1998

Neurochemical and behavioral tolerance to cocaine following treatment with cocaine analogs

Tobias Tong-Po Lee

Yale University

Follow this and additional works at: http://elischolar.library.yale.edu/ymtdl

NEUROCHEMICAL AND BEHAVIORAL TOLERANCE TO COCAINE FOLLOWING TREATMENT WITH COCAINE ANALOGS

TOBIAS TONG-PO LEE

Yale University

1998

Neurochemical and Behavioral Tolerance to Cocaine Following Treatment with Cocaine Analogs

A Thesis Submitted to the
Yale University School of Medicine
in Partial Fulfillment of the Requirement for the
Degree of Doctor of Medicine

By

Tobias Tong-Po Lee

1998

NEUROCHEMICAL AND BEHAVIORAL TOLERANCE TO COCAINE FOLLOWING TREATMENT WITH COCAINE ANALOGS. Tobias T. Lee, Peter Jatlow, and Charles W. Bradberry. Departments of Laboratory Medicine and Psychiatry, Yale University , School of Medicine, New Haven, CT.

ABSTRACT: The search for a pharmacologic treatment of cocaine addiction has lead to the study of possible cocaine analogs to be used as substitution therapy, much like methadone is used in heroin addiction. The goal is to identify compounds which may produce cross-tolerance to cocaine and thus blunt cocaine's euphoric effects. The effects of acute (three hours) and chronic (seven days) exposure to intravenous cocaine, cocaethylene, or isopropyl cocaine on the response to a subsequent cocaine challenge were examined. Neurochemical (microdialysis nucleus accumbens dopamine level measurements) and behavioral (locomotor activity) measures were compared. The relative potency of isopropyl cocaine compared to cocaine in the anesthetized rat was also examined. The results of the acute tolerance experiments showed no behavioral tolerance in any group; however, there was a decrease in dopamine response to a cocaine challenge in the cocaine- and cocaethylene-pretreated groups. Though acute neurochemical tolerance was suggested, these data need to be interpreted with caution as the cocaine pretreatment group had a sustained, high baseline during the three hour maintenance which may be the animals' maximal response to cocaine. Cocaethylene seemed to produce acute tolerance to itself during the three hour maintenance phase as neither the neurochemical nor the behavioral effects were sustained. Chronically, cocaine- and cocaethylene-pretreated animals demonstrated a reduced response to a subsequent cocaine challenge compared to placebo; the isopropyl cocaine pretreated animals behaved no differently from the placebo group. Isopropyl cocaine is much less potent than cocaine in its ability to elevate dopamine in both awake and anesthetized animals. Most significantly, these studies show that behavioral tolerance was achieved after one week of cocaethylene pretreatment. Cocaethylene or its related analogs shows some promise as a possible pharmacological treatment of cocaine addiction as chronic treatment blunts the subsequent effects of cocaine.

Neurochemical and Behavioral Tolerance to Cocaine Following Treatment with Cocaine Analogs

By Tobias T. Lee

Yale University School of Medicine

Faculty Advisors

Peter Jatlow, MD

Charles Bradberry, PhD

14 March 1998

Table of Contents

1. INTRODUCTION

The 1985 National Household Survey on Drug Abuse estimated the number of individuals over the age of 12 that have tried cocaine to be approximately 25 million in the United States (1988). This increasing problem has lead to continued interest in research in both cocaine and its analogs, both to understand the nature and effects of cocaine and also to search for possible pharmacological modalities to treat cocaine dependence (Leshner, 1996; Self *et al.*, 1996).

Currently there are no effective treatments for cocaine addiction. Studies evaluating the treatment of this illness with nonpharmacological methods such as talk therapy or acupuncture have yielded poor results so far. While effective medications have yet to be identified, the approaches for finding a pharmacological treatment for cocaine addiction have been many: some researchers search for an agent that would treat the addiction by acting as a cocaine antagonist much like naloxone acts in opioid overdoses, others explore the use of antidepressants to decrease craving and subsequent drug-seeking behavior as with buproprion for treatment of nicotine addiction. In this thesis, compounds which can induce cross-tolerance to subsequent cocaine use are examined to see if these cocaine analogs have potential for therapy. If tolerance to cocaine can be successfully shown to occur with chronically administered cocaethylene or isopropyl cocaine, then there may be a potential benefit if these or other cocaine analog can be used to treat cocaine abusers much in the same way that methadone is used currently to maintain heroin addicts.

