, pons-medulla, midbrain, pituitary, and olfactory bulbs. These brain areas were frozen on dry ice and stored at −80°C until neurochemical analyses were performed.

Brain monoamine measurement. Levels of the monoamines dopamine, noradrenaline, and serotonin and their metabolites, homovanillic acid (HVA), 3,4-dihydroxyphenylacetic acid (DOPAC), normetane-

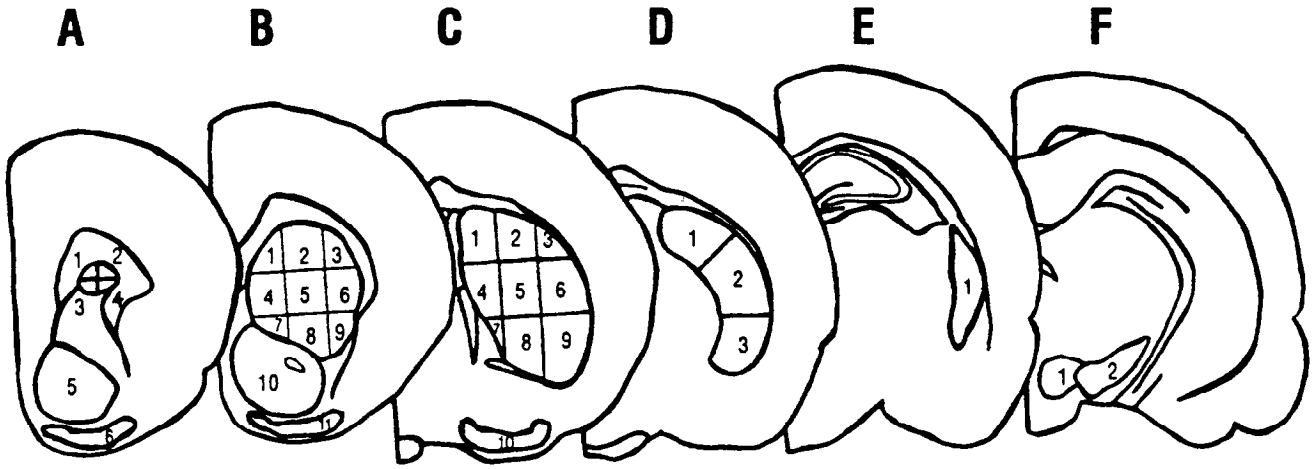

Figure 1. Representative coronal sections through the rat brain. Plates correspond to the rat brain atlas of Paxinos and Watson (1986). *A*, Plate 10, rostral pole of striatum: *1*, dorsomedial; *2*, dorsolateral; *3*, ventromedial; *4*, ventrolateral; anterior nucleus accumbens (*5*) and anterior olfactory tubercle (*6*). *B*, Plate 12, subdivisions of the rostral body of the striatum: *1*, dorsomedial; *2*, dorsointermediate; *3*, dorsolateral; *4*, intermediate-medial; *5*, intermediate-intermediate; *6*, intermediate-lateral; *7*, ventromedial; *8*, ventrointermediate; *9*, ventrolateral; posterior nucleus accumbens (*10*) and posterior olfactory tubercle (*11*). *C*, Plate 19, subdivisions of the intermediate body of the striatum (*1–9*, as above) and posterior olfactory tubercle (*10*). *D*, Plate 24, subdivisions of the caudal body of the striatum: *1*, dorsal; *2*, intermediate; *3*, ventral. *E*, Plate 30, caudate tail (*1*). *F*, Plate 37, ventral tegmental area (*1*) and substantia nigra, pars compacta and pars reticulata (*2*).

phrine, 3-methoxy-4-hydroxyphenylglycol, and 5-hydroxyindoleacetic acid, were determined using HPLC with electrochemical detection. An ESA Coulochem II EC detector was connected to a Spectra Physics SP8000 with a 25 cm × 4.6 mm Spherisorb ODS2 (5 μm) column. Samples were injected into a six port Rheodyne valve fitted with a 100 μl loop. The mobile phase was composed of 0.1 M sodium acetate buffer (pH 3.9) containing 130 mg of octane sulfonate per liter and 3% methanol, with a flow rate of 1 ml/min. Tissue was sonicated in 1.0 ml ice-cold H_2O and 100 μl was removed for determination of protein content (Lowry et al., 1951). Immediately following sonication, 100 μl of perchloric acid (1.0 *N*, containing 2 μM sodium bisulfite, prepared daily) was added to the tissue and resonicated. The samples were centrifuged at 35,000 × *g* for 30 min and the supernatant was removed and passed through a Conz filter (0.45 μm) and injected directly onto the column. For quantification, every third sample was spiked with a cocktail containing standard amounts of each of the compounds being measured.

Dopamine transporter autoradiography. Minor modifications of the procedure of Richfield (1991) were used to determine ^{3}H-GBR 12,935 binding to the dopamine transporter. Brain sections were preincubated in 25 mM sodium phosphate buffer (pH 7.4), containing 100 mM NaCl, 0.001% ascorbate, and 0.025% bovine serum albumen, at 0–4°C for 20 min to remove any residual cocaine that might be present. Sections were incubated in the same buffer containing 0.25 nM ^{3}H-GBR 12,935 (44.6 Ci/mmol; New England Nuclear Research Products, Boston, MA) for 30 hr, to assess total binding. Adjacent sections were incubated under identical conditions in the presence of 1 μM mazindol to measure nonspecific binding. The sections were rinsed for 2 hr in cold buffer followed by a quick rinse in distilled water and left to dry.

^{3}H-WIN 35,428 binding to the dopamine transporter was assessed using the procedure of Canfield et al. (1990) with minor modifications. Brain sections were preincubated for 20 min at 0–4°C in 50 mM sodium phosphate buffer (pH 7.4) containing 50 mM NaCl to remove any residual cocaine that might be present, and then incubated for 2 hr in the same buffer containing 10 nM ^{3}H-WIN 35,428 (85.9 Ci/mmol; New England Nuclear Research Products, Boston, MA) in the presence (nonspecific binding) or absence (total binding) of 30 μM cocaine. Sections were washed in two quick rinses of fresh buffer, and one rinse in distilled water before drying.

Autoradiography and densitometry. Dried sections were apposed to tritium-sensitive film (Kodak Hyperfilm) at 0–4°C for 2 weeks (^{3}H-WIN 35,428) and 6 weeks (^{3}H-GBR 12,935) in the presence of tritium-calibrated ^{14}C-labeled standards. Films were developed using Kodak D19 developer and densitometric analysis of autoradiograms was performed using a camera-based computerized imaging device (MCID, Imaging Research Inc., St. Catharines, Ontario, Canada). As shown in Figure 1, brain areas were subdivided in anteroposterior, dorsoventral, and mediolateral planes where appropriate.

Pharmacological characterization of binding to the dopamine transporter. The competition between various displacing drugs and ^{3}H-GBR 12,935 and ^{3}H-WIN 35,428 binding was determined in rat striatal membranes according to minor modifications of the procedures of Andersen (1987) and Madras et al. (1989). Briefly, rat striatum was homogenized in 100 vol (w/v) sodium phosphate buffer (25 mM, pH 7.7, at 4°C, final sodium concentration 48 mM) and centrifuged at 35,000 × *g* for 10 min. The resulting pellet was resuspended in 100 vol of buffer and the wash procedure repeated. The membrane preparation was diluted to 3 mg tissue/ml buffer and incubated (0.2 ml) in triplicate with ^{3}H-GBR 12,935 (1 nM) or ^{3}H-WIN 35,428 (4 nM) in the presence of increasing concentrations of displacer (0.2 ml, 10 fM to 1 mM) for 4 hr at 0–4°C, in a final volume of 0.6 ml. Incubation was terminated by rapid filtration using a 12-well cell harvester and glass fiber filter mats presoaked in 0.1% bovine serum albumen. The filters were washed twice with 5 ml of cold buffer and residual radioactivity measured by scintillation counting.

Data analysis. Unless otherwise stated, a one-way analysis of variance (ANOVA), at the 0.05 criterion level, followed by Dunnett's multiple comparison test was used for statistical comparisons.

