Pergamon

Pharmacology Biochemistry and Behavior, Vol. 49, No. 3, pp. 711–728, 1994

0091-3057/94 $6.00 + .00

0091-3057(94)00202-9

Ibogaine Modulates Cocaine Responses Which Are Altered Due to Environmental Habituation: In Vivo Microvoltammetric and Behavioral Studies[1]

PATRICIA A. BRODERICK,*†[2] FRANK T. PHELAN,[3] FRANK ENG* AND ROBERT T. WECHSLER[4]

*Department of Pharmacology, The City University of New York Medical School
†Departments of Biology and Psychology, CUNY Graduate School, New York, NY 10031

Received 23 December 1993

BRODERICK, P. A., F. T. PHELAN, F. ENG AND R. T. WECHSLER. *Ibogaine modulates cocaine responses which are altered due to environmental habituation: In vivo microvoltammetric and behavioral studies.* PHARMACOL BIOCHEM BEHAV 49(3) 711–728, 1994.—Ibogaine, a serotonergic (5-HTergic) indole alkaloid, was studied for cocaine modulatory effects on four parameters of behavior by computerized infrared photocell beam detection. The behavioral parameters were: a) locomotor activity (ambulations), b) rearing, c) stereotypy (fine movements, primarily grooming), and d) agoraphobia [(thigmotaxis) a natural tendency to avoid the center of the behavioral chamber]. With each behavioral data point, dopamine (DA) release, and serotonin (5-HT) release were detected within seconds in nucleus accumbens (NAcc) of the same behaving male Sprague–Dawley rats, using in vivo electrochemistry (voltammetry). Ibogaine was administered (40 mg/kg IP) for 4 consecutive days. Importantly, the DAergic and the 5-HTergic responses to (SC) cocaine and two behavioral responses, ambulations and central ambulations, were reduced in intensity due to extended time spent in the novel behavioral chamber (habituated). Rearing and fine movement patterns were not habituated. The results show that ibogaine downmodulated the (SC) cocaine-induced increase in NAcc DA release ($p < 0.0001$) and potentiated the (SC) cocaine-induced decrease in NAcc 5-HT release ($p < 0.0001$). Concurrently, ibogaine downmodulated cocaine-induced ambulation ($p < 0.0001$) and central ambulation behavior ($p < 0.0001$). On the other hand, the behavioral parameters that did not exhibit habituation, i.e., rearing behavior and fine movement behavior, were not downmodulated by ibogaine ($p < 0.1558$) ($p < 0.3763$), respectively. Furthermore, ibogaine itself did not significantly alter NAcc DA release over the 2-h period studied ($p < 0.9113$) although individual time points were significantly affected bidirectionally. Concurrently ibogaine significantly increased 5-HT release ($p < 0.0155$). Behaviorally, ibogaine appears to be a weak psychostimulant. The data show a critical modulatory role for 5-HT in ibogaine–cocaine interactions. Also elucidated as critical is the efficacy of ibogaine when the response to (SC) cocaine is decreased due to the habituation of the animals to their environment.

Ibogaine Cocaine Psychostimulant behavior Nucleus accumbens (NAcc) Dopamine (DA) Serotonin (5-HT) In vivo electrochemistry (Voltammetry) Stearate microelectrode Freely moving rat paradigm Locomotor activity Central locomotor activity (agoraphobic inhibition) Rearing behavior Stereotypy

IBOGAINE, as a putative treatment for cocaine addiction, has a rather unique secular and historical perspective. Ibogaine has been purported to end the craving process for psychostimulants, such as cocaine, and for narcotics, such as heroin; the anecdotal reports have been given substance by the issuance of a use patent by the U.S. Office of Patents and

[1] These results were originally presented at the Second Annual International Behavioral Neuroscience Society Conference held in Clearwater Beach, FL, April 22–25, 1993.

[2] Requests for reprints should be addressed to Dr. P. A. Broderick, Department of Pharmacology, Rm. J-910, The City University of NY Medical School, Convent Ave. & W. 138th St., New York, NY 10031.

[3] Present address: NYC, Department of Environmental Protection, Department of Limnology, Ben Nesin Lab., 28A, Shokan, NY.

[4] Present address: Chicago Medical School, 333 Green Bay Road, N. Chicago, IL 60064-3095.

Trademarks (39). Presently, Phase 1 clinical trials to determine the safety and pharmacokinetics of ibogaine (HCl) are progressing (44) and the National Institute on Drug Abuse (NIDA) is reported to be actively supporting studies to ascertain the clinical importance of ibogaine (67).

Ibogaine also has a rather unique scientific and historical perspective as well, in its pharmacognosy, chemistry, and in its pharmacology. The pharmacognosy of ibogaine describes the plant as the yellowish root of the African shrub *Tabernanthe iboga* Baillon of the family *Apocynaceae*. The plant is cultivated in Gabon, Africa. Interestingly, either rarely known or emphasized, is the fact that the alkaloids of the genera *Apocynaceae* have found extensive use in modern medicine, an example of which is the *Rauwolfia* alkaloids, from which the well-known drug reserpine is derived. The chemistry of ibogaine places this compound as an indole alkaloid, similar in structure to the neurotransmitter, serotonin (5-HT). L-Tryptophan is the precursor for ibogaine, which places ibogaine in the same biochemical pathway as 5-HT. Ibogaine was first isolated in 1901 by Dybowski and Landrin [cited in (21)]. The chemical structure of ibogaine is known; its molecular formula is ($C_{20}H_{26}N_2O$) (23). Brain concentrations of ibogaine can be determined by thin layer chromatography, and concentrations of ibogaine in blood and urine can be determined by ultraviolet spectrophotometric assay (22). The structural formula of ibogaine, which was suggested by Taylor in 1957 (66), has been confirmed by x-ray crystallography (1). The structure of many of the other iboga alkaloids have been deduced from that of ibogaine (23). Interestingly, the origin of the indole alkaloid structure per se is predicated on yohimbine, a well known α_2-adrenoreceptor antagonist, which is actually derived from *l*-tryptophan, phenylalanine, and formaldehyde by two Mannich condensations (65). There are other structural types of alkaloids of Apocynaceae, e.g., the isomeric quinindolines and quinindoles, such as quinolinic acid, are representative of one of the other basic structural types.

The studies of the peripheral pharmacology of ibogaine to date have been mainly concerned with the cardiovascular system. Ibogaine was found to decrease blood pressure in anesthetized animals and to increase blood pressure in unanesthetized animals (27). Ibogaine was found to produce bradycardia; the bradycardic effect was potentiated by both domperidone and sulpiride, D_2 receptor antagonists and by atropine, a muscarinic cholinergic antagonist (35). There have been reports that tabernanthine, an ibogaine congener, is a calcium entry blocker that also affects cellular calcium metabolism by inhibiting norepinephrine (NE) and calcium (Ca^{2+})-induced vascular contractions (34). In other similar studies, tabernanthine was found to have a dose-dependent potentiation (low dose) and inhibition (high dose) of contractions elicited by NE in the mesenteric artery so that it has actions related to the turnover of intracellular Ca^{2+} releasable by NE (45). Peripherally, tabernanthine has been observed to potentiate Ca^{2+} responses and produce a catecholamine sensitization in isolated rat duodenum (70). Finally, ibogaine itself produces hypoglycemia (21).

Studies on the central nervous system pharmacology of ibogaine have shown that ibogaine can produce tremors in mice (62,75,76) and in rats (63). The nontremorigenic doses of ibogaine have been reported to be 5 and 10 mg/kg IP (61). Cappendijk and Dzoljic (15) have reported that the tremorigenic dose of ibogaine is 80 mg/kg and a lower dose of 40 mg/kg produces less prominent and shorter-lasting behavioral effects than the higher dose. Although, it has been shown that ibogaine itself did not interact at either the benzodiazepine receptor or the closely related $GABA_A$ receptor (19), the tremor induced by a related compound, tabernanthine, has been studied; tabernanthine acted as a benzodiazepine receptor inverse agonist in vitro in a discriminant binding assay. In addition, a tabernanthine-induced tremor was functionally inhibited by the benzodiazepine antagonist, RO-15,1788 (69).

Further studies on the central nervous system pharmacology of ibogaine have shown that ibogaine increased arousability (57). These results are consistent with data from sleep studies that show that the ibogaine congeners, tabernanthine tartrate and tabernanthine parachlorophenoxyacetate (SAD 103), provoked an increase in wakefulness level, a reduction in the level of slow wave sleep, and a transient blocking of paradoxical sleep (17,18). Both ibogaine and its congener tabernanthine produced an increase in the energy of the theta band frequency (5–6 Hz) and increased the duration of wakefulness in rats and the level of vigilance in the human. Indeed, ibogaine was reported to act like an antidepressant in these sleep paradigms (20,33). Studies on the hallucinatory effects of ibogaine in humans have been reported [(58) and cited by Dhahir, 1971 (21)]. According to the latter citation (21), Dr. Sigg has described his own experiences with ibogaine as the following: "visual hallucinations, blue disks appearing only in the dark, accompanied by many other symptoms common to hallucinogenic intoxication, but no undesirable after effects, [such as] exhaustion or depression were noted."

Controlled studies on the toxic effects of ibogaine began in 1971 (21), where tissue distribution and excretion studies were done in rats. The findings showed that ibogaine (50 mg/kg IP) was located in high concentrations in the liver, kidneys, and brain. Concentrations of ibogaine, however, were not cumulative and ibogaine disappeared at a rate of 4% of the injected dose per hour. About 5% of the injected dose of ibogaine was excreted unchanged in urine. The tissue : blood ratio was high, suggesting that the blood may not be the specimen of choice for postmortem toxicological analysis. Further, toxicological studies using chronic doses of ibogaine showed that ibogaine (10 and 50 mg/kg IP) did not cause any significant pathologic changes in the liver, kidney, heart, and brain when ibogaine was administered for 30 consecutive days, and no pathologic changes were observed in the liver or kidneys after ibogaine (40 mg/kg) was administered for 12 consecutive days. By comparison, when 5-HT was administered in doses of 10 mg/kg (base) for 12 consecutive days, severe damage to the kidney characteristic of ischemic coagulation necrosis occurred (21). More recent findings show that ibogaine at high doses (100 mg/kg IP) and administered chronically, activates glial cells in distinct longitudinal zones of the cerebellum, and causes degeneration of Purkinje cells, the major neurons of the cerebellar cortex. Interestingly, prior destruction of the inferior olive blocked Purkinje cell destruction (47,48). However, in many other brain areas, O'Hearn and Molliver found no evidence of ibogaine toxicity [citation in (67)].

Insofar as ibogaine and the pharmacology of addiction is concerned, studies on the ability of ibogaine to block cocaine self-administration were positive in male rats (15). In addition, the ability of ibogaine to block morphine self-administration was also positive, but in some cases somewhat discrepant in female rats (29,31). Results show that the ability of ibogaine to alleviate opiate withdrawal are equivocal, some are positive, some are negative (24,26,30,61). Moreover, ibogaine at a very low dose (3.5–7.0 mg/kg) did not significantly affect cocaine discrimination (55).

The ibogaine reports on the neurochemistry of DA and locomotor behavior are varied, probably as much as the pa-

rameters and paradigms are varied. Ibogaine did not affect extracellular concentrations of DA in NAcc of male rats as monitored by the microdialysis technique (41). Importantly, in postmortem studies though, ibogaine decreased DA levels in female rats (43). Moreover, ibogaine increased cocaine-induced and amphetamine-induced extracellular concentrations of DA in NAcc in female rats (40,42), whereas ibogaine decreased the usually increased extracellular concentrations of DA after morphine administration in male rats (41). In mice, ibogaine reduced amphetamine (59) and cocaine-induced locomotor stimulations (60).

Very few studies have been done on the interaction between ibogaine and the neurochemistry of the neurotransmitter serotonin (5-HT) except for early studies focussed on hypoxia (46). Importantly, ibogaine produces side-to-side head weave (63), a classical 5-HTergic syndrome (68).

We have published a series of articles that show that cocaine acts on presynaptic release mechanisms to alter synaptic concentrations of DA and 5-HT in a colocalized fashion in A_{10} pathways (7-11,13). Our light microscopic immunocytochemical evidence for 5-HT in A_{10} nerve terminals shows that the core of NAcc contains a dense terminal field of tyrosine hydroxylase (TH) axons that have an extensive overlap with 5-HT axons in the periphery within the core (51,52). Importantly, we have studied the region of NAcc that is ventral and lateral to the anterior commissure. This region has been referred to as ventrolateral NAcc (vlNAcc) by Swanson and Cowan (64); it has been suggested by these authors that vlNAcc has reciprocal connections with ventral tegmental area (VTA). Our findings of 5-HT axons in close proximity to DA neurons in vlNAcc are consistent with ultrastructural studies that show 5-HT immunoreactive terminals in the core and shell of medial NAcc (72) and with very recent studies that show a prominence of asymmetric junctions formed by 5-HT-labeled terminals on neurons projecting to the NAcc with those containing tyrosine hydroxylase in the VTA (71).

Therefore, the purpose of the present study was to assess the effect of ibogaine both alone and on cocaine-induced alterations in DA and 5-HT release in vlNAcc of freely moving and behaving male rats, concurrently with a study of the effect of ibogaine on locomotor activity and other parameters of cocaine-induced psychostimulant behaviors. Changes in synaptic concentrations of DA and 5-HT reflect presynaptic release mechanisms because in vivo microvoltammetric studies using γBL, have shown that cocaine does not increase DA concentrations in the presence of an impulse flow blocker (8). The results of the in vivo microvoltammetric studies are consistent with earlier studies that have described the dopamine releasing properties of cocaine (56). The well-known action of cocaine on reuptake inhibition processes (53,54) are not precluded.

A paradigm of altered responses to cocaine due to extended habituation of the animal in a novel environment was chosen. This paradigm more closely mimics clinical conditions.