It is presently believed that cocaine and other psychoactive drugs cause reinforcement in animals and presumably euphoria in humans through the elevation of dopamine levels in the nucleus accumbens; cocaine is also known to elevate extracellular concentrations of other monoamine neurotransmitters (Bradberry *et al.*, 1993; Koob and Bloom, 1988; Ritz *et al.*, 1987). The increases in extracellular neurotransmitter concentrations are believed to be due to cocaine's binding to monoamine transporters thereby preventing dopamine, norepinephrine, and serotonin reuptake after initial release (Hammer and Cooke, 1994).

Drug tolerance is defined as an attenuation of drug responsiveness resulting from prior exposure to the drug (Pan *et al.*, 1991). By producing tolerance in an experimental subject, a constant amount of cocaine will produce a reduced euphoric effect. Numerous studies have demonstrated tolerance in the rat model with chronic cocaine administration (Emmett-Oglesby *et al.*, 1993; Frank *et al.*, 1992; Katz *et al.*, 1992; Masserano *et al.*, 1994).

Between 70% and 80% of cocaine abusers concurrently use ethanol (Grant and Harford, 1990). Cocaethylene is a cocaine analog and metabolite formed *in vivo* following concurrent cocaine and ethanol consumption as a result of enzymatic transesterification (Bailey, 1993; Boyer and Petersen, 1992; Dean *et al.*, 1991; Jatlow *et al.*, 1991). It is pharmacologically active and produces a euphoric effect in humans that is similar to cocaine but is eliminated more slowly (Bradberry *et al.*, 1993; Hearn *et al.*, 1991; McCance *et al.*, 1995; McCance-Katz *et al.*, 1993). Cocaethylene binds equipotently to cocaine at dopamine transporters but exhibits a lower binding affinity to serotonin transporters than cocaine (Elsworth *et al.*, 1993; Hearn *et al.*, 1991). Isopropyl cocaine is an analog of cocaine with an isopropyl moiety substituted for the methyl group. Many of the properties of isopropyl cocaine are currently under study. This analog is even more selective for the dopamine transporter than cocaethylene (Elsworth *et al.*, 1993; Jatlow, 1993). Differences in monoamine transporter selectivity may alter the neurotransmitter pattern of neurochemical tolerance resulting from effects of cocaine analogs, thus altering the subjective effects of subsequent cocaine. The structures of cocaine, cocaethylene, and isopropyl cocaine are presented in *Figure 1*.

Three groups of experiments have been conducted in this study. Acute tolerance was examined in the first experiment. The effects of an intravenous bolus and constant infusion of cocaine or cocaine analog on a rat that is free to move about a cage followed by a cocaine challenge were examined. Extracellular dopamine levels in the nucleus accumbens were measured using the technique of microdialysis. These data were collected along with photocell measurements of rat locomotor activity. Second, the dopamine response in the nucleus accumbens of an anesthetized rat to challenges of cocaine or cocaine analog were

measured to compare the potency of isopropyl cocaine at increasing extracellular dopamine. Finally, the effects of a continuous seven day constant infusion of cocaine, cocaethylene, and isopropyl cocaine on the behavioral activation in animals given a subsequent challenge of cocaine were determined. This experiment addressed the issue of behavioral response to a cocaine challenge after a chronic pretreatment with an analog.

The hypothesis to be tested was that continuous infusion of cocaine, cocaethylene, or isopropyl cocaine would blunt response to later cocaine challenge. Two different lengths of time for the maintenance phase were examined: one study included a three hour constant infusion phase while the other a week long period of pre-treatment. Also, further characterization of cocaine, cocaethylene, and isopropyl cocaine was performed.

All experiments and procedures (including the construction and stereotactic insertion of the microdialysis probes) for this thesis were performed by me except for the post-mortem plasma drug analysis using extraction and HPLC which was performed in Dr. Jatlow's laboratory and the tail vein injections during the anesthetized rat studies which were performed by Dr. Charles Bradberry.