Results

Cocaine self-administration

After an initial period of acquisition, in which responding increased steadily for approximately 10–20 d, there was typically a variable pattern of cocaine intake with high and low numbers of infusions on alternating days. The mean daily cocaine intake in rats given unlimited access was 36.8 ± 4.2 mg/d (approximately 90 mg/kg/d). Although the pattern of cocaine self-administration varied from day to day for each individual animal (see Fig. 2), there were no significant differences (using Student's two-tailed *t* test) in mean daily intake (on cocaine, 42 ± 4 mg; cocaine withdrawn, 30 ± 8 mg; $p > 0.05$), total cocaine intake (on cocaine, 1700 ± 290 mg; cocaine withdrawn, 1690 ± 350 mg; $p > 0.05$), maximum daily intake (on cocaine, 132 ± 18 mg; cocaine withdrawn, 111 ± 26 mg; $p > 0.05$), and duration of cocaine access (on cocaine, 41 ± 6 d; cocaine withdrawn, 60 ± 7 d; $p > 0.05$) between the on-cocaine and cocaine-withdrawn groups. Of the 10 rats in the on-cocaine group, seven had self-administered substantial amounts (32–112 mg) during the 24 hr period prior to death; the remaining three rats self-administered 0.1–1.2 mg. However, no correlations were observed

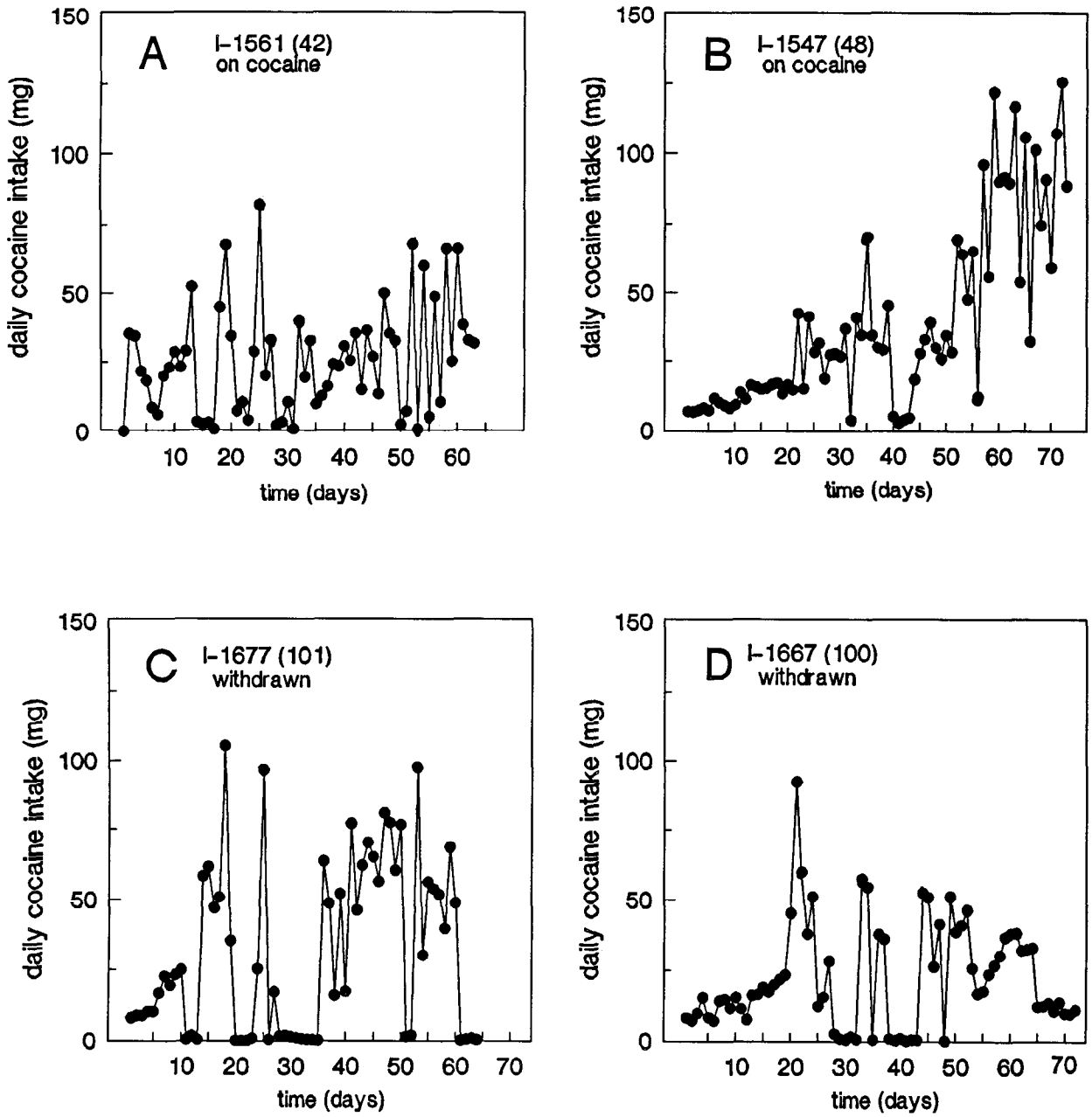

Figure 2. Daily amounts of cocaine (mg) self-administered by rats during unlimited access cocaine exposure. *A* and *B* illustrate daily cocaine intake for two representative rats from the group killed on the last day of cocaine access. *C* and *D* represent daily cocaine intake for two rats from the cocaine-withdrawn group.

between the amount of cocaine self-administered during the last 24 hr period and any of the dopaminergic markers examined. We observed 30% mortality in rats given unlimited access to cocaine. This was related primarily to cocaine overdose, but it also included illness and surgical complications.

Dopamine transporter distribution in control brain

Specific binding of ^{3}H-GBR 12,935 and ^{3}H-WIN 35,428 was restricted to nigrostriatal (substantia nigra and striatum) and mesolimbic (ventral tegmental area, nucleus accumbens, and olfactory tubercle) areas, with the highest binding for both ligands occurring in the striatum, followed in decreasing order of magnitude by the nucleus accumbens, olfactory tubercle, ventral tegmental area, and substantia nigra (Table 1, Fig. 3). These data are consistent with previous reports of lower levels of binding of various radioligands to the dopamine transporter in nucleus accumbens relative to striatum (Richfield, 1991; Sharpe et al., 1991; Benmansour et al., 1992; Cass et al., 1992).

A subregional analysis of the binding data revealed a heterogeneous pattern of binding in the nerve terminal areas with rostrocaudal and, for striatum, dorsoventral and mediolateral gradients that were dependent upon the specific dopamine transporter ligand. Thus, ^{3}H-GBR 12,935 binding was more dense in anterior than posterior regions of striatum (rostral pole, 67 pmol/μg tissue; caudal body, 55 pmol/μg tissue) and nucleus accumbens (anterior, 44 pmol/μg tissue; posterior, 29 pmol/μg tissue). The densities of ^{3}H-WIN 35,428 binding, however, were similar in anterior and posterior portions of the nucleus accum-

Table 1. ^{3}H-WIN 35,428 and ^{3}H-GBR 12,935 binding in rat brain: influence of chronic, unlimited access to self-administration of cocaine