METHOD

Drug Protocol

Ibogaine (40 mg/kg IP) was administered in suspension, each consecutive day for 4 days before the in vivo voltammetric and behavioral studies were performed. Ibogaine (Sigma, St. Louis, MO) was suspended in doubly deionized water and stirred for at least 40–50 min on a Thermolyne stirring plate. On each experimental day (the fourth day of ibogaine injection), after stable in vivo electrochemical signals for DA and 5-HT were evident, ibogaine (40 mg/kg IP) was administered and studied for 2 h after exploratory behavior was completed and baseline neurotransmitter release was stabilized. Then, 2 h after the administration of ibogaine, cocaine HCl (Sigma) (20 mg/kg SC), dissolved in doubly deionized water, was injected and the effects of cocaine (20 mg/kg SC) were studied for 4 h.

In another group of male Sprague–Dawley rats, physiological saline (1 cc/kg IP) was administered instead of ibogaine; the (SC) cocaine protocol and the neurochemical and behavioral protocols remained the same. The results were then compared with the effect of (SC) cocaine on DA and 5-HT release in vlNAcc in freely moving and behaving rats when cocaine (SC) injection was not preceded by a 2-h saline study (7). It is important to note that in the latter study, animals had completed exploratory behavior in their novel environment before cocaine injection.

Extended Habituation Protocol

In the present studies, animals were allowed a 2-h extended time period in the novel behavioral chamber before either injection of cocaine (20 mg/kg SC) or injection of saline (bodyweight in kg IP) was given. Due to the resulting extended habituation of the animal in the novel behavioral chamber, the DAergic responses, the 5-HTergic responses, and the behavioral responses to (SC) cocaine of hyperactivity and antiagoraphobia were significantly changed. Therefore, ibogaine was studied for its effects on (SC) cocaine responses, which were environmentally habituated. Also, results on rearing behavior and stereotypic behavior after cocaine injection that were not affected by environmental habituation, are reported.

Animals

The studies were done in unrestrained, freely moving, male, virus-free Sprague–Dawley rats (Charles River, Kingston, NY) (weight range 327 to 394 g at the time of the in vivo electrochemical 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. The rats were tested free from the following viruses: 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.

Surgical Protocol

Male Sprague–Dawley rats were anesthetized with pentobarbital (Na) (50 mg/kg IP). Body temperature was continuously maintained at 37 ± 0.5°C with an aquamatic K module heating pad (Amer. Hosp. Supply, Edison, NJ). Booster shots of pentobarbital (Na) (0.05 cc) were administered to maintain adequate anesthesia. Corneal, pinnal, and leg flexion responses were absent throughout the surgery. Rats were stereotaxically implanted with stearate working microelectrodes in ventrolateral (vl) NAcc (AP = +2.6, ML = +2.5, DV = −7.3) (50), which is the ventrolateral region of the posterior part of NAcc, as has been previously described (Swanson and Cowen (64)). Ag/AgCl reference microelectrodes and stainless steel auxiliary microelectrodes were placed in contact with cortex. The working, reference, and auxiliary microelectrode assembly was held in place with dental acrylic (Kadon Cavity Liner, Caulk, Becker Parkin Dental Supply Co. Inc., NY).

In Vivo Voltammetry: Notes on the Stearate (1.24 cc Nujol) Working Microelectrode

The stearate 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, TN), and 99% stearic acid (100 mg) (Sigma, St. Louis, MO). The stearate microelectrode was fabricated by pulling the Teflon envelope surrounding the stainless steel wire (Medwire Corp., Mt. Vernon, NY) over the edge to form a microcavity inside the Teflon well. The length of the resulting Teflon well was 500 μm; the diameter of the Teflon well was 200 μm. The detailed methodology for both the microelectrode assembly and the synthesis of the paste mixture is published by this laboratory (4-6). The microelectrode specifications are different from those published by others (2,3). Also importantly, electrochemical (EC) macroelectrodes have different surface EC properties than microelectrodes and, thus, such EC phenomenon as catalysis cannot be directly compared (73).

Notes on the Interpretation of In Vivo Electrochemical Voltammetric Signals

Dopamine and 5-HT were detected with the 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 10-12 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}). Figure 1 shows a voltammogram of sequentially detected DA and 5-HT release in vlNAcc of freely moving and behaving Sprague-Dawley rats. Even at high concentrations, the anionic metabolite of DA, i.e., 3,4 dihydroxyphenylacetic acid (DOPAC), cannot be detected. At high concentrations ascorbic acid (AA), the cofactor in the DA biosynthetic pathway, can be detected. However, AA can be detected at 0.050 V, a different peak potential (E_{ap}) than that for DA or 5-HT. The neurotransmitter, 5-HT was detected

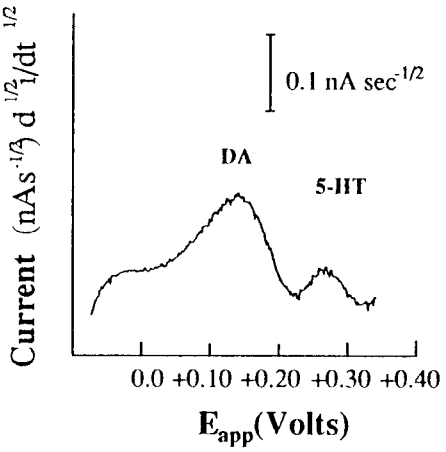

FIG. 1. A semiderivative voltammogram showing the in vivo electrochemical detection of DA and 5-HT in freely moving and behaving male Sprague-Dawley rats. Peak potentials for DA and 5-HT were 0.140 ± 0.015 V and 0.290 ± 0.015 V, respectively. Dopamine and 5-HT release were detected with a temporal resolution of 10-15 s and 10-12 s, respectively.

within a 10-12 s time period, at an E_{ap} of +0.290 V ± 0.015 V. The metabolite of 5-HT, that is, 5-hydroxyindoleacetic acid (5-HIAA) cannot be detected. Uric acid (UA), which is a constituent of brain with similar electroactive E_{ap} properties to those of 5-HT, can be detected but not at the same peak potential as that for 5-HT. Increasing the concentrations of DA and 5-HT did not significantly affect either signal. Each voltammogram was completed within 60 s. Each voltammogram, consisting of detection of faradaic in vivo electrochemical signals for DA and 5-HT, was completed in 25 s. A calibration curve for the detection of DA and 5-HT with the stearate (1.24 cc Nujol) microelectrode is previously published by this laboratory (11).

Medium exchange technique for stearate microelectrodes. A medium exchange technique was performed on each microelectrode in vitro before surgical insertion and implantation of the microelectrode in vivo. This procedure consisted 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.

In Vivo Voltammetric Protocol

In vivo voltammetric (semiderivative) studies on conscious rats were begun approximately 9-14 days after the aseptic surgical procedures were performed. On each experimental day, an animal was placed in a Faradaic and Plexiglas chamber (dimensions: 24 × 18 × 23.5″). The three microelectrode assembly, enclosed within the animal's prosthetic acrylic cap, was connected to a CV37 detector (BAS, West Lafayette, IN) by means of a mercury commutator (Br. Res. Instr., Princeton, NJ), a flexible cable, and a mating connector (BJM Electronics, Staten Island, NY). The CV37 was electrically connected to a Minigard surge suppressor (Jefferson Electric, Magnetek, NY), which was then connected to an isolated electrical ground. Potentials were applied to the working microelectrode with respect to a Ag/AgCl microelectrode (0.16 M saline) by a CV37 detector-potentiostat. 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. Initial applied potential (E_i) was −0.200 V. The scan rate was 10 mV sec^{-1}.

Behavioral Assessment

Activity pattern analysis simultaneously monitored several different responses both as they occurred spatially and temporally. The APM measured multiple concurrent measures of ambulations (locomotor activity), central ambulations, rearing behavior, and fine movements (primarily grooming). The movement of each male Sprague-Dawley rat was detected by a 16 × 16 array of infrared photobeams that surrounded the behavioral chamber; the photobeams were held in place by an aluminum frame. The aluminum frame was placed 3/4 inch above the Plexiglas floor of the behavioral cage (24 × 18 × 23.5″). The infrared photobeams were sampled by an IBM computer that defined the x-y position of the animal within a 1.5 inch resolution. An outer exterior layer of the behavioral chamber consisted of copper that provided a faradaic shield

for the in vivo monitoring of electrochemical (voltammetric) signals.

The definition of the specific x-y position of the animal was important because when an x-y position was calculated, it was used to define an animal's position in one of 16 equally sized sectors and one of 9 unequally sized regions. These regions were then used for more descriptive measures of, e.g., entries spent in the center of the cage. This central entries parameter of locomotor behavior becomes a reliable measure of thigmotaxia, i.e., the agoraphobic response, which is considered to be equivalent to fear (28). Moreover, rearing, wherein both forepaws of the animal are away from the floor for a period of at least 1 s, was measured by another series of infrared photobeams situated about 6 inches from the floor of the behavioral chamber. An IBM computer sampled the status of all the beams and the circuits in the behavioral chamber every 100 ms. The system is a custom modified version of an Activity Pattern Monitor (APM) (San Diego Instruments, San Diego, CA). Each parameter of behavior was monitored concurrently with the online detection of DA release and the separate yet concurrent detection of 5-HT release.

Statistical Protocol

Each component of the behavior monitored online, in addition to the DA and 5-HT release detected on line, was tested for statistically significant differences between pre- and post-ibogaine-cocaine and pre- and postsaline-cocaine, by analysis of variance (ANOVA) (Statview, Brain Power Inc., Calabasas, CA). Moreover, differences between ibogaine and control and saline and control were also determined by ANOVA. ANOVAs were followed by post hoc tests, Fisher PLSD (least square differences), and the Scheffe F-test (Statview, Brain Power Inc., Calabasas, CA), to determine hourly statistically significant differences. The hourly data was a composite of the individual 10-min interval time course data points. 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 drug or saline 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%.

Because the actual detection time for DA was 10–15 s, the percent change in synaptic concentrations of DA at each data point represents a 10–15 s current change (pA) from baseline. A similar principle of in vivo electrochemical detection applies for 5-HT. Because the actual detection time for 5-HT at each data point is 10–12 s, the percent change in synaptic concentrations of 5-HT, at each data point, represents a measurement of electrical current (in pA) within the same discrete synaptic environment as that for DA, within vlNAcc within a 10–12 s time period.

Finally, basal (endogenous) saline and cocaine-altered DA and 5-HT release in vlNAcc and concurrent parameters of behaviors were studied for statistically significant correlative value by the Pearson Product Moment Coefficient of Correlation (r) (polynomial distribution) (Statview, Brain Power Inc., Calabasas, CA). Corresponding z values were derived from the table of z for values of r from 0.0 to 1.0.

Neuroanatomical Location of Indicator (Working) Microelectrode

Placement of working microelectrodes in vlNAcc was confirmed by the potassium ferrocyanide blue dot method (10% formalin-transcardial perfusion with 80 ml saline). Current was passed at an amperage of 50 μA in a time period of 30 s. Examination of the brain revealed that virtually no damage to brain tissue took place.

RESULTS

Figure 1 shows a semiderivative voltammogram of basal DA and 5-HT release, detected concurrently in vlNAcc. The peak potential for the in vivo electrochemical signal for DA was 0.140 ± 0.015V and that for 5-HT was 0.290 ± 0.015V. Lowest detection limits, currently possible for DA and 5-HT, are 5 nmol and 1 nmol, respectively.

DA release in vlNAcc: Animals Habituated (Extended) to Novel Environment: Extended Time Period: Saline-Cocaine Group

Figure 2 shows the effect of saline (1 cc/kg IP) on DA release in vlNAcc, in addition to showing the effects of cocaine (20 mg/kg SC) on DA release in vlNAcc which follow the 2-h saline study. The studies were done in freely moving and behaving male Sprague-Dawley rats.

(a) Phase 1: Effect of saline. Injection of saline did not

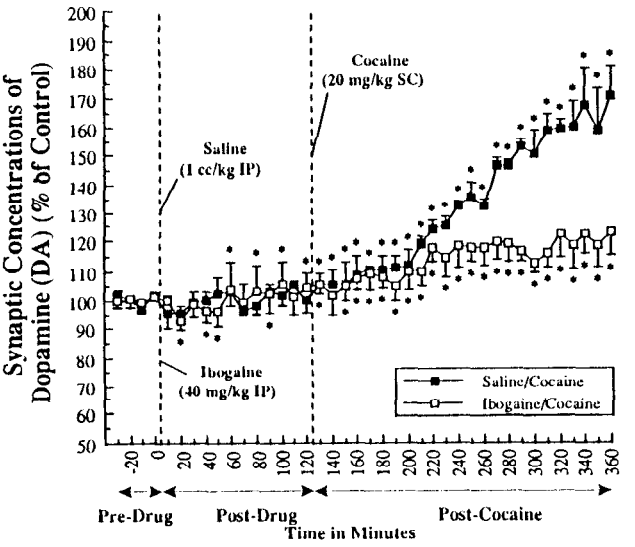

FIG. 2. DA release in vlNAcc: Animals habituated (extended) to novel environment: extended time period: saline-cocaine group and ibogaine-cocaine group. Phase 1: the effect of saline (■) on synaptic concentrations of dopamine (DA) in vlNAcc of freely moving and behaving male Sprague-Dawley rats ($N = 4$) as compared with the effects of ibogaine (□) on synaptic concentrations of DA in vlNAcc of a different group of freely moving and behaving male Sprague-Dawley rats ($N = 5$). Phase 2: the effect of cocaine (20 mg/kg SC) on synaptic concentrations of DA in vlNAcc of the same freely moving and behaving male Sprague-Dawley rats in which saline's effects on vlNAcc DA synaptic concentrations were studied ($n = 4$) (■), as compared with the effect of cocaine (20 mg/kg SC) on synaptic concentrations of DA in vlNAcc of the same freely moving and behaving male Sprague-Dawley rats in which ibogaine's effects on vlNAcc DA synaptic concentrations were studied ($n = 5$) (□). The saline group and the ibogaine group each had injections of either saline or ibogaine respectively for 3 consecutive days before the day of and on the day of the study. $^*p < 0.05$; 95% confidence limits (CL). See text for ANOVA statistics.

significantly affect DA release for the 2-h time course study [ANOVA: $F(1, 16) = 0.028$, $p < 0.8703$]. The hourly data was a composite of the individual 10-minute interval time course data points.