2. MATERIALS AND METHODS

2.1 Introduction for Acute Tolerance Studies

Male Sprague-Dawley rats (obtained from CAMM) weighing between 250–450 grams were used in these studies. Food and water were provided *ad libitum* at all times. All animal use procedures were in strict accordance with the NIH *Guide for the Care and Use of Laboratory Animals* (1985) and were approved by the local Animal Studies Committee. For the Acute Tolerance Studies, the animals were anesthetized during which time both a microdialysis guide cannula and a jugular catheter were implanted. The rat was allowed to recover for at least two full days but not longer than one week prior to studies. Preparation for an experiment included stereotactic insertion of the microdialysis probe in the nucleus accumbens through the guide cannula and connecting the animal to a liquid swivel in the cages; this procedure required minimal halothane administration. The rats were allowed to recover overnight during which behavioral data were collected. The

next day, extracellular dopamine concentrations in the nucleus accumbens were measured with *in vivo* microdialysis prior to, during, and subsequent to drug challenge at 20 minute intervals, and the number of non-consecutive breaks of infrared photo beams using an infrared detector was recorded at five minute intervals for behavioral measurements; both were continued for the entire experiment. After a baseline of two hours, a bolus followed by a constant infusion of drug was administered through the jugular catheter. This first bolus and infusion of drug was cocaine, cocaethylene, or isopropyl cocaine respectively. Three hours later, a challenge of cocaine was given by bolus and dialysis samples were collected for another hour with no more drug administered. At the end of the hour the animal was sacrificed following equithesin anesthesia. Blood was collected, centrifuged, and the plasma was later analyzed. Probe placement was verified by analyzing postmortem, formaldehyde-fixed brains stained with cresyl violet and sliced in 100 μm sections.

2.2 Surgery for Acute Tolerance Studies

Rats were placed in an induction box and given 5% halothane mixed with 80% oxygen. Upon induction, it was immediately removed and given an intraperitoneal injection of equithesin (0.35 mL/100 gm) (see *Table 1: Equithesin*). The equithesin was allowed at least five minutes to take effect before the surgery began. Additional anesthetic was given as needed throughout the procedure to insure surgical anesthesia. Attention was paid to sterile technique. Rat body temperature was maintained with an electric heating pad set at 37° C.

TABLE 1: EQUITHESIN		
nembutal (50 mg/mL pentobarbital)	16.20	mL
chloral hydrate	4.25	gm
propane-1,2-diol (propylene glycol)	39.60	mL
absolute ethanol	10.00	mL
add to volume of 100 mL with H_2O		

The fur above the skull and in the upper portions of the thorax was shaved; care was taken not to damage the whiskers so as not to affect behavior. An incision approximately 1.5–2 cm in length was made along the right midclavicular line above the point where pulsation was found in the supine animal. Careful dissection yielded the jugular vein which was exposed. The vein was tied off with 2–0 silk surgical thread. The animal was then turned over, and an incision approximately 2 cm long was made midsagitally along the top of the skull. Using scissors, a subcutaneous path was made from the caudal aspect of the incision at the head to the exposed jugular vein. The jugular catheter was then passed through the subcutaneous path. A small nick was made in the jugular vein proximal to the occlusion and the jugular catheter was inserted to the right atrium of the heart.

The jugular catheter was made with 13 cm of silastic medical grade tubing (Dow Corning, 0.020 inch i.d., 0.037 inch o.d.). At the end to be inserted into the jugular vein, two small beads of 6382 RTV Silicone Elastomer (Factor II, AZ) were placed 3.5 cm from the end to hold the catheter in position *in vivo*. The other end consisted of a piece of 23 gauge tubing bent to form an L shape attached to the silastic tubing by multipurpose sealant (Dow Corning 732 RTV). Before surgery, heparinized saline was run into the jugular catheter to prevent air emboli.

Once inserted to the beads, the jugular catheter was tied in place with more surgical thread. Proper placement was checked by syringe aspiration. A small amount of heparinized saline was then injected into the rat to clear the jugular cannula of blood and ensure patency. The rat was then placed in a stereotaxic frame after the thoracic wound was closed.

After the rat was placed in the stereotaxic frame in the flat skull position, the skull was exposed from the previous incision by spreading the layers of the scalp using cotton swabs. An 18 gauge stainless-steel guide cannula of 8 mm length was implanted 1.0 mm below the skull surface over the nucleus accumbens using the coordinates AP +1.7 mm, L −1.5 mm, and V −8.3 mm from bregma (Paxinos and Watson, 1982). Two stainless-steel screws were placed in the skull to secure the dental acrylic which anchored in place the microdialysis guide cannula, the jugular catheter L piece, and the slotted screw used by the

liquid swivel for awake animal experiments. The rats were then returned to their cages to recover from one to six days with free access to water and food before being placed in the experimental set-up.