	^{3}H-WIN 35,428 binding (pmol/μg tissue)			^{3}H-GBR 12,935 binding (pmol/μg tissue)		
Brain region	Control	On cocaine	Withdrawn	Control	On cocaine	Withdrawn
Nucleus accumbens						
Whole	48.0 ± 2.6	80.9 ± 6.4**	33.7 ± 3.4*	39.8 ± 2.9	53.1 ± 6.9	28.9 ± 4.9
Anterior	45.7 ± 2.9	85.3 ± 11.2**	34.7 ± 4.7	43.7 ± 4.1	72.1 ± 9.2*	37.3 ± 7.4
Posterior	48.5 ± 0.3	66.8 ± 7.6*	32.4 ± 3.2	29.4 ± 4.1	41.3 ± 5.8	24.4 ± 4.3
Olfactory tubercule						
Whole	35.2 ± 2.7	41.2 ± 4.3	28.3 ± 6.3	34.6 ± 3.1	40.2 ± 5.7	23.6 ± 2.6
Anterior	37.3 ± 3.2	41.1 ± 5.4	28.2 ± 6.2	36.7 ± 3.6	48.8 ± 7.9	25.5 ± 3.0
Posterior	33.0 ± 3.1	32.0 ± 5.8	27.5 ± 6.7	31.4 ± 3.4	29.8 ± 4.0	21.7 ± 3.4
Striatum						
Whole	85.3 ± 3.9	140.5 ± 8.7**	76.1 ± 4.7	56.9 ± 3.5	65.3 ± 6.9	37.6 ± 6.1*
Rostral pole	71.0 ± 5.8	156.2 ± 13.4**	55.2 ± 6.8	66.9 ± 6.7	111.1 ± 13.6*	62.5 ± 8.8
Dorsomedial	69.4 ± 6.2	155.9 ± 12.0**	53.6 ± 6.3	71.0 ± 7.2	128.0 ± 16.1*	66.6 ± 11.7
Dorsolateral	69.5 ± 6.4	164.6 ± 15.8**	56.6 ± 8.3	64.6 ± 6.3	109.8 ± 14.5*	70.2 ± 11.9
Ventromedial	72.3 ± 4.9	149.0 ± 11.8**	55.9 ± 7.7	68.4 ± 8.4	110.7 ± 14.0*	62.5 ± 8.4
Ventrolateral	68.9 ± 5.1	154.1 ± 15.6**	55.5 ± 7.7	59.3 ± 6.0	93.6 ± 10.7*	51.8 ± 6.1
Rostral body	98.6 ± 4.5	164.7 ± 9.7**	89.7 ± 6.1	64.3 ± 4.1	77.0 ± 10.2	45.8 ± 6.3
Dorsomedial	78.8 ± 4.6	133.2 ± 10.6**	77.2 ± 5.2	71.3 ± 5.0	99.3 ± 13.5*	56.2 ± 7.1
Dorsointermed.	100.6 ± 4.6	170.2 ± 10.4**	94.2 ± 7.1	72.0 ± 4.7	91.8 ± 12.9	55.1 ± 6.6
Dorsolateral	107.3 ± 4.7	172.6 ± 10.5**	94.6 ± 7.9	64.2 ± 4.0	71.5 ± 11.3	45.6 ± 6.5
Intermed.–medial	88.0 ± 5.1	152.8 ± 11.6**	79.0 ± 4.7	67.5 ± 5.1	87.8 ± 9.8	49.3 ± 6.8
Intermed.–intermed.	106.2 ± 5.0	178.2 ± 13.4**	96.2 ± 5.0	62.4 ± 4.0	70.1 ± 9.5	41.5 ± 6.8
Intermed.–lateral	107.5 ± 4.4	175.2 ± 8.5**	98.8 ± 7.3	56.5 ± 3.7	58.0 ± 8.7	36.7 ± 6.2*
Ventromedial	86.9 ± 5.7	144.0 ± 9.1**	72.7 ± 4.5	61.9 ± 4.9	75.4 ± 8.9	45.0 ± 6.8
Ventrointermed.	105.1 ± 4.6	174.2 ± 13.1**	91.3 ± 6.2	64.0 ± 4.9	71.4 ± 9.4	42.2 ± 6.1
Ventrolateral	104.4 ± 4.2	177.1 ± 16.7**	93.4 ± 9.7	58.5 ± 4.2	62.0 ± 9.4	39.6 ± 6.8
Intermediate body	96.7 ± 4.0	139.4 ± 8.6**	90.2 ± 5.0	57.4 ± 5.1	54.7 ± 6.5	34.7 ± 4.3*
Dorsomedial	74.5 ± 4.6	108.3 ± 6.7**	68.2 ± 5.5	66.0 ± 6.6	72.5 ± 6.6	41.9 ± 5.2*
Dorsointermed.	90.0 ± 3.5	134.4 ± 6.9**	93.2 ± 6.9	60.7 ± 4.7	62.7 ± 6.4	38.9 ± 4.4*
Dorsolateral	109.1 ± 5.0	160.9 ± 8.8**	114.2 ± 8.9	55.2 ± 4.3	51.1 ± 6.4	32.0 ± 4.1*
Intermed.–medial	67.6 ± 3.7	99.5 ± 6.7**	61.4 ± 3.7	51.6 ± 5.4	51.2 ± 4.5	31.3 ± 4.6*
Intermed.–intermed.	96.1 ± 4.4	142.8 ± 8.6**	88.1 ± 4.3	52.3 ± 4.5	45.9 ± 5.6	31.0 ± 4.2*
Intermed.–lateral	122.6 ± 5.4	179.7 ± 11.7**	116.6 ± 6.4	48.9 ± 3.9	42.2 ± 5.5	27.8 ± 4.1*
Ventromedial	72.6 ± 5.3	88.9 ± 6.6	49.6 ± 8.2	50.2 ± 5.9	41.7 ± 5.4	28.1 ± 5.4
Ventrointermed.	113.4 ± 6.2	161.4 ± 11.3*	98.0 ± 6.7	62.7 ± 6.9	58.4 ± 8.5	39.9 ± 5.2
Ventrolateral	135.7 ± 6.8	194.8 ± 13.9**	125.0 ± 6.0	59.2 ± 5.6	60.3 ± 9.4	35.1 ± 4.5*
Caudal body	66.0 ± 4.6	126.5 ± 12.4**	60.0 ± 4.8	55.4 ± 3.1	66.0 ± 6.9	46.8 ± 4.7
Dorsal	41.1 ± 2.9	78.3 ± 9.1**	39.9 ± 3.5	42.7 ± 3.8	51.7 ± 5.5	41.3 ± 4.5
Intermed.	69.1 ± 5.0	131.8 ± 13.1**	66.9 ± 6.4	55.7 ± 3.3	63.3 ± 7.0	47.0 ± 4.8
Ventral	92.1 ± 7.6	179.0 ± 16.7**	84.6 ± 9.9	67.6 ± 4.1	87.2 ± 10.8	53.3 ± 5.3
Caudate tail	33.2 ± 3.6	84.6 ± 8.5**	26.5 ± 4.5	44.8 ± 3.2	54.0 ± 5.5	29.6 ± 5.1*
Substantia nigra	13.0 ± 1.2	22.9 ± 2.9	11.2 ± 1.8	19.7 ± 2.7	36.4 ± 2.2**	15.7 ± 2.8
Ventral tegmental area	21.7 ± 2.6	36.1 ± 5.9	13.9 ± 4.3	33.7 ± 4.3	55.8 ± 1.7**	25.2 ± 3.7

Data are subregional distribution (mean ± SEM) of ^{3}H-WIN 35,428 and ^{3}H-GBR 12,935 binding in brain of control rats (n = 15) and of rats killed on the last day of (n = 10) or 3 weeks after withdrawal from (n = 8) chronic, unlimited access to self-administration of cocaine. One-way ANOVA, followed by Dunnett's test (**, $p < 0.001$; *, $p < 0.05$).

bens (46 and 49 pmol/μg tissue, respectively) and, unlike ^{3}H-GBR 12,935 binding, showed increased binding from the rostral pole of the striatum (71 pmol/μg tissue) to rostral (99 pmol/μg tissue) and intermediate body (97 pmol/μg tissue). Throughout the striatum, neither ^{3}H-GBR 12,935 nor ^{3}H-WIN 35,428 binding showed any clear dorsoventral gradient, except in the caudal body of the striatum in which the ventral subdivisions showed the highest binding. A mediolateral gradient for striatal ^{3}H-GBR 12,935 was not observed, whereas ^{3}H-WIN 35,428 binding showed a consistent increasing mediolateral density gradient that was especially pronounced in the intermediate and ventral subdivisions of the intermediate striatum.