(b) Phase 2: Effect of (SC) cocaine with saline pretreatment. Injection of cocaine (20 mg/kg SC) following the 2-h saline study caused a significant increase in DA release over baseline [ANOVA: $F(4, 25) = 113.993$, $p < 0.0001$] over the 4 h of study. DA release was significantly increased ($p < 0.05$, 95% CL) to 106% within 10 min following injection and maximally increased to 171% ($p < 0.05$, 95% CL) 4 h after cocaine (SC) injection (baseline = 100%). Post hoc analyses show statistically significant differences between basal DA release and cocaine-induced DA release for each of the 4 h following cocaine injection (Fisher's PLSD = 7.043 and Scheffe's $F = 1.311$, 9.018, 41.261, 82.794, first to fourth hours, respectively).

DA Release in vlNAcc: Animals Habituated (Extended) to Novel Environment: Extended Time Period: Ibogaine-Cocaine Group

Figure 2 also shows a 2-h time course study on the effect of ibogaine (40 mg/kg IP) on DA release in vlNAcc, in addition to showing the effect of cocaine (20 mg/kg SC) on DA release in vlNAcc for the 4-h period that follows the 2-h ibogaine study. The studies were done in a separate group of freely moving and behaving male Sprague-Dawley rats.

(a) Phase 1: Effect of ibogaine. Injection of ibogaine did not significantly affect DA release for the 2-h time course study [ANOVA: $F(1, 16) = 0.013$, $p < 0.9112$]. The hourly data was a composite of the individual 10-min interval time course data points. There were, however, small but statistically significant ($p < 0.05$ (95% CL)) initial decreases in DA release followed by small but statistically significant increases in DA release, at specific time intervals during the time course studies. DA release at the time points (20, 40, and 50 min) following ibogaine injection were significantly decreased below basal DA release (95% CL) and DA release at time points (60, 80, 90, and 100 min) following ibogaine injection were significantly increased over basal DA release (95% CL). The temporal resolution of in vivo electrochemistry allowed the selective effects of ibogaine on DA release to be detected.

(b) Phase 2: Effect of (SC) cocaine with ibogaine pretreatment. Injection of cocaine (20 mg/kg SC) following the 2-h ibogaine study, produced a significant increase in DA release in vlNAcc over baseline [ANOVA: $F(4, 25) = 41.619$, $p < 0.0001$]. DA release was significantly increased to 106% at 130 min ($p < 0.05$, 95% CL) and maximally increased to 122% ($p < 0.05$, 95% CL) at 320 min (baseline = 100%). Post hoc analyses show that the statistically significant differences between basal and cocaine-induced DA release were significant for each of the 4 h following cocaine injection (Fisher's PLSD = 3.659 and Scheffe's $F = 3.183$, 12.755, 23.225, 31.485, first to fourth hours, respectively). It is important to note that saline or ibogaine injections were administered chronically for 4 days. The day of the study was the fourth day of saline or ibogaine injection.

Comparison Between the Saline and Ibogaine Groups

A comparison between the effect of saline and the effect of ibogaine on DA release in vlNAcc shows that there were no significant differences in vlNAcc DA release between saline-treated and ibogaine-treated animals for either hour of the 2-h study [ANOVA: $F(1, 5) = 0.925$, $p < 0.3803$, ANOVA: $F(1, 5) = 1.967$, $p < 0.2197$, first and second hours, respectively].

A comparison between the effect of cocaine on DA release with saline pretreatment vs. ibogaine pretreatment shows that ibogaine significantly downmodulated cocaine-induced DA release below saline [ANOVA: $F(1, 23) = 31.638$, $p < 0.0001$]. Ibogaine significantly reduced cocaine's effect on vlNAcc DA release in the first hour [ANOVA: $F(1, 5) = 10.128$, $p < 0.0245$], in the second hour [ANOVA: $F(1, 5) = 19.91$, $p < 0.0066$], in the third hour [ANOVA: $F(1, 5) = 50.795$, $p < 0.0008$], and in the fourth hour [ANOVA: $F(1, 5) = 753.705$, $p < 0.0001$]. The intensity of the reduction in cocaine-induced DA release in vlNAcc by ibogaine progressed to a maximum decrease of 42% by the fourth hour of study.

5-HT Release in vlNAcc: Animals Habituated (Extended) to Novel Environment: Extended Time Period: Saline-Cocaine Group

Figure 3 shows the effect of saline (1 cc/kg IP) on serotonin (5-HT) release in vlNAcc (a 2-h study) followed by a 4-h study on the effect of cocaine (20 mg/kg SC) on 5-HT release in vlNAcc in the same freely moving and behaving male Sprague-Dawley rats in which DA release was studied. Dopa-

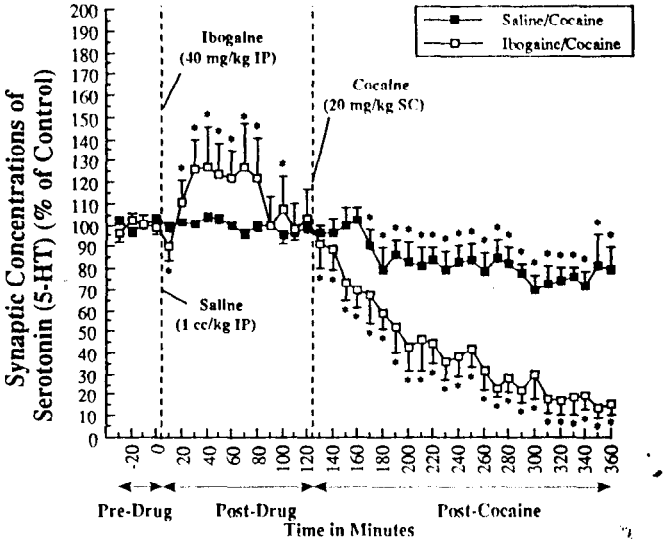

FIG. 3. 5-HT release in vlNAcc: Animals habituated (extended) to novel environment: extended time period: saline-cocaine group and ibogaine-cocaine group. Phase 1: the effect of saline (■) on synaptic concentrations of 5-HT in vlNAcc of freely moving and behaving male Sprague-Dawley rats ($n = 4$) as compared with the effects of ibogaine (□) on synaptic concentrations of 5-HT in vlNAcc of freely moving and behaving male Sprague-Dawley rats ($N = 5$). Phase 2: the effect of cocaine (20 mg/kg SC) on synaptic concentrations of 5-HT in vlNAcc of the same freely moving and behaving male Sprague-Dawley rats in which saline's effects on vlNAcc synaptic concentrations of 5-HT were studied ($N = 4$) (■) as compared with the effect of cocaine (20 mg/kg SC) on synaptic concentrations of 5-HT in vlNAcc of the same freely moving and behaving male Sprague-Dawley rats in which ibogaine's effects were studied ($n = 5$) (□). In vivo electrochemical signals for DA and 5-HT were detected concurrently. Both the saline group and the ibogaine group each had injections of either saline or ibogaine respectively for 3 consecutive days before the day of and on the day of the study. *$p < 0.05$; 95% confidence limits (CL). See text for ANOVA statistics.

mine and 5-HT release were studied concurrently with a temporal resolution of 10–15 s and 10–12 s, respectively. In vivo electrochemical signals for DA and 5-HT peaks were separate, distinct and on-line.

(a) Phase 1: Effect of saline. Injection of saline did not significantly affect 5-HT release for the 2-h time course study [ANOVA: $F(1, 16) = 0.4$, $p < 0.5359$]. The hourly data was a composite of the individual 10-min interval time course data points.

(b) Phase 2: Effect of (SC) cocaine with saline pretreatment. Injection of cocaine (20 mg/kg SC) following the 2-h saline study caused a significant decrease in 5-HT release below baseline [ANOVA: $F(4, 25) = 26.544$, $p < 0.0001$]. 5-HT release was significantly decreased beginning at 170 min to 10% below baseline ($p < 0.05$, 95% CL) and maximally decreased to 30% below baseline ($p < 0.05$, 95% CL) at 300 min (baseline = 100%). Post hoc analyses show that the statistically significant differences between basal and cocaine-altered release of 5-HT were significant for each of the 4 h following cocaine (20 mg/kg SC) injection (Fisher's PLSD = 5.85 and Scheffe's $F = 1.236$, 9.71, 13.556, 18.71, first to fourth hours, respectively).

5-HT Release in vlNAcc: Animals Habituated (Extended) to Novel Environment: Extended Time Period: Ibogaine-Cocaine Group

Figure 3 also shows a 2 h time course study on the effect of ibogaine (40 mg/kg IP) on 5-HT release followed by a study of the effect of cocaine (20 mg/kg SC) on 5-HT release in vlNAcc which follows the 2-h ibogaine study. The study was done in the same freely moving and behaving male Sprague-Dawley rats in which DA release was studied. However, the studies were done in a separate group of animals from those in which the saline studies were performed.

(a) Phase 1: Effect of ibogaine. Injection of ibogaine significantly increased 5-HT release for the 2-h time course study [ANOVA: $F(1, 16) = 6.206$, $p < 0.0241$]. The hourly data was a composite of the individual 10-min interval time course data points. Further analysis showed that ibogaine induced a significant increase of 5-HT release in the first hour of the 2 h following baseline [ANOVA: $F(1, 10) = 8.484$, $p < 0.0155$]. Ibogaine significantly increased 5-HT release beginning at the 20 min mark to 110% ($p < 0.05$, 95% CL) and maximally increased 5-HT release at the 40-min mark to 127% ($p < 0.05$, 95% CL) (baseline = 100%).

(b) Phase 2: Effect of (SC) cocaine with ibogaine pretreatment. Injection of cocaine (20 mg/kg SC) following the 2-h ibogaine study produced a significant decrease in 5-HT release below baseline [ANOVA: $F(4, 25) = 140.652$, $p < 0.0001$]. 5-HT release was significantly decreased beginning at 130 min by 9% ($p < 0.05$, 95% CL) and maximally decreased by 86% ($p < 0.05$, 95% CL), i.e., at 350 min (baseline = 100%). Post hoc analyses of the data show that the statistically significant differences were significant for each of the 4 h following cocaine injection (Fisher's PLSD = 8.317 and Scheffe's $F = 9.287$, 48.504, 75.198, 104.385, first to fourth hours, respectively).

Comparison Between the Saline and Ibogaine Groups

A comparison between the effect of saline and the effect of ibogaine on 5-HT release shows that ibogaine significantly increased 5-HT release over saline in the first hour following baseline [ANOVA: $F(1, 5) = 7.96$, $p < 0.0371$, ANOVA: $F(1, 5) = 5.82$, $p < 0.0607$, first and second hours, respectively, following baseline).

A comparison between the effect of cocaine on 5-HT release with saline pretreatment vs. ibogaine pretreatment shows that ibogaine significantly potentiated the cocaine-induced decrease in 5-HT release [ANOVA: $F(1, 23) = 150.16$, $p < 0.0001$] over the 4 h following cocaine injection. Ibogaine significantly potentiated the cocaine-induced decrease in 5-HT release by 19% in the first hour [ANOVA: $F(1, 5) = 19.921$, $p < 0.0066$], by 39% in the second hour [ANOVA: $F(1, 5) = 499.818$, $p < 0.0001$], by 50% in the third hour [ANOVA: $F(1, 5) = 218.567$, $p < 0.0001$], and by 58% in the fourth hour [ANOVA: $F(1, 5) = 606.681$, $p < 0.0001$]. The potentiation effect progressed over the 4-h study by 39%.

DA and 5-HT Release in vlNAcc: Effect of (SC) Cocaine Without Saline or Ibogaine Pretreatment: Animals Not Habituated (Extended) to Novel Environment: Time Period Not Extended

Figure 4 shows the effect of cocaine (20 mg/kg SC) on DA and 5-HT release in vlNAcc when cocaine was injected without an immediately preceding 2-h study of either saline or ibogaine, i.e., immediately after a steady-state baseline of endogenous DA and 5-HT release was achieved. Cocaine significantly increased DA release over baseline [ANOVA: $F(4, 25) = 77.214$, $p < 0.0001$]. DA release was significantly increased beginning at 10 min following baseline to 107% ($p < 0.05$, 95% CL) and maximally increased to 176% ($p < 0.05$, 95% CL) at 190 min (baseline = 100%). Cocaine concurrently and significantly decreased 5-HT release below baseline [ANOVA: $F(4, 25) = 52.23$, $p < 0.0001$]. 5-HT release was

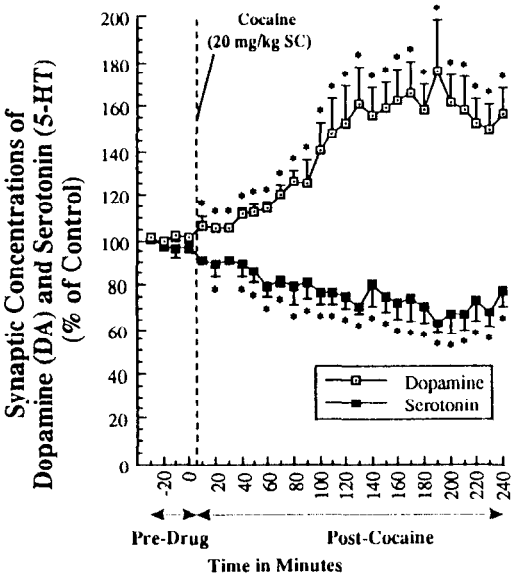

FIG. 4. DA and 5-HT release in vlNAcc: Effect of (SC) cocaine without saline or ibogaine pretreatment: animals not habituated (not extended habituation) to novel environment: time period not extended. The effect of cocaine (20 mg/kg SC) on synaptic concentrations of DA and 5-HT in vlNAcc when cocaine was injected without an immediately preceding 2-h study of either saline or ibogaine. $*p < 0.05$; 95% confidence limits (CL). See text for ANOVA statistics ($N = 5$). Modified from Broderick (7). Reprinted with permission of Elsevier Science, Ltd.

significantly decreased beginning at 20 min to 11% below baseline ($p < 0.05$, 95% CL) and maximally decreased to 37% below baseline ($p < 0.05$, 95% CL) at 190 min (baseline = 100%).

vlNAcc DA Release: Comparisons Between the Habituated and NonHabituated (SC) Cocaine Responses

A statistical comparison between the effect of cocaine on DA release with repeated environmental stimulation (extended time period in novel behavioral chamber) and that effect without, shows that the repeated stimulation response to (SC) cocaine was significantly reduced [ANOVA: $F(1, 23) = 9.994$, $p < 0.0044$]. Therefore, cocaine-induced DA release was significantly habituated and downmodulated by the number of hours the animal spent in the behavioral chamber, even in a novel behavioral chamber. Importantly, the most prominent habituation effects on DA release were seen in the second hour [ANOVA: $F(1, 5) = 32.185$, $p < 0.0024$] and in the third hour [ANOVA: $F(1, 5) = 36.127$, $p < 0.0018$].

vlNAcc 5-HT Release: Comparisons Between the Habituated and Nonhabituated (SC) Cocaine Responses

A statistical comparison between the effect of cocaine on 5-HT release in vlNAcc with repeated environmental stimulation and that without shows that the response to (SC) cocaine was significantly reduced in intensity [ANOVA: $F(1, 23) = 35.475$, $p < 0.0001$]. Thus, the (SC) cocaine-induced decrease in 5-HT release in vlNAcc was habituated and upmodulated by the extended time spent in the novel behavioral chamber. Importantly, the most prominent habituation effects were seen in the first hour [ANOVA: $F(1, 5) = 12.662$, $p < 0.0162$], in the second hour [ANOVA: $F(1, 5) = 13.435$, $p < 0.0145$], and in the fourth hour [ANOVA: $F(1, 5) = 7.924$, $p < 0.0373$].

