RAPID COMMUNICATION

Cocaine's Colocalized Effects on Synaptic Serotonin and Dopamine in Ventral Tegmentum in a Reinforcement Paradigm

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BRODERICK, P. A. *Cocaine's colocalized effects on synaptic serotonin and dopamine in ventral tegmentum in a reinforcement paradigm.* PHARMACOL BIOCHEM BEHAV **42**(4), 889-898, 1992.—The effect of subcutaneous (SC) cocaine (20 mg/kg) on synaptic concentrations of the biogenic amines, dopamine (DA), and serotonin (5-HT) in Ventral Tegmental Area, (VTA-[A_{10}]) was studied in freely moving and behaving rats (*rattus norvegicus*) with in vivo voltammetry (in vivo electrochemistry). The actual detection of the biogenic amines was on-line and within a temporal resolution of seconds. Simultaneously, the psychostimulant behavior induced by cocaine was studied by infrared photocell beam detection. The results show that cocaine concurrently and significantly increased synaptic concentrations of DA ($p < 0.0001$) and 5-HT ($p < 0.004$) in VTA. Serotonin changes were accompanied by a notable oscillatory pattern. Importantly, DA and 5-HT changes in VTA were significantly and positively correlated ($p < 0.01$). Moreover, psychostimulant behaviors induced by cocaine were significantly increased over control values ($p < 0.0001$). Psychostimulant behaviors were significantly correlated with concurrently changing synaptic concentrations of DA ($p < 0.01$) and also with 5-HT to a lesser degree. Additionally, behavioral data indicate that cocaine may exhibit an anxiolytic effect during acute administration because agoraphobic behavior, as shown by increased central ambulatory behavior, was dramatically reduced by cocaine. Summarily, the present findings show that cocaine increased synaptic concentrations of DA in VTA, an action that is correlated with cocaine-induced psychostimulant behavior. The DA-ergic effect appears to be tonically maintained. Furthermore, new findings demonstrate a colocalized, cocaine induced 5-HT-ergic effect in VTA, which keeps pace with cocaine-induced alterations in DA-ergic neurotransmission. Thus, 5-HT may be a relay or a gating mechanism for a DA reward signalling pathway for cocaine.

Cocaine   Dopamine   Serotonin   Ventral tegmentum   In vivo voltammetry (electrochemistry)
Psychostimulant behavior   Reinforcement

VARIOUS types of reinforcement capabilities for cocaine have been empirically and systematically reported during the past 25 years (4,17,24,26,37). Furthermore, commensurate neurochemical evidence points to a distinct cocaine-induced mesolimbic dopaminergic influence in nucleus accumbens (NAcc) (10-12,23,25,29,34,35,44); the nerve terminals for discrete neuronal circuits thought responsible for brain reward phenomena (38). Although early scientific papers debated the importance of a dopaminergic mediation in somatodendritic ventral tegmental area (VTA-[A_{10}]) in neurophysiological reinforcement processes (cf. (36) for review), a critical paper clearly emphasized a mediation for DA in VTA in cocaine-induced reinforcement (39). Electrophysiologically, cocaine suppresses impulse frequency in VTA somatodendrites (16). Since lesions of NAcc and rostral VTA attenuate a cocaine-induced suppression of somatodendritic cell firing (16), it is thought that both presynaptic nerve terminals and somatodendritic autoreceptors regulate cocaine's action in VTA. Indeed, a current report shows that cocaine increased the availability of dopamine (DA) in situ, in VTA in the anesthetized rat; the report supports the latter hypotheses (7). Interestingly, cocaine does not act on DA in the parallel A_9 somatodendrites in substantia nigra (22).

The involvement of serotonin (5-HT) in reinforcement pro-

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cesses has historically been empirically equivocal (cf. [43] for review). Provocatively, 5-HT per se has been shown to decrease intracranial self-stimulation (ICSS)(47). Amidst the studies on cocaine reinforcement though, two recent scientific papers have been foremost in demonstrating that cocaine acts through a 5-HT-ergic component (10,27). Early on, cocaine was shown mechanistically to produce a potent inhibition of active 5-HT reuptake processes in whole brain (40). More recent studies, however, have further shown that cocaine inhibits 5-HT reuptake (46) and decreases 5-HT synthesis in the discrete neuroanatomical substrate NAcc (18). Electrophysiologically, cocaine suppresses impulse frequency rates in vivo and in vitro in the somatodendritic cell bodies for 5-HT; that is, the dorsal raphe magnus (DR) (14,31). Taken together with in vivo and in vitro evidence that shows (SC) cocaine suppresses 5-HT release in NAcc (10,11,15), the emerging 5-HT-ergic role for cocaine is that of a centrally acting agonist.

Interestingly, although DA neurons account for almost two thirds of neurons in VTA (45), radioautographic evidence with [³H] leucine shows that the majority of ascending efferent 5-HT-ergic projections from raphe nuclei are located in VTA and are further located rostrally in medial forebrain bundle (6). Moreover, VTA contains high levels of 5-HT and tryptophan hydroxylase activity (41), and exhibits high affinity uptake for 5-HT (3). Radioautographic (32) and immunohistochemical (42) studies have further shown that VTA contains a dense network of 5-HT axonal varicosities. More recent ultrastructural evidence from light and electron microscopy after intraventricular infusion of [³H] 5-HT, shows that 5-HT neurons innervate DA-ergic neurons in VTA through demonstrated synaptic junctions (20).

The purpose of the present study was to elucidate cocaine-induced DA and 5-HT mechanisms simultaneously in VTA in the freely moving animal. The effects of cocaine on synaptic concentrations of DA and 5-HT were detected in vivo, sequentially, and on-line with in vivo electrochemistry within a psychomotor stimulant paradigm of reinforcement (48).

METHOD

In Vivo Electrochemical (Voltammetric) Biotechnology

Semidifferential (semiderivative) electroanalysis, a modification of linear scanning electrochemical techniques, was used in vivo with stearate modified graphite paste indicator (working) microelectrodes to detect DA and 5-HT usually sequentially, in two separate waveforms on the in vivo voltammogram. Dopamine was the first signal to be detected in the time course of the applied potential (Eapp), signifying a faster rate of electron transfer for this cation, vis-à-vis the cation 5-HT. Dopamine was detected in 10–15 s at a peak potential (Eap) of +0.140 V ±0.015 V. Dopamine was detected without electrode capacitance to detect at the same Eap, the metabolite of DA, 3–4 dihydroxyphenylacetic acid (DOPAC), and ascorbic acid (AA). Moreover, the neurotransmitter 5-HT was detected at an Eap of +0.290 V ±0.015 V within 10 to 13 s during each in vivo voltammogram. The electrochemical signal for 5-HT was detected, without interference at the same Eap from either the metabolite of 5-HT, that is, 5-hydroxyindoleacetic acid (5-HIAA) or uric acid (UA), which is a constituent of brain with similar electroactive properties to those of 5-HT. One voltammetric scan spanned 60 s. There was an 8.5-min interval between scans. The indicator microelectrode was activated by a 2-min cell deposition before each scan. Nonfaradaic charging current (C_{dl}) was eliminated in the first 25 s of

each scan. Using a medium exchange technique, the analytes, DA, and 5-HT were selectively preconcentrated (conditioned) onto the working microelectrode surface in saline phosphate (PO_4) buffer (0.01 M), pH 7.4, in a closed semidifferential circuit, daily for 3 days before its surgical insertion in VTA. This provides a controlled, increased sensitivity, and selectivity for DA and 5-HT detection in the electrolyte environment of the brain. A Ag/AgCl microelectrode served the reference function for the electrochemical circuit and a stainless steel microelectrode served the auxiliary function in this circuit in vitro and in vivo. Detailed procedures for the formulation of each of the microelectrodes in the three-microelectrode assembly are previously published by our laboratory (8–11). In addition, specifications for synthesizing the modified carbon paste, which actually constitutes the microelectrode detection device, are published by our laboratory (8). As shown by others (5), possible stearic-acid electrocatalytic interactions between AA and DA, or possible nucleophilic reactions between glutathione (GSH) and DA, through dopamine-o-quinone (DOQ) intermediates are insignificant in physiological neuronal tissue.