Dopamine transporter distribution in cocaine-exposed brain: on-cocaine group

In rats killed on the last day of cocaine access, ^{3}H-GBR 12,935 binding was markedly and significantly elevated in substantia nigra (+85%, $p < 0.001$) and the ventral tegmental area (+66%,

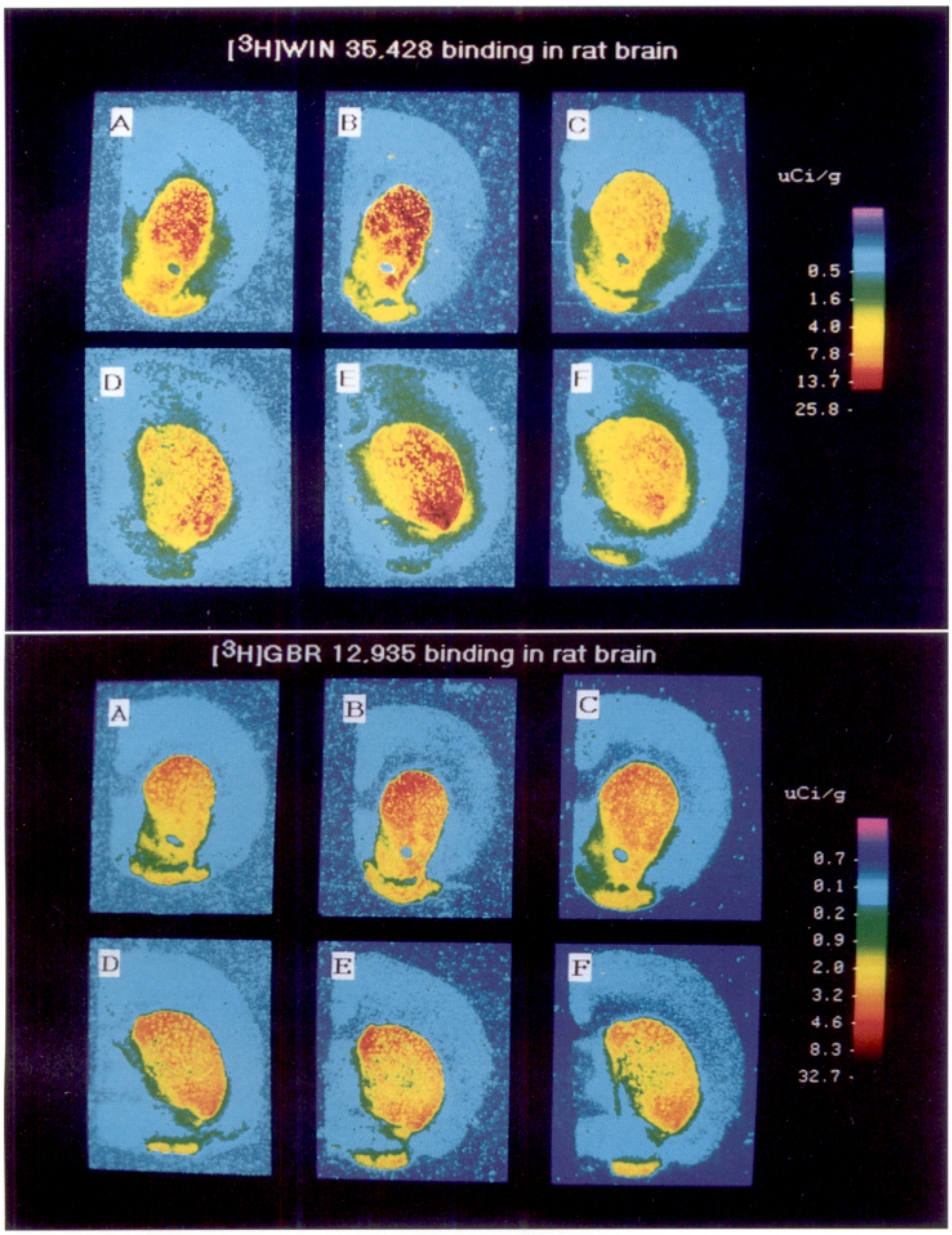

Figure 3. Color-enhanced autoradiograms showing localization of ^{3}H-WIN 35,428 and ^{3}H-GBR 12,935 binding in rat brain. *Top,* Plates *A* and *D* illustrate ^{3}H-WIN 35,428 binding sites in control rat brain, at levels that correspond to the brain areas depicted in Figure 1*B* (anterior nucleus accumbens, olfactory tubercle, and striatum) and Figure 1*C* (posterior olfactory tubercle and intermediate striatum), respectively. Plates *B* and *E*, and plates *C* and *F* are equivalent sections from on-cocaine and cocaine-withdrawn rats, respectively. *Bottom,* Plates *A–F* (as above) illustrate ^{3}H-GBR 12,935 binding sites in rat brain.

$p < 0.001$), whereas no statistically significant changes were observed in olfactory tubercle (Table 1). Although no statistically significant changes were observed in whole nucleus accumbens or striatum of the on-cocaine group, a trend toward increased binding of ^{3}H-GBR 12,935 was observed in several subdivisions of the two nuclei, reaching significance in the anterior nucleus accumbens (+65%, $p < 0.05$), all four subdivisions of the rostral pole of the striatum (+58 to +80%, $p < 0.05$), and the dorsomedial subdivision of the rostral body of the striatum (+39%, $p < 0.05$).

On the last day of cocaine access ^{3}H-WIN 35,428 binding was significantly elevated (Table 1) in both subdivisions of the nucleus accumbens (anterior: +86%, $p < 0.001$; posterior: +38%, $p < 0.05$) and, unlike ^{3}H-GBR 12,935 binding, in almost all examined subdivisions of the striatum (+65% for whole striatum, $p < 0.001$), whereas the increased binding in the substantia nigra (+76%), ventral tegmental area (+66%), and olfactory tubercle (+17%) failed to reach statistical significance ($p > 0.05$). Within the nucleus accumbens and striatum the most marked increases occurred in the anterior portions of both nuclei and the caudal body and tail of the striatum.

Dopamine transporter distribution in cocaine-exposed brain: cocaine-withdrawn group

Following 3 weeks of withdrawal from cocaine, ^{3}H-GBR 12,935 binding was reduced in whole striatum (−34%, $p < 0.05$) with slight (−20 to −32%) but statistically nonsignificant reductions in substantia nigra, ventral tegmental area, nucleus accumbens, and olfactory tubercle. A subregional analysis of the striatum revealed that the reductions in ^{3}H-GBR 12,935 binding were primarily limited to portions of the intermediate striatum (−36 to −43%) and the caudate tail (−34%).

Mean ^{3}H-WIN 35,428 binding was significantly reduced in whole nucleus accumbens (−30%, $p < 0.05$) but was unaltered in any other brain area examined.

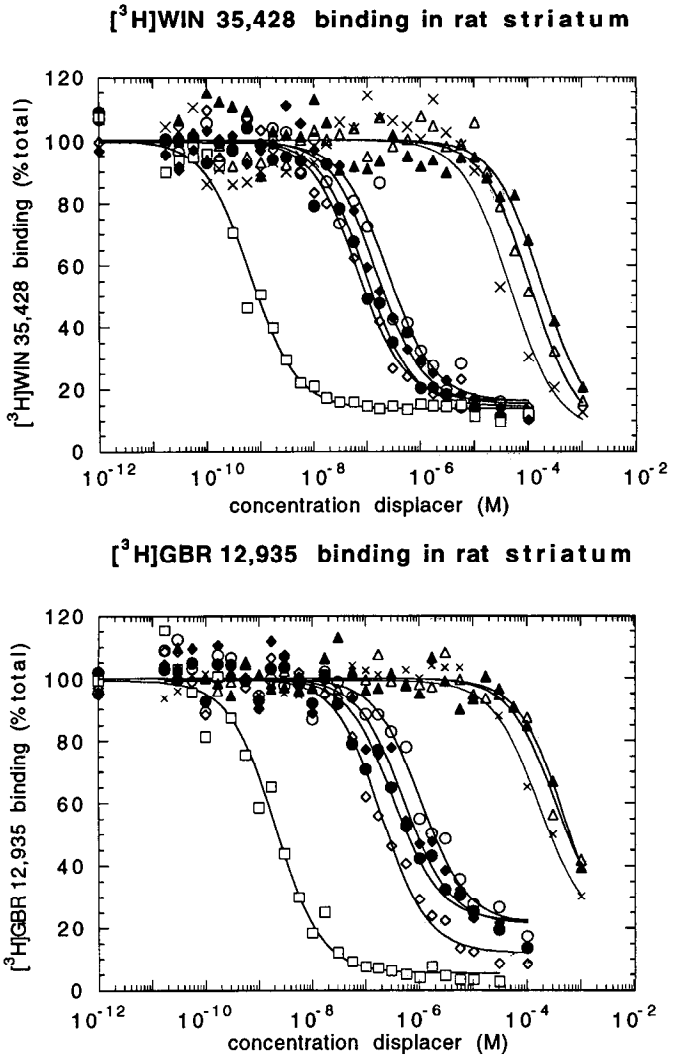

Figure 4. Pharmacological characterization of ^{3}H-WIN 35,428 (*top*) and ^{3}H-GBR 12,935 (*bottom*) binding in rat striatal membranes. The same rank order potency for displacement of both ligands was obtained: GBR 12,909 (□) > mazindol (◇) > WIN 35,428 (●) > methylphenidate (◆) > cocaine (○) > dopamine (×) > noradrenaline (△) > serotonin (▲). The data are from a single experiment that was repeated with similar results.