Behavioral Assessment: Ambulatory Behavior: Animals Habituated (Extended) to Novel Environment: Extended Time Period: Saline-Cocaine Group

Figure 5 shows the effect of saline (1 cc/kg IP) on the frequency of ambulatory behavior, in addition to showing the effect of cocaine (20 mg/kg SC) on ambulation frequency which follows the 2-h saline study. The study was done in the same freely moving and behaving male Sprague–Dawley rats in which the selective and concurrent neurotransmitter study on DA and 5-HT release was done.

(a) Phase 1: Effect of saline. Injection of saline did not significantly affect ambulation frequency for the 2-h time course study [ANOVA: $F(1, 16) = 1.021$, $p < 0.3274$]. The hourly data was a composite of the individual 10-min interval time course data points. However, initially a significant increase did occur due to an initial arousal of the animal when exploratory behavior had been completed.

(b) Phase 2: Effect of (SC) cocaine with saline pretreatment. Injection of cocaine (20 mg/kg SC) following the 2-h saline study produced a significant increase in ambulation frequency over baseline [ANOVA: $F(4, 25) = 45.03$, $p < 0.0001$]. Ambulation frequency was significantly increased from 166 ambulations at baseline to 713 ambulations ($p < 0.05$, 95% CL), beginning at 130 min. The maximal increase to 1459 ambulations ($p < 0.05$, 95% CL) occurred at 270 min. Post hoc analyses show that the statistically significant differences between basal and cocaine-induced ambulation frequency were significant for each of the 4 h following cocaine injection (Fisher's PLSD = 206.639 and Scheffe's F = 3.785, 23.613, 34.214, 18.012, first to fourth hours, respectively).

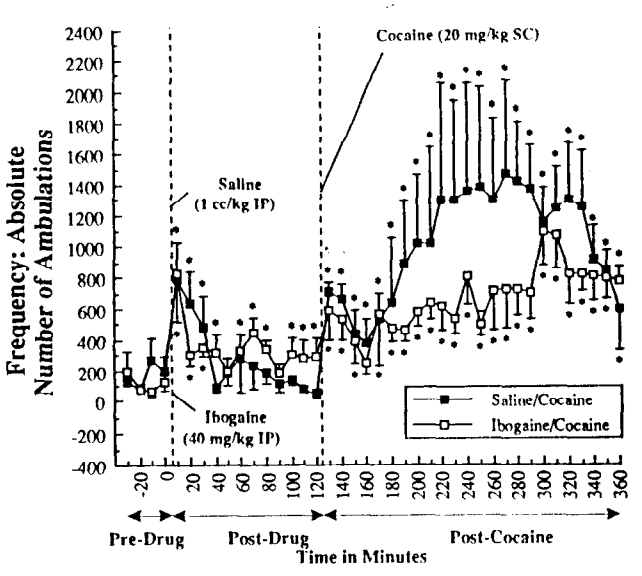

FIG. 5. Ambulatory behavior: Animals habituated (extended) to novel environment: extended time period: saline-cocaine group and ibogaine-cocaine group. Phase 1: the effect of saline (■) on ambulatory behavior (locomotor behavior) ($N = 4$) as compared with the effects of ibogaine (□) ($N = 5$). The studies were done in the same group in which vlNAcc DA and 5-HT release were studied in terms of saline or ibogaine pretreatment. Ambulations are reported in terms of frequency or number of ambulatory events. Phase 2: the effect of cocaine (20 mg/kg SC) on ambulatory behavior continued from the saline or ibogaine studies. $*p < 0.05$; 95% confidence limits (CL). See text for ANOVA statistics.

Ambulatory Behavior: Animals Habituated (Extended) to Novel Environment: Extended Time Period: Ibogaine-Cocaine Group

Figure 5 also shows the effect of ibogaine (40 mg/kg IP) on ambulation frequency, in addition to showing the effects of cocaine (20 mg/kg SC) on ambulation frequency which follow the 2-h ibogaine study. The study was done in the same freely moving and behaving male Sprague–Dawley rats in which the selective and concurrent neurotransmitter study on DA and 5-HT release was done.

(a) Phase 1: Effect of ibogaine. Injection of ibogaine significantly increased ambulation frequency for the 2-h time course study [ANOVA: $F(1, 16) = 10.555$, $p < 0.005$]. The hourly data was a composite of the individual 10-min interval time course data points. Ambulation frequency significantly increased for both hours of the 2 h following baseline [ANOVA: $F(1, 10) = 8.298$, $p < 0.0164$]. Within the first 10 min, ambulations increased fourfold, an effect that was seen in the saline group as well, due to arousal of animals after completed exploratory behavior and pursuant rest.

(b) Phase 2: Effect of (SC) cocaine with ibogaine pretreatment. Injection of cocaine (20 mg/kg SC) following the 2-h ibogaine study caused a significant increase in cocaine-induced ambulation frequency over baseline [ANOVA: $F(4, 25) = 28.812$, $p < 0.0001$]. Ambulation frequency was significantly increased from 118 ambulations at baseline to 583 ambula-

tions ($p < 0.05$, 95% CL), beginning at 130 min. Ambulation frequency was maximally increased to 1090 ambulations ($p < 0.05$, 95% CL) at 300 min. Post hoc analyses show that the statistically significant differences between basal and cocaine-induced ambulation frequency were significant for each of the 4 h following cocaine injection (Fisher's PLSD = 153.305 and Scheffe's F = 5.309, 10.666, 17.35, 23.785, first to fourth hours, respectively).

Comparison Between the Saline and Ibogaine Groups

A comparison between the effect of saline and the effect of ibogaine on ambulation frequency shows that ibogaine significantly increased ambulation frequency in the second hour following baseline [ANOVA: $F(1, 5) = 0.084$, $p < 0.7836$, ANOVA: $F(1, 5) = 46.82$, $p < 0.001$, first and second hours, respectively, following baseline).

A comparison between the effect of cocaine with saline pretreatment vs. the effect of cocaine with ibogaine pretreatment shows that ibogaine significantly reduced cocaine-induced ambulation frequency [ANOVA: $F(1, 23) = 34.054$, $p < 0.0001$]. Ibogaine significantly reduced cocaine-induced ambulation frequency in the first hour [ANOVA: $F(1, 5) = 9.059$, $p < 0.0298$], in the second hour [ANOVA: $F(1, 5) = 74.105$, $p < 0.0003$], and in the third hour [ANOVA: $F(1, 5) = 26.844$, $p < 0.0035$].

Frequency of Ambulatory Behavior: Effect of (SC) Cocaine Without Saline or Ibogaine Pretreatment: Animals Not Habituated (Extended) to Novel Environment: Time Period Not Extended

Figure 6 shows the effect of cocaine on ambulation behavior when cocaine (20 mg/kg SC) was injected without an immediately preceding 2-h study of either saline or ibogaine. Cocaine (20 mg/kg SC) significantly increased ambulation frequency [ANOVA: $F(4, 25) = 58.845$, $p < 0.0001$]. Ambulation frequency was significantly increased from a baseline of 253 ambulations to 707.25 ambulations, beginning at the 10-min mark ($p < 0.05$, 95% CL). Ambulation frequency was maximally increased to 1578 ambulations ($p < 0.05$, 95% CL) at the 210-min mark.

Frequency of Ambulatory Behavior: Comparisons Between the Habituated and Nonhabituated (SC) Cocaine Responses

A statistical comparison between the effect of cocaine on ambulation frequency with repeated environmental stimulation and that without shows that the repeated stimulation response to (SC) cocaine was significantly reduced [ANOVA: $F(1, 23) = 14.994$, $p < 0.0008$]. Therefore, a subsequent habituated and downmodulated response to (SC) cocaine was seen with the ambulatory behavior parameter, particularly in the first [ANOVA: $F(1, 5) = 19.873$, $p < 0.0067$], and fourth hours [ANOVA: $F(1, 5) = 13.972$, $p < 0.0135$].

Rearing Behavior: Animals Not Exhibiting Extended Habituation Response in Novel Environment Although Time Period Was Extended: Saline-Cocaine Group

Figure 7 shows the effect of saline (1 cc/kg IP) on rearing behavior, in addition to showing the effect of cocaine (20 mg/kg SC) on rearing behavior which follows the 2-h saline study. The study was done in the same freely moving and behaving male Sprague–Dawley rats in which vlNAcc DA release and 5-HT release and ambulatory behavior were studied, i.e., the saline-cocaine group.

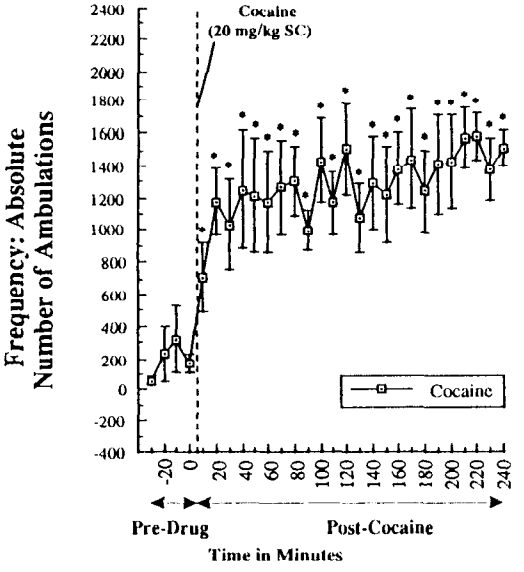

FIG. 6. Frequency of ambulatory behavior: effect of (SC) cocaine without saline or ibogaine pretreatment: animal group not habituated (not extended habituation) to novel environment: time period not extended. The effect of cocaine (20 mg/kg SC) on ambulatory behavior when cocaine was administered without a 2-h saline or ibogaine study preceding cocaine injection. vlNAcc DA and 5-HT release was studied concurrently (Fig. 4). Ambulatory (locomotor) behavior is reported in terms of frequency (number) of events. *$p < 0.05$; 95% confidence limits (CL). See text for ANOVA statistics ($N = 6$).

(a) Phase 1: Effect of saline. Injection of saline had no significant effect on rearing frequency for the 2-h time course study [ANOVA: $F(1, 16) = 0.292$, $p < 0.5966$]. The hourly data was a composite of the individual 10-min interval time course data points. Within the first 10 min, rearing increased due to arousal of animals after exploratory behavior was completed.

(b) Phase 2: Effect of (SC) cocaine with saline pretreatment. Injection of cocaine (20 mg/kg SC) following the 2-h saline study significantly increased rearing frequency over baseline [ANOVA: $F(4, 25) = 22.852$, $p < 0.0001$]. Rearing frequency was significantly increased from 2 rears at baseline to 12 rears ($p < 0.05$, 95% CL), beginning at 190 min, and was maximally increased to 58 rears ($p < 0.05$, 95% CL) at 300 min. Post hoc analyses show that the statistically significant differences between basal and cocaine-induced rearing frequency were significant for the second to fourth hours following cocaine (20 mg/kg SC) injection (Fisher's PLSD = 11.63 and Scheffe's F = 5.557, 12.13, 13.341, second to fourth hours, respectively).

Rearing Behavior: Animal Group Not Exhibiting Extended Habituation Response in Novel Environment Although Time Period Was Extended: Ibogaine-Cocaine Group

Figure 7 also shows the effect of ibogaine (40 mg/kg IP) on rearing behavior, in addition to showing the effect of cocaine (20 mg/kg SC) on rearing behavior which follows a 2-h ibogaine study. The study was done in the same freely moving and behaving male Sprague–Dawley rats in which DA and 5-HT release and ambulatory behavior were concurrently detected and monitored, i.e., the ibogaine-cocaine group.

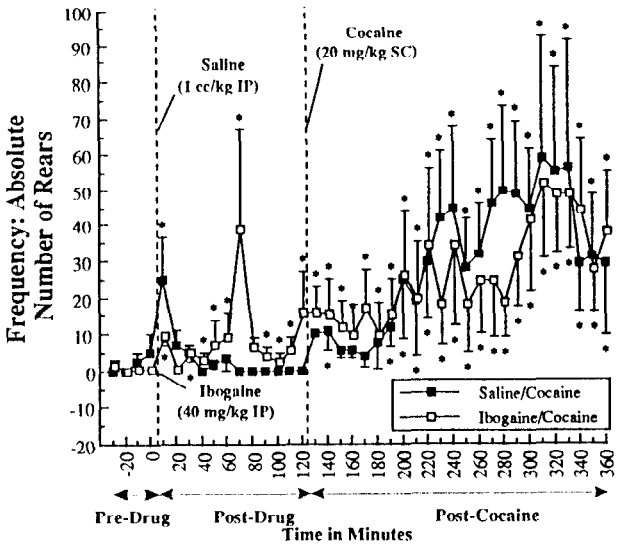

FIG. 7. Rearing behavior: Animals not habituated (not extended habituation) to novel environment although time period was extended: saline-cocaine group and ibogaine-cocaine group. Phase 1: the effect of saline (■) or ibogaine (□) on rearing behavior. Either saline (1 cc/kg IP) or ibogaine (40 mg/kg IP) was injected after exploratory behavior was essentially completed and at the time when a stable baseline for DA and 5-HT release in vlNAcc had been achieved. Phase 2: the effect of cocaine (20 mg/kg SC) on rearing behavior in the same freely moving and behaving male Sprague-Dawley rats in which the 2-h saline study had taken place (■) ($N = 4$), as compared with the effects of cocaine (20 mg/kg SC) on rearing behavior in the same freely moving and behaving male Sprague-Dawley rats that were pretreated with ibogaine 2 h before (□) ($N = 5$). *$p < 0.05$; 95% confidence limits (CL). See text for ANOVA statistics.