2.3 Microdialysis

Concentric-style microdialysis probes (Bradberry *et al.*, 1993; Bradberry and Roth, 1989; Johnson and Justice, 1983) were used. A hollow dialysis fiber (300 μm i.d., 330 μm o.d.) with one end epoxy-sealed and containing a fused silica fiber (102 μm i.d., 163 μm o.d.) was housed in a 23 gauge (0.64 mm o.d.), thin-walled hypodermic tube. The outlet opening was through another piece of fused silica placed into the hypodermic tube. Epoxy was applied as sealant at the end of the dialysis fiber and spread generously along both open ends of the hypodermic tube to provide a water-tight seal around the fused silica. This forced the perfusion buffer to travel in a path from the inlet opening along the dialysis membrane and out the outlet openings.

The standard probe was housed in a cut 100 μL pipette tip (not shown in *Figure 2: Microdialysis Probe*) filled with epoxy to prevent damage to the probe body both during placement and also during the experiments for the Acute Tolerance studies. The rat was allowed to run freely in a standard cage. The pipette tip, fixed at a measured distance from the microdialysis tip, also provided a guide as to how far the probe needed to be inserted in the experimental animal: full insertion of the probe in the guide cannula (placed during surgery) to the pipette tip determined the precise dorsal-ventral distance of 7 mm below the skull. The length of the exposed dialysis membrane in the probe was 2 mm which allowed collection of dopamine along the entire dorsal-ventral aspect of the nucleus accumbens.

The microdialysis probe was inserted into the study animal under anesthesia (2% halothane and 80% oxygen) one day prior to the experiment. Then the animal was attached to the liquid swivel and placed in the experimental cage. Probes were perfused overnight with artificial cerebral spinal fluid (ACSF), containing in mM: KCl 2.4, NaCL 137, $CaCl_2$ 1.2, $MgCl_2$ 1.2, NaH_2PO_4 0.9, and ascorbic acid 0.08, at pH 7.4 at a flow rate of 1 μL/min by infusion pump. On the day of the experiment the flow rate was increased to

2 μL/min for at least half an hour prior to collection of the first sample. Samples were collected through fused silica lines by a fraction collector every 20 minutes for the duration of the experiment and were analyzed using HPLC.

2.4 Behavioral Studies for Acute Tolerance Studies

Rat behavioral data were collected in the standard experimental cages which were modified by the addition of four infrared detectors spaced evenly along the length 1.75 inches from the cage floor. The Med-PC program (MED Associates & Thomas A. Tatham version 2) running on an IBM compatible computer recorded the number of non-consecutive beam breaks a rat made at five minute intervals until the conclusion of the experiment. On the night previous to the experiment day (after microdialysis probe insertion) behavioral data were recorded for the rats placed in the cages to assess any circadian rhythm variations.

2.5 Schedule and Drug Administration for Acute Tolerance Studies

On the morning of the study day, the ACSF was increased from the overnight flow rate of 1 μL/min to 2 μL/min for at least half an hour prior to collection of the first microdialysis baseline sample. Collection of baseline behavioral information and baseline dopamine concentration levels at the nucleus accumbens was begun at the same time and continued for two hours prior to the first bolus of study drug. As previously mentioned, behavioral data were collected at five minute intervals, and microdialysis samples were collected at 20 minute intervals.

After a two hour baseline period, the first bolus of 3 μmol/kg of study drug—cocaine, cocaethylene, or isopropyl cocaine—was infused into the jugular catheter over 30 seconds. This was immediately followed by a maintenance flow rate of the bolused drug at 17 μL/min of the same 3 μmol/mL solution. The maintenance rate was calculated to achieve an approximate blood plasma concentration goal of 200 ng/mL and was based on a reported clearance rate of 73.5 ± 9.5 mL/min and 82.8 ± 7.4 mL/min for intravenous cocaine and cocaethylene respectively (Nobiletti et al., 1994). These clearance rates were not significantly different. Clearance was estimated as 80 ml/min for both compounds. 200 ng/ml x 80 ml/min = 16 μg/min infusion rate. Isopropyl cocaine clearance rates were

estimated to be similar though currently no studies have yet been done to quantify the pharmacokinetic properties of this compound.