Pharmacological characterization of ^{3}H-GBR 12935 and ^{3}H-WIN 35,428 binding

As shown in Figure 4, binding of ^{3}H-GBR 12,935 and ^{3}H-WIN 35,428 to rat striatal membranes displayed a rank order of potency suggestive of the sodium-dependent dopamine transporter. The rank order profiles for the displacing drugs were similar for both ligands (GBR 12,909 > mazindol > WIN 35,428 > methylphenidate > cocaine > dopamine > noradrenaline > serotonin).

Brain monoamines

As shown in Table 2, rats killed on the last day following chronic cocaine self-administration had, as compared with controls, a slight reduction in concentration of dopamine in striatum (−15%, $p < 0.05$) together with a marked increase in levels of the dopamine metabolite HVA (+103%, $p < 0.001$). No change, however, was observed in striatal concentration of a second dopamine metabolite, DOPAC. HVA levels were similarly increased in the on-cocaine group in medial prefrontal cortex (+56%, $p < 0.01$) and olfactory bulb (+55%, $p < 0.01$), whereas levels were reduced in cerebellum (−40%, $p < 0.05$). A 27% reduction in dopamine levels in nucleus accumbens failed to reach statistical significance (Fig. 5). No other statistically significant changes in dopamine and metabolite levels were observed in any other brain area in the on-cocaine group.

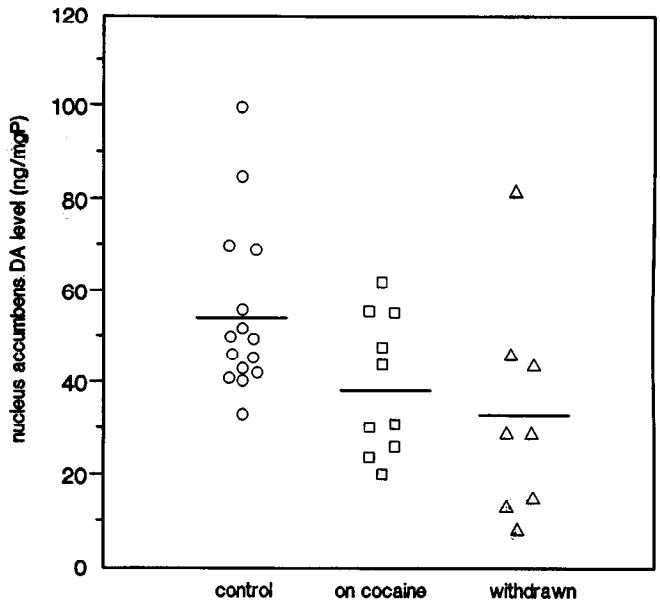

Figure 5. Influence of chronic, unlimited access self-administration of cocaine on nucleus accumbens dopamine (*DA*) levels in rats killed on the last day of cocaine access (□; $n = 10$) and following 3 weeks of cocaine abstinence (△; $n = 8$), as compared with controls (○, $n = 15$).

Following 3 weeks of withdrawal from chronic cocaine self-administration, a 40% reduction in the level of dopamine ($p < 0.05$) was observed in the nucleus accumbens (Fig. 5), together with normal levels of HVA and DOPAC. No significant changes in dopamine and metabolite concentrations were observed in any other brain area of the cocaine-withdrawn group.

Changes in levels of noradrenaline and serotonin (Table 3) were restricted to the on-cocaine group, with an increase in noradrenaline concentration in the pituitary (+59%, $p < 0.05$) and an increase in serotonin in hypothalamus (+36%, $p < 0.05$). There were no changes in the levels of the noradrenaline metabolites normetanephrine or 3-methoxy-4-hydroxyphenylglycol in any of the brain areas examined. The concentration of the serotonin metabolite 5-hydroxyindoleacetic acid was elevated in striatum (+22%, $p < 0.05$) and nucleus accumbens (+27%, $p < 0.05$) in the on-cocaine group.

Discussion

To our knowledge, this is the first experimental animal study of the effects of chronic, unlimited access cocaine self-administration on whole-tissue dopamine and dopamine transporter levels. The major findings of this investigation are different subregional binding patterns of ^{3}H-GBR 12,935 and ^{3}H-WIN 35,428 in normal rat brain and differential regulation of these binding sites following unlimited access to cocaine self-administration and withdrawal. In addition, a nucleus accumbens dopamine reduction was observed in the cocaine-withdrawn animals.

Table 2. Levels of dopamine and metabolites in rat brain: influence of chronic, unlimited access to self-administration of cocaine

Brain area	Group	Dopamine	Homovanillic acid	DOPAC
Striatum	Control	84.5 ± 3.1	6.1 ± 0.3	16.1 ± 1.5
	On cocaine	71.7 ± 4.1*	12.4 ± 1.7**	18.4 ± 1.4
	Withdrawn	73.3 ± 2.7	5.7 ± 0.5	11.9 ± 0.6
Nucleus accumbens	Control	55.0 ± 4.8	5.4 ± 0.4	18.1 ± 2.0
	On cocaine	39.8 ± 4.8	6.4 ± 0.6	14.1 ± 2.0
	Withdrawn	33.2 ± 8.5*	4.3 ± 0.8	11.2 ± 2.5
Hypothalamus	Control	3.2 ± 0.29	0.73 ± 0.12	1.43 ± 0.19
	On cocaine	2.9 ± 0.26	0.49 ± 0.05	0.75 ± 0.05
	Withdrawn	3.2 ± 0.45	0.49 ± 0.11	0.89 ± 0.19
Medial prefrontal cortex	Control	0.63 ± 0.08	0.53 ± 0.05	0.36 ± 0.06
	On cocaine	0.67 ± 0.04	0.83 ± 0.09**	0.33 ± 0.05
	Withdrawn	0.76 ± 0.09	0.54 ± 0.04	0.37 ± 0.07
Lateral prefrontal cortex	Control	0.26 ± 0.03	0.24 ± 0.02	0.26 ± 0.04
	On cocaine	0.19 ± 0.02	0.39 ± 0.06	0.16 ± 0.02
	Withdrawn	0.30 ± 0.07	0.43 ± 0.13	0.32 ± 0.08
Amygdala	Control	2.7 ± 0.18	0.74 ± 0.08	1.44 ± 0.22
	On cocaine	3.1 ± 0.46	0.71 ± 0.12	0.83 ± 0.17
	Withdrawn	2.6 ± 0.52	0.57 ± 0.09	0.88 ± 0.19
Cerebellum	Control	0.07 ± 0.01	0.20 ± 0.03	0.08 ± 0.01
	On cocaine	0.06 ± 0.01	0.12 ± 0.01*	0.08 ± 0.01
	Withdrawn	0.07 ± 0.01	0.13 ± 0.02	0.07 ± 0.01
Olfactory bulb	Control	0.60 ± 0.04	0.82 ± 0.08	0.35 ± 0.04
	On cocaine	0.69 ± 0.04	1.27 ± 0.08**	0.46 ± 0.04
	Withdrawn	0.52 ± 0.06	0.87 ± 0.10	0.34 ± 0.02
Pons-medulla	Control	0.39 ± 0.03	0.38 ± 0.04	0.21 ± 0.03
	On cocaine	0.44 ± 0.02	0.38 ± 0.04	0.24 ± 0.01
	Withdrawn	0.39 ± 0.04	0.34 ± 0.02	0.24 ± 0.03
Hippocampus	Control	0.12 ± 0.01	0.18 ± 0.03	0.09 ± 0.01
	On cocaine	0.16 ± 0.04	0.12 ± 0.02	0.11 ± 0.02
	Withdrawn	0.18 ± 0.04	0.15 ± 0.02	0.10 ± 0.01
Septum	Control	5.3 ± 0.9	3.3 ± 0.8	4.5 ± 0.8
	On cocaine	7.2 ± 1.6	3.3 ± 1.3	7.7 ± 3.4
	Withdrawn	7.6 ± 1.7	2.2 ± 0.7	4.5 ± 1.0
Thalamus	Control	1.6 ± 0.08	0.48 ± 0.02	0.56 ± 0.04
	On cocaine	1.5 ± 0.16	0.49 ± 0.06	0.63 ± 0.05
	Withdrawn	3.1 ± 1.58	0.70 ± 0.27	1.65 ± 1.09
Pituitary	Control	1.1 ± 0.07	0.42 ± 0.03	0.40 ± 0.08
	On cocaine	1.6 ± 0.16*	0.43 ± 0.06	0.26 ± 0.02
	Withdrawn	1.3 ± 0.23	0.55 ± 0.06	0.54 ± 0.14
Midbrain	Control	1.0 ± 0.11	0.34 ± 0.03	0.50 ± 0.06
	On cocaine	1.1 ± 0.09	0.46 ± 0.02	0.48 ± 0.04
	Withdrawn	1.1 ± 0.11	0.43 ± 0.07	0.52 ± 0.07

