*Pharmacology Biochemistry and Behavior*, Vol. 46, pp. 973–984, 1993
Printed in the U.S.A. 

0091-3057/93 $6.00 + .00


RAPID COMMUNICATION

# In Vivo Electrochemical Studies of Gradient Effects of (SC) Cocaine on Dopamine and Serotonin Release in Dorsal Striatum of Conscious Rats

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Received 9 July 1993

BRODERICK, P. A. *In vivo electrochemical studies of gradient effects of (SC) cocaine on dopamine and serotonin release in dorsal striatum of conscious rats.* PHARMACOL BIOCHEM BEHAV **46**(4) 973–984, 1993.—Cocaine (20 mg/kg) was administered subcutaneously (SC) to conscious male Sprague–Dawley rats after exploration in a novel chamber. (SC) cocaine was studied for its influence on in vivo dopamine (DA) and serotonin (5-HT) release in dorsal striatum (STr), with a further study of an anterior–posterior dorsal subdivision in a range of ±400 μm. Semiderivative voltammetry, a circuit for in vivo electrochemical biotechnologies, was used in combination with a stearate microelectrode to concurrently detect in separate electrochemical signals the electroactive species for DA and 5-HT in dorsal STr. The temporal resolution for detection was in the order of seconds. Concomitantly, cocaine-induced psychostimulant behaviors were studied with infrared photobeam detection. Psychostimulant behaviors classically thought to depend on DA—that is, hyperactivity (increased locomotor activity or ambulations), rearing, and finally stereotypy (fine movements of grooming and head bob)—and a 5-HT-ergic behavior, central ambulations, were monitored. The results showed that (SC) cocaine significantly ($p < 0.0001$) increased DA release in dorsal STr, whereas the overall effect of (SC) cocaine on 5-HT release was a significant increase ($p < 0.0001$) followed by an overall small (13%) but statistically significant decrease ($p < 0.05$). A dramatic cocaine-induced gradient effect on 5-HT release was seen in anterior–posterior dorsal STr, where 5-HT release was significantly ($p < 0.0001$) increased throughout the entire time period of study. Classically DA-dependent behaviors were significantly and positively correlated with increased DA release in dorsal STr and anterior–posterior dorsal STr ($p < 0.001$) in the 4-h period of study. However, 5-HT release after cocaine in the anterior–posterior dorsal STr was significantly and positively correlated with the classically DA-dependent behaviors as well ($p < 0.001$), implicating a role for 5-HT in the effectuation of cocaine-induced psychostimulant behavior. Generally, the 5-HT-ergic response to cocaine was enhanced before the DA-ergic response. Therefore, the data show that 5-HT as well as DA plays a role in the underlying mechanism of action of cocaine in dorsal STr. The data suggest that 5-HT may play a compensatory or adaptive role in the modulation of cocaine-induced nigrostriatal DA-ergic regulation.

Cocaine Dopamine Serotonin $A_9$ nerve terminals Dorsal striatum (STr) Nigrostriatum
In vivo electrochemistry (voltammetry) Psychostimulant behavior Agoraphobia (thigmotaxis)
Stearate microelectrode

CONTEMPORARY thinking on cocaine's effects in basal ganglia posits the ($A_9$) dorsal striatum (dorsal STr) (caudate putamen) as the substrate mediating behavioral stereotypy and the ($A_{10}$) ventral STr (nucleus accumbens [NAcc] and tuberculum olfactorium [TO]) as the substrates mediating brain reward. It is thought that the biogenic amine dopamine (DA) communicates information in $A_9$ nerve terminals (dorsal STr) to execute motoric responses and in $A_{10}$ nerve terminals (ventral STr) to modulate motivational responses [cf. (30,92)]. Further support for the hypothesis comes from data showing

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that cocaine altered levels of tyrosine hydroxylase immunoreactivity and phosphorylation states in $A_{10}$ but not in $A_9$ brain areas (7).

However, there is an abundance of evidence showing that cocaine binds to the DA transporter in dorsal STr to inhibit DA reuptake from the synapse (28,49,50,72,74,84,96) and that the potency of cocaine binding to the DA transporter in dorsal STr highly correlates with its ability to cause self-administration of the drug (77,89). A recent report shows that the DA reuptake blockers, cocaine and GBR 12909, and the DA releasers, amfonelic acid (1,59) and methylphenidate (43), affected DA reuptake in both dorsal and ventral STr in the same way (51). Although some reports disagree (25,60) it has also been shown that [$^3$H] cocaine binding is the same in both dorsal and ventral STr (13). Therefore, dorsal STr remains important for cocaine-induced brain reward.

Dorsal STr is believed to be important in sensitization processes, and suggestions have been made that it is DA in STr that underlies the repetitive stereotypic behavior which occurs preclinically and clinically in subjects on chronic (long-term) cocaine (37,52,54,55,102). It has been suggested that chronic cocaine may work in STr through a subsensitivity of release-modulating terminal DA autoreceptors (100).

Moreover, the neurotransmitter serotonin (5-HT) has been shown to be involved in cocaine's presynaptic mechanism of action concurrently with DA (18–22). Specifically in dorsal STr, $NA^+$-independent [$^3$H] cocaine binding has been shown to be associated with 5-HT-ergic nerve terminals (73,75,76), and cocaine was shown to bind to a 5-HT transporter, although in STr, cocaine binding to the DA transporter was predominant (14). Serotonin in dorsal STr was found to play a prominent role in cocaine-induced activation of transcription factor genes (c fos and zif 268) (9). In dorsal raphe (DR), the cell bodies for 5-HT neurons, cocaine blocked cell firing rates (27). Considering the endogenous interplay between DA and 5-HT in the catecholamine and indoleamine pathways (33, 66,81) and the reported demonstration of 5-HT-ergic projections from DR to striatum (5), it seems reasonable that a role for 5-HT in cocaine's action is unfolding.

The purpose of the present studies was to address the effects of (SC) cocaine concurrently on synaptic concentrations of DA and 5-HT in dorsal STr in the conscious rat, while simultaneously monitoring cocaine-induced psychostimulant behaviors including stereotypy. In vivo electrochemistry, specifically semiderivative electroanalysis, was used to detect DA and 5-HT within seconds and on line over a period of 4 h after (SC) cocaine administration. Administration of (SC) cocaine has been found to produce cocaine levels comparable to human values (90).

The electroactive species for DA and 5-HT studied by in vivo electrochemistry is believed to be primarily reflective of presynaptic release mechanisms because the electroactive species is continuously provided to the microelectrode surface in vivo, a process strongly dependent on active depolarization of neurons. Since the enhanced effects of cocaine on DA neurotransmission in dorsal STr have been shown to be calcium-dependent (45,65), tetrodotoxin (TTX)-dependent (63), and gamma-butyrolactone (γ-BL) (i.e., impulse flow)-dependent (24), it is highly likely that synaptic concentrations of DA and 5-HT in STr can be a result of presynaptic release mechanisms. Cocaine's effects on DA neurons have long been and are currently thought to originate from a releasable vesicular storage pool (45,83). An interpretation which includes presynaptic reuptake inhibitory mechanisms, however, is not ruled out because mechanisms that alter the release of DA further alter cocaine-induced reuptake inhibition of DA with and without cocaine (101).

## METHODS

### *In Vivo Electrochemistry*

*General description.* Current is the essence of the in vivo electrochemical measurement. The electrochemical working (indicator) microelectrodes do not sense membrane potentials; they pass small, finite currents because molecules close to the microelectrode surface undergo oxidation and/or reduction. Electrochemical measurements are called faradaic because the amount of the oxidative/reductive species detected at the microelectrode surface is calculated by Faraday's Law, which shows that a direct proportionality exists between the charge and the mass of a chemical (2). The proportionality between charge and mass is described by the Cottrell equation, in which the amount of neurotransmitter detected is dependent on the size of the electrode. Importantly, electrocatalytic interactions between DA and ascorbic acid (AA) are also dependent on the size of the electrode. For example, such reactions have been reported to occur with macroelectrodes in vitro (35), but more recent reports show that these interactions are not found in vivo when a microelectrode is used (10,98).

*Semiderivative electroanalysis.* Basically, the in vivo electrochemical studies described here are based on a semiderivative circuit, which is a linear circuit modified with a ladder network of resistors and capacitors (64) acting to produce sharper resolution between peaks. A potential (in mV) is applied between a stearate microelectrode and a Ag/AgCl reference microelectrode; the latter maintains a stable potential at relative zero. A stainless steel auxiliary microelectrode additionally serves to maintain a stable starting potential. Current is formed at the working microelectrode from the reduction of the electroactive species for DA and 5-HT (Patent #4, 883,057).

The coulombic efficiency of the stearate microelectrode for

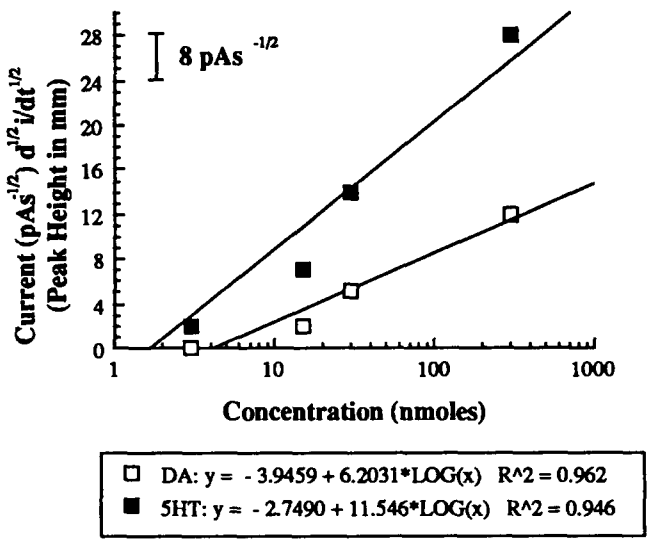


FIG. 1. Calibration curve: A logarithmic regression analysis showing the response of the stearate microelectrode to nmol amounts of DA and 5-HT. Detection limits as low as 5 nmol and 1 nmol, respectively, are currently possible with this biotechnology (standard errors are virtually zero or too low to be seen in the graph).