TABLE 2: STUDY DRUGS			
drug	*salt MW*	*active component MW*	*infusion rate*
cocaine • HCl	339.9	303.4	17.3 μg/min (51 nmol/min)
cocaethylene • fumarate	491.5	317.4	25.1 μg/min (51 nmol/min)
isopropyl cocaine • HCl	368.0	331.5	18.7 μg/min (51 nmol/min)
MW: molecular weight			

Three hours after the initial bolus, the constant infusion of drug was stopped and a challenge of 3 μmol/kg cocaine in a 3 μmol/mL solution was given intravenously with microdialysis and behavioral data continuing for one hour. The rats were then immediately anesthetized with 0.12 mL/100 gm equithesin intravenously through the jugular catheter and transported in a closed box with halothane to be sacrificed. The plasma was collected in a vacutainer containing potassium oxalate and sodium fluoride, vortexed for 10 seconds, and centrifuged for three minutes. The plasma was then removed and frozen for later extraction and analysis. The brains were removed and placed in formalin; later, 100 μm coronal sections were made by vibratome and stained with cresyl violet to verify probe placement. Drug concentrations in the rat during the experiment were verified by plasma analysis using extraction, and HPLC was performed in Dr. Jatlow's laboratory using a method previously described (Jatlow and Nadim, 1990).

2.6 Chromatography

Determination of dialysate dopamine content was performed using liquid chromatography with microbore columns packed in the laboratory with 3 μm Spherisorb ODS2 particles and electrochemical detection (Eapp: +0.6 V *vs* Ag/AgCl reference, Bioanalytical System, West Lafayette, IN). The output was to a strip chart recorder. Mobile phase consisted of 9 mg/L sodium monophosphate, 320 mg/L sodium octanesulfonate, 120 mg/L methanol, 0.1 mM disodium EDTA, and 300 μL triethylamine.

The pH was adjusted with sodium hydroxide or phosphoric acid until its value was 5. Mobile phase was pumped using an ESA 580 pump at a flow rate of 0.200 mL/min and samples were injected by ESA 465 autosampler.

2.7 Isopropyl Cocaine Dosing Studies in the Anesthetized Rat

Dopamine response studies comparing various dosages of isopropyl cocaine to cocaine were conducted with chloral hydrate anesthetized rats using a microdialysis procedure previously described (Bradberry *et al.*, 1993). An intraperitoneal injection of 400 mg/kg chloral hydrate was given to the rat; additional chloral hydrate supplement was provided as needed to maintain surgical anesthesia for the entire duration of the experiment. After chloral hydrate administration, the rat was placed in the flat skull position, and the skull was exposed as described in section 2.2, *Surgery for Acute Tolerance Studies*. No guide cannula was placed as in the awake animal experiments; rather, a standard probe was lowered slowly to the nucleus accumbens—AP +1.7 mm, L –1.5 mm, and V –8.3 mm from bregma. The probe position was secured with methacrylate adhesive. The rat was then removed from the stereotaxic frame for the experiment. Rat body temperature was maintained with an electric heating pad set at 37° C. The perfusion buffer for microdialysis was identical to that used in the experiment described earlier (section 2.3, *Microdialysis*) and flowed at 2 μL/min. Samples were collected every 20 minutes. A stable baseline dopamine level was confirmed by a two hour collection period before drug infusion. After the baseline period, cocaine at 3 μmol/kg; isopropyl cocaine at 6, 12, or 24 μmol/kg; or both cocaine at 3 μmol/kg and isopropyl cocaine at 12 μmol/kg was infused in the rat by tail vein injection over 30 seconds. Dopamine concentrations in the dialysate were measured for another two hours as described in section 2.6, *Chromatography*.