Data are levels (mean ± SEM; ng/mg protein) of dopamine and metabolites homovanillic acid and DOPAC (3,4-dihydroxyphenylacetic acid) in brain areas of control rats (n = 15) and of rats killed on the last day of (n = 10) or 3 weeks after withdrawal from (n = 8) chronic, unlimited access to self-administration of cocaine. One-way ANOVA, followed by Dunnett's test (**, $P < 0.01$, *, $p < 0.05$).

The dopamine transporter in normal rat brain

Dopamine uptake sites have been studied and identified by radioligand binding techniques with the use of several radioactive probes, including ^{3}H-cocaine (Reith et al., 1981; Kennedy and Hanbauer, 1983; Schoemaker et al., 1985; Calligaro and Eldefrawi, 1988; Madras et al., 1989) and analogs ^{3}H-BTCP, ^{3}H-WIN 35,428, and ^{125}I-RTI-55 (Vignon et al., 1988; Filloux et al., 1989; Madras et al., 1989; Ritz et al., 1990; Boja et al., 1991); ^{3}H-mazindol (Javitch et al., 1983); ^{3}H-methylphenidate (Janowsky et al., 1985); ^{3}H-nomifensine (Dubocovich and Zahniser, 1985); and the aryldialkylpiperazine (GBR) series that are specific for inhibiting dopamine uptake (Van der Zee et al., 1980; Bonnet and Costentin, 1986; Pileblad and Engberg, 1986; Berger et al., 1990) with little effect on noradrenergic or serotonergic uptake (Andersen, 1987). The evidence that the binding site for ^{3}H-GBR 12,935 and various cocaine analogs, including ^{3}H-WIN 35,428, is on the dopamine transporter itself is presumptive and consists of the observations that (1) sodium ion dependence is characteristic of both the dopamine uptake site

Table 3. Levels of noradrenaline, serotonin, and 5-HIAA in rat brain: influence of chornic, unlimited access to self-administration of cocaine

Brain area	Group	Noradrenaline	Serotonin	5-HIAA
Striatum	Control	2.1 ± 0.30	3.5 ± 0.26	5.0 ± 0.22
	On cocaine	1.4 ± 0.18	3.0 ± 0.40	6.1 ± 0.34*
	Withdrawn	1.4 ± 0.24	3.4 ± 0.33	4.8 ± 0.41
Nucleus accumbens	Control	4.4 ± 0.93	3.7 ± 0.41	4.4 ± 0.27
	On cocaine	5.7 ± 0.87	3.5 ± 0.39	5.6 ± 0.27*
	Withdrawn	5.6 ± 1.2	3.8 ± 0.61	5.3 ± 0.61
Hypothalamus	Control	12.7 ± 0.46	4.4 ± 0.44	5.8 ± 0.41
	On cocaine	13.1 ± 0.82	6.0 ± 0.38*	5.3 ± 0.44
	Withdrawn	14.2 ± 1.2	5.3 ± 0.36	4.5 ± 0.23
Medial prefrontal cortex	Control	3.0 ± 0.16	3.1 ± 0.28	3.5 ± 0.24
	On cocaine	3.1 ± 0.17	3.5 ± 0.23	3.7 ± 0.25
	Withdrawn	3.3 ± 0.13	3.5 ± 0.12	3.6 ± 0.32
Lateral prefrontal cortex	Control	2.7 ± 0.19	4.0 ± 0.33	3.9 ± 0.36
	On cocaine	2.4 ± 0.15	3.6 ± 0.19	3.2 ± 0.20
	Withdrawn	2.8 ± 0.17	4.5 ± 0.50	4.3 ± 0.55
Amygdala	Control	4.5 ± 0.19	4.4 ± 0.34	5.3 ± 0.44
	On cocaine	3.9 ± 0.48	4.5 ± 0.44	4.0 ± 0.51
	Withdrawn	4.6 ± 0.13	4.5 ± 0.38	5.0 ± 0.62
Cerebellum	Control	1.5 ± 0.10	0.44 ± 0.07	0.77 ± 0.07
	On cocaine	1.3 ± 0.11	0.26 ± 0.02	0.71 ± 0.03
	Withdrawn	1.3 ± 0.10	0.58 ± 0.08	0.96 ± 0.15
Olfactory bulb	Control	3.0 ± 0.17	1.7 ± 0.11	2.5 ± 0.15
	On cocaine	3.3 ± 0.27	1.9 ± 0.16	2.9 ± 0.31
	Withdrawn	3.0 ± 0.18	1.5 ± 0.12	2.1 ± 0.22
Pons-medulla	Control	4.3 ± 0.14	3.2 ± 0.21	3.4 ± 0.24
	On cocaine	4.4 ± 0.14	3.2 ± 0.19	4.2 ± 0.30
	Withdrawn	4.6 ± 0.49	3.0 ± 0.23	3.3 ± 0.39
Hippocampus	Control	2.5 ± 0.19	2.0 ± 0.15	3.4 ± 0.26
	On cocaine	3.0 ± 0.23	2.3 ± 0.15	3.8 ± 0.18
	Withdrawn	3.0 ± 0.25	2.4 ± 0.13	3.9 ± 0.36
Septum	Control	8.8 ± 1.5	4.0 ± 0.55	6.2 ± 0.59
	On cocaine	8.6 ± 0.93	4.2 ± 0.39	7.6 ± 1.60
	Withdrawn	8.7 ± 1.8	4.6 ± 0.52	5.8 ± 0.68
Thalamus	Control	3.8 ± 0.19	4.2 ± 0.20	5.4 ± 0.20
	On cocaine	3.6 ± 0.35	3.8 ± 0.30	5.9 ± 0.35
	Withdrawn	4.3 ± 0.23	4.1 ± 0.22	5.6 ± 0.34
Pituitary	Control	0.48 ± 0.05	0.86 ± 0.11	0.47 ± 0.06
	On cocaine	0.77 ± 0.10*	0.60 ± 0.07	0.32 ± 0.03
	Withdrawn	0.71 ± 0.12	0.85 ± 0.15	0.64 ± 0.15
Midbrain	Control	3.8 ± 0.25	5.4 ± 0.42	7.7 ± 0.58
	On cocaine	3.7 ± 0.18	5.3 ± 0.27	7.4 ± 0.42
	Withdrawn	3.8 ± 0.17	4.4 ± 0.21	6.6 ± 0.77

Levels (mean ± SEM; ng/mg protein) of noradrenaline, serotonin, and 5-hydroxyindoleacetic acid (5-HIAA) in brain areas of control rats (n = 15) and of rats killed on the last day of (n = 10) or 3 weeks after withdrawal from (n = 8) chronic, unlimited access to self-administration of cocaine. One-way ANOVA, followed by Dunnett's test (*, $p < 0.05$).