DA and 5-HT is shown in Fig. 1. The coulombic responses of the stearate microelectrode to both DA and 5-HT in vitro in saline phosphate buffer pH 7.4 are linear; at high concentrations, the stearate microelectrode is 2–3× more sensitive to 5-HT than to DA. The lowest detection limits currently possible with this biotechnology are 5 nmol for DA and 1 nmol for 5-HT. A change in the applied potential is accompanied by a current called a background or charging current. Charging current is a current pulse which flows through the capacitance double layer ($C_{dl}$) of the working microelectrode that allows potential to accumulate at the surface of the microelectrode. This is a necessary requirement to get to the point where faradaic electron transfer can begin. Since charging current is proportional to electrode surface area, microelectrodes have the advantage of decreasing its effects. Also, potentials are applied from −0.2V to further minimize effects of charging current.

*Microelectrodes.* The microelectrode paste mixture consisted of U.S.P. ultra superior purity carbon (1.5 g) (Ultra-Carbon Corp., Bay City, MI), extra heavy Nujol (1.24 cc) (Plough, Memphis) and +99% stearic acid (100 mg) (Sigma, St. Louis). The stearate microelectrode (surface diameter = 200 μm) was fabricated by pulling the Teflon coat of stainless steel wire (Medwire Corp., Mt. Vernon, NY) 500 μm over the edge to form a microcavity inside the Teflon well. The detailed methodology for microelectrode construction and the synthesis of the paste mixture is published by this laboratory (16,17). The microelectrode specifications are different from those published by others (11).

*Medium exchange technique.* A medium-exchange technique was performed on each microelectrode in vitro before surgical insertion and implantation of the microelectrode in vivo. This procedure consists of performing a selective preconcentration of the analytes DA and 5-HT onto the microelectrode surface in saline phosphate buffer (0.01 M) pH 7.4 in a closed semidifferential circuit scanning from −0.2 V to +0.4 V at 2 nA/V for three to five exposures with a 2-min cell deposition. This method achieves optimum and stable preconcentration of analytes and improves the selectivity, the efficacy, and the sensitivity of the microelectrode for analytes in an electrolyte environment.

*Pre- and postcalibration procedures.* Working microelectrodes were precalibrated in vitro in a fresh, deaerated saline (0.16 M) phosphate buffer ($PO_4$) solution pH 7.4 (0.01 M) containing aliquots of nmol solutions of DA and 5-HT (DA [purity, 99%]: Sigma, and 5-HT [purity, 99%]: Aldrich, Milwaukee). The buffer was deareated with prepurified nitrogen gas ($N_2$) extra dry grade 5 psi (T.W. Smith Co., Brooklyn, NY). $N_2$ gas was perfused through the $PO_4$ buffer, which was regulated by a variable area flowmeter (Purgemaster, Fisher Scientific, Fadem, NJ). The variable area flowmeter was calibrated at 100 cc/min. Deaeration took place for one minute before each cell activation period and the subsequent voltammetric scan from $E_i$ began. Working microelectrodes were also postcalibrated in vitro in a freshly deoxygenated $PO_4$ buffer solution pH 7.4 (0.01 M) made exactly as the precalibration buffer, after each study was completed. Peak area of each DA and 5-HT signal was calculated by multiplying the peak height (mm) of each electrochemical signal by the width (mm) of each electrochemical signal at one half the peak height (mm).

*Interpretation of electrochemical signals.* Dopamine and 5-HT were detected with a stearate microelectrode at peak potentials of +0.14 ± 0.015 V and +0.29 ± 0.015 V, respectively. Dopamine and 5-HT were detected within 10–15 s and 10–13 s, respectively, and appear sequentially in two separate waveforms on both in vivo and in vitro voltammograms. Dopamine was the first signal and 5-HT was the second signal to be detected in the time course of the applied potential ($E_{app}$). Even at high concentrations, the anionic metabolite of DA, 3,4-dihydroxyphenylacetic acid (DOPAC), was not detected. At high concentrations, however, ascorbic acid (AA), the cofactor in the DA biosynthetic pathway, was detectable, but at 0.050 V, a different peak potential ($E_{ap}$) than that for DA or 5-HT. The neurotransmitter 5-HT was detected within a 10–13-s time period, at an $E_{ap}$ of +0.290 ± 0.015 V. Neither the metabolite of 5-HT (i.e., 5-hydroxyindoleacetic acid [5-HIAA]) nor uric acid (UA), which is a constituent of the brain with similar electroactive $E_{ap}$ properties to those of 5-HT, was part of the 5-HT signal. Increasing the amounts of DA and 5-HT did not significantly affect either signal. Each voltammogram is completed within 60 s in vivo. Initial potential ($E_{app}$) was −0.200 V. The scan rate was 10 mV/s. Potentials were applied to the working microelectrode with respect to a Ag/AgCl microelectrode (with 0.16 M saline) by a CV37 detector-potentiostat (BAS, West Lafayette, IN). The CV37 was electrically connected to a Minigard Surge Suppressor (Jefferson Electric, Magnetek, NY), which was then connected to an isolated electrical ground. There was a 6-min interval between the completion of one voltammogram and the 2-min cell activation for the next voltammogram. Nonfaradaic charging current was eliminated in the first 20 s of each scan. A semiderivative voltammogram of DA and 5-HT detection in dorsal STr in the behaving, conscious rat is shown in Fig. 2.

*Animals*

The studies were done in conscious, male, virus free [cf. (18)] Sprague-Dawley rats (Charles River, Kingston, NY) (weight range 370 to 476 g at the time of the in vivo electro-

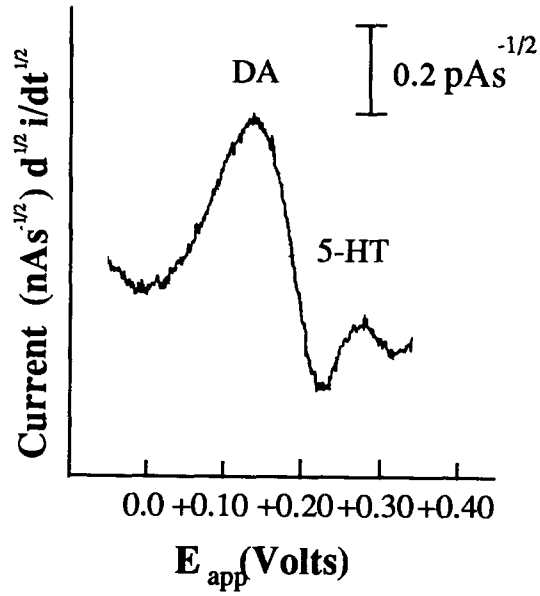


FIG. 2. A semiderivative voltammogram showing in vivo detection of dopamine (DA) and serotonin (5-HT) in dorsal STr in the conscious and behaving rat. The peak potential for DA is +0.140 ± 0.015 V, and that for 5-HT is 0.290 ± 0.015 V. (See text for description of interpretation of electrochemical signals.)

chemical and behavioral studies). The animals were fed Purina Rat Chow and water ad lib and were group housed before surgery and individually housed after surgery. A 12-h dark/light cycle was maintained both during the housing of the laboratory rats and throughout the experimental studies.

*Surgery*

Pentobarbital Na (50 mg/kg IP) with attendant boosters was the general anesthetic employed to produce surgical anesthesia. Rats were tested for an absence of corneal, pinnal, and leg flexion responses throughout the surgery. Body temperature was continuously monitored with a rectal probe and thermometer (Fisher Scientific) and was maintained at 37 ± 0.5°C with an aquamatic K module heating pad (American Hospital Supply, Edison, NJ). Rats were stereotaxically (Kopf Stereotaxic, Tujunga, CA) implanted with stearate working microelectrodes in dorsal STr (AP = +2.6, ML = +2.5, DV = −4.0) (67). Anterior-posterior subdivisions of dorsal STr were also delineated to assess the resultant bidirectional 5-HT-ergic responses to cocaine. Posterior dorsal striatal placements were AP = +2.2 and anterodorsal STr placements were AP = +2.8 and AP = +3.0 (Figure 3 shows a schematic representation of sensor [working microelectrode] placements). Ag/AgCl reference microelectrodes and stainless steel auxiliary microelectrodes were placed in contact with the cortex. The working (indicator), reference, and auxiliary microelectrodes were held in place with dental acrylic (Kadon Cavity Liner, Caulk, Becker Parkin Dental Supply Co. Inc., NY). Animals recovered in an appropriately bedded Plexiglas chamber (dimensions 12″ × 12″ × 18″). Physiological saline was administered (IP) postsurgery. In vivo voltammetric studies were begun 10–15 days later. Stable in vivo electrochemical signals for DA and 5-HT were evident before cocaine (20 mg/kg SC) was administered. Cocaine (Sigma) was dissolved in doubly distilled water and solutions were made fresh on the day of each study. The behavioral chamber was novel for each animal, but the animal was habituated before (SC) cocaine injection. Each animal was treated with care throughout the surgical procedures and the studies.