The neurotransmitters and the behavioral movements of the animal were concurrently detected in vivo in behaving and freely moving, virus-free, male, Sprague-Dawley rats (Charles River, Kingston, NY). The weight range of the animals at the time of study was 290–310 g. A priori, rats were determined free of specific viral organisms, that is, Sendai Virus, Kilham Rat Virus, Reo Virus Type 3, Sialodacryoadenitis Virus, Rat Corona Virus, Toolan's H1 Virus, Micro Plasma Pulmonis Virus, Lymphocytic Choriomeningitis Virus, Hantaan Virus and Encephalitozoon Cuniculi Virus. Although rats were group housed in Plexiglas cages (dimensions: l = 21″ × w = 11.5″ × h = 8″) before surgery, each rat was provided recovery, individually after surgery in a taller Plexiglas cage (l = 12″ × w = 12″ × h = 18″). In addition, rats were treated with physiological saline (0.5 ml) immediately and for 2 days after surgery. Indicator microelectrodes were implanted in VTA-(A_{10}), under aseptic surgery with pentobarbital Na anesthesia (50 mg/kg IP: dilute solution); booster injections of pentobarbital Na were administered once after the first 2 h of surgery in one increment of 0.1 ml and once every subsequent hour (0.05 ml) to continue to maintain the appropriate depth of anesthesia. Specifically, the surgical procedure consisted of gently inserting the indicator microelectrode into VTA using a stereotaxic guide (Kopf Stereotaxic, Tujunga, CA). The stereotaxic coordinates were from Bregma, AP = 2.8, ML = 0.9, and DV = −8.6 (33). Importantly, the Ag/AgCl reference microelectrode and the stainless steel auxiliary microelectrode were simply placed in contact with cortex; they were not actually inserted into a specific neuroanatomical substrate. The three-microelectrode assembly was held in place with dental acrylic (Kadon Cavity Liner, Caulk, Becker Parkin Dental Supply Co. Inc., NY). The total time for the surgical procedure was 3–4 h. Throughout surgery, body temperature was continuously monitored with a rectal probe attached to a thermometer (Fisher Scientific, Fadem, NJ) and was continuously maintained at 37 ± 0.5°C with an aquamatic K module heating pad (American Hospital Supply, Edison, NJ). The reason for this was twofold: (a) to prevent anesthetic-induced hypothermia and (b) to prevent electrochemical Eap shifts, since Eap is temperature-dependent. Animals were provided a period of 7 to 14 days recovery after aseptic surgical operations were performed, although animals were obviously recovered from surgical anesthesia within the same day. Purina Rat Chow and water were available ad libitum and a 12-h,

dark–light cycle was continuously maintained for the animals. Each animal was treated with a great deal of care throughout the surgical procedure and throughout the studies.

On each day of the cocaine study, each animal was placed in a novel copper-enclosed Plexiglas chamber (dimensions: l = 24″ × w = 18″ × h = 23.5″). The faradaic-Plexiglas chamber provided space for facile movement of the animals, while deterring possible electrical interference. The three-microelectrode assembly, enclosed within the animal's prosthetic acrylic cap, was connected to a CV37 BAS detector (BAS, West Lafayette, IN) by means of a mercury commutator (Brain Research Instruments, Princeton, NJ), a flexible cable, and a mating connector (BJM Electronics, Staten Island, NY). The CV37 detector-potentiostat was electrically connected to a Minigard Surge Suppressor (Jefferson Electric, Magnetek, NY), which was then connected to an isolated electrical ground. Each animal was studied for effects of cocaine (20 mg/kg SC) on its own neurotransmitters, DA, and 5-HT in VTA. Cocaine HCl (Sigma, St. Louis, MO) was dissolved in deionized, organic-free water (resistance 5–10 MΩ). Cocaine was administered (SC) after stable in vivo electrochemical signals for DA and for 5-HT were evident. The effect of (SC) cocaine HCl on DA and 5-HT neurotransmission was studied for 4 h, in keeping with its reported prolonged effects and existence in brain after (SC) administration (30).

Each implanted stearate graphite indicator microelectrode (200 μm; 500 μm [length]) was studied for its pre- and postcalibration sensitivity to DA and 5-HT in vitro in saline phosphate buffer solution, pH 7.4 (0.01 M). Specific details for pre- and postcalibration procedures have been previously published by this laboratory (10). In vivo electrochemical signals, and in vitro pre- and postcalibration electrochemical data were measured in units of peak area. Peak area 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).

Statistically significant changes between synaptic concentrations of DA and 5-HT in VTA before and after (SC) cocaine administration were determined by standard repeated measures, analysis of variance (ANOVA) (Statview, Brain Power Inc., Calabasas, CA). ANOVAs were followed by post-hoc tests, that is, Fisher PLSD (least square differences) and the Scheffe F test (Statview, Brain Power Inc., Calabasas, CA), to determine hourly statistically significant differences in neurotransmitter alterations. Statistically significant differences were also calculated on the individual time-course data points by 95% Confidence Limits (CL), setting the p value at <0.05. Changes in DA and 5-HT values after (SC) cocaine treatment vis-à-vis untreated (same animal) controls are presented as percentage of change, to minimize normal between animal variations. Control is represented as 100%. Basal concentrations of DA and 5-HT, as low as 5 nmol and 2 nmol, respectively, are currently possible with this biotechnology.

Histological placement of indicator microelectrodes in VTA were confirmed by the potassium ferrocyanide blue dot method. The precise electrical specifications for deposition of the blue dot in VTA was 50 μA, current in a 30-s time period. Virtually no damage to brain tissue occurred.

Behavior

On each day of the cocaine study, each animal was placed in the faradaic copper-enclosed Plexiglas chamber described above. The behavioral chamber was novel to each animal. Moreover, the behavioral chamber was equipped with side-by-

side double doors (dimensions: w = 15.75″ × h = 23.5″) to enable a facile injection procedure. A series of infrared photocell beams were encased in aluminum frames around the chamber's perimeter. When activated with an IBM computerized circuit box, these infrared photocell beams detected the animal's position in the behavioral chamber on an x-y axes positional basis. Thus, multiple concurrent measures of the animal's activity were simultaneously assayed. The specific activities of each animal assayed were (a) ambulations (locomotor activity), (b) central ambulations (locomotor activity in the central part of the chamber), (c) rearing behavior (wherein both forepaws of the animal are raised away from the floor), and (d) fine movements (stereotypic movements of sniffing and grooming). The status of the infrared photocell beams was sampled every 100 ms. The system is a modified version of an Activity Pattern Monitor (San Diego Instruments, San Diego, CA). Data were collected as measures of concurrent and separate activities for 10-min time periods.