and the binding site for the tritiated dopamine uptake inhibitors (Janowsky et al., 1986); (2) regional brain localization of tritiated uptake inhibitor binding is selective for areas with dopamine nerve terminals (Dawson et al., 1986; Canfield et al., 1990); (3) dopamine uptake/binding and axonal transport of tritiated uptake inhibitors are decreased by surgical or 6-hydroxydopamine destruction of dopaminergic afferents (Janowsky et al., 1985; Schoemaker et al., 1985; Ciliax et al., 1990; Richfield, 1991) and in Parkinson's disease (Schoemaker et al., 1985; Janowsky et al., 1987; Brooks et al., 1990; Kaufman and Madras, 1991); and (4) a correlation of the rank order of potency for displacement of tritiated ligands at the binding site and the dopamine uptake carrier (Bonnet et al., 1986; Andersen, 1987). Whether GBR 12,935 and WIN 35,428, two of the most characterized dopamine transporter ligands, interact with the substrate recognition site on the dopamine transporter or with distinct but allosterically linked binding sites remains uncertain (Calligaro and Eldefrawi, 1988; Johnson et al., 1992; Reith and Selmeci, 1992; Reith et al., 1992). The rank order of potency for displacement of ^{3}H-GBR 12,935 and ^{3}H-WIN 35,428 binding in rat striatum reported in this investigation (GBR 12,909 > mazindol > WIN 35,428 > methylphenidate > cocaine > dopa-

mine > noradrenaline > serotonin) confirms earlier observations of similar rank orders of potency for displacement of ^{3}H-WIN 35,428 (Madras et al., 1989; Canfield et al., 1990), its analog ^{3}H-WIN 35,065-2 (Ritz et al., 1990), and ^{3}H-GBR 12,935 binding (Berger et al., 1985; Janowsky et al., 1986; Kula and Baldessarini, 1991; Richfield, 1991) and is similar to the rank order for inhibition of ^{3}H-dopamine uptake (Berger et al., 1985; Janowsky et al., 1986; Andersen, 1987), suggesting that the two ligands recognize a similar binding site associated with the dopamine uptake site. However, our autoradiographic study in normal rat brain, which to our knowledge is the first to compare the binding patterns of ^{3}H-WIN 35,428 and ^{3}H-GBR 12,935 systematically in the same brain samples, demonstrates different subregional patterns of the two ligand binding sites in the basal ganglia. Thus, while ^{3}H-GBR 12,935 binding was most dense in dorsomedial striatum, which is the main target site for nigrostriatal cells originating in the substantia nigra (Gerfen, 1987), ^{3}H-WIN 35,428 binding was most dense in ventrolateral striatum, which receives dopaminergic innervation from the ventral tegmental area (Gerfen, 1987). The different anatomical localization of these ligand binding sites suggests that the dopaminergic neurons originating from the substantia nigra and the ventral tegmental area might express different forms of the dopamine transporter with different relative proportions of GBR 12,935- and WIN 35,428-sensitive sites. The possibility that the two ligands recognize different sites or states of the dopamine transporter is consistent with a preliminary study showing different responses of ^{3}H-cocaine and ^{3}H-GBR 12,935 binding following destruction of corticostriatal glutamatergic neural projections (Grilli et al., 1988) and a more recent study demonstrating that tolerance to the effects of cocaine conferred cross-tolerance to the effects of WIN 35,428 but not to the effects of GBR 12,909 (Katz et al., 1993). Thus, the two dopamine transporter ligands might be (1) binding to two distinct (e.g., splice variants) but pharmacologically homologous populations of the dopamine transporter, (2) recognizing different functional states (e.g., phosphorylated vs unphosphorylated) of the dopamine transporter, (3) binding differentially to nondopamine transporters (e.g., serotonin, noradrenaline, or orphan transporters), or (4) recognizing transporters that could be expressed in nondopaminergic terminals (postsynaptic neurons) or even glial cells (Uhl, 1992). The relative proportions of such subtypes/affinity states or recognition sites could confer differential sensitivity to the acute and chronic effects of cocaine.

Cocaine and the dopamine transporter

The results of our study show that on the last day of chronic exposure to self-administered cocaine, binding of ^{3}H-WIN 35,428 and ^{3}H-GBR 12,935 to the dopamine transporter was elevated in various subdivisions of the striatum and nucleus accumbens. Since all animals were recently exposed to cocaine, the increase in binding density could be related to the acute effects of the drug. However, consistent with our findings, Alburges et al. (1993) recently demonstrated elevated binding of ^{3}H-cocaine in striatum of rats exposed to cocaine for 14 or 21 d, but not after exposure for only 1 d, suggesting that the increase in binding is associated with repeated drug administration. Since during cocaine self-administration synaptic dopamine levels typically increase 350–400% above basal levels even after prolonged exposure (Pettit and Justice, 1991), our observation of increased binding to the dopamine transporter could reflect a functional compensatory mechanism (involving increased transporter affinity or number) to enhance removal of dopamine from the synaptic cleft. These results are consistent with a report of increased striatal ^{3}H-dopamine uptake during chronic cocaine administration, although in this study increased transporter function was detected in the absence of increased ^{3}H-GBR 12,935 binding (Yi and Johnson, 1990).

Our observation of reduced ^{3}H-WIN 35,428 and ^{3}H-GBR 12,935 binding in some subdivisions of the striatum and/or nucleus accumbens following 3 weeks of withdrawal from self-administered cocaine is consistent with similar observations of other investigators showing reduced ^{3}H-mazindol binding in nucleus accumbens (10 d withdrawal, Sharpe et al., 1991) and reduced ^{3}H-GBR 12,935 binding in striatum (14 d withdrawal, Farfel et al., 1992) and medial prefrontal cortex (42 d withdrawal, Hitri et al., 1989). In contrast, no change in dopamine transporter concentration was observed in rodents killed 24 hr (^{3}H-nomifensine, Peris et al., 1990), 2–3 d (^{3}H-GBR 12,935, Kula and Baldessarini, 1991), or 7 d (^{3}H-GBR 12,935, Yi and Johnson, 1990) following repeated injection of cocaine. Function of the dopamine transporter as evidenced by ^{3}H-dopamine uptake following repeated cocaine injection has been reported as being reduced (24 hr withdrawal, Izenwasser and Cox, 1990), elevated (24 hr, Ng et al., 1991), or normal (24 hr, Peris et al., 1990; 2 d, Missale et al., 1985; 7 d, Yi and Johnson, 1990) during the cocaine abstinence period. Although the reduced binding observed 3 weeks after cocaine withdrawal in the present investigation suggests that a long-lasting neuroadaptive change has taken place, it is possible that a more dramatic reduction might have been detected at a shorter withdrawal time when the behavioral correlates of withdrawal are more severe (Gawin and Ellinwood, 1990). Nonetheless, the long-lasting decreases in ^{3}H-GBR 12,935 and ^{3}H-WIN 35,428 binding could reflect dopamine transporter downregulation and reduced dopamine uptake, in order to maximize the synaptic efficacy of the remaining neurotransmitter during a dopamine-deficient state (see below). However, upon cocaine rechallenge, the diminished capacity for dopamine reuptake in nucleus accumbens and striatum might be associated with an even greater potentiation of dopaminergic neurotransmission, which could underlie the development of behavioral sensitization to the locomotor and stereotypic effects of repeated cocaine (Post and Rose, 1976; Robinson and Becker, 1986). Dopamine transporter concentration could be regulated directly by cocaine itself, or, as suggested above, indirectly by synaptic dopamine levels, and might reflect a compensatory homeostatic process. In support of this hypothesis, chronic treatment with deprenyl, a monoamine oxidase inhibitor that increases synaptic dopamine levels, is associated with increased binding of ^{3}H-mazindol to the dopamine transporter in mouse striatum (Wiener et al., 1989), and treatment with reserpine (which depletes intracellular dopamine stores) reduces *in vivo* binding of ^{18}F-GBR 13119 in mouse striatum (Kilbourn et al., 1992; but see Maurice et al., 1991). Although in our study there was no statistically significant correlation between whole nucleus accumbens and striatal tissue dopamine levels and dopamine transporter concentration ($p > 0.1$; $-0.023 < r < -0.242$), a significant correlation might have been apparent had we been able to measure dopamine levels within the individual striatal and nucleus accumbens subdivisions. Finally, the observations in the rodent of similar cocaine-induced changes in the brain serotonin transporter (increased concentration while on cocaine, Cunningham et al., 1992; decreased concentration during cocaine withdrawal, Tung et al.,

1990; but see Benmansour et al., 1992) suggest that compensatory homeostatic changes in transporter concentration might also be a feature of nondopaminergic monoamine neurotransmitter transporters.