*Psychostimulant Behaviors*

Behaviors were studied by infrared photobeam detection. The system used was a modified version of the Activity Pattern Monitor (APM) (San Diego Instruments, San Diego). The system permits simultaneous monitoring of several different responses both as they occur in space and in time with a resolution of 0.1 s in time and 1.5 in. in space (36). The behavioral chamber is made of Plexiglas with an outer layer of copper. A 16 × 16 array of infrared photobeams directed by a computer are used to define the xy position of the animal

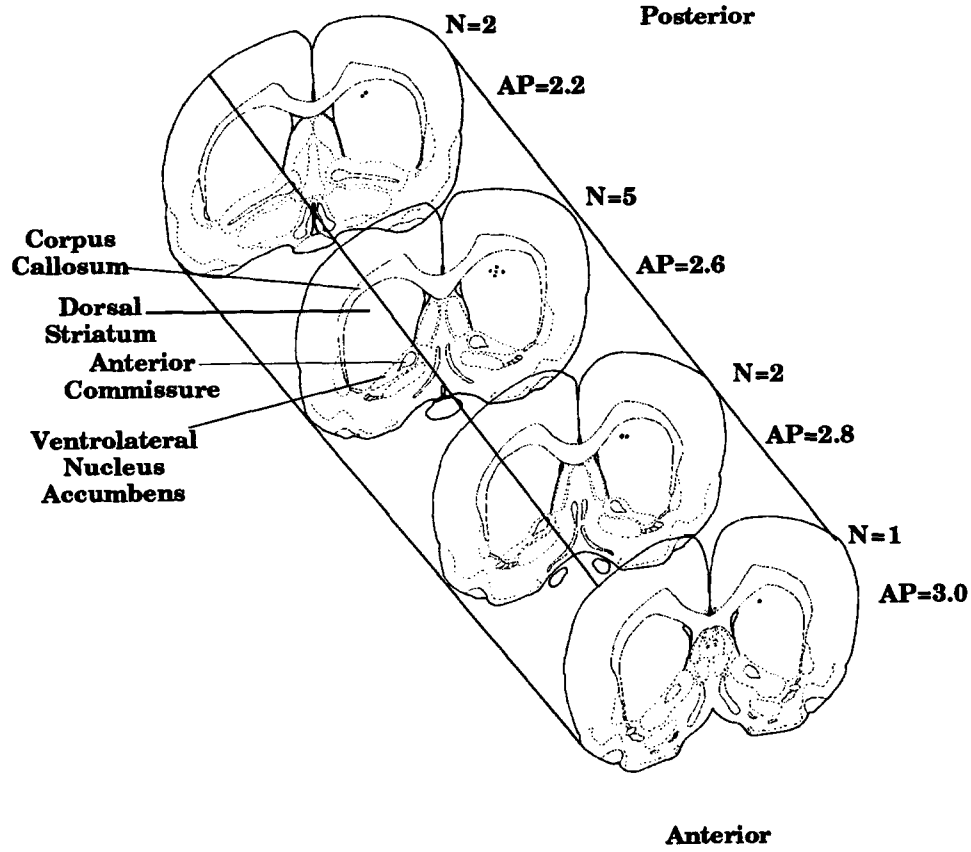


FIG. 3. A schematic diagram of dorsal striatal placements of the microelectrode sensor (AP = +2.6 ± 400 μm sections anterior and posterior to AP = +2.6). Adapted by permission from Pellegrino, L. J.; Pellegrino, A. S.; Cushman, A. J. A stereotaxic atlas of the rat brain. New York: Plenum Press; 1979:17–21.

within a 1.5-in. resolution. When an xy position is calculated, 1 of 16 equally sized sectors and 1 of 9 unequally sized regions are used for more descriptive measures—for example, of entries spent in the center of the chamber (a reliable measure of agoraphobia [fear of open spaces]). Every 100 ms, the computer samples the status of all the photobeams and circuits in the cage. If any change has occurred from the previously stored reading for the cage, the current status of all beams then is stored together. The data are then stored on disk files and reduced to variances such as 1) rearings, 2) corner entries, 3) ambulations, 4) fine movements, 5) central ambulations, and 6) peripheral ambulations.

Thus, multiple concurrent measures of the animal's activity were simultaneously assayed. The specific activities of each animal assayed were the "classically DA-dependent" behaviors: 1) ambulations (locomotor activity or running ["running" is forward locomotion interacting with the maintenance of a horizontal position of the head, without lateral turning (94)]), 2) rearing behavior [maximal upward vertical movement of the head involving recruitment of the body, without any forward or lateral movement (94)], 3) fine movements (combined stereotypic movements of head bob, sniffing, and grooming), and 4) a 5-HT-ergic behavior, central ambulations [locomotor activity in the central part of the chamber which indicates movements away from the walls; central ambulatory behavior is called agoraphobic (thigmotactic) inhibition and indicates reduced fear on the part of the animal (36)].

### *Confirmation of Microelectrode Placement*

Following the completion of the study, the prosthetic acrylic cap was removed from the skull while the animal was under Na pentobarbital anesthesia. The working (indicator) microelectrode was postcalibrated for in vitro electrochemical detection of DA and 5-HT. Placement of working microelectrodes in STr was confirmed by the potassium ferrocyanide in 10% formalin, blue dot method with transcardial perfusion (80 ml saline). The electrical specifications for deposition of the blue dot in STr was 50 $\mu$A current in a 30-s time period. Virtually no damage to brain tissue occurred.

### *Statistics*

Each component of the psychostimulant behavior monitored, in addition to the DA and 5-HT release assayed, was tested for statistically significant differences due to cocaine by analysis of variance (ANOVA) (Statview, Brain Power Inc., Calabasas, CA). ANOVAs were followed by post hoc tests, Fisher PLSD (least square differences), and the Scheffe $F$ test (Statview, Brain Power Inc.), to determine hourly statistically significant differences. Statistically significant differences were also calculated on the individual time course data points by 95% confidence limits (95% CL), setting the $p$ value at $p < 0.05$. Changes in DA and 5-HT values after (SC) cocaine treatment vis-à-vis untreated (same animal) controls are presented as percent change, whereas behavioral data are presented as frequency or number of behavioral events. Control is represented as 100%.

Cocaine-induced effects on the electrochemical signals for DA and 5-HT in dorsal STr and consequent psychostimulant behavior were studied for statistically significant correlative value by the Pearson product-moment coefficient of correlation ($r$) polynomial regression analyses (Statview, Brain Power Inc.); corresponding $z$ values were derived from the table of $z$ for values of $r$ from 0.0 to 1.0.

## RESULTS

Figure 4 shows the concurrent effect of cocaine (20 mg/kg SC) on synaptic concentrations of DA and 5-HT release in dorsal STr. The in vivo electrochemical signal for DA was significantly increased, ANOVA $F(4, 23) = 123.891$, $p < 0.0001$, $N = 8$. Post hoc analysis further showed that there were statistically significant differences from baseline in the second, third, and fourth hours of the 4-h study (Fisher PLSD = 12.623, Scheffe $F = 15.06$, 51.481, and 70.46 for second, third, and fourth hours, respectively). DA release was significantly increased 113% ($p < 0.05$, 95% CL) over baseline within 30 min and was maximally increased 200–205% ($p < 0.05$, 95% CL) over baseline (baseline = 100%) within about 160 min after cocaine administration. A plateau effect at 200–205% above baseline was seen in the last 80 min of the study.

Figure 4 also shows the effect of (SC) cocaine on 5-HT release in dorsal STr. Cocaine (20 mg/kg SC) significantly increased and then decreased the in vivo electrochemical signal for 5-HT, ANOVA $F(4, 23) = 32.464$, $p < 0.0001$, $N = 10$, when one considers the overall 4-h (SC) cocaine effect. (SC) cocaine produced a significantly increased 5-HT release (111%, $p < 0.05$, 95% CL) 30 min after cocaine injection. The effect remained significant for 50 min and was coincident with the cocaine-induced DA effect at the 30- and 40-min marks. In the first hour, the increase in 5-HT release occurred in a bell-shaped curve, returned to baseline, and then showed an overall slight but statistically significant ($p < 0.05$) decrease (maximally by 13%) and finally showed a trend toward baseline (baseline = 100%). Post hoc analysis showed that these small but statistically significant differences from baseline occurred in the third and fourth hours of the four hours tested (Fisher PLSD = 3.675, Scheffe $F = 7.824$ and 7.461 for third and fourth hours, respectively). The increased 5-HT effect preceded the increased DA effect by 30 min.

Figure 5 shows the gradient effects of (SC) cocaine on concurrently, yet separately detected DA and 5-HT release when specific anterior dorsal and posterior dorsal differences in dorsal STr were delineated.

Figure 5A shows that cocaine (20 mg/kg SC) simultane-

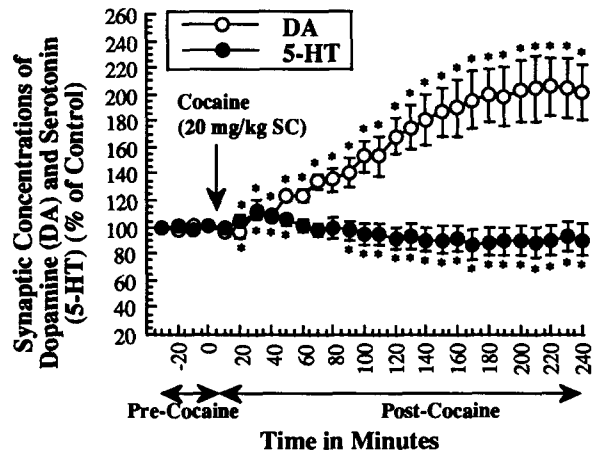


FIG. 4. The effects of cocaine (20 mg/kg SC) on concurrent DA and 5-HT in dorsal striatum in conscious and behaving male Sprague-Dawley rats ($N = 8$–10). *$p < 0.05$ (95% confidence limits) (cf. text for ANOVA statistics).