Activity pattern analysis was also performed. With activity pattern analysis, the various motoric responses of the animal can be teased apart temporally and spatially. With a spatial picture of cocaine-induced psychostimulant behavior, the classical thigmotaxic (agoraphobic) response of the animal, that is, the resistance of the animal to venture into the central region of the chamber, was studied. This parameter can discern fear responses in animal behavior (19) and can be used as a measure of anxiety.

Statistically significant differences between each of the four behavioral parameters assayed, that is, (a) locomotor, (b) central locomotion, (c) rearing behavior, and (d) fine movements (stereotypy), before and after acute (SC) cocaine treatment (same animal control), were determined by standard repeated measures, ANOVA. ANOVAs were followed by post-hoc tests, that is, the Fisher PLSD and the Scheffe F-test, to determine hourly statistically significant differences. Each behavioral data point was also subjected to 95% CL, setting the significance level at $p < 0.05$. Changes in behavioral parameters are presented as frequency data, that is, the actual number of behavioral events that occurred are shown.

In vivo electrochemical and behavioral parameters were studied for statistically significant correlative value by the Pearson Product Moment Coefficient of Correlation (r) (Statview, Brain Power Inc., Calabasas, CA); corresponding z values were derived from the Table of z for values of r from 0.0 to 1.00 (2).

RESULTS

A representative semidifferential (semiderivative) voltammogram showing the in vivo detection of the electroactive species for DA and for 5-HT in VTA-(A_{10}) in the freely moving and behaving rat is shown in Fig. 1(a). Figure 1(b) shows the effect of (SC) cocaine at the 40-min mark after injection; the electroactive species for DA and 5-HT in vivo are equally and significantly increased 118% above basal values ($p < 0.05$). Figure 1(c) shows a representative in vivo voltammogram, at the 100-min mark, post (SC) cocaine administration. Dopamine was significantly increased to 126% ($p < 0.05$, 95% CL) above basal values (basal values = 100%), whereas 5-HT, at this time point, remained significantly increased, but reduced in time and amount to 111% ($p < 0.05$) above basal values (100%).

Figure 2 shows the temporal effects of cocaine on synaptic concentrations of DA in VTA in freely moving rats. Cocaine, at a dose of 20 mg/kg (SC), significantly increased synaptic
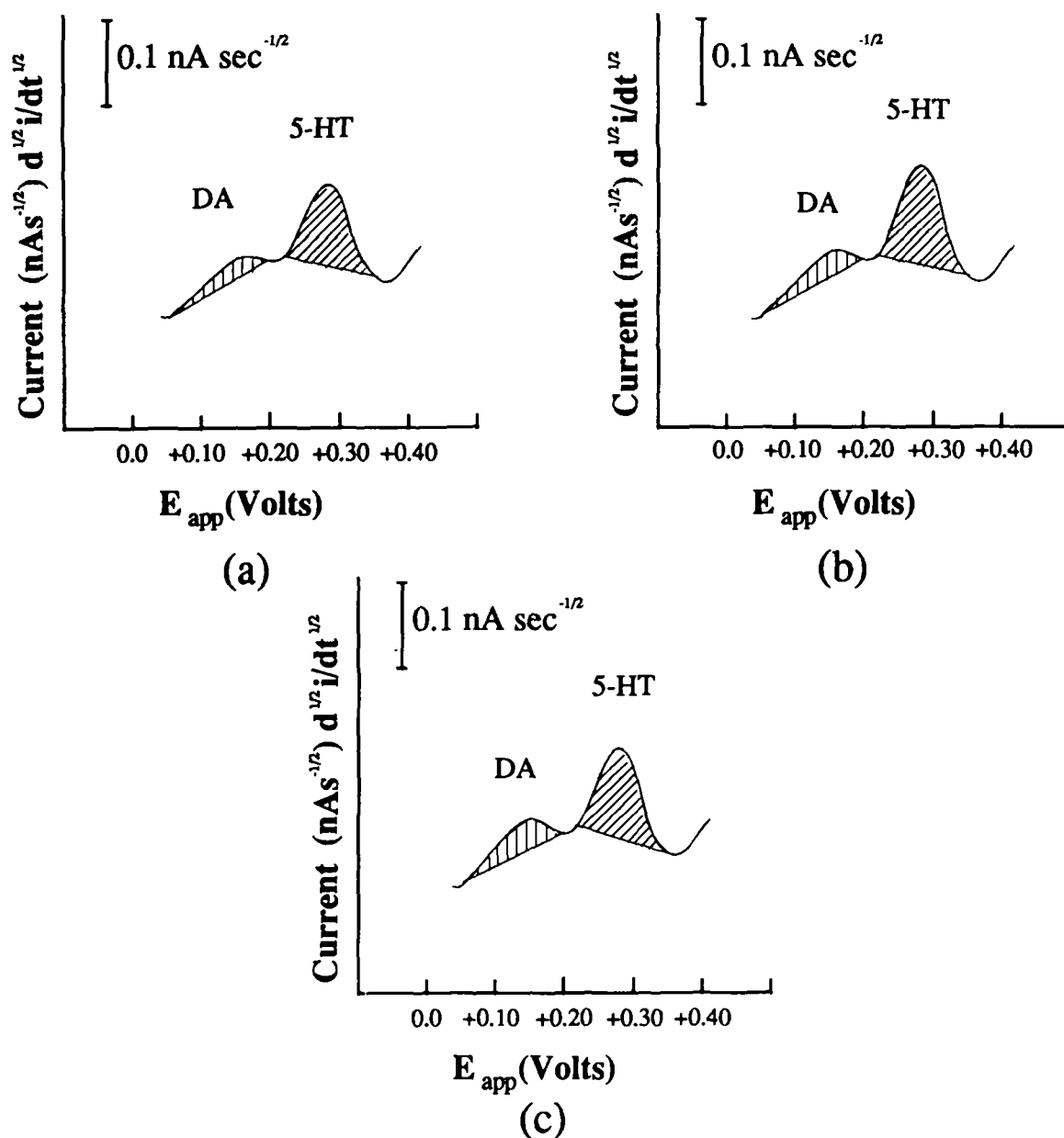
FIG. 1.(a) In vivo semidifferential (semiderivative) voltammogram, which shows the detection of the electroactive species for basal dopamine (DA) and serotonin (5-HT) in Ventral Tegmental Area (A_{10}) in freely moving and behaving, virus free, male, Sprague-Dawley rats (*rattus norvegicus*); (b) In vivo voltammogram showing the detection of the electroactive species for DA and 5-HT 40 min after (SC) cocaine administration; and (c) In vivo voltammogram that shows the detection of the electroactive species for DA and 5-HT, 1 h and 40 min after (SC) cocaine administration.

concentrations of DA over the 4-h period of testing [ANOVA: $F(4, 20) = 84.54; p < 0.0001; N = 4$]. Post-hoc analysis further revealed that statistically significant differences occurred hourly (Fisher PLSD = 6.739; Scheffe F = 9.822, 35.425, 49.733, and 63.545, respectively; $p < 0.01$; $N = 4$). Dopamine increased to 112% above basal values ($p < 0.05$, 95% CL) immediately after (SC) cocaine. Synaptic concentrations of DA did not significantly differ in the third vis-à-vis the fourth hour after cocaine, (Scheffe F = 0.845); nor, did DA synaptic concentrations differ in the second hour vis-à-vis the third hour after cocaine (Scheffe F = 1.211). Therefore, syn-

aptic concentrations of DA plateaued in a small stepwise progression in VTA in the second through the fourth hour of the study.