(a) Phase 1: Effect of ibogaine. Injection of ibogaine significantly increased rearing frequency for the first hour of the 2 h following baseline [ANOVA: $F(1, 10) = 11.001$, $p < 0.0078$]. The second hour, though statistically not significant [ANOVA: $F(1, 10) = 4.085$, $p < 0.0708$] may well be physiologically significant. Ibogaine significantly increased rearing frequency at the 10, 30–120 min marks ($p < 0.05$, 95% CL).

(b) Phase 2: Effect of (SC) cocaine with ibogaine pretreatment. Injection of cocaine (20 mg/kg SC) following the 2-h ibogaine study significantly increased rearing frequency over baseline [ANOVA: $F(4, 25) = 31.036$, $p < 0.0001$]. Rearing frequency was significantly increased from 1 rear at baseline to 16 rears ($p < 0.05$, 95% CL), beginning at 130 min. Rearing behavior was maximally increased to 51 rears ($p < 0.05$, 95% CL) at 310 min. Post hoc analyses show that the statistically significant differences between basal and cocaine-induced rearing frequency were significant for each of the 4 h following cocaine injection (Fisher's PLSD = 8.213 and Scheffe's $F = 2.418$, 8.808, 10.198, 27.871, first to fourth hours, respectively).

Comparison Between the Saline and Ibogaine Groups

A comparison between the effect of saline and the effect of ibogaine on rearing behavior shows that there were no significant differences in rearing frequency for either hour of the 2 h following baseline [ANOVA: $F(1, 5) = 0.179$, $p < 0.6899$, ANOVA: $F(1, 5) = 4.533$, $p < 0.0865$, first and second hours, respectively, following baseline].

A comparison between the rearing effects of cocaine with saline pretreatment vs. ibogaine pretreatment shows that there was no significant difference between saline and ibogaine on cocaine's effect on rearing frequency [ANOVA: $F(1, 23) = 2.154$, $p < 0.1558$] over the 4 h following cocaine injection.

Rearing Behavior: Effect of (SC) Cocaine Without Saline or Ibogaine Pretreatment: Animals Not Habituated (Extended) to Novel Environment: Time Period Not Extended

Figure 8 shows the effect of cocaine on rearing behavior when cocaine (20 mg/kg SC) was injected without the immediately preceding studies (2 h) of either saline or ibogaine. Cocaine significantly increased rearing frequency over baseline [ANOVA: $F(4, 25) = 48.371$, $p < 0.0001$]. Rearing frequency was significantly increased from a baseline of 4 rears to 16 rears beginning at 10 min ($p < 0.05$, 95% CL). Rearing behavior was maximally increased to 49 rears ($p < 0.05$, 95% CL) at 100 min.

Rearing Behavior: Comparison

A statistical comparison between the rearing effects of cocaine when studied with repeated environmental stimulation and that without, shows that there was no significant difference between the two [ANOVA: $F(1, 23) = 0.236$, $p < 0.6318$]. Therefore, the psychostimulant rearing behavior induced by cocaine did not exhibit the habituated response to cocaine that was seen with both neurochemical responses to cocaine and the behavioral ambulatory response to (SC) cocaine. Therefore, ibogaine did not downmodulate cocaine-induced rearing behavior a response that was not habituated.

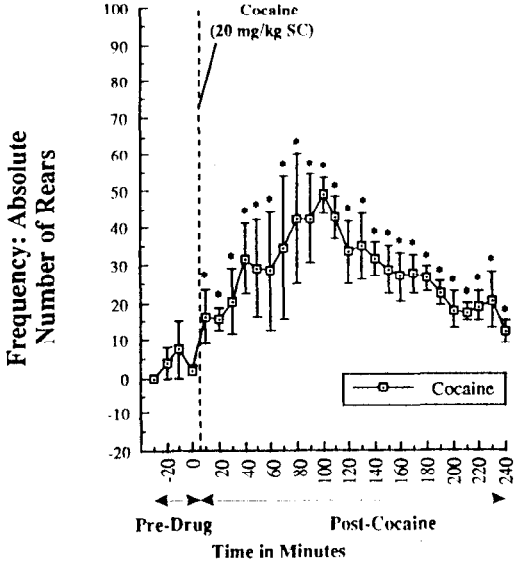

FIG. 8. Rearing behavior: Effect of (SC) cocaine without saline or ibogaine pretreatment: animals not habituated to (not extended habituation) novel environment: time period not extended. The effect of cocaine (20 mg/kg SC) on rearing behavior in freely moving and behaving male Sprague-Dawley rats when cocaine was administered (SC) without an immediately preceding saline or ibogaine study. vlNAcc DA and 5-HT release and ambulatory behavior were studied concurrently (Figs. 4 and 6). *$p < 0.05$; 95% confidence limits (CL). See text for ANOVA statistics ($N = 6$).

Fine Movement Behavior: Animals Not Exhibiting Extended Habituation Response in Novel Environment Although Time Period Was Extended: Saline-Cocaine Group

Figure 9 shows the effect of saline (1 cc/kg IP) on frequency of stereotypy (fine movement behavior), in addition to showing the effects of cocaine (20 mg/kg SC) on fine movement behavior which follow a 2-h saline study. The study was done in the same freely moving and behaving male Sprague-Dawley rats in which vlNAcc DA and 5-HT release were concurrently detected as well as the same animals in which the psychostimulant cocaine-induced behaviors of ambulations and rearing behavior were concurrently monitored.

(a) Phase 1: Effect of saline. Injection of saline had no significant effect on frequency of fine movement behavior for the 2-h time course study [ANOVA: $F(1, 16) = 0.867$, $p < 0.3657$]. The hourly data was a composite of the individual 10-min interval time course data points. Saline had an effect within the first 10 min due to arousal of the animal since exploratory behavior was completed and rest had ensued.

(b) Phase 2: Effect of (SC) cocaine with saline pretreatment. Injection of cocaine (20 mg/kg SC) following the 2-h saline study significantly increased fine movement frequency over baseline [ANOVA: $F(4, 25) = 27.294$, $p < 0.0001$]. Fine movement frequency was significantly increased from 3 fine movements at baseline to 26 fine movements ($p < 0.05$, 95% CL), beginning at 190 min, and was maximally increased to 78 fine movements ($p < 0.05$, 95% CL) at 240 min following baseline. Post hoc analyses show that the statistically significant difference between fine movement frequency and cocaine-induced fine movement frequency was significant for the second to fourth hours following cocaine injection (Fisher's PLSD = 15.264 and Scheffe's $F = 11.405$, 15.123, 13.673, second to fourth hours, respectively).

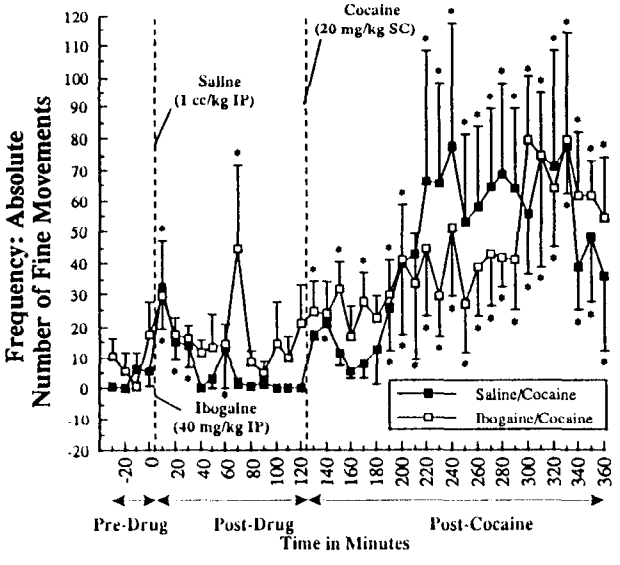

FIG. 9. Fine movement behavior: Animals not habituated (extended) to novel environment although time period was extended: saline-cocaine group and ibogaine-cocaine group. Phase 1: the effect of saline (■) ($N = 4$) or ibogaine (□) ($N = 5$) on fine movements (stereotypic movements of grooming behavior). Either saline (1 cc/kg IP) or ibogaine (40 mg/kg IP) was injected after exploratory behavior was essentially completed and at the time when a stable baseline for DA and 5-HT release in vlNAcc had been achieved. Phase 2: all parameters are exactly the same as described in Figs. 2, 3, 5, and 7 because vlNAcc DA and 5-HT release, ambulations, rearing behavior, and fine movements were separately and concurrently monitored. *$p < 0.05$; 95% confidence limits (CL). See text for ANOVA statistics.

Fine Movement Behavior: Animals Not Exhibiting Extended Habituation Response in Novel Environment Although Time Period Was Extended: Ibogaine-Cocaine Group

Figure 9 also shows the time course effects of ibogaine (40 mg/kg IP) on fine movement frequency, in addition to showing the effect of cocaine (20 mg/kg SC) on fine movement frequency which follows the 2-h ibogaine study. The study was done in the same freely moving and behaving male Sprague-Dawley rats in which vlNAcc DA and 5-HT release, ambulatory, and rearing behavior were studied concurrently (ibogaine-cocaine group).

(a) Phase 1: Effect of (SC) ibogaine. Ibogaine significantly increased fine movement frequency for the first hour of the 2 h following baseline [ANOVA: $F(1, 10) = 5.637$, $p < 0.039$]. Ibogaine significantly increased fine movement frequency at the 10 and 70 min marks ($p < 0.05$, 95% CL). Ibogaine had a similar effect to saline within the first 10 min due to arousal of the animal since exploratory behavior was completed and rest had ensued.

(b) Phase 2: Effect of (SC) cocaine with ibogaine pretreatment. Injection of cocaine (20 mg/kg SC) following the 2-h ibogaine study produced a significantly increased fine movement frequency over baseline [ANOVA: $F(4, 25) = 25.6762$, $p < 0.0001$]. Fine movement frequency was significantly increased from 9 fine movements at baseline to 24 fine movements ($p < 0.05$, 95% CL), beginning at 130 min. Fine movements were maximally increased to 80 fine movements ($p < 0.05$, 95% CL) at 300 min. Post hoc analyses show that the statistically significant differences between basal and cocaine-induced fine movement frequency were significant for each of the 4 h following cocaine injection (Fisher's PLSD = 12.425 and Scheffe's $F = 1.748$, 6.19, 9.313, 22.545, first to fourth hours, respectively).

Comparison Between the Saline and Ibogaine Groups

A comparison between the effect of saline and the effect of ibogaine on fine movement frequency shows that ibogaine significantly increased fine movement frequency over saline in the second hour [ANOVA: $F(1, 5) = 3.095$, $p < 0.1389$, ANOVA: $F(1, 5) = 8.004$, $p < 0.0367$, first and second hours, respectively).

However, a comparison between the effect of cocaine with saline pretreatment vs. ibogaine pretreatment, shows that there was no significant difference between saline and ibogaine on cocaine induced fine movement behavior [ANOVA: $F(1, 23) = 0.814$, $p < 0.3763$] over the 4 h following cocaine injection. However, ibogaine significantly increased cocaine's (20 mg/kg SC) effect on fine movement frequency by 12 fine movements in the first hour [ANOVA: $F(1, 5) = 17.403$, $p < 0.0087$].

Fine Movement Behavior: Effect of (SC) Cocaine Without Saline or Ibogaine Pretreatment: Animals Not Habituated (Extended) to Novel Environment: Time Period Not Extended

Figure 10 shows the effect of cocaine on fine movement behavior when cocaine (20 mg/kg SC) was injected without an immediately preceding 2 h study of either saline or ibogaine.

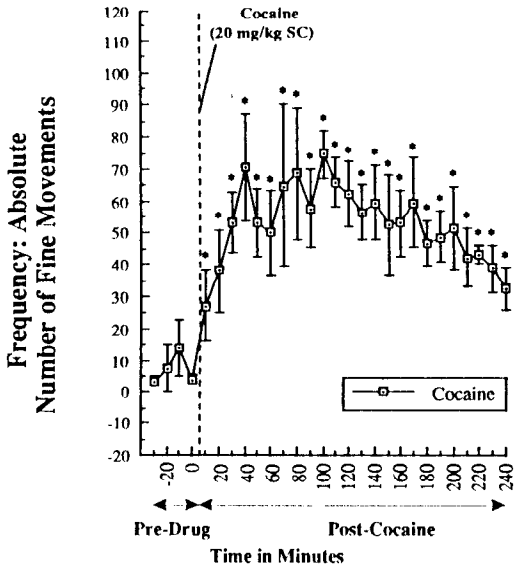

FIG. 10. Fine movement behavior: effect of (SC) cocaine without saline or ibogaine pretreatment: animals not habituated (not extended habituation) to novel environment: time period not extended. The effect of cocaine (20 mg/kg SC) on fine movement behavior when cocaine was administered without an immediately preceding 2-h saline or ibogaine study. vlNAcc DA and 5-HT release and ambulatory behavior were studied concurrently (Figs. 4, 6, and 8). $^*p < 0.05$; 95% confidence limits (CL). See text for ANOVA statistics ($N = 6$).

Cocaine significantly increased fine movement behavior over baseline [ANOVA: $F(4, 25) = 38.817$, $p < 0.0001$]. Fine movement frequency was significantly increased from a baseline of 9 fine movements to 27 fine movements, beginning at the 10 min mark ($p < 0.05$, 95% CL), and fine movements were maximally increased to 74 fine movements ($p < 0.05$, 95% CL) at 100 min.