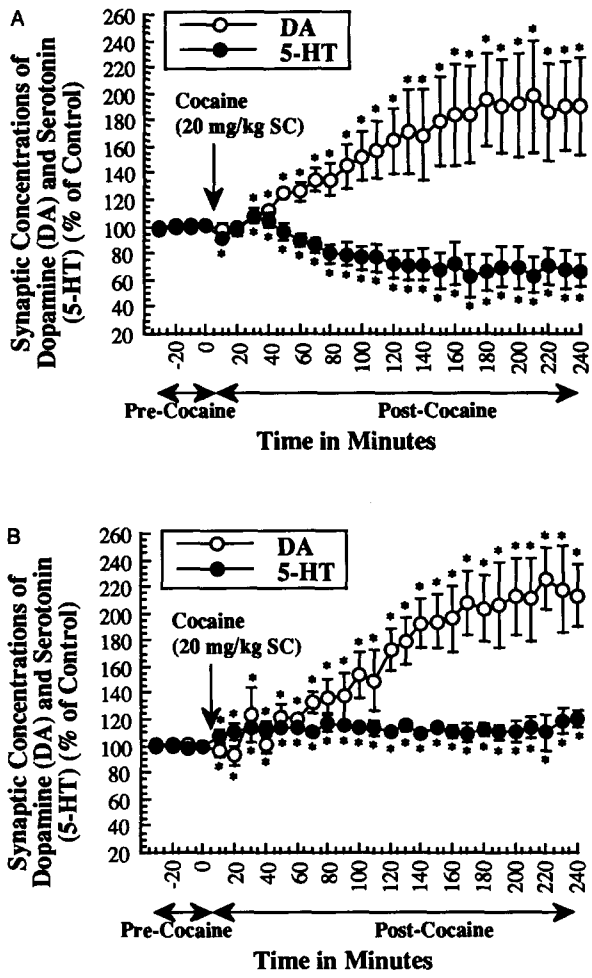


FIG. 5. The effects of cocaine (20 mg/kg SC) on concurrent DA and 5-HT in (A) dorsal striatum (at AP = 2.6) and (B) anterior-posterior dorsal striatum (at AP = 2.2, 2.8, and 3.0) in conscious and behaving male Sprague-Dawley rats ($N$ = 4–5). *$p < 0.05$ (95% confidence limits) (cf. text for ANOVA statistics).

ously and significantly increased the in vivo electrochemical signal for DA, ANOVA $F(4, 23) = 98.959$, $p < 0.0001$, $N = 4$, in dorsal STr (AP = +2.6). Post hoc analysis further showed that there were statistically significant differences from baseline in the second, third, and fourth hours of study (Fisher PLSD = 12.482, Scheffe $F = 15.829$, 43.911, and 56.606 for second, third, and fourth hours, respectively). DA release was significantly increased to 108% ($p < 0.05$, 95% CL) above baseline within 30 min, was increased 126% above baseline within 60 min ($p < 0.05$, 95% CL), and was maximally increased to 195–197% ($p < 0.05$, 95% CL) above baseline within 180–200 min after cocaine administration, after which a plateau effect was seen (baseline = 100%).

Figure 5A also shows the effect of (SC) cocaine on 5-HT release in dorsal STr (AP = +2.6). Cocaine (20 mg/kg SC) significantly increased and then decreased the in vivo electrochemical signal for 5-HT, ANOVA $F(4, 23) = 64.273$, $p < 0.0001$, $N = 5$, in dorsal STr (AP = +2.6). Serotonin was significantly increased to 108% above baseline values within 30 min after injection. The cocaine-induced 5-HT release was coincident with concurrently detected DA release 20, 30, and 40 min after cocaine administration. Post hoc analysis further showed that there were statistically significant decreases from baseline in the second, third, and fourth hours of study (Fisher PLSD = 6.049, Scheffe $F = 13.54$, 29.232, and 30.361 for second, third, and fourth hours, respectively). In fact, 5-HT was significantly decreased maximally by 37% ($p < 0.05$, 95% CL) below baseline within 170 min after cocaine administration, at which time a plateau effect was seen.

Figure 5B shows that cocaine (20 mg/kg SC) simultaneously and significantly increased the in vivo electrochemical signal for DA, ANOVA $F(4, 23) = 112.568$, $p < 0.0001$, $N = 4$, in the anterior-posterior dorsal STr (AP = 2.2, AP = +2.8 and AP = +3.0). Post hoc analysis further showed that there were statistically significant differences from baseline in the second, third, and fourth hours of study (Fisher PLSD = 14.832, Scheffe $F = 10.602$, 44.033, and 63.29 for second, third, and fourth hours, respectively). DA was significantly increased to 124% ($p < 0.05$, 95% CL) over baseline within 30 min and was maximally increased to 225% ($p < 0.05$, 95% CL) over baseline within 220 min after cocaine administration (baseline = 100%). Increased DA release after cocaine resembled more a stepwise progression than a plateau-like effect. Interestingly, the cocaine-induced effect on DA release in the anterior and posterior portions of dorsal STr was significantly greater than those effects seen in dorsal STr per se in the third and fourth hours of study, ANOVA $F(1, 10) > 7.149$, $p < 0.0233$, $N = 4$. Thus (SC) cocaine shows a gradient effect on DA neurons in dorsal STr.

Figure 5B also shows the 5-HT-ergic effect of (SC) cocaine. Cocaine (20 mg/kg SC) significantly increased 5-HT release in the anterior-posterior dorsal STr (AP = 2.2, AP = +2.8 and AP = +3.0), ANOVA $F(4, 23) = 15.915$, $p < 0.0001$, $N = 5$. Post hoc analysis further revealed that there were statistically significant differences from baseline in each of the four hours of study (Fisher PLSD = 4.017, Scheffe $F = 9.79$, 12.0, 9.044, and 12.535 for first, second, third, and fourth hours, respectively). 5-HT was significantly increased to 107% ($p < 0.05$, 95% CL) over baseline within 10 min and was maximally increased to 120% ($p < 0.05$, 95% CL) over baseline within 30 min after cocaine administration. Increased 5-HT release remained 120% above baseline for the 2-h period of study (baseline = 100%). Coincident DA and 5-HT points in the time course occurred at 10, 30, 40, 50, and 60 min after (SC) cocaine. Cocaine-induced effects on 5-HT release were more prominent than its DA effects immediately and generally up to 40 min after cocaine injection. Moreover, the cocaine-induced effect on 5-HT release in dorsal STr ($\pm 400$ $\mu$m) was significantly greater than those 5-HT-ergic effects which occurred in dorsal STr per se, ANOVA $F(1, 10) > 19.416$, $p < 0.0013$, $N = 5$. Thus, cocaine produces a gradient effect on 5-HT neurons in dorsal STr as well. The gradient effect was more dramatic for 5-HT than for DA. Enhanced 5-HT release produced by cocaine preceded enhanced DA release by 30 min.

Cocaine's colocalized and gradient effects on DA and 5-HT release in anterior-posterior dorsal STr were most highly and positively correlated in the first hour of study (Pearson product: $r_{(a)} > 0.801$, $z_{(f)} > 1.0986$, $p < 0.001$). Interestingly, the cocaine-induced 5-HT response in anterior-posterior dorsal STr was significantly and positively correlated with each of the cocaine-induced gradient effects on DA neurons (Pearson product: $r_{(a)} > 0.603$, $z_{(f)} > 0.6931$, $p < 0.05$) throughout the 4-h period of study.

Figure 6 shows the effect of cocaine (20 mg/kg SC) on

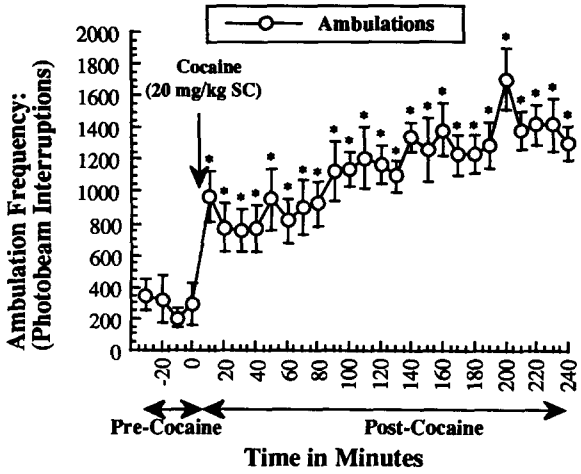


FIG. 6. The effect of cocaine (20 mg/kg SC) on the frequency of ambulations (locomotor activity or running behavior) in the same group of conscious and behaving male Sprague-Dawley rats in which neurochemistry was assayed in vivo. *$p < 0.05$ (95% confidence limits) (cf. text for ANOVA statistics). Baseline photobeam interruptions were 291 ± 57.36, mean ± SE (habituated behavior before cocaine injection).

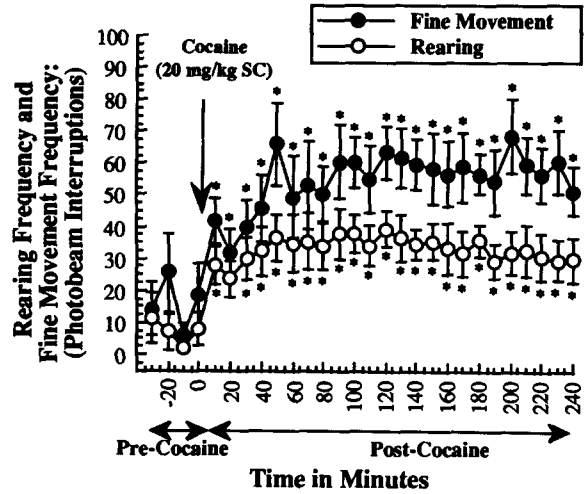


FIG. 7. The effect of cocaine (20 mg/kg SC) on frequency of rearing behavior and frequency of stereotypic fine movement behavior (head bob, sniff, and groom) in the same group of conscious and behaving male Sprague-Dawley rats in which neurochemistry was assayed in vivo. *$p < 0.05$ (95% confidence limits) (cf. text for ANOVA statistics). Baseline photobeam interruptions were 7 ± 2.69 and 16 ± 4.51, respectively, mean ± SE (habituated behavior before cocaine injection).