Figure 3 shows the temporal effects of cocaine on synaptic concentrations of 5-HT in VTA in freely moving rats. Cocaine, at a dose of 20 mg/kg SC, significantly increased synaptic concentrations of 5-HT over the 4-h period of testing [ANOVA: $F(4, 20) = 5.367; p < 0.004; N = 6$]. The temporal pattern was oscillatory. Post-hoc analysis shows that the hourly statistically significant differences in synaptic concentrations of 5-HT occurred in the initial hour after cocaine
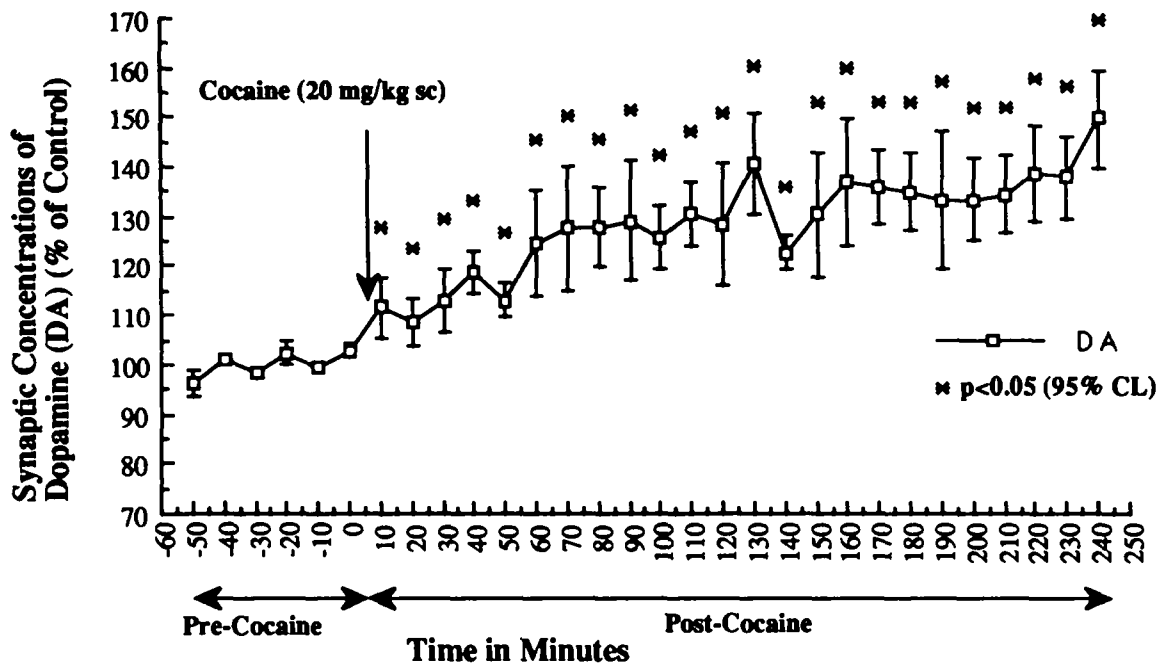
FIG. 2. The effect of cocaine (20 mg/kg SC) on synaptic concentrations of dopamine (DA) in Ventral Tegmental Area (A_{10}) in freely moving and behaving rats. Cocaine significantly increased synaptic DA [ANOVA: $F(4, 20) = 84.542$; $p < 0.0001$; $N = 4$]. The cocaine effect was statistically significant immediately after (SC) cocaine administration ($p < 0.05$, 95% CL).

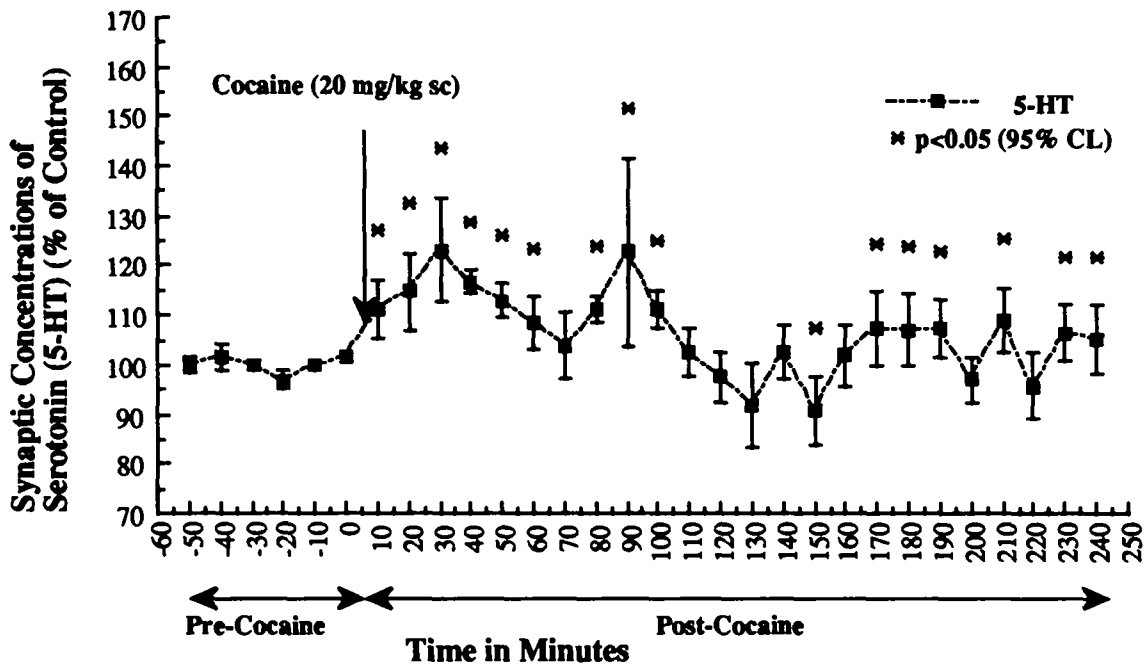FIG. 3. The effect of cocaine (20 mg/kg SC) on synaptic concentrations of serotonin (5-HT) in Ventral Tegmental Area (A_{10}) in freely moving and behaving rats. Serotonin was measured in the same in vivo voltammogram as was dopamine (DA). Cocaine significantly increased synaptic 5-HT [ANOVA: $F(4, 20) = 5.367$; $p < 0.004$; $N = 4$]. The effect was immediately significant after (SC) cocaine administration ($p < 0.05$, 95% CL).

administration (Fisher PLSD = 9.455; Scheffe F = 3.771; p < 0.02; N = 6). Moreover, significant data points appear in the second through the fourth hour after cocaine administration (p < T0.05, 95% CL). Serotonin increased to 111% above basal values (p < 0.05) immediately after (SC) cocaine. Although synaptic concentrations of 5-HT actually decreased below basal values in the beginning of the third hour after cocaine administration, later temporal data do not indicate a trend toward baseline. Dopamine and 5-HT changes in VTA were significantly and positively correlated for 3 h after (SC)

cocaine (Pearson Product: $r_{(a)}$ = 0.721; $z_{(a)}$ = 0.9076; p < 0.01: $r_{(a)}$ = 0.378; $z_{(a)}$ = 0.4001; p < 0.01: $r_{(a)}$ = 0.828; $z_{(a)}$ = 1.1881; p < 0.01, hours 1-3, respectively). There was a 10-min lag in the 5-HT component of the bimodal temporal pattern.