It has generally been assumed that the dopamine transporter proteins are static presynaptic markers and have thus been used as indices of monoaminergic neuronal integrity in positron emission tomography studies of human brain disorders such as Parkinson's disease (Brooks et al., 1990), as well as in brain of experimental animals following insult with dopaminergic neurotoxins (Richfield, 1991). However, to function as a neuronal marker for quantitative purposes, the transporter should not be influenced by transient alterations in endogenous neurotransmitter levels, or by chronic treatment with agonists or antagonists. Such adaptive processes have been demonstrated for many types of neurotransmitter receptors, including dopamine receptors (Burt et al., 1976; List and Seeman, 1979). The results of our investigation suggest that the concentration of the dopamine transporter might, in fact, change as a function of drug exposure or differences in dopamine neurotransmitter levels, and therefore, that dopamine transporter concentration might not provide an accurate estimation of nerve terminal integrity.

Brain monoamines in the on-cocaine group

Although cocaine inhibits reuptake of dopamine (Heikkila et al., 1975; Kennedy and Hanbauer, 1983), noradrenaline (Koe, 1976; Schlicker et al., 1983), and serotonin (Ross and Renyi, 1969; Reith et al., 1985), the major neurochemical changes in this investigation were primarily restricted to the levels of dopamine and its metabolite HVA, suggesting a specificity of the effect of chronic cocaine exposure on the dopaminergic neurotransmitter system.

Rats killed on the last day of chronic, unlimited access self-administration of cocaine had slightly reduced striatal and nucleus accumbens dopamine levels and increased levels of HVA in striatum, medial prefrontal cortex, and olfactory bulbs. This resulted in significantly elevated HVA/dopamine "turnover" ratio in the striatum (+134%, $p < 0.001$) with a trend toward an increased ratio in the nucleus accumbens (+68%, $p = 0.11$). These data, suggesting increased activity of the dopamine neurons innervating the striatum and nucleus accumbens during chronic cocaine self-administration, are consistent with the electrophysiological observations of increased firing rate and number of spontaneously active dopamine cells immediately after the discontinuation of repeated cocaine treatment (Henry et al., 1989; Ackerman and White, 1990). This could be explained by a subsensitivity of inhibitory impulse-regulating somatodendritic autoreceptors, as evidenced by increased electrophysiological responses to iontophoretic administration of dopamine following repeated cocaine administration (Henry et al., 1989). Such neuronal adaptation could also be related both to the biochemical finding of markedly increased (fivefold) basal extracellular dopamine levels in nucleus accumbens of cocaine self-administering rats (Weiss et al., 1992) and to the behavioral observation of sensitization to the motor stimulant actions of cocaine (Post and Rose, 1976; Robinson and Becker, 1986). Such behavioral parameters were not examined in the present study. To the extent that HVA and DOPAC represent extra- and intraneuronal metabolites of dopamine, respectively (Roffler-Tarlov et al., 1971), our observation of a significantly reduced DOPAC:HVA ratio in striatum (−38%, $p < 0.01$) and nucleus accumbens (−31%, $p < 0.01$) of rats killed while still on cocaine suggests a shift to more extraneuronal dopamine breakdown. Our finding of elevated levels of striatal HVA but unaltered DOPAC is consistent with similar extracellular striatal HVA and DOPAC levels previously reported in a microdialysis study following continuous cocaine exposure via osmotic minipumps (Inada et al., 1992) and also could be explained by enhanced extraneuronal catabolism of dopamine during cocaine-induced elevation of synaptic dopamine levels during blockade of dopamine reuptake (Pettit and Justice, 1991).

Brain monoamines in cocaine-withdrawn group

Our observation of reduced nucleus accumbens dopamine (and transporter levels) 3 weeks after cocaine withdrawal indicates that the influence of sustained, unlimited access to self-administration of cocaine on the mesolimbic dopamine system is long lasting. These data differ from the results of other studies which report no change (Kalivas et al., 1988; Kleven et al., 1988), transient increases (Roy et al., 1978), or more sustained increases (Hurd et al., 1990) in terminal field tissue dopamine levels following repeated injection of cocaine, but are consistent with other investigations showing reduced nucleus accumbens dopamine levels following 3 d withdrawal from limited (2 hr/d) cocaine self-administration (Bozarth, 1989) and tyrosine hydroxylase immunoreactivity following withdrawal from repeated cocaine administration (Trulson et al., 1987). Our observation that nucleus accumbens dopamine was reduced to a greater extent following a period of cocaine abstinence than on the last day of cocaine exposure is consistent with the "rebound" decrease in basal extracellular dopamine levels during cocaine withdrawal (Parsons et al., 1991; Robertson et al., 1991; Rosetti et al., 1992; Weiss et al., 1992; but see Kalivas and Duffy, 1993) and also with decreased dopamine synthesis (nucleus accumbens, Kalivas et al., 1988; striatum, Trulson and Ulissey, 1987) and decreased release (nucleus accumbens, Kalivas and Duffy, 1988) following a 10 d or longer abstinence period. The subnormal dopamine levels [with a statistically nonsignificant trend for lowered HVA (−20%) and DOPAC (−38%) levels] could be explained by a long-term reduction in dopamine synthesis, as demonstrated following repeated experimenter-delivered cocaine (Kalivas et al., 1988; Brock et al., 1990; Beitner-Johnson and Nestler, 1991). Although most evidence now suggests that cocaine, unlike amphetamine, is not neurotoxic to dopamine neuron endings (Ryan et al., 1988; Seiden and Kleven, 1988; Bennett et al., 1993a,b), the alternate possibility, which has not been addressed in this study, of actual degeneration of the dopaminergic nerve terminals cannot be ruled out. No other significant changes in dopamine levels occurred in any other brain area examined (including dopamine terminal areas of striatum, medial prefrontal cortex, hippocampus, and amygdala), suggesting that the mesolimbic dopamine neurons innervating the nucleus accumbens might be more susceptible to the long-term effects of cocaine than other dopaminergic neurons. An analysis of the individual dopamine levels (Fig. 5) revealed that of the eight animals withdrawn from cocaine, five had nucleus accumbens dopamine levels below the lower limit of the control range, but three had values distinctly within the range of the controls. This indicates that under the conditions of our unlimited access cocaine self-administration paradigm, a nucleus accumbens dopamine reduction is not a constant feature of cocaine withdrawal. Although the variability in dopamine levels is likely due to different patterns of self-administration, we could not identify, by correlational analysis, the normal versus low nucleus

accumbens dopamine levels on the basis of mean daily or total cocaine intake, or by the pattern of self-administration.

Functional significance

The data presented provide support for the "dopamine depletion hypothesis of cocaine addiction" (Dackis and Gold, 1985), which suggests that the drug craving and depression/anhedonia experienced during cocaine withdrawal (Gawin and Ellinwood, 1990) are consequent to reduced brain dopaminergic function. With the dopaminergic system in a compromised state during cocaine withdrawal, the downregulation of the dopamine transporter could reflect a homeostatic response whereby reduced capacity for dopamine reuptake would maintain neurotransmission at more normal levels. Thus, our data suggest that the dopamine transporter might actively participate in modulating the behavioral consequences of chronic cocaine. In addition, this compensatory mechanism might underlie the development of behavioral sensitization (Post and Rose, 1976; Robinson and Becker, 1986) or cocaine-induced paranoid psychosis (Post, 1975), which have been reported following repeated cocaine administration or prolonged binges, since reduced dopamine reuptake upon cocaine challenge would further intensify dopaminergic neurotransmission. Finally, the different anatomical localization of ^{3}H-GBR 12,935 and ^{3}H-WIN 35,428 binding sites in normal rat brain suggests that dopaminergic cells originating from substantia nigra and the ventral tegmental area might express different forms of the dopamine transporter with different relative proportions of GBR 12,935- and WIN 35,428-sensitive sites; these might confer differential regional sensitivities and behavioral responses to the acute and chronic effects of cocaine. Further investigation, using the unlimited access self-administration model, comparing the temporal pattern of the brain dopamine neurotransmitter system changes and the type and extent of dysphoric behavior observed during cocaine abstinence, is likely to help clarify the critical neurochemical correlates of the cocaine withdrawal syndrome.

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