*ambulations* (locomotor activity) described in terms of frequency or number of events in the same group of animals in which the neurochemistry of cocaine was assayed. Cocaine (20 mg/kg SC) significantly increased ambulation frequency, ANOVA $F(4, 23) = 67.683$, $p < 0.0001$, $N = 10$. Post hoc analysis further showed that there were statistically significant differences from baseline in each of the four hours of study tested (Fisher PLSD = 154.076, Scheffe $F = 13.444, 27.867, 41.867$, and 56.761 for first, second, third, and fourth hours, respectively). Ambulation frequency significantly increased from a baseline of 291 ± 57.36 photobeam interruptions within 10 min to 965 ± 153.88 ($p < 0.05$, 95% CL) and maximally increased to 1698 ± 192.03 ($p < 0.05$, 95% CL) within 200 min after cocaine administration. Ambulation frequency tended toward baseline at the end of the 4-h period of study but was still significantly above baseline.

Figure 7 shows the concurrent effect of cocaine (20 mg/kg SC) on *rearing* frequency. Cocaine (20 mg/kg SC) significantly increased rearing frequency, ANOVA $F(4, 23) = 68.026$, $p < 0.0001$, $N = 10$. Furthermore, post hoc analysis showed that there were statistically significant differences from baseline in all four hours of study (Fisher PLSD = 4.024, Scheffe $F = 37.775, 57.022, 49.643$, and 36.573 for first, second, third, and fourth hours, respectively). Rearing frequency was significantly increased to 28 ± 5.88 photobeam interruptions ($p < 0.05$, 95% CL) from a baseline of 7 ± 2.69 within 10 min and maximally increased to 39 ± 5.93 ($p < 0.05$, 95% CL) within 120 min after cocaine administration. The parameter rearing showed a general tendency toward reaching baseline at the end of the 4-h period of study but was still significantly above baseline.

Figure 7 also shows the concurrent effect of cocaine (20 mg/kg SC) on *fine movement* (stereotypy) frequency. Cocaine (20 mg/kg SC) significantly increased fine movement frequency, ANOVA $F(4, 23) = 28.211$, $p < 0.0001$, $N = 10$. Post hoc analysis further showed that there were statistically significant differences from baseline in all four hours of study (Fisher PLSD = 9.593, Scheffe $F = 10.155, 19.375, 20.923$, and 20.612 for first, second, third, and fourth hours, respectively). Frequency of fine movements was significantly increased to 42 ± 7.22 photobeam interruptions ($p < 0.05$, 95% CL) from a baseline of 16 ± 4.51 within 10 min and was maximally increased to 66 ± 12.72 ($p < 0.05$, 95% CL) within 50 min after cocaine administration. The frequency of fine movements also showed a trend toward returning to baseline but had not reached baseline at the end of the 4-h period of study.

Figure 8 shows the concurrent effect of cocaine (20 mg/kg SC) on the frequency (number) of *central ambulations*. Cocaine (20 mg/kg SC) significantly increased the frequency of central ambulations, ANOVA $F(4, 23) = 39.245$, $p < 0.0001$, $N = 10$. Post hoc analysis further showed that there were statistically significant differences from baseline in the second, third, and fourth hours of study (Fisher PLSD = 4.318, Scheffe $F = 6.487, 14.053$, and 29.019 for the second, third, and fourth hours, respectively). Frequency of central ambulations was significantly increased to 6 ± 2.37 photobeam interruptions ($p < 0.05$, 95% CL) from a baseline of 1 ± 0.69 within 10 min and maximally increased to 32 ± 8.80 ($p < 0.05$, 95% CL) within 200 min after cocaine administration. The parameter, central ambulations, inclined toward baseline but had not yet reached baseline at the end of the 4-h period of study.

Increased DA release in dorsal STr and anterior-posterior dorsal STr was significantly and positively correlated with the increased classically DA-dependent behaviors (Pearson product: $r_{(a)} > 0.824$, $z_{(f)} > 1.1568$, $p < 0.001$) and with the 5-HT-ergic behavior, central ambulations (Pearson product: $r_{(a)} > 0.912$, $z > 1.539$, $p < 0.001$) for the 4-h period of study. Among the cocaine-induced 5-HT-ergic gradients, cocaine-enhanced release of 5-HT in anterior-posterior dorsal STr was most highly correlated with classically DA-dependent behav-

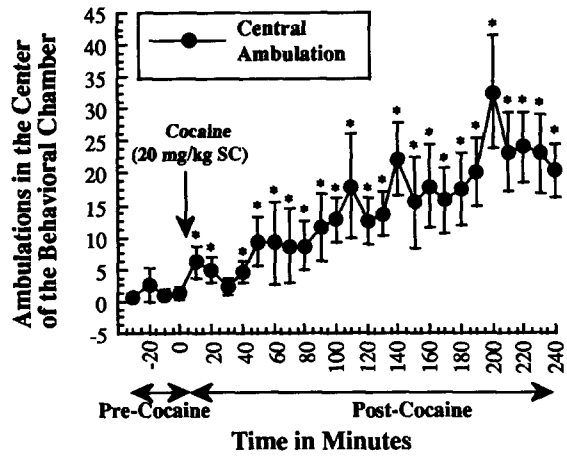


FIG. 8. The effect of cocaine (20 mg/kg SC) on frequency of central ambulation behavior (agoraphobic inhibition) in the same group of conscious Sprague–Dawley rats in which neurochemistry was assayed in vivo. *$p < 0.05$ (95% confidence limits) (cf. text for ANOVA statistics). Baseline photobeam interruptions were 1 ± 0.69, mean ± SE (habituated behavior before cocaine injection).

iors (Pearson product: $r_{(a)} > 0.825$, $z_{(f)} > 1.1881$, $p < 0.001$) in the first hour of study.

## DISCUSSION

The present findings show that (SC) cocaine administration produced a significant enhancement of DA release in dorsal STr of freely moving and behaving male Sprague–Dawley rats. The results are consistent with many other reports of increased extracellular DA in dorsal STr after cocaine administration using the dialysis technique (4,24,26,31,37,44,45, 47,63,65,79) and using radioimmunoassay postmortem (87). These previous reports concern either the acute or chronic administration of cocaine. One article reports that a day-to-day assay of a daily continuous infusion of cocaine produced equivocal results on extracellular concentrations of DA in STr, which was reportedly due to the inherent limitations of the dialysis assay (79). In the present studies, enhanced DA release in dorsal STr was detected after cocaine administration, concomitantly with infrared photobeam detection of cocaine-induced psychostimulant behavior which has been called a psychostimulant model of reinforcement (99). Taken together with other studies which show that extracellular concentrations of DA in STr are increased during acute cocaine self-administration (46), the present data further support a role for enhanced presynaptic DA-ergic function in dorsal STr in the putative underlying mechanism of action of brain reward.

When considering the present report, in context with others (noted above), it is of interest, mechanistically, that DA release, reuptake inhibition, and/or overflow are significantly increased in rat dorsal STr after cocaine, independently of route of administration. There is a dependence on the route of administration of cocaine though, under the altered physiological conditions of chloral hydrate anesthesia (15,20). Another important consideration concerning the mechanism of action of cocaine derives from recent data showing that medial forebrain bundle (MFB) stimulation, dependent on efferent projections from the substantia nigra (SN) (23), produced enhanced extracellular concentrations of DA in caudal dialysates (58). Since other data show that SN per se did not respond to the presence of direct administration of cocaine by releasing DA (45), perhaps a strionigral feedback DA circuit (56) is operative in cocaine's action.

Although there have been many reports of increased extracellular concentrations of—and release of—DA in STr after cocaine, only one previous article by Manley et al. (58) reports an assessment of what happens to the release and/or overflow (including release and reuptake inhibition) of the neurotransmitter serotonin (5-HT) in dorsal STr after cocaine. Actually, the reported original purpose of the Manley et al. studies was to assess in dorsal STr whether or not MFB activation would have stimulating effects on caudate dialysate DA and 5-HT, and cocaine was used appropriately in its very well-known role as reuptake inhibitor of both DA and 5-HT neurons (42,80). The results showed, as did the present results, that extracellular concentrations of 5-HT were enhanced by cocaine in anterior STr. It is noteworthy though that the Manley et al. studies were not able to assay endogenous or basal 5-HT in the absence of cocaine. Also, in the latter studies, it is likely that the 4-mm-length dialysis probe was drawing from extracellular fluid in the central portion of the rostral-most anterior caudate putamen complex, whereas in the present studies, the 200-$\mu$m electrochemical sensor was detecting changes in basal vis-à-vis cocaine-induced 5-HT release in the caudal-most anterior portion of dorsal STr.