The psychostimulant behavioral effects of cocaine (20 mg/kg SC) are shown in Fig. 4(a) with an activity pattern plot. Additionally, Table 1 shows these activity patterns in the form of a digital readout. The activity pattern analysis, shown in Fig. 4(a), is representative of the integrated effect of cocaine

(a)

Pre-Cocaine   1st hr Post-Cocaine   2nd hr Post-Cocaine

3rd hr Post-Cocaine   4th hr Post-Cocaine

(b)

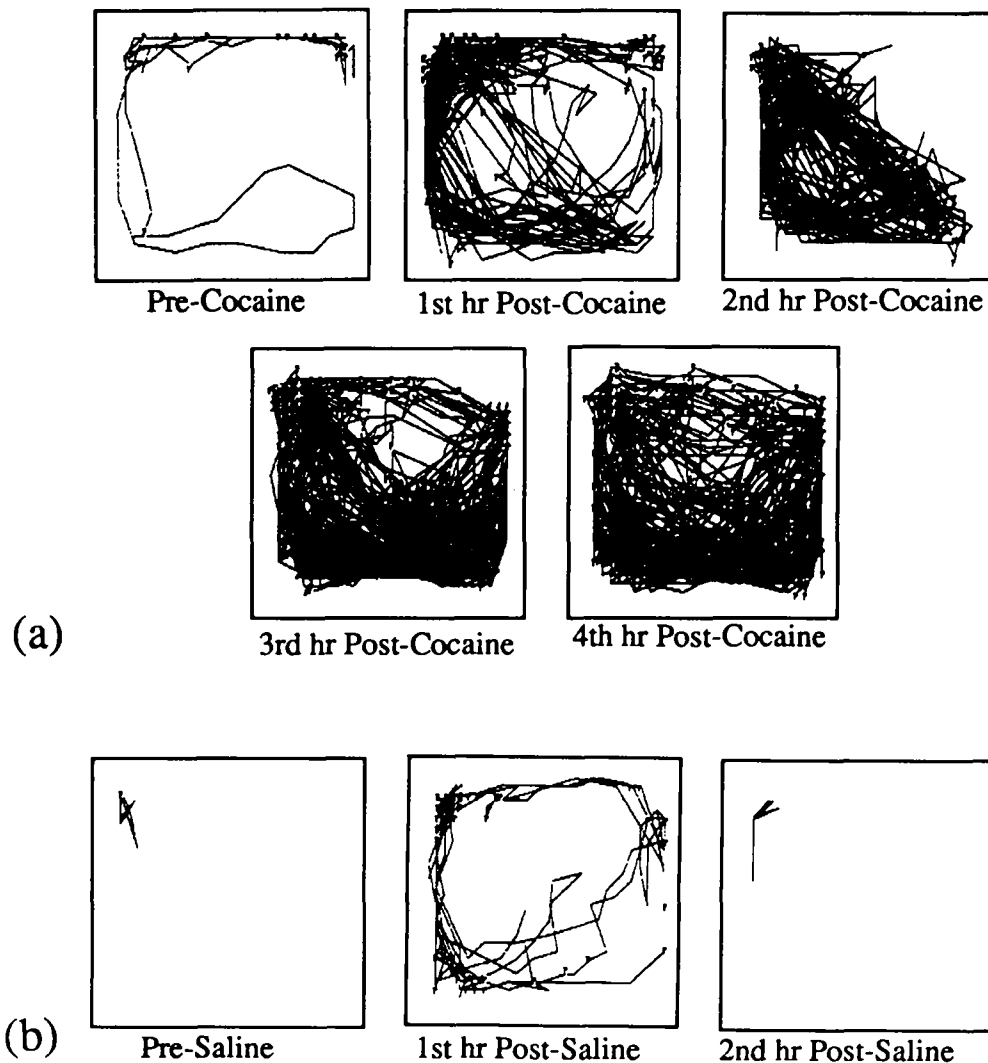Pre-Saline   1st hr Post-Saline   2nd hr Post-Saline

FIG. 4. (a) Representative activity pattern plots which show the effect of cocaine (20 mg/kg SC) on spatial and temporal patterns of animal movements after (SC) cocaine administration (20 mg/kg). Each square-shaped schematic diagram reflects the floor of the behavioral chamber. The activity markings (plots) within the chamber integrate ambulatory, central ambulatory, and rearing behaviors. Behavioral data are presented in terms of frequency. Behavioral data was detected on-line and simultaneously with dopamine (DA) and serotonin (5-HT). Cocaine significantly increased ambulatory, central ambulatory, rearing, and fine movements (sniffing and grooming) [ANOVA, $F(4, 20)$ > 17.474; p < 0.0001; N = 5]. Each of these behaviors began to significantly increase immediately after (SC) cocaine injection (p < 0.05, 95% CL). (b) Comparative and representative activity pattern plots, which shows the effect of physiological saline (1 ml/kg IP) on spatial and temporal patterns of animal movements. (Pre-cocaine and pre-saline activity plots show post exploratory behavior.)