Fine Movement Behavior: Comparison

A statistical comparison between the stereotypic effects of cocaine when studied with repeated environmental stimulation and that without, show that there were no significant differences [ANOVA: $F(1, 23) = 1.791$, $p < 0.1939$] over the 4 h following cocaine injection. Therefore, the cocaine-induced fine movement response does not exhibit habituation. The effect is similar to rearing. Thus, ibogaine did not downmodulate the cocaine-induced stereotypic behavioral response, a response that was not habituated.

Central Ambulatory Behavior: Animals Habituated (Extended) to Novel Environment: Extended Time Period: Saline-Cocaine Group

Figure 11 shows the effect of saline (1 cc/kg IP) on central ambulation frequency, in addition to showing the effect of cocaine (20 mg/kg SC) on central ambulation frequency which follows the 2-h saline study. The study was done in the same freely moving and behaving male Sprague-Dawley rats in which vlNAcc DA and 5-HT release, ambulatory, rearing, and fine movement behaviors were studied concurrently.

(a) Phase 1: Effect of saline. Injection of saline had no significant effect on central ambulation frequency for the 2-h time course study following baseline [ANOVA: $F(1, 16) = 0.225$, $p < 0.6418$]. The hourly data was a composite of the individual 10- min interval time course data points. Saline had an effect within the first 10 min due to arousal of the animal since exploratory behavior was completed and rest had ensued.

(b) Phase 2: Effect of (SC) cocaine with saline pretreatment. Injection of cocaine (20 mg/kg SC) following the 2-h saline study significantly increased central ambulation frequency over baseline [ANOVA: $F(4, 25) = 38.867$, $p < 0.0001$]. Central ambulation frequency was significantly increased from 0 central ambulations at baseline to 7 ($p < 0.05$, 95% CL), beginning at 170 min. Central ambulatory activity was maximally increased to 28 central ambulations ($p < 0.05$, 95% CL) at 330 min. Post hoc analyses show that the statistically significant differences between basal and cocaine-induced central ambulation frequency were significant for the second to fourth hours following cocaine (20 mg/kg SC) injection (Fisher's PLSD = 4.618 and Scheffe's $F = 12.844$, 22.079, 19.373, second to fourth hours, respectively).

Central Ambulatory Behavior: Animals Habituated (Extended) to Novel Environment: Extended Time Period: Ibogaine-Cocaine Group

Figure 11 also shows the effects of ibogaine (40 mg/kg IP) on central ambulation frequency, in addition to showing the effect of cocaine (20 mg/kg SC) on central ambulation frequency which follows the 2-h ibogaine study. The study was done in the same freely moving and behaving male Sprague-Dawley rats in which vlNAcc DA and 5-HT release, ambula-

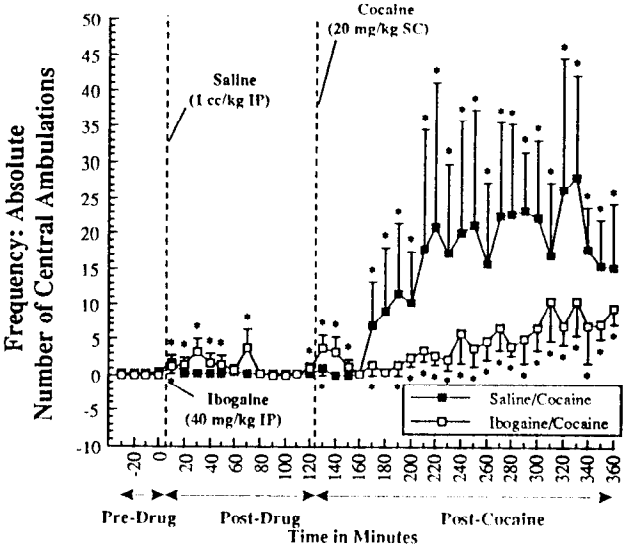

FIG. 11. Central ambulatory behavior: Animals habituated (extended) to novel environment: extended time period: saline-cocaine group and ibogaine-cocaine group. Phase 1: the effect of saline (■) or ibogaine (□) on central ambulations (agoraphobia inhibitions) ($N = 4, 5$, respectively). vlNAcc DA and 5-HT release, ambulations, rearing behavior, fine movements, and central ambulations were concurrently monitored (Figs. 2, 3, 5, 7, and 9). Phase 2: all parameters are exactly the same as described in Figs. 2, 3, 5, 7, and 9 because cocaine-induced effects on DA and 5-HT release and all parameters were concurrently monitored. $^*p < 0.05$; 95% confidence limits (CL). See text for ANOVA statistics.

tory, rearing, and fine movement behaviors were studied concurrently.

(a) Phase 1: Effect of ibogaine. Injection of Ibogaine significantly increased central ambulation frequency for the 2-h time course study [ANOVA: $F(1, 16) = 6.144$, $p < 0.0247$]. The hourly data was a composite of the individual 10-min interval time course data points. Ibogaine significantly increased central ambulation frequency primarily for the first hour of the 2 h following baseline [ANOVA: $F(1, 10) = 21.355$, $p < 0.0009$].

(b) Phase 2: Effect of (SC) cocaine with ibogaine pretreatment. Injection of cocaine (20 mg/kg SC) following the 2-h ibogaine study, significantly increased central ambulation frequency over baseline [ANOVA: $F(4, 25) = 34.319$, $p < 0.0001$]. Central ambulation frequency was significantly increased from 0 central ambulations at baseline to 4 central ambulations ($p < 0.05$, 95% CL), beginning at 130 min and was maximally increased to 10 central ambulations ($p < 0.05$, 95% CL) at 310 min. Post hoc analyses show that the statistically significant differences between basal and cocaine-induced central ambulation frequency were significant for each of the 4 h following cocaine injection (Fisher's PLSD = 1.625 and Scheffe's $F = 1.206, 3.776, 10.581, 28.785$, first to fourth hours, respectively).

Comparison Between the Saline and Ibogaine Groups

A comparison between the effect of saline and the effect of ibogaine on central ambulation frequency shows that there were no significant differences in central ambulation frequency for either hour of the 2 h following baseline [ANOVA: $F(1, 5) = 5.076$, $p < 0.074$, ANOVA: $F(1, 5) = 1.601$, $p < 0.2615$, first and second hours, respectively, following baseline].

However, a comparison between the effect of cocaine with saline pretreatment vs. ibogaine pretreatment shows that ibogaine significantly reduced cocaine induced central ambulation frequency [ANOVA: $F(1, 23) = 52.155$, $p < 0.0001$]. Ibogaine significantly reduced cocaine's effect on central ambulation frequency in the second hour [ANOVA: $F(1, 5) = 74.18$, $p < 0.0003$], in the third hour [ANOVA: $F(1, 5) = 194.786$, $p < 0.0001$], and in the fourth hour [ANOVA: $F(1, 5) = 23.917$, $p < 0.0045$].

Central Ambulatory Behavior: Effect of (SC) Cocaine Without Saline or Ibogaine Pretreatment: Animals Not Habituated (Extended) to Novel Environment: Time Period Not Extended

Figure 12 shows the effect of cocaine on central ambulation behavior when cocaine (20 mg/kg SC) was injected without an immediately preceding study (2 h) of either saline or ibogaine. Cocaine significantly increased central ambulation frequency over baseline [ANOVA: $F(4, 25) = 28.804$, $p < 0.0001$]. Central ambulation frequency was significantly increased from a baseline of two central ambulations to four central ambulations beginning at the 10 min mark ($p < 0.05$, 95% CL). Central ambulations were maximally increased to 51 central ambulations ($p < 0.05$, 95% CL) at 220 min.

Comparisons between the Habituated and Nonhabituated (SC) Cocaine Responses: Central Ambulatory Behavior.

A statistical comparison between the effects of cocaine on central ambulatory behavior when studied with repeated environmental stimulation and that effect without, shows that the repeated stimulation central ambulatory response was significantly reduced in intensity [ANOVA: $F(1, 23) = 128.936$, $p < 0.0001$]. Thus, cocaine-induced central ambulatory or antiagoraphobic behavior undergoes an habituation response. The habituated response was prominent in all 4 h [ANOVA: $F(1, 5) = 15.664$, $p < 0.0108$] [ANOVA: $F(1, 5) = 25.091$, $p < 0.0041$] [ANOVA: $F(1, 5) = 40.28$, $p < 0.0014$] [ANOVA: $F(1, 5) = 118.848$, $p < 0.0001$], first to fourth hours, respectively. Therefore, antiagoraphobic or antithigmotactic behavior, which is thought to represent or reflect an anxiolytic (anxiety-reducing) behavior, undergoes habituation such as that which was seen with the DA and 5-HT response and with the ambulatory response.

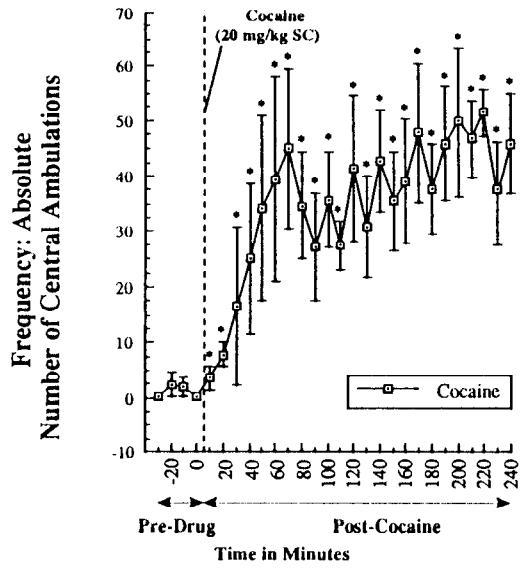

FIG. 12. Central ambulatory behavior: Effect of (SC) cocaine without saline and ibogaine pretreatment: animals not habituated (not extended habituation) to novel environment: time period not extended. The effect of cocaine (20 mg/kg SC) on central ambulatory behavior when cocaine was administered (SC) without the immediately preceding 2-h saline or ibogaine study. vlNacc DA and 5-HT release and psychostimulant behaviors were monitored concurrently (Figs. 4, 6, 8, and 10). *$p < 0.05$; 95% confidence limits (CL). See text for ANOVA statistics ($N = 6$).

Importantly, ibogaine downmodulated the habituated behavioral responses to (SC) cocaine, whereas the behavioral responses that were not diminished by habituation were not significantly affected by ibogaine.

Figure 13 shows activity patterns for the habituated saline-cocaine paradigm described in Figs. 5 and 11, i.e., when cocaine was administered after a 2-h saline study. Ambulatory and central ambulatory moments are shown. The initial stimulation caused by saline injection due to arousal is shown in the first hour postsaline pattern. The habituation response to cocaine is clear as compared with Fig. 14 which describes the nonhabituated response to (SC) cocaine, i.e., when no additional time is spent by the animal in the novel behavioral chamber.

Figure 15 shows activity patterns for the habituated ibogaine-cocaine paradigm, i.e., when cocaine is administered after a 2-h ibogaine study. Ambulatory and central ambulatory moments are shown. Ibogaine itself appears to be a weak psychostimulant; the effects are ongoing in the second hour.

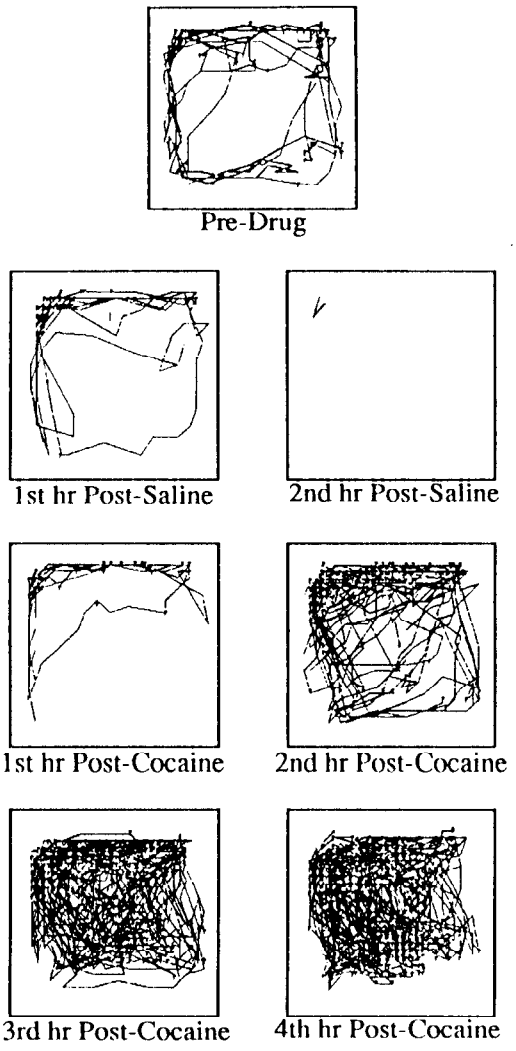

FIG. 13. Representative activity pattern plots showing the effects of saline (1 cc/kg IP) followed by cocaine (20 mg/kg SC) on the spatial and temporal movement patterns of the same male Sprague–Dawley rats in which neurotransmitter release was studied. Each square shaped schematic diagram reflects the floor of the behavioral chamber. The activity markings (plots) within the chamber are integrated ambulatory and central ambulatory behaviors. Rearings are marked with an r. Behavioral data are presented in frequency or number of events.

A comparison of Figs. 13 and 15 shows that although cocaine-induced ambulations are reduced after ibogaine pretreatment, some activity remains which is clearly greater than that seen in the saline-cocaine group.

Tables 1 and 2 show the highly significant correlative values between cocaine-induced 5-HT release in vlNAcc and cocaine-induced psychostimulant behaviors as well as the highly correlative values between cocaine-induced DA release and psychostimulant behaviors, both in the ibogaine-cocaine group (Table 1) and in the saline-cocaine group (Table 2).

DISCUSSION

New findings show that ibogaine significantly downmodulates the cocaine-induced increase in DA release in vlNAcc in the freely moving and behaving environmentally habituated rat model. The important distinction between the present studies and previous studies is that the present data are derived from a freely moving and behaving rat paradigm in which DA responses to (SC) cocaine have been diminished due to environmental habituation, i.e., each animal had a reduced responsiveness to DA release after (SC) cocaine, presumably due to the repeated environmental stimulation in the novel behavioral chamber. Moreover, this effect of (SC) cocaine on DA release on vlNAcc in an extended habituated environment was compared with previous studies in which the paradigm was completely the same with the one exception, that the protocol did not have an extended habituation component (7). The response to cocaine in the habituated environment fits the classical definition of neurochemical habituation, i.e., that repeated action potentials in nerve terminals can accompany behavioral habituation by decreasing the number of open CA^{2+} channels and inactivating synapses (36).