The experimental design used in the Manley et al. studies may well be critical to the understanding of the interrelationship between DA and 5-HT and the interaction of these biogenic amines in the presence of cocaine. These studies used the known single-spike and bursting cell firing characteristics of DA neurons (39,40) and the known slow frequency, single-spike, nonbursting characteristics of 5-HT neurons (3,82) to show that extracellular concentrations of DA responded to both the single-spike and bursting pattern modes of DA neurons, whereas 5-HT responded only to single-spike, regular interval stimulation. The results are relevant to the present results because it is the bursting cell firing patterns of DA neurons that are known to enhance active DA release mechanisms presynaptically, either by electrical coupling of DA neurons or by switching of DA neurons from one single-spike mode to another (34,38). Interestingly, even in the presence of reuptake blockers like cocaine, these bursting cell firing patterns of DA neurons are known to cause DA release. For this reason, the interburst interval is thought to determine the quantity of released DA after cocaine (58). Taken together with the present findings, one might consider that the evenly spaced, single-spike characteristics of 5-HT neurons could well play a role in the modulation of DA neurons during DA interburst intervals. An emerging body of evidence in the literature speaks of a hypothesis which circumscribes an integral interrelationship between DA and 5-HT in STr (8,12,57,71, 88,91,93). Since exogenous stimuli seem generally necessary to activate a DA–5-HT interplay within STr, a putative role for cocaine, then, could be the provision of such an external stimulus. Morphologically differentiated synaptic contacts do exist in the cellular microenvironment of the hyperinnervated 5-HT varicosities in STr, which result from DA depletion (29).

The STr is not a homogeneous black box, but rather a remarkably heterogeneous neuroanatomical substrate with gradient zones of variable DA (7,41,53,62,70,97) and 5-HT innervation (6,68,71,91,93,95) which provide a functional compartmentalization within the STr. Immunocytochemical-electron microscopic studies have shown a DA immunoreactive compartmentalization in dorsal STr (97), and a clear gra-

dient of the $D_2$ receptor subtype localization was observed along the rostro-caudal axis of STr (32). A rostro-caudal gradient for both biogenic amines DA and 5-HT was found, showing that DA was highest in rostral STr and 5-HT was highest in caudal STr (6,95). However, whereas previous studies describe larger neuroanatomical areas, our studies seem to involve a micromanagement between DA and 5-HT in more neuroanatomically discrete areas in dorsal STr.

Our new findings show that cocaine's colocalized effects on dorsal striatal DA and 5-HT release were ones of enhancement. Due to the sensitive temporal and spatial resolution of in vivo electrochemical techniques, we were able to uncover both a DA-ergic and a 5-HT-ergic gradient, 200–400 $\mu$m anterior and posterior to dorsal STr vis-à-vis dorsal STr at the level of AP = 2.6. The important DA-ergic gradient effects consisted of a greater cocaine-induced DA-release in anterior-posterior dorsal STr vis-à-vis dorsal STr. The important 5-HT-ergic gradient differences consisted of a significantly increased 5-HT release in anterior and posterior portions of dorsal STr which remained for the entire period of study, whereas the cocaine-induced increase in 5-HT release in dorsal STr (AP = +2.6) was short-acting and followed by a decrease in 5-HT release. Previous data showing that (SC) cocaine decreased 5-HT release in the ventral striatal substrate (vlNAcc) at AP = +2.6 (18) may lend an explanatory note. Perhaps significant then is the specific positioning of the sensor in dorsal STr directly above vlNAcc, where tyrosine hydroxylase (TH) and 5-HT immunoreactive neurons have been shown to converge (69). The present data are generally consistent with current concepts that 5-HT can exert profound effects on DA neurons, dependent on the variability of neuronal innervation. The present data reflect support for this hypothesis even when the DA–5-HT interplay is induced by cocaine. The gradient effects of cocaine on DA and 5-HT release seen in the present results further suggest that the role for 5-HT in cocaine's mechanism of action may consist of a very precise modulation, perhaps even a replacement role on a functional level.

Sherrington has described the motor individual as being driven by both the exterior and the interior world (85), and it is generally agreed that the basal ganglia adequately describe the inner world. Basal ganglia consist of the ventral mesencephalon (SN and VTA) and DA-ergic projections to the dorsal (body of caudate-putamen) and ventral (anterior-ventral caudate, NAcc and TO) STr. It is the function of the basal ganglia to subserve sensory motor integration, and selective depletion of DA impairs an animal's ability to integrate sensory input with motor input (48). Although a chemical heterogeneity contributes mechanistically to the DA-ergic function of basal ganglia (53,61,68,86), the present article addresses only DA and 5-HT behaviors. Expectedly, the present findings show that the cocaine-induced increase in DA release highly correlated with increased production of DA-ergic behaviors. Each of the DA-ergic psychostimulant behaviors was correlated with increased DA release, though it is noteworthy that DA in dorsal STr is known to be associated more with stereotypy than with the other behavioral components (78). Also as expected, cocaine-induced 5-HT-ergic effects in anterior-posterior dorsal STr were correlated with the 5-HT-ergic behavior, central ambulations. Unexpectedly, the anterior-posterior dorsal STr 5-HT-ergic release was highly correlated with the classically DA-dependent behaviors. These results implicate a role for 5-HT in the effectuation of cocaine-induced psychostimulant behavior. In addition, the patterns of rearing and stereotypy behaviors induced by cocaine were almost superimposable, consistent with the Teitelbaum definition of rearing [a simple extension of stereotypic head movement (94)].

To summarize the results, cocaine produced concurrent general enhancements of DA and 5-HT release in dorsal STr, concomitantly with increased psychostimulant behaviors. Significant gradient effects of cocaine on DA and 5-HT release were seen, of which the anterior-posterior dorsal 5-HT-ergic gradient was most prominent. Thus, the neuroanatomical substrate, dorsal STr, remains a viable candidate in the ultimate search for the underlying mechanism of action of cocaine in reinforcement and addictive processes.

ACKNOWLEDGEMENTS

This research was supported by National Institute on Drug Abuse Award R01-04755-01, by Professional Staff Congress/CUNY Awards RF 661188, 669201, 663202, and by DHHS PHS Grant 2-S07-RR07132. The author thanks Frank T. Phelan, Frank Eng, and Eugene P. Kornak, Jr. for excellent research assistance.

## REFERENCES

1. Aceto, M. D.; Botton, I.; Martin, R.; Levitt, M.; Bentley, H. C.; Speight, P. T. Pharmacologic properties and mechanism of action of amfonelic acid. Eur. J. Pharmacol. 10:344–354; 1970.
2. Adams, R. N.; Marsden, C. A. New techniques in psychopharmacology. In: Iversen, L. L.; Iversen, S. D.; Snyder, S. H., eds. Handbook of psychopharmacology. New York: Plenum Press; 1982.
3. Aghajanian, G. K.; Foote, W. E.; Sheard, M. H. Lysergic acid diethylamide: Sensitive neuronal units in the midbrain raphe. Science 161:706–708; 1968.
4. Akimoto, K.; Hamamura, T.; Otsuki, S. Subchronic cocaine treatment enhances cocaine-induced dopamine efflux, studied by in vivo intracerebral dialysis. Brain Res. 490:339–344; 1989.
5. Azmitia, E. C.; Segal, M. An autoradiographic analysis of the differential ascending projections of the dorsal and median raphe nuclei in the rat. J. Comp. Neurol. 179:641–667; 1978.
6. Beal, M. F.; Martin, J. B. Topographical dopamine and serotonin distribution and turnover in rat striatum. Brain Res. 358: 10–15; 1985.
7. Beitner-Johnson, D.; Nestler, E. J. Morphine and cocaine exert common chronic actions on tyrosine hydroxylase in dopaminergic brain reward regions. J. Neurochem. 57:344–347; 1991.
8. Benloucif, S.; Galloway, M. P. Facilitation of dopamine release in vivo by serotonin agonists: Studies with microdialysis. Eur. J. Pharmacol. 200:1–8; 1991.
9. Bhat, R. V.; Cole, A. J.; Baraban, J. M. Role of monoamine systems in activation of zif268 by cocaine. J. Psychiat. Neurosci. 17:94–102; 1992.
10. Blaha, C. D.; Jung, M. E. Electrochemical evaluation of stearate modified graphite paste electrodes: Selective detection of dopamine maintained after exposure to brain tissue. J. Electroanal. Chem. 310:317–334; 1991.
11. Blaha, C. D.; Lane, R. F. Chemically modified electrode for in vivo monitoring of brain catecholamines. Brain Res. Bull. 10: 861–864; 1983.
12. Blandina, P.; GoldFarb, J.; Green, J. P. Activation of a 5-$HT_3$ receptor releases dopamine from rat striatal slice. Eur. J. Pharmacol. 155:349–350; 1988.
13. Boja, J. W.; Kuhar, M. J. [$^3$H] Cocaine binding and inhibition of [$^3$H] dopamine uptake is similar in both the rat striatum and nucleus accumbens. Eur. J. Pharmacol. 173:215–217; 1989.