TABLE 1
THE EFFECT OF COCAINE (20 mg/kg SC) ON BEHAVIORAL PARAMETERS: TIME COURSE

	Ambulations	Central Ambulations	Rearing Behavior	Fine Movements
Pre-cocaine	574.60 ± 267.05	1.46 ± 0.85	8.10 ± 5.48	9.02 ± 4.84
	472.20 ± 197.61	0.48 ± 0.38	2.54 ± 2.37	5.64 ± 2.31
	290.40 ± 71.69	0.10 ± 0.00	0.56 ± 0.36	11.82 ± 9.58
	127.40 ± 32.77	0.10 ± 0.00	4.56 ± 4.36	8.24 ± 4.39
	254.80 ± 155.97	0.28 ± 0.18	5.96 ± 5.76	11.05 ± 7.75
	103.20 ± 34.15	0.10 ± 0.00	0.18 ± 0.02	9.46 ± 6.79
	303.77 ± 76.51	**0.42 ± 0.22**	**3.65 ± 1.28**	**9.21 ± 0.89**
Post-cocaine 1st Hour	*979.60 ± 142.38	*4.04 ± 2.37	*14.40 ± 4.83	*28.80 ± 7.34
	*1516.20 ± 302.19	*8.60 ± 4.25	*26.60 ± 7.76	*33.40 ± 12.47
	*1801.60 ± 355.41	*7.62 ± 2.98	*16.44 ± 5.24	*25.42 ± 7.96
	*1655.60 ± 235.08	*5.82 ± 2.88	*23.00 ± 8.32	*25.00 ± 10.44
	*1617.20 ± 354.80	*11.02 ± 4.15	*33.24 ± 13.02	*30.40 ± 11.87
	*1848.40 ± 612.72	*14.40 ± 6.90	*28.80 ± 9.16	*36.80 ± 21.22
	1569.77 ± 128.08	**8.58 ± 1.52**	**23.75 ± 2.97**	**29.97 ± 1.87**
Post-cocaine 2nd Hour	*1831.80 ± 324.95	*20.80 ± 9.55	*21.60 ± 9.04	*36.60 ± 14.14
	*1487.00 ± 245.56	*15.40 ± 3.41	*25.60 ± 7.11	*31.20 ± 7.05
	*1464.40 ± 264.26	*15.20 ± 5.83	*23.20 ± 6.53	*29.80 ± 11.45
	*1648.40 ± 287.94	*20.40 ± 5.56	*22.80 ± 8.96	*37.40 ± .55
	*1720.80 ± 327.94	*12.20 ± 4.49	*21.00 ± 8.15	*31.20 ± 6.72
	*1807.00 ± 173.77	*18.60 ± 7.80	*20.00 ± 8.58	*43.20 ± 6.37
	1659.90 ± 64.07	**17.10 ± 1.38**	**22.37 ± 0.80**	**34.90 ± 2.09**
Post-cocaine 3rd Hour	*1650.80 ± 354.77	*27.80 ± 10.91	*19.80 ± 5.52	*36.40 ± 10.78
	*2217.40 ± 192.01	*32.00 ± 10.67	*31.00 ± 7.43	*60.20 ± 10.54
	*2364.00 ± 255.74	*47.42 ± 21.10	*36.00 ± 8.94	*61.40 ± 11.57
	*1879.40 ± 217.73	*31.00 ± 13.01	*27.60 ± 7.73	*47.60 ± 14.35
	*2283.80 ± 294.74	*39.60 ± 10.57	*30.40 ± 8.63	*54.60 ± 14.81
	*2012.40 ± 182.42	*33.20 ± 4.33	*28.24 ± 10.34	*45.00 ± 9.73
	2067.97 ± 110.94	**35.17 ± 2.92**	**28.84 ± 2.18**	**50.87 ± 3.94**
Post-cocaine 4th Hour	*2130.20 ± 239.36	*35.80 ± 2.18	*26.24 ± 8.02	*57.60 ± 7.53
	*1821.60 ± 212.04	*34.80 ± 10.47	*28.84 ± 11.12	*52.20 ± 10.43
	*2006.60 ± 254.27	*34.20 ± 11.85	*21.40 ± 5.23	*58.40 ± 7.12
	*1911.00 ± 322.66	*34.20 ± 9.65	*12.40 ± 3.12	*52.20 ± 12.80
	*1798.60 ± 249.80	*22.80 ± 8.13	*19.04 ± 7.38	*46.40 ± 14.36
	*1303.20 ± 326.40	*16.64 ± 9.71	9.80 ± 4.41	*39.60 ± 10.15
	1828.53 ± 116.39	**29.74 ± 3.28**	**19.62 ± 3.06**	**51.07 ± 2.90**

*$p < 0.05$, 95% confidence limits of the mean (cf. text for ANOVA statistics). **Bold**: Hourly mean ± SE (N = 5) from each accumulated 10 min mean ± SE (N = 5). Behavioral data are expressed as frequency (i.e., the numbers of behavioral events that occurred are shown).

on ambulatory, central ambulatory, and rearing behavior. Each square-shaped, schematic diagram represents the floor of the behavioral chamber. A comparison with representative activity pattern analysis plots from saline treated animals (Fig. 4[b]) serves to emphasize the dramatic cocaine-induced psychostimulant behavior shown in Fig. 4(a).

Table 1 shows that cocaine (20 mg/kg SC) significantly increased ambulations [ANOVA, $F(4, 20) = 38.964$; $p < 0.0001$; $N = 5$]. Post-hoc analysis discloses that data in each of the 4-h testing periods was statistically significant (Fisher PLSD = 443.249; Scheffe $F = 16.513, 18.948, 32.066$, and 23.953, hours 1–4, respectively; $p < 0.01$; $N = 5$). A greater than threefold increase in ambulatory behavior was evidenced within the first 10 min after cocaine administration. A fivefold increase in ambulatory behavior was seen in the first hour after cocaine. No statistically significant differences were seen between any of the individual postcocaine groups (Scheffe

F-test < 1.716). The data show that a profound increase in hyperactivity occurred immediately after cocaine, which was then followed by a plateau effect.

Central ambulations (Table 1) were significantly increased by (SC) cocaine [ANOVA: $F(4, 20) = 38.366$; $p < 0.0001$; $N = 5$]. Post-hoc analysis indicates that hours 2, 3, and 4 after cocaine were highly significant (Fisher PLSD = 9.364; Scheffe $F = 6.423, 27.877$, and 19.846 in respective hours; $p < 0.01$; $N = 5$). There was a trend toward basal values in the fourth hour after cocaine. Thigmotaxic (agoraphobic) behavior was dramatically reduced by cocaine, tenfold in the first 10 min and twentyfold in the first hour after cocaine.

Rearing behavior (Table 1) was significantly increased by (SC) cocaine [ANOVA: $F(4, 20) = 17.474$; $p < 0.0001$; $N = 5$]. Post-hoc analysis showed that the significant increase took place in each of the 4 hours tested after cocaine (Fisher

PLSD = 9.191; Scheffe F = 9.678, 8.394, 15.205 and 6.111, in respective hours; $p < 0.01$; N = 5). Further analysis shows that the postcocaine hourly data did not significantly differ (Scheffe $F < 2.037$), creating a plateau effect. Rearing behavior was increased by fourfold in the first 10 min and greater than sevenfold in the first hour after administration.

Fine movements (sniffing and grooming) (Table 1) were significantly increased by (SC) cocaine [ANOVA, $F(4, 20)$ = 38.823; $p < 0.0001$; N = 5]. Post-hoc analysis reveals that the data derived in each of the four hours tested, was statistically significant (Fisher PLSD = 11.188; Scheffe F = 6.973, 10.677, 28.068, and 28.338, in respective hours; $p < .01$; N = 5). Fine movements increased threefold immediately after (SC) cocaine. The increases in fine movements were generally progressive. A plateau effect was reached in the fourth hour of study.

An examination of the neurochemical vis-à-vis behavioral data showed that statistically significant correlations were found between cocaine-induced DA alterations in VTA and (a) ambulatory behavior (Pearson Product: $r_{(a)} > 0.773$; $z_{(a)} > 1.0203$; $p < 0.01$); (b) central ambulatory behavior (Pearson Product: $r_{(a)} > 0.789$; $z_{(a)} > 1.0714$; $p < 0.01$;); (c) rearing behavior (Pearson Product: $r_{(a)} > 0.508$; $z_{(a)} > 0.5627$; $p < 0.01$); and (d) fine movements (Pearson Product: $r_{(a)} > 0.807$; $z_{(a)} > 1.1270$; $p < 0.01$). Moreover, positive correlates between each of the four behavioral parameters and synaptic concentrations of 5-HT were significant for 2 h after (SC) cocaine administration (Pearson Product: $r_{(a)} > 0.318$; $z_{(a)} > 0.3316$; $p < 0.01$). Rearing behavior continued to be positively correlated with synaptic concentrations of 5-HT in VTA in later hours (Pearson Product: $r_{(a)} > 0.259$; $z_{(a)} > 0.2661$; $p < 0.01$), whereas the correlation between central ambulatory behavior and 5-HT reversed to a negative but significant correlation (Pearson Product: $r_{(a)} < -0.215$; $z_{(a)} < -0.2237$; $p < 0.01$).