These findings of the habituation aspects of ibogaine-cocaine interactions point to the necessity of a crucial assessment of the previous ibogaine-cocaine literature within the context of the varied experimental paradigms. Although there has been a recent surge of interest in preclinical research on the mechanism of action of ibogaine, there are only two previous papers specifically on ibogaine-cocaine DAergic interactions. One article is from the laboratory of Glick in the U.S. (40), and the other is from the laboratory of Dzoljic in The Netherlands (15). There are relevant differences in paradigms, i.e., a) Glick's laboratory utilized female rats and Dzoljic's

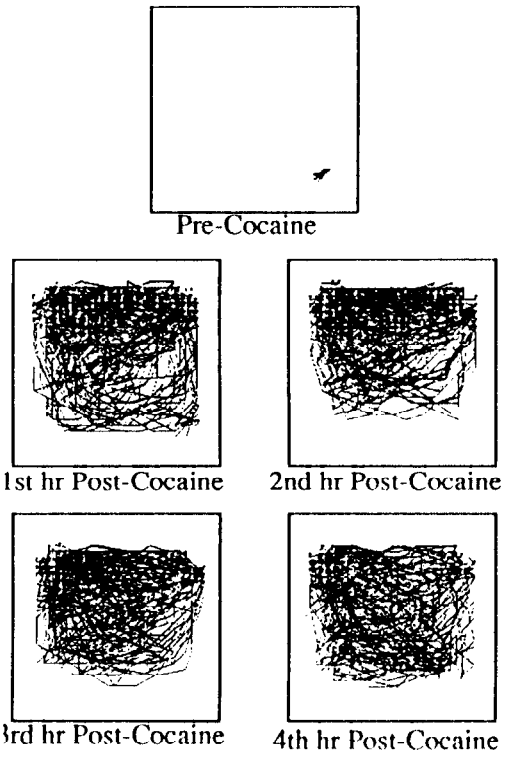

FIG. 14. Representative activity pattern plots when (SC) cocaine was administered without an immediately preceding 2-h saline and ibogaine study. Reprinted from Broderick (7), with permission of Elsevier Science, Ltd.

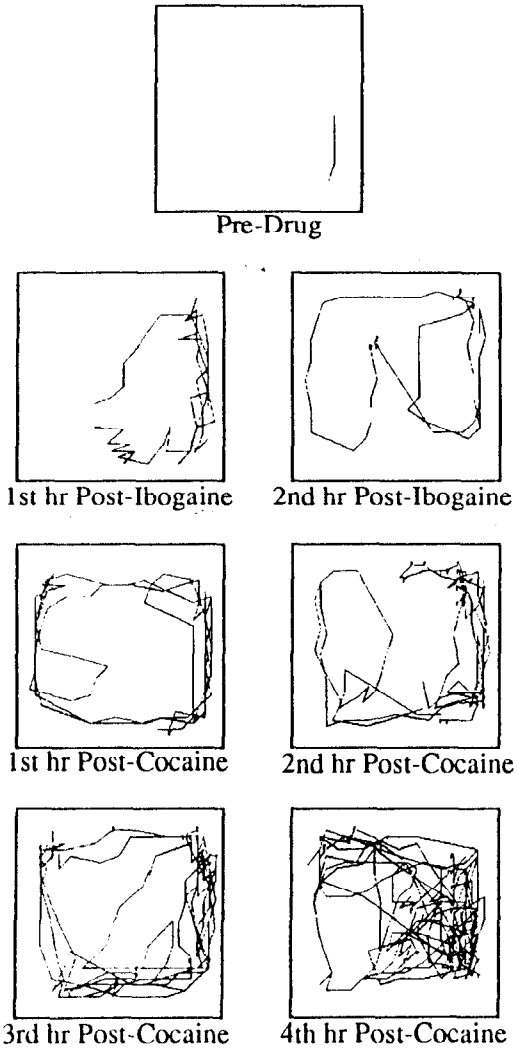

FIG. 15. Representative activity pattern plots showing the effects of cocaine (20 mg/kg SC) on the spatial and temporal movement patterns when a 2-h ibogaine study preceded the (SC) injection of cocaine. All parameters are the same as in Fig. 13.

TABLE 1

(IBOGAINE-COCAINE GROUP) 5-HT RELEASE: PEARSON'S PRODUCT MOMENT OF CORRELATION COEFFICIENT VIS-À-VIS COCAINE-ALTERED DA AND PSYCHOSTIMULANT BEHAVIOR

	5-HT	
	(r)	(z_t)
Dopamine	0.9360	1.7047*
Ambulation	0.8490	1.2562*
Rearing	0.8800	1.3758*
Fine movement	0.8570	1.2933*
Central ambulation	0.8520	1.2562*

*$p < 0.001$.

TABLE 2

(SALINE-COCAINE GROUP) 5-HT RELEASE: PEARSON'S PRODUCT MOMENT OF CORRELATION COEFFICIENT VIS-À-VIS COCAINE-ALTERED DA AND PSYCHOSTIMULANT BEHAVIOR

	5-HT	
	(r)	(z_t)
Dopamine	0.8310	1.1881*
Ambulation	0.8190	1.1568*
Rearing	0.8780	1.3758*
Fine movement	0.8020	1.0986*
Central ambulation	0.8080	1.1270*

*$p < 0.001$.

laboratory utilized male rats. b) Glick's paradigm consisted of administering a single dose of ibogaine (40 mg/kg IP), 19 h before the administration of cocaine, and Dzoljic's laboratory utilized both single doses of ibogaine (10-40 mg/kg IP) and repetitive doses of ibogaine (40 mg/kg IP), and c) Glick's laboratory studied cocaine-induced extracellular concentrations of DA and locomotor behavior, whereas Dzoljic's laboratory studied the ability of ibogaine to affect the self-administration of cocaine. The conclusions were consistent in that a potentiation of the cocaine-induced increase in extracellular concentrations of DA by ibogaine occurred (the ibogaine was administrated 19 h earlier) (40), and there was a long-lasting effect of ibogaine in its reported inhibition of cocaine self-administration (15). Because DA antagonists classically increase the rate of cocaine self-administration (74), yet, given certain caveats, e.g., various rates of responding measures, the previous data may suggest that ibogaine does not act on cocaine self-administration through a direct DA receptor blockade.

Consistent with the present findings is a previous study that reported that ibogaine per se did not significantly affect DA release in NAcc (41). That ibogaine had no effects on extracellular concentrations of DA, as studied by dialysis, was again reported in 1992 by Maisonneuve and Glick (40). It is important to note that postmortem HPLC-EC assays of ibogaine's effects on DA levels later showed that ibogaine decreased DA levels by 50% 1 h after ibogaine injection in NAcc, but 1 month later, DA was entirely unaffected by ibogaine (43). Expectedly, the postmortem assay may have had additional variables as compared with results derived from in vivo microvoltammetry or dialysis studies. Nonetheless, the empirical data, to date, show that the general overall effects of ibogaine per se on NAcc DA release over time are not prominent.

Further support for ibogaine's inability to affect DA presynaptically in central nervous system is gleaned from reuptake studies, i.e., a) ibogaine at concentrations as high as 100 μM did not significantly inhibit [^{3}H] GBR-12935 binding to the DA transporter protein and 1 μM of ibogaine had no effect on in vitro striatal DA reuptake by a P_2 membrane fraction (14), b) in vivo treatment with ibogaine did not affect the binding of [^{3}H] WIN 35,248 to the cocaine binding site in striatal tissue measured in vitro (60), and (c) ibogaine added, in vitro, had a weak affinity for the WIN 35,248 binding site (60). Too, although the data, based on the action of ibogaine on DA receptors are incomplete, neither the D_1 or the D_2 receptors in rat striatum and calf caudate, as studied by radioligands SCH 23390 and *N*-methyl spiperone respectively, were affected by ibogaine (19). Thus, ibogaine may not act through

DA receptors to downmodulate the cocaine induced increase in DA release in vlNAcc at the synapse. Ibogaine may act to downmodulate DA release at the synapse only under conditions of increased DA release and/or reuptake inhibition. Under the usual hypoxic conditions of increased DA release (12), studies of ibogaine and DA function further support the latter hypothesis (16). Thus, ibogaine's action on cocaine-induced DA release may well depend on the availability of DA at the synapse at least in an animal model of environmental habituation. Preliminary data from our laboratory show that ibogaine may not downmodulate DA release in vlNAcc after cocaine administration, when the animal has not undergone an extended habituation to its environment. An habituated environment may be a key to the underlying mechanism of action of cocaine on DA function.

New findings on the interaction of ibogaine and cocaine on 5-HT release in vlNAcc are reported here. Changes in 5-HT release in vlNAcc after cocaine were detected on line and concurrently with the action of cocaine on in vivo detection of DA release. Importantly, the (SC) cocaine response to 5-HT was also habituated; 5-HT release in vlNAcc was reduced by more than 35%. The administration of ibogaine subsequently potentiated the habituated, reduced 5-HT response in vlNAcc seen after (SC) cocaine administration. This is an unexpected finding because ibogaine per se significantly increased 5-HT release in vlNAcc, as shown in Fig. 3. Interestingly, in the presence of (SC) cocaine, it appears that ibogaine acts at the synapse to further decrease 5-HT when 5-HT is less available a priori.

The effects of cocaine on 5-HT release when administered (SC) vs. (IP) in vlNAcc are different. Whereas (SC) cocaine decreases 5-HT release in vlNAcc of freely moving and behaving rats (7), (IP) cocaine increases 5-HT release in vlNAcc of freely moving and behaving rats (13). Whereas ibogaine potentiated the decreased 5-HT release induced by (SC) cocaine, ibogaine also appears to potentiate the (IP) cocaine increase in 5-HT release in vlNAcc as well (preliminary data from this laboratory). Thus, ibogaine seems also to act on 5-HT release, dependent on the availability of 5-HT in the synapse. The mechanism, unlike DA though, is bidirectional.

As far as direct receptor subtype for 5-HT is concerned, it is presently unclear which specific 5-HT receptor subtype is involved in the action of ibogaine. Recent behavioral studies suggest the functional involvement of the 5-HT_2 receptor and the possible functional involvement of the 5-HT_{1A} receptor in the stimulus properties of ibogaine (49). However, direct radioligand binding studies, using DPAT for 5-HT_{1A}, 5-HT for 5-HT_{1B}, mesulergine for 5-HT_{1C}, 5-HT for 5-HT_{1D}, ketanserin for 5-HT_2 and GR65630 for 5-HT_3 show that ibogaine did not bind to any of these receptor subtypes (19). Future studies of ibogaine's interaction with subclassifications of the $5\text{-HT}_{4,5,6 \text{ and } 7}$ receptors may clarify the 5-HT receptor binding properties of ibogaine. Studies of ibogaine's interaction with subclassifications of the 5-HT_2 receptor are currently underway in this laboratory.

A great deal of discussion has evolved about the possibility of ibogaine exerting a neurophysiological influence through a metabolite. Metabolites of iboga have already been studied; antileukemic activity (37) and antitumoral activity have been reported (38). Although, the present studies do not directly address the metabolic aspect of ibogaine, we found that ibogaine's effects on cocaine mechanisms were more dramatic in the later hours. Too, the studies by Dzoljic, Glick, and Sershen point to a study of the possible metabolites of ibogaine, as deserving. Along these lines, it is interesting that the dimeric Vinca alkaloid vinblastine, the well-known antineoplastic agent, undergoes metabolic transformation to iboga by the human serum copper oxidase ceruloplasmin, using chlorpromazine as the shuttle oxidant (25). On the other hand, the dimeric Vinca alkaloid, leurosine, consists of an Iboga substructure which oxidizes to 15-hydroxycatharine (32). Thus, further study on the active metabolites of ibogaine is importantly underway (44).

Summarily, the present data, show that ibogaine per se releases 5-HT in vlNAcc, potentiates the 5-HTergic actions of cocaine, and exhibits highly correlative values with both cocaine-induced DA release and psychostimulant behavior. Therefore, the data suggest that ibogaine acts to regulate cocaine through 5-HTergic neuronal activity in the A_{10} DA circuit. Critical though to ibogaine's manipulation of cocaine effects on biogenic amines and behavior is that the cocaine response had to be habituated for ibogaine to be effective. A role for the habituated response to cocaine, presumably environmental, then, is emphasized, for ibogaine-cocaine interactions.

ACKNOWLEDGEMENTS

The cocaine research was supported in part by NIDA Award R01 DA04755 and PSC/CUNY Awards RF 669201. The ibogaine and ibogaine-cocaine research was supported by PSC/CUNY Awards RF 661188 and 664033 and DHHS BRSG PHS Award 2-S07-RR07132-20 to P. A. Broderick.

REFERENCES

1. Arai, G.; Coppola, J.; Jeffrey, G. A. The structure of ibogaine. Acta Cryst. 13:553–564; 1960.
2. Blaha, C. D.; Lane, R. F. Chemically modified electrode for in vivo monitoring of brain catecholamines. Brain Res. Bull. 10: 861–864; 1983.
3. 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.
4. Broderick, P. A. Distinguishing in vitro electrochemical signatures for norepinephrine and dopamine. Neurosci. Lett. 95:275–280; 1988.
5. Broderick, P. A. Characterizing stearate probes in vitro for the electrochemical detection of dopamine and serotonin. Brain Res. 495:115–121; 1989.
6. Broderick, P. A. State-of-the-Art microelectrodes for in vivo voltammetry. Electroanalysis 2(3):241–251; 1990.
7. Broderick, P. A. Cocaine-on-line analysis of an accumbens amine neural basis for psychomotor behavior. Pharmacol. Biochem. Behav. 40:959–968; 1991.
8. Broderick, P. A. In vivo voltammetric studies on release mechanisms for cocaine with γ-butyrolactone. Pharmacol. Biochem. Behav. 40:969–975; 1991.
9. 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.
10. 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.
11. 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. 46:973–984; 1993.