14. Boja, J. W.; Mitchell, W. M.; Patel, A.; Kopajtic, T. A.; Carroll, F. I.; Lewin, A. H.; Abraham, P.; Kuhar, M. J. High-affinity binding of [125I]RTI-55 to dopamine and serotonin transporters in rat brain. Synapse 12:27–36; 1992.
15. Broderick, P. A. Rat striatal dopamine release mechanisms of cocaine. NIDA Res. Monogr. 75:367–370; 1986.
16. Broderick, P. A. Characterizing stearate probes in vitro for the electrochemical detection of dopamine and serotonin. Brain Res. 495:115–121; 1989.
17. Broderick, P.A. State-of-the-Art microelectrodes for in vivo voltammetry. Electroanalysis 2:241–251; 1990.
18. Broderick, P. A. Cocaine-on-line analysis of an accumbens amine neural basis for psychomotor behavior. Pharmacol. Biochem. Behav. 40:959–968; 1991.
19. Broderick, P. A. In vivo voltammetric studies on release mechanisms for cocaine with γ-butyrolactone. Pharmacol. Biochem. Behav. 40:969–975; 1991.
20. Broderick, P. A. Cocaine's colocalized effects on synaptic serotonin and dopamine in ventral tegmentum in a reinforcement paradigm. Pharmacol. Biochem. Behav. 42:889–898; 1992.
21. Broderick, P. A. Distinguishing effects of cocaine (IV) and (SC) on mesoaccumbens dopamine and serotonin release with chloral hydrate anesthesia. Pharmacol. Biochem. Behav. 43:929–937; 1992.
22. Broderick, P. A.; Kornak, E. P., Jr.; Eng, F.; Wechsler, R. Real time detection of acute (IP) cocaine-enhanced dopamine and serotonin release in ventrolateral nucleus accumbens of the behaving Norway rat. Pharmacol. Biochem. Behav. 46:715–722; 1993.
23. Bunney, B. S.; Aghajanian, G. K. The precise localisation of nigral afferents in the rat as determined by a retrograde tracing technique. Brain Res. 117:423–435; 1976.
24. Carboni, E.; Imperato, A.; Perezzani, L.; DiChiara, G. Amphetamine, cocaine, phencyclidine and nomifensine increase extracellular dopamine concentrations preferentially in the nucleus accumbens of freely moving rats. Neuroscience 28:653–661; 1989.
25. Cass, W. A.; Gerhardt, G. A.; Mayfield, R. D.; Curella, P.; Zahniser, N. R. Differences in dopamine clearance and diffusion in rat striatum and nucleus accumbens following systemic cocaine administration. J. Neurochem. 59:259–266; 1992.
26. Church, W. H.; Justice, J. B., Jr.; Byrd, L. D. Extracellular dopamine in rat striatum following uptake inhibition by cocaine nomifensine and benztropine. Eur. J. Pharmacol. 139:345–348; 1987.
27. Cunningham, K. A.; Lakoski, J. M. Electrophysiological effects of cocaine and procaine on dorsal raphe serotonin neurons. Eur. J. Pharmacol. 148:457–462; 1988.
28. Davies, H. M.; Saikali, E.; Sexton, T.; Childers, S. R. Novel 2-substituted cocaine analogs: Binding properties at dopamine transport sites in rat striatum. Eur. J. Pharmacol. 244:93–97; 1993.
29. Descarries, L.; Soghomonian, J. J.; Garcia, S.; Doucet, G.; Bruno, J. P. Ultrastructural analysis of the serotonin hyperinnervation in adult rat neostriatum following neonatal dopamine denervation with 6-hydroxydopamine. Brain Res. 569:1–13; 1992.
30. Di Chiara, G.; Morelli, M.; Acquas, E.; Carboni, E. Functions of dopamine in the extrapyramidal and limbic systems. Clues for the mechanism of drug actions. Arzneimittelforschung 42: 231–237; 1992.
31. Di Paolo, T.; Rouillard, C.; Morissette, M.; Levesque, D.; Bedard, P. J. Endocrine and neurochemical actions of cocaine. Can. J. Physiol. Pharmacol. 67:1177–1181, 1989.
32. Dubois, A.; Scatton, B. Heterogenous distribution of dopamine $D_2$ receptors within the rat striatum as revealed by autoradiography of [$^3$H] N-n-propylnorapomorphine binding sites. Neurosci Lett. 57:7–12; 1985.
33. Fibiger, H. C.; Miller, J. J. An anatomical and electrophysiological investigation of the serotonergic projection from the dorsal raphe nucleus to the substantia nigra in the rat. Neuroscience 2: 975–987; 1977.
34. Freeman, A. S.; Meltzer, L. T.; Bunney, B. S. Firing properties of substantia nigra dopaminergic neurons in freely moving rats. Life Sci. 36:1983–1994; 1985.
35. Gelbert, M. B.; Curran, D. J. Alternating current voltammetry of dopamine and ascorbic acid at carbon paste and stearic acid modified carbon paste electrodes. Anal. Chem. 58:1028–1032; 1986.
36. Geyer, M. A.; Russo, P. V.; Masten, V. L. Multivariate assessment of locomotor behavior: Pharmacological and behavioral analyses. Pharmacol. Biochem. Behav. 25:277–286; 1986.
37. Gifford, A. N.; Johnson, K. M. Effect of chronic cocaine treatment on $D_2$ receptors regulating the release of dopamine and acetylcholine in the nucleus accumbens and striatum. Pharmacol. Biochem. Behav. 41:841–846; 1992.
38. Gonon, F. G.; Buda, M. J. Regulation of dopamine release by impulse flow and by autoreceptors as studied by in vivo voltammetry in rat striatum. Neuroscience 14:765–774; 1985.
39. Grace, A. A.; Bunney, B. S. The control of firing pattern in nigral dopamine neurons: Single spike firing. J. Neurosci. 4: 2866–2876; 1984.
40. Grace, A. A.; Bunney, B. S. The control of firing pattern in nigral dopamine neurons: Burst firing. J. Neurosci. 4:2877–2890; 1984.
41. Graybiel, A. M.; Moratalla, R. Dopamine uptake sites in the striatum are distributed differentially in striosome and matrix compartments. Proc. Natl. Acad. Sci. U. S. A. 86:9020–9024; 1989.
42. Heikkila, R. E.; Orlansky, H.; Cohen, G. Studies on the distinction between uptake inhibition and release of $^3$H-dopamine in rat brain tissue slices. Biochem. Pharmacol. 24:847–852; 1975.
43. Hoffman, B. B.; Lefkowitz, R. J. Catecholamines and sympathomimetic drugs. In: Gilman, A. G.; Rall, T. W.; Nies, A. S.; Taylor, P., eds. Goodman and Gilman's pharmacological basis of therapeutics, 8th ed. Elmsford, NY: Pergamon Press; 1990: 187–220.
44. Hurd, Y. L.; Kehr, J.; Ungerstedt, U. In vivo microdialysis as a technique to monitor drug transport: Correlation of extracellular cocaine levels and dopamine overflow in the rat brain. J. Neurochem. 51:1314–1316; 1988.
45. Hurd, Y. L.; Ungerstedt, U. Cocaine: An in vivo microdialysis evaluation of its acute action on dopamine transmission in rat striatum. Synapse 3:48–54; 1989.
46. Hurd, Y. L.; Weiss, F.; Koob, G.; Ungerstedt, U. The influence of cocaine self administration on in vivo dopamine and acetylcholine neurotransmission in rat caudate putamen. Neurosci. Lett. 109:227–233; 1990.
47. Inada, T.; Polk, K.; Purser, C.; Hume, A.; Hoskins, B.; Ho, I. K.; Rockhold, R. W. Behavioral and neurochemical effects of continuous infusion of cocaine in rats. Neuropharmacology 31: 701–708; 1992.
48. Iversen, S. D. Behavioural effects of manipulation of basal ganglia neurotransmitters. CIBA Found. Symp. 107:183–200; 1984.
49. Izenwasser, S.; Cox, B. M. Inhibition of dopamine uptake by cocaine and nicotine: Tolerance to chronic treatments. Brain Res. 573:119–125; 1992.
50. Izenwasser, S.; Rosenberger, J. G.; Cox, B. M. Inhibition of [$^3$H] dopamine and [$^3$H] serotonin uptake by cocaine: Comparison between chopped tissue slices and synaptosomes. Life Sci. 50:541–547; 1992.
51. Izenwasser, S.; Werling, L. L.; Cox, B. M. Comparison of the effects of cocaine and other inhibitors of dopamine uptake in rat striatum, nucleus accumbens, olfactory tubercle, and medial prefrontal cortex. Brain Res. 520:303–309; 1990.
52. Jaffe, J. Drug addiction and drug abuse. In: Gilman, A. G.; Rall, T. W.; Nies, A. S.; Taylor, P., eds. Goodman and Gilman's pharmacological basis of therapeutics, 8th ed. Elmsford, NY: Pergamon Press; 1990:522–573.
53. Joyce, J. N. Differential response of striatal dopamine and muscarinic cholinergic receptor subtypes to the loss of dopamine. I. Effects of intranigral or intracerebroventricular 6-hydroxydopamine lesions of the mesostriatal dopamine system. Exp. Neurol. 113:261–276; 1991.