DISCUSSION

The present studies are in agreement with previous studies, which show that cocaine increased extracellular concentrations of DA in VTA-(A_{10}) (7). However, the present studies further elucidate DA-ergic mechanisms in VTA by assessing cocaine's action on DA in the freely moving and behaving animal, in a psychomotor stimulant reinforcement paradigm (48). The present data support previous data that DA-ergic neurons in VTA-(A_{10}) mediate reinforcement processes (39). The present findings indicate that a tonic-type relationship may exist between the DA-ergic response to cocaine in VTA and time. Such a response may lend an explanatory note to the hypothesis that favors cocaine-induced inhibition of impulse frequency rates by somatodendritic autoreceptor regulation. The data do not rule out, however, the well-known alternative hypothesis, that is, that activation of a negative feedback pathway from enhanced DA at the nerve terminal, can cause a suppression of cocaine-induced cell firing. What may be most contributive from the present studies though, is the actual demonstration that there is an apparent failure on the part of somatodendrites to completely suppress cocaine-induced enhancement of DA, despite a cocaine-induced suppression of impulse frequency. Thus, DA release mechanisms in VTA after cocaine are to some extent independent from somatodendritic cell firing components. Perhaps, such a disassociation could enable cocaine's potent abuse potential. The behavioral psychostimulant effects of cocaine were as expected, as were the highly correlative relationships that were found between DA and each of the cocaine-induced behavioral parameters studied (10). The capacity of cocaine to cause an anxiolytic response acutely, may also be important in defining its abuse potential.

New findings show that (SC) cocaine increased synaptic concentrations of 5-HT in VTA. It is likely that the 5-HT-ergic effects of cocaine in VTA are derived from neighboring 5-HT somatodendrites. Supporting this suggestion is reported evidence of a 5-HT-ergic axon fiber pathway that relays efferent ascending 5-HT projections through VTA en route to medial forebrain bundle (6). Consequently, cocaine-induced 5-HT-ergic effects in VTA may be influenced by cell body impulse frequency rates. In this way, an increase in synaptic 5-HT in VTA may occur as a negative feedback compensatory response to a cocaine-induced decrease in dorsal raphe firing rates (14) and/or to a (SC) cocaine-induced decrease in 5-HT release in nucleus accumbens (10,11). However, another plausible explanation for the present data is that cocaine might induce an action on 5-HT that is localized in VTA. Supporting this hypothesis are biochemical, radioautographic, immunohistochemical, and ultrastructural electron microscopic data that show or suggest that 5-HT axons not only pass through VTA, but terminate in VTA (3,32,41,42) and/or form distinct synaptic junctions to directly innervate DA-ergic neurons in VTA (20). Therefore, 5-HT-ergic release and reuptake inhibitory processes can occur within the VTA itself and cocaine may affect these processes either separately or in an integrative fashion. Simplistically then, cocaine-induced 5-HT reuptake inhibition may explain the present data.

Importantly, the colocalization of DA and 5-HT in VTA and the resultant coaction of cocaine on DA and 5-HT in VTA may elucidate new functional implications for its mechanism of action. The present findings provide evidence that the 5-HT-ergic effects of cocaine mimic its enhanced DA-ergic effects. Indeed, both 10 min and 40 min after (SC) cocaine administration, synaptic concentrations of the two biogenic amines, DA and 5-HT are significantly increased to the same extent. Moreover, cocaine-induced 5-HT-ergic changes maintain a positive correlation with the cocaine-induced DA-ergic effects. The data suggest that the oscillatory nature of the 5-HT-ergic response may be modulatory to the DA-ergic tonic response to cocaine. Accordingly, 5-HT may act as a relay or a gating mechanism for DA-ergic circuitry. Thus, cocaine may relay its biogenic amine effects through raphe nuclei. Biochemical evidence of DA neurons in raphe nuclei (41) and pharmacological evidence for a modulation of extracellular DA by 5-HT in nucleus accumbens (13), in addition to lesioning studies that evidence a 5-HT-DA relationship between raphe and NAcc (21), would argue for the relay hypothesis. Alternatively, cocaine may gate its biogenic amine effects through a colocalized action in VTA per se. The fact that 5-HT neurons have been found to directly innervate DA-ergic neurons in VTA (20), in addition to pharmacological evidence that DA neurons might be directly regulated by 5-HT in VTA (3), considerably enhances the gating hypothesis.

The present paper also presents new findings that the psychostimulant behavioral effects of cocaine were not as highly correlated with cocaine-induced changes in synaptic concentrations of 5-HT, as was the case with DA. Although a mediation for DA-ergic VTA-(A_{10}) cell bodies has been previously shown to be important in the 5-HT behavioral syndrome (i.e., e.g., hindlimb abduction, and forepaw tread) (1), cocaine-induced hyperactivity does not generally assimilate the 5-HT

behavioral syndrome and is generally thought to be a primarily DA-ergic phenomenon. Indeed, classical behavioral hyperactivity, even when the 5-HT syndrome participates, has been basically associated with activation of DA systems (28). The present data support a role for 5-HT in cocaine that may act in a contributory nature, relative to DA, in cocaine-induced locomotor activity behavior.

Summarily, it is likely then, that the DA cocaine reward signalling pathway may consist of impinging 5-HT components, which may factor into the abuse potential aspects of cocaine use and into subsequent treatment strategies.