12. Broderick, P. A.; Gibson, G. E. Dopamine and serotonin in rat striatum during in vivo hypoxic-hypoxia. Metab. Brain Dis. 4: 143-153; 1989.
13. Broderick, P. A.; Kornak, E. P.; Eng, F.; Wechsler, R. W. 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.
14. Broderick, P. A.; Phelan, F. T.; Berger, S. P. Ibogaine alters cocaine-induced biogenic amine and psychostimulant dysfunction but not [^{3}H]GBR-12935 binding to the dopamine transporter protein. NIDA Res. Mono. Series 119:285; 1992.
15. Cappendijk, S. L. T.; Dzoljic, M. R. Inhibitory effects of ibogaine on cocaine self administration in rats. Eur. J. Pharmacol. 241:261-265; 1993.
16. Cretet, E.; Prioux-Guyonneau, M.; Jacquot, C.; Sentenac, H.; Wepierre, J. Effect of tabernanthine on the turnover time of brain catecholamines in normal and hypobaric hypoxic rats. Naunyn Schmiedebergs Arch. Pharmacol. 313:119-123; 1980.
17. DaCosta, L.; Sulklaper, I.; Naquet, R. Modification of awake-sleep equilibrium by tabernanthine and some of its derivatives in the cat. Rev. D Electroencephalogr. Neurophysiol. Clin. 10:105-112; 1980.
18. DaCosta-Rochette, L.; Sulklaper, I.; Tomei, C.; Naquet, R. Restoration of sleep in cats pretreated with tabernanthine *p*-chlorophenoxyacetate (SAD 103). Rev. D Electroencephalogr. Neurophysiol. Clin. 11:147-154; 1981.
19. Deecher, D. C.; Teitler, M.; Soderlund, D. M.; Bornmann, W. G.; Kuehne, M. E.; Glick, S. D. Mechanism of action of ibogaine and harmaline congeners based on radioligand binding studies. Brain Res. 571:242-247; 1992.
20. Depoortere, H. Neocortical rhythmic slow activity during wakefulness and paradoxical sleep in rats. Neuropsychobiology 18: 160-168; 1987.
21. Dhahir, H. I. A comparative study on the toxicity of ibogaine and serotonin. Ph.D. Thesis. Indiana University, IN; 1971.
22. Dhahir, H. I.; Jain, N. C.; Thornton, J. I. The identification of ibogaine in biological material. J. Forensic Sci. Soc. 12:309-313; 1972.
23. Downing, D. F. The chemistry of the psychotomimetic substances. Q. Rev. 16:133-162; 1962.
24. Dzoljic, E. D.; Kaplan, C. D.; Dzoljic, M. R. Effects of ibogaine on naloxone-precipitated withdrawal syndrome in chronic morphine-dependent rats. Arch. Int. Pharmacodyn. Ther. 294:64-70; 1988.
25. Elmarakby, S. A.; Duffel, M. W.; Rosazza, J. P. In vitro metabolic transformations of vinblastine: Oxidations catalyzed by human ceruloplasmin. J. Med. Chem. 32:2158-2162; 1989.
26. Frances, B.; Gout, R.; Cros, J.; Zajac, J. M. Effects of ibogaine on naloxone-precipitated withdrawal in morphine-dependent mice. Fund. Clin. Pharmacol. 6:327-332; 1992.
27. Gershon, S.; Lang, W. J. A psychopharmacological study of some indole alkaloids. Arch. Int. Pharmacodyn. 135:31-56; 1962.
28. 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: A. R. Liss; 1990:81-99.
29. Glick, S. D.; Maisonneuve, I. M.; Carlson, J. N.; Keller, R. W. Interactions of ibogaine with morphine in rats: Drug self-administration and in vivo microdialysis. NIDA Res. Monogr. Series 119:283; 1992.
30. Glick, S. D.; Rossman, K.; Rao, N. C.; Maisonneuve, I. M.; Carlson, J. N. Effects of ibogaine on acute signs of morphine withdrawal in rats: Independence from tremor. Neuropharmacology 31:497-500; 1992.
31. Glick, S. D.; Rossman, K.; Steindorf, S.; Maisonneuve, I. M.; Carlson, J. N. Effects and after effects of ibogaine on morphine self-administration in rats. Eur. J. Pharmacol. 195:341-345; 1991.
32. Goswami, A.; Macdonald, T. L.; Hubbard, C.; Duffel, M. W.; Rosazza, J. P. Leurosine biotransformations: An unusual ring-fission reaction catalyzed by peroxidase. Chem. Res. Toxicol. 1: 238-242; 1988.
33. Grünberger, J.; Saletu, B.; Linzmayer, L.; Stöhr, H. Objective measures in determining the central effectiveness of a new antihypoxidotic SL 76188: Pharmaco-EEG, psychometric and pharmacokinetic analyses in the elderly. Arch. Gerontol. Geriatr. 1:261-285; 1982.
34. Hajo-Tello, N.; Dupont, C.; Wepierre, J.; Cohen, Y.; Miller, R.; Godfraind, T. Effects of tabernanthine on calcium and catecholamine stimulated contractions of isolated vascular and cardiac muscle. Arch. Int. Pharmacodyn. Ther. 276:35-43; 1985.
35. Hamon, G.; Castillon, A.; Gaignault, J. C.; Worcel, M. Peripheral cardiovascular effects of tabernanthine tartrate in anaesthetized rats. Arch. Int. Pharmacodyn. Ther. 276:60-72; 1985.
36. Kandel, E. R. Environmental determinants of brain architecture and of behavior: Early experience and learning. In: Kandel, E. R.; Schwartz, J. H.. eds. Principles of neuroscience. Amsterdam: Elsevier North Holland Inc.; 1981:620-632.
37. Kingston, D. G.; Sami, S. M. Cytotoxicity of modified indole alkaloids. J. Pharmaceut. Sci. 68:1403-1405; 1979.
38. Kutney, J .P. Studies on the total synthesis of bisindole alkaloids in the vinblastine-vincristine series. Lloydia 40:107-126 1977.
39. Lotsof, H. Rapid method for interrupting the cocaine and amphetamine abuse syndrome, Patent No. 4,587,253; 1986.
40. Maisonneuve, I. M.; Glick, S. D. Interactions between ibogaine and cocaine in rats: In vivo microdialysis and motor behavior. Eur. J. Pharmacol. 212:263-266; 1992.
41. Maisonneuve, I. M.; Keller, R. W., Jr.; Glick, S. D. Interactions between ibogaine, a potential anti-addictive agent and morphine: An in vivo microdialysis study. Eur. J. Pharmacol. 199:35-42; 1991.
42. Maisonneuve, I. M.; Keller, R. W., Jr.; Glick, S. D. Interactions of ibogaine, and D-amphetamine: In vivo microdialysis and motor behavior in rats. Brain Res. 579:87-92; 1992.
43. Maisonneuve, I. M.; Rossman, K. L.; Keller, R. W., Jr.; Glick, S. D. Acute and prolonged effects of ibogaine on brain dopamine metabolism and morphine-induced locomotor activity in rats. Brain Res. 575:69-73; 1992.
44. Mash, D. C.; Sanchez-Ramos, J.; et al. Ibogaine (HCl) (Endabuse®): Pharmacokinetic/pharmacodynamic and safety trial. Investigational new drug study. US IND #39680; 1993.
45. Miller, R. C.; Godfraind, T. The action of tabernanthine on noradrenaline-stimulated contractions and 45 Ca movements in rat isolated vascular smooth muscle. Eur. J. Pharmacol. 96:251-259; 1983.
46. Mocaër-Cretet, E.; Prioux-Guyonneau, M.; Redjimi, F.; Cohen, Y.; Jacquot, C. Evidence for an antagonistic action of tabernanthine on hypoxia-induced changes in brain serotonin levels. Naunyn Schmiedebergs Arch. Pharmacol. 326:287-290; 1984.
47. O'Hearn, E.; Long, D. B.; Molliver, M. E. Ibogaine induces glial activation in parasagittal zones of the cerebellum. Neuroreport 4: 299-302; 1993.
48. O'Hearn, E.; Molliver, M. E. Degeneration of Purkinje cells in parasagittal zones of the cerebellar vermis after treatment with ibogaine or harmaline. Neuroscience 55:303-310; 1993.
49. Palumbo. P. A.; Winter, J. C. Stimulus effects of ibogaine in rats trained with yohimbine, DOM or LSD. Pharmacol. Biochem. Behav. 43:1221-1226; 1992.
50. Pellegrino, L. J.; Pellegrino, A. S.; Cushman, A. J. A stereotaxic atlas of the rat brain. New York: Plenum Press; 1979.
51. Phelix, C. F.; Jackson, T.; Broderick, P. A.; Wayner, M. J. Immunohistochemical analysis of serotonin, dopamine and GABA in dorsal and ventral striatum. Physiologist 34:A11; 1993.
52. Phelix, C. F.; Tshoepe, L.; Broderick, P. A. Convergence of serotonin and dopamine in ventrolateral nucleus accumbens: Anatomical and in vivo electrochemical analysis. Soc. Neurosci. Abstr. 19:1827; 1993.
53. 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.
54. Ross, S. B.; Renyi, A. L. Inhibition of the uptake of tritiated 5-hydroxytryptamine in brain tissue. Eur. J. Pharmacol. 7:270-277; 1969.
55. Schechter, M. D. Cocaine discrimination is attenuated by isradipine and CGS 10746B. Pharmacol. Biochem. Behav. 44:661-664; 1993.
56. Scheel-Kruger, J.; Baestrup, C.; Nielson, M.; Golembiowska, K.;

Mogilnicka, E. Cocaine: Discussion on the role of dopamine in the biochemical mechanism of cocaine action. Adv. Behav. Biol. 21:373–407; 1977.
57. Schneider, J. A.; Sigg, E. B. Neuropharmacological studies on ibogaine, an indole alkaloid with central stimulant properties. Ann. NY Acad. Sci. 66:765–766; 1957.
58. Schultes, R. E. The plant kingdom and hallucinogens. Bull. Narcotics XXI(3):4; 1969.
59. Sershen, H.; Harsing, L. G., Jr.; Hashim, A.; Lajtha, A. Ibogaine reduces amphetamine-induced locomotor stimulation in C57 BL/6 By mice but stimulates locomotor activity in rats. Life Sci. 51:1003–1011; 1992.
60. Sershen, H.; Hashim, A.; Harsing, L.; Lajtha, A. Ibogaine antagonizes cocaine-induced locomotor stimulation in mice. Life Sci. 50:1079–1086; 1992.
61. Sharpe, L. G.; Jaffe, J. H. Ibogaine fails to reduce naloxone-precipitated withdrawal in the morphine dependent rat. Neuroreport 1:17–19; 1990.
62. Singbartl, G.; Zetler, G; Schlosser, L. Structure-activity relationships of intracerebrally injected tremorigenic indole alkaloids. Neuropharmacology 12:239–244; 1973.
63. Sloviter, R. S.; Drust, E. G.; Damiano, B. P.; Conner, J. D. A common mechanism for lysergic acid, indolealkylamine and phenethylamine hallucinogens: Serotonergic medication of behavioral effects in rats. J. Pharmacol. Exp. Ther. 214:231–238; 1980.
64. Swanson, L. W.; Cowan, W. M. A note on the connections and development of the nucleus accumbens. Brain Res. 92:324–330; 1975.
65. Taylor, W. I. Indole alkaloids, an introduction to the enamine chemistry of natural products. New York: Pergamon Press; 1966.
66. Taylor, W. I. Iboga alkaloids. The structure of ibogaine, ibogamine and tabernanthine. J. Am. Chem. Soc. 79:3298; 1957.
67. Touchette, N. Ibogaine neurotoxicity raises new questions in addiction research. J. NIH Res. 5:50–55; 1993.
68. Tricklebank, M. D.; Forler, C.; Fozard, J. R. The involvement of subtypes of the 5-HT_1 receptor and of catecholaminergic systems in the behavioral response to 8-hydroxy-2-(di-n-propylamino)-tetralin in the rat. Eur. J. Pharmacol. 106:271–282; 1984.
69. Trouvin, J. H.; Jacqmin, P.; Rouch, C.; Lesne, M.; Jacquot C. Benzodiazepine receptors are involved in tabernanthine-induced tremor: in vitro and in vivo evidence. Eur. J. Pharmacol. 140: 303–309; 1987.
70. Valette, G.; Leclair, M. F. The effect of alkaloids from tabernanthe iboga H. Bn. on the response of isolated organs to catecholamines and the possible role of calcium exchange. C. R. Acad. Sci. [D] 285:591–594; 1977.
71. Van Bockstaele, E. J.; Cestari, D. M.; Pickel, V. Synaptic structure and connectivity of serotonin terminals in the ventral tegmental area: Potential sites for modulation of mesolimbic dopamine neurons. Brain Res. 647:307–322; 1994.
72. Van Bockstaele, E. J.; Pickel, V. Ultrastructure of serotonin-immunoreactive terminals in the core and shell of the rat nucleus accumbens: Cellular substrates for interactions with catecholamine afferents. J. Comp. Neurol. 334:603–617; 1993.
73. Wightman, R. M. Microvoltammetric electrodes. Anal. Chem. 53:1125A–1134A; 1981.
74. Woods, J. H.; Herling, S.; Winger, G. Chlorpromazine and haloperidol-induced changes in some behavioral effects of cocaine and amphetamine. In: Deniker, P., Radouco-Thomas, C.; Villeneuve, A., eds. Proceedings of the Tenth congress of the Collegium International Neuropsychopharmacologium. New York: Pergamon Press; 1978:1485–1502.
75. Zetler, G. Cholecystokinin octapeptide (CCK-8), ceruletide and analogues of ceruletide: Effects on tremors induced by oxotremorine, harmine and ibogaine, a comparison with prolyl-leucyl-glycine amide (MIF), anti-parkinsonian drugs and clonazepam. Neuropharmarmacology 22:757–766; 1983.
76. Zetler, G.; Singbartl, G.; Scholsser, L. Cerebral pharmacokinetics of tremor-producing harmala and iboga alkaloids. Pharmacology 7:237–248; 1972.