54. Kalivas, P. W.; Duffy, P.; DuMars, L. A.; Skinner, C. Behavioral and neurochemical effects of acute and daily cocaine administration in rats. J. Pharmacol. Exp. Ther. 245:485–492; 1988.
55. Kalivas, P. W.; Weber, B. Amphetamine injection into the ventral mesencephalon sensitizes rats to peripheral amphetamine and cocaine. J. Pharmacol. Exp. Ther. 245:1095–1102; 1988.
56. Kemp, J. M. The termination of strio-pallidal and strio-nigral fibres. Brain Res. 17:125–128; 1970.
57. Luthman, J.; Bolioli, B.; Tsutsumi, T.; Verhofstad, A.; Jonsson, G. Sprouting of striatal serotonin nerve terminals following selective lesions of nigro-striatal dopamine neurons in neonatal rat. Brain Res. Bull. 19:269–274; 1987.
58. Manley, L. D.; Kuczenski, R.; Segal, D. S.; Young, S. J.; Groves, P. M. Effects of frequency and pattern of medial forebrain bundle stimulation on caudate dialysate dopamine and serotonin. J. Neurochem. 58:1491–1498; 1992.
59. Miller, H. H.; Shore, P. A. Effects of amphetamine and amfonelic acid on the disposition of striatal newly synthesized dopamine. Eur. J. Pharmacol. 78:33–44; 1982.
60. Missale, C.; Castelletti, L.; Govoni, S.; Spano, P. F.; Trabucchi, M.; Hanbauer, I. Dopamine uptake is differentially regulated in rat striatum and nucleus accumbens. J. Neurochem. 45: 51–56; 1985.
61. Moghaddam, B.; Gruen, R. J.; Roth, R. H.; Bunney, B. S.; Adams, R. N. Effect of L-glutamate on the release of striatal dopamine: In vivo dialysis and electrochemical studies. Brain Res. 518:55–60; 1990.
62. Moratalla, R.; Robertson, H. A.; Graybiel, A. M. Dynamic regulation of NGFI-A(zif 268, egrl) gene expression in the striatum. J. Neurosci. 12:2609–2622; 1992.
63. Nomikos, G. G.; Damsma, G.; Wenkstern, D.; Fibiger, H. C. In vivo characterization of locally applied dopamine uptake inhibitors by striatal microdialysis. Synapse 6:106–112; 1990.
64. Oldham, K. Semi-integral electroanalysis: Analog implementation. Anal. Chem. 45:39–50; 1973.
65. Pani, L.; Kuzmin, A.; Diana, M.; DeMontis, G.; Gessa, G. L.; Rossetti, Z. L. Calcium receptor antagonists modify cocaine effects in the central nervous system differently. Eur. J. Pharmacol. 190:217–221; 1990.
66. Pazos, A.; Cortés, R.; Palacios, J. M. Quantitative autoradiographic mapping of serotonin receptors in the rat brain: II. Serotonin-2 receptors. Brain Res. 346:231–249; 1985.
67. Pellegrino, L. J.; Pellegrino, A. S.; Cushman, A. J. A stereotaxic atlas of the rat brain. New York: Plenum Press; 1979.
68. Phelix, C. F.; Jackson, T.; Broderick, P. A.; Wayner, M. J. Immunohistochemical analysis of serotonin, dopamine and GABA in dorsal and ventral striatum. Am. Physiol. Soc.; in press.
69. Phelix, C. F.; Tshoepe, L.; Broderick, P. A. Convergence of serotonin and dopamine in ventolateral nucleus accumbens: Anatomical and in vivo electrochemical analysis. Soc. Neurosci. Abstr. 19:1827; 1993.
70. Pycock, C. J. Turning behavior in animals. Neuroscience 5:461–514; 1980.
71. Radja, F.; Descarries, L.; Dewar, K. M.; Reader, T. A. Serotonin 5-$HT_1$ and 5-$HT_2$ receptors in adult rat brain after neonatal destruction of nigrostriatal dopamine neurons: A quantitative autoradiographic study. Brain Res. 606:273–285; 1993.
72. Reith, M. E.; de Costa, B.; Rice, K. C.; Jacobson, A. E. Evidence for mutually exclusive binding of cocaine, BTCP, GBR 12935, and dopamine to the dopamine transporter. Eur. J. Pharmacol. 227:417–425; 1992.
73. Reith, M. E.; Meisler, B. E.; Sershen, H.; Lajtha, A. Sodium-independent binding of [$^3$H]cocaine in mouse striatum is serotonin related. Brain Res. 342:145–148; 1985.
74. Reith, M. E.; Selmeci, G. Radiolabeling of dopamine uptake sites in mouse striatum: Comparison of binding sites for cocaine, mazindol and GBR 12935. Naunyn Schmiedebergs Arch. Pharmacol. 345:309–318; 1992.
75. Reith, M. E.; Sershen, H.; Lajtha, A. Binding sites for [$^3$H]cocaine in mouse striatum and cerebral cortex have different dissociation kinetics. J. Neurochem. 46:309–312; 1986.
76. Reith, M. E. A.; Meisler, B. E.; Sershen, H.; Lajtha, A. Structural requirements for cocaine congeners to interact with dopamine and serotonin uptake sites in mouse brain and to induce stereotyped behavior. Biochem. Pharmacol. 35:1123–1129; 1986.
77. Ritz, M. C.; Lamb, R. J.; Goldberg, S. R.; Kuhar, M. J. Cocaine receptors on dopamine transporters are related to self-administration of cocaine. Science 237:1219–1223; 1987.
78. Robbins, T. W.; Mittelman, G.; O'Brien, J.; Winn, P. The neuropsychological significance of stereotypy induced by stimulant drugs. In: Cooper, S. J.; Dourish, C. T., eds. Neurobiology of stereotyped behavior. New York: Oxford Science Publishers; 1990:25–63.
79. Robinson, T. E.; Camp, D. M. The effects of four days of continuous striatal microdialysis on indices of dopamine and serotonin neurotransmission in rats. J. Neurosci. Methods 40: 211–222; 1991.
80. Ross, S. B.; Renyi, A. L. Inhibition of the uptake of tritiated 5-hydroxytryptamine in brain tissue. Eur. J. Pharmacol. 7:270–277; 1969.
81. Saavedra, J. M. Distribution of serotonin and synthesizing enzymes in discrete areas of the brain. Fed. Proc. 36:2134–2141; 1977.
82. Sawyer, S. F.; Tepper, J. M.; Young, S. J.; Groves, P. M. Antidromic activation of dorsal raphe neurons from neostriatum: Physiological characterization and effects of technical autoreceptor activation. Brain Res. 332:15–28; 1985.
83. Scheel-Kruger, J. Behavioural and biochemical comparison of amphetamine derivatives, cocaine, benztropine and tricyclic anti-depressant drugs. Eur. J. Pharmacol. 18:63–73; 1972.
84. Schoemaker, H.; Pimoule, C.; Arbilla, S.; Scatton, B.; Javoy-Agid, F.; Langer, S. Z. Sodium dependent [$^3$H]cocaine binding associated with dopamine uptake sites in the rat striatum and human putamen decrease after dopaminergic denervation and in Parkinsons disease. Naunyn Schmiedebergs Arch. Pharmacol. 329:227–335; 1985.
85. Sherrington, C. S. Man and his nature. Cambridge, UK: Cambridge University Press; 1940.
86. Shimizu, N.; Duan, S. M.; Hori, T.; Oomura, Y. Glutamate modulates dopamine release in the striatum as measured by brain microdialysis. Brain Res. Bull. 25:99–102; 1990.
87. Sivam, S. P. Cocaine selectively increases striatonigral dynorphin levels by a dopaminergic mechanism. J. Pharmacol. Exp. Ther. 250:818–824; 1989.
88. Snyder, A. M.; Zigmond, M. J.; Lund, R. D. Sprouting of serotonergic afferents into striatum after dopamine-depleting lesions in infant rats: A retrograde transport and immunocytochemical study. J. Comp. Neurol. 245:274–281; 1986.
89. Spealman, R. D.; Madras, B. K.; Bergman, J. Effects of cocaine and related drugs in nonhuman primates. II. Stimulant effects on schedule-controlled behavior. J. Pharmacol. Exp. Ther. 251: 142–149; 1989.
90. Spear, L. P.; Frambes, N. A.; Kirstein, C. L. Fetal and maternal brain and plasma levels of cocaine and benzoylecgonine following chronic SC administration of cocaine during gestation in rats. Psychopharmacology 97:427–431; 1989.
91. Stachowiak, M. K.; Bruno, J. P.; Snyder, A. M.; Stricker, E. M.; Zigmond, M. J. Apparent sprouting of striatal serotonergic terminals after dopamine-depleting brain lesions in neonatal rats. Brain Res. 291:164–167; 1984.
92. Stein, E. A.; Fuller, S. A. Selective effects of cocaine on regional blood flow in the rat. J. Pharmacol. Exp. Ther. 262:327–334; 1992.
93. Stotz, E. H.; Triarhou, L. C.; Ghetti, B.; Simon, J. R. Serotonin content is elevated in the dopamine deficient striatum of the weaver mutant mouse. Brain Res. 606:267–272; 1993.
94. Teitelbaum, P.; Pellis, S. M.; DeVietti, T. L. Disintegration into stereotypy induced by drugs or brain damage: A microdescriptive behavioral analysis. In: Cooper, S. J.; Dourish, C. T., eds. Neurobiology of stereotyped behavior. New York: Oxford University Press; 1990:169–199.
95. Ternaux, J. P.; Héry, F.; Bourgoin, S.; Adrien, J.; Glowinski, J.; Hamon, M. The topographical distribution of serotonergic

terminals in the neostriatum of the rat and the caudate nucleus of the cat. Brain Res. 121:311–326; 1977.
96. Uhl, G. R.; Wang, J. B.; Kitayama, S. Dopamine transporter residues differentially important for dopamine transport and cocaine recognition: Implications for medications development. Paper presented at the fifty-fifth annual meeting of College on Problems of Drug Dependence Inc., Toronto; 1993.
97. Voorn, P.; Jorritsma-Byham, B.; Van Dijk, C.; Buijs, R. M. The dopaminergic innervation of the ventral striatum in the rat: A light and electron-microscopical study with antibodies against dopamine. J. Comp. Neurol. 251:84–99; 1986.
98. Wightman, R. M. Voltammetric electrodes. Anal. Chem. 53: 1125A–1134A; 1981.
99. Wise, R. A.; Bozarth, M. A. A psychomotor stimulant theory of addiction. Psychol. Rev. 94:469–492; 1987.
100. Yi, S. J.; Johnson, K. M. Chronic cocaine treatment impairs the regulation of synaptosomal $^3$H-DA release by $D_2$ autoreceptors. Pharmacol. Biochem. Behav. 36:457–461; 1990.
101. Yi, S. J.; Johnson, K. M. Effects of acute and chronic administration of cocaine on striatal uptake, compartmentalization and release of [$^3$H]dopamine. Neuropharmacology 29:475–486; 1990.
102. Zahniser, N. R.; Peris, J.; Dwoskin, L. P.; Curella, P.; Yasuda, R. P.; O'Keefe, L.; Boyson, S. J. Sensitization to cocaine in the nigrostriatal dopamine system. NIDA Res. Monogr. 88:55–77; 1988.