REFERENCES

1. Andrews, C. D.; Fernando, J. C. R.; Curzon, G. Differential involvement of dopamine-containing tracts in 5-hydroxytryptamine-dependent behaviours caused by amphetamine in large doses. Neuropharmacology 21:63–68; 1982.
2. Arkin, H.; Colton, R. R. Tables for Statisticians. New York: Harper and Row Publishers; 1963:16–17.
3. Beart, P. M.; McDonald, D. 5-Hydroxytryptamine and 5-hydroxytryptaminergic-dopaminergic interactions in the ventral tegmental area of rat brain. J. Pharm. Pharmacol. 34:591–593; 1982.
4. Bedford, J. A.; Bailey, L. P.; Wilson, M. C. Cocaine reinforced progressive ratio performance in the rhesus monkey. Pharmacol. Biochem. Behav. 9:631–638; 1978.
5. Blaha, C. D.; Jung, M. E. Electrochemical evaluation of stearate-modified graphite paste electrodes: Selective detection of dopamine is maintained after exposure to brain tissue. J. Electroanal. Chem. 310:317–334; 1991.
6. Bobillier, P.; Seguin, S.; Degueurce, A.; Lewis, B. D.; Pujol, J. F. The efferent connections of the nucleus raphe centralis superior in the rat as revealed by radioautography. Brain Res. 166:1–8; 1979.
7. Bradberry, C. W.; Roth, R. H. Cocaine increases extracellular dopamine in rat nucleus accumbens and ventral tegmental area as shown by in vivo microdialysis. Neurosci. Lett. 103:97–102; 1989.
8. Broderick, P. A. Characterizing stearate probes in vitro for the electrochemical detection of dopamine and serotonin. Brain Res. 495:115–121; 1989.
9. Broderick, P. A. State-of-the-Art microelectrodes for in vivo voltammetry. Electroanalysis 2:241–251; 1990.
10. Broderick, P. A. Cocaine-on-line analysis of an accumbens amine neuronal basis for psychomotor behavior. Pharmacol. Biochem. Behav. 40:959–968; 1991.
11. Broderick, P. A. In vivo voltammetric studies on release mechanisms for cocaine with γ-butyrolactone. Pharmacol. Biochem. Behav. 40:969–975; 1991.
12. 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.
13. Chen, J.; van Praag, H. M. ; Gardner, E. L. Activation of 5-HT₃ receptor by 1-phenylbiguanide increases dopamine release in the rat nucleus accumbens. Brain Res. 543:354–357; 1991.
14. Cunningham, K. A.; Lakoski, J. M. Electrophysiological effects of cocaine and procaine on dorsal raphe serotonin neurons. Eur. J. Pharmacol. 148:457–462; 1988.
15. Drescher, K.; Hetey, L. Influence of antipsychotics and serotonin antagonists on presynaptic receptors modulating the release of serotonin in synaptosomes of the nucleus accumbens of rats. Neuropharmacology 27:31–36; 1988.
16. Einhorn, L. C.; Johansen, P. A.; White, F. J. Electrophysiological effects of cocaine in the mesoaccumbens dopamine system: Studies in the ventral tegmental area. J. Neurosci. 8:100–112; 1988.
17. Emmett-Oglesby, M. W.; Wurst, M.; Lal, H. Discriminative stimulus properties of a small dose of cocaine. Neuropharmacology 22:97–101; 1983.
18. Galloway, M. P. Regulation of dopamine and serotonin synthesis by acute administration of cocaine. Synapse 6:63–72; 1990.
19. Geyer, M. A. Approaches to the characterization of drug effects on locomotor activity in rodents. In: Adler, M. W.; Cowan, A., eds. Testing and evaluation of drugs of abuse. New York: Alan R. Liss; 1990:81–99.
20. Herve, D.; Pickel, V. M.; Joh, T. H.; Beaudet, A. Serotonin axon terminals in the ventral tegmental area of the rat: fine structure and synaptic input to dopaminergic neurons. Brain Res. 435: 71–83; 1987.
21. Herve, D.; Simon, H.; Blanc, G.; LeMoal, M.; Glowinski, J.; Tassin, J. P. Opposite changes in dopamine utilization in the nucleus accumbens and the frontal cortex after electrolytic lesion of the median raphe in the rat. Brain Res. 216:422–428; 1981.
22. 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.
23. Hurd, Y. L.; Weiss, F.; Koob, G. F.; Anden, N. E.; Ungerstedt, U. Cocaine reinforcement and extracellular dopamine overflow in rat nucleus accumbens: An in vivo microdialysis study. Brain Res. 498:199–203; 1989.
24. Johanson, C. E.; Schuster, C. R. A choice procedure for drug reinforcers: Cocaine and methylphenidate in the rhesus monkey. J. Pharmacol. Exp. Therap. 193:676–688; 1975.
25. Kalivas, P. W.; Duffy, P. Effect of acute and daily cocaine treatment on extracellular dopamine in the nucleus accumbens. Synapse 5:48–58; 1990.
26. Kornetsky, C.; Esposito, R. U. Reward and detection thresholds for brain stimulation: Dissociative effects of cocaine. Brain Res. 209:496–500; 1981.
27. Loh, E. A.; Roberts, D. C. Break-points on a progressive ratio schedule reinforced by intravenous cocaine increase following depletion of forebrain serotonin. Psychopharmacology (Berlin) 101:262–266; 1990.
28. Marsden, C. A. Involvement of 5-hydroxytryptamine and dopamine neurons in the behavioral effects of α-methyltryptamine. Neuropharmacology 19:691–698; 1980.
29. Moghaddam, B.; Bunney, B. S. Differential effect of cocaine on extracellular dopamine levels in rat medial prefrontal cortex and nucleus accumbens: Comparison to amphetamine. Synapse 4: 156–161; 1989.
30. Nayak, P. K.; Misra, A. L.; Mule, S. J. Physiological disposition and biotransformation of [³H] cocaine in activity and chronically treated rats. J. Pharmacol. Exp. Ther. 196:556–569; 1976.
31. Pan, Z. Z.; Williams, J. T. Differential actions of cocaine and amphetamine on dorsal raphe neurons in vitro. J. Pharmacol. Exp. Therap. 251:56–62; 1989.
32. Parent, A.; Descarries, L.; Beaudet, A. Organization of ascending serotonin systems in the adult rat brain. A radioautographic study after intraventricular administration of [³H] 5-hydroxytryptamine. Neuroscience 6:115–138; 1981.
33. Pelligrino, L. J.; Cushman, A. J. A stereotaxic atlas of the rat brain. New York: Appleton-Century-Crofts; 1967: p. 46.
34. Peris, J.; Boyson, S. J.; Cass, W. A.; Curella, P.; Dwoskin, L. P.; Larson, G.; Lin, L. H.; Yasuda, R. P.; Zahniser, N. R. Persistence of neurochemical changes in dopamine systems after repeated cocaine administration. J. Pharmacol. Exp. Therap. 253:38–44; 1990.

35. Pettit, H. O.; Justice, J. B., Jr. Effect of dose on cocaine self-administration behavior and dopamine levels in the nucleus accumbens. Brain Res. 539:94-102; 1991.
36. Phillips, A. G.; Fibiger, H. C. Neuroanatomical basis of intracranial self stimulation: Untangling the Gordian knot. In: Liebman, J. M.; Cooper, S. J., eds. The neuropharmacological basis of reward. New York: Oxford University Press; 1989:66-105.
37. Pickens, R.; Thompson, T. Cocaine reinforced behavior in rats: Effects of reinforcement magnitude and fixed ratio size. J. Pharmacol. Exp. Therap. 161:122-129; 1968.
38. Porrino, L. J.; Esposito, R. U.; Seeger, T. F.; Crane, A. M.; Pert, A.; Sokoloff, L. Metabolic mapping of the brain during rewarding self-stimulation. Science 224:306-309; 1984.
39. Roberts, D. C. S.; Koob, G. F. Disruption of cocaine self-administration following 6-hydroxydopamine lesions of the ventral tegmental area in rats. Pharmacol. Biochem. Behav. 17:901-904; 1982.
40. Ross, S. B.; Renyi, A. L. Inhibition of the uptake of tritiated 5-hydroxytryptamine in brain tissue. Eur. J. Pharmacol. 7:270-277; 1969.
41. Saavedra, J. M. Distribution of serotonin and synthesizing enzymes in discrete areas of the brain. Fed. Proc. 36:2134-2141; 1977.
42. Steinbusch, H. W. M. Distribution of serotonin-immunoreactivity in the central nervous system of the rat-cell bodies and terminals. Neuroscience 6:557-618; 1981.
43. Stellar, J. R.; Rice, M. B. Pharmacological basis of intracranial self-stimulation reward. In: Liebman, J. M.; Cooper, S. J., eds. The neuropharmacological basis of reward. New York: Oxford University Press; 1989:14-65.
44. Sulzer, D.; Rayport S. Amphetamine and other psychostimulants reduce pH gradients in midbrain dopaminergic neurons and chromaffin granules: A mechanism of action. Neuron 5:797-808; 1990.
45. Swanson, L. W. The projections of the ventral tegmental area and adjacent regions: A combined fluorescent retrograde tracer and immunofluorescence study in the rat. Brain Res. Bull. 9:321-353; 1982.
46. Uchimura, N.; North, R. A. Actions of cocaine on rat nucleus accumbens neurons in vitro. Br. J. Pharmacology 99:736-740; 1990.
47. Wise, C. D.; Berger, B. D.; Stein, L. Evidence of α-noradrenergic reward receptors and serotonergic punishment receptors in the rat brain. Biol. Psychiatry 6:3-21; 1973.
48. Wise, R. A.; Bozarth, M. A. A psychomotor stimulant theory of addiction. Psychol. Rev. 94:469-492; 1987.