2.8 Chronic Tolerance Studies

These rats were prepared with a jugular catheter as described in section 2.2, *Surgery for Acute Tolerance Studies*. In addition, an Alzet 2ML1 osmotic pump was implanted subcutaneously on the back, midline and slightly posterior to the scapulae. The 2ML1 pump solution was released at a rate of 10.0 μL per hour for seven days which delivered

drug into the free jugular vein via silastic medical grade tubing. Thus bilateral jugular veins were catheterized; venous blood flow occurred through collaterals. Care was paid to correct filling, incubation, and sterile handling of the pump as prescribed by Alzet. A free loop of silastic tubing was left exposed at the back of the neck after pump implantation to allow ligation and termination of continuous fluid infusion during the experiment. The minipump was filled with sterile water (control), cocaine solution, cocaethylene solution, or isopropyl cocaine solution in amounts given in the following *Table 3: Minipump Study Drugs*. Desired plasma levels were 85 to 90 ng/ml for the cocaine and cocaethylene animals. As can be seen, isopropyl cocaine was infused at one half the rate of the other two test drugs because of limited availability of this specially synthesized compound. The experimental design did not permit verification of plasma levels.

TABLE 3: MINIPUMP STUDY DRUGS			
drug	*salt MW*	*active component MW*	*infusion rate*
cocaine • HCl	339.9	303.4	7.7 μg/min (22.6 nmol/min)
cocaethylene • fumarate	491.5	317.4	11.1 μg/min (22.6 nmol/min)
isopropyl cocaine • HCl	368.0	331.5	4.6 μg/min (11.3 nmol/min)
MW: molecular weight	*1.68 mL total delivered by pump for week at 10 μL per hour*		

No microdialysis was conducted on these animals. Behavioral data were collected overnight on the sixth day after implantation of mini-pump. The next morning an hour of baseline behavior was collected after which the continuous infusion by minipump was stopped by ligation of tubing running to the jugular catheter. A washout period of two hours to clear plasma of the chronically infused drug was followed by a bolus of 3 μmol/kg cocaine via jugular catheter and one more hour of behavioral data collection. The rats were then sacrificed with an overdose of equithesin or chloral hydrate.

During the week of constant infusion, the jugular catheter connecting to the outside by way of the L piece and dental acrylic was flushed daily with heparinized saline to maintain patency. Animals that appeared to be in distress after the surgery were sacrificed

before the scheduled experiment date. Rats were housed individually and given free access to food and water.

2.9 Data Analysis

Dopamine levels were calculated as femtomoles per microliter for statistical analysis (uncorrected for probe recovery). One way repeated measures analysis of variance (ANOVA) was used to determine if administration of drug produced a significant change in dopamine levels or behavioral response within a group receiving a particular treatment. Two way repeated measures ANOVA was used to determine if there was a significant difference between experimental groups. An unpaired, two-tailed, Student's t-test was used to compare activity after cocaine challenge between groups. The level of statistical significance was set at $p < 0.05$. All graphs represent dopamine data as a percentage of baseline. The mean of the three points prior to drug administration was used to determine the pre-drug baseline.

3. RESULTS

3.1 Results from Acute Tolerance Studies

Some animals were sacrificed before drug challenge at the end of continuous cocaine infusion in order to measure plasma drug levels. Mean plasma cocaine levels were 481 ± 66 ng/mL cocaine ($n = 9$) which were different from initially calculated. The target plasma drug concentration was 200 ng/mL which is slightly less than half of measured plasma cocaine levels. This difference in plasma drug levels may be due in part to differences in anesthesia which the rats in this experiment were exposed to compared to the one on which we based our calculations (Nobiletti *et al.*, 1994).

The acute tolerance studies consisted of an initial bolus of cocaine, cocaethylene, or isopropyl cocaine followed immediately by a three hour infusion phase; all rats received a cocaine challenge at the end of the infusion. Microdialysis results from the group receiving a constant infusion of cocaine from times 0 to 180 minutes after bolus injection are presented in *Figure 3*. Results from the group receiving a constant infusion of cocaethylene is shown by *Figure 4*, and those for the isopropyl cocaine group are seen in *Figure 5. Table 4:*

Absolute Microdialysis Basal Levels presents basal dopamine values for the three groups which did not differ significantly.

Large increases in dopamine levels were seen with an initial bolus of drug (3 μmol/kg) in the animals receiving cocaine and cocaethylene but not with isopropyl cocaine. Using repeated measures ANOVA, there was a statistically significant increase in dopamine concentration in the nucleus accumbens starting from 40 minutes prior until 20 minutes following bolus 1 administration of cocaine (F = 11.174, p = 0.0006); dopamine levels reached 163.5 $\pm$ 19.0% of baseline levels. Comparing over time starting from the same 40 minutes of baseline samples immediately prior to drug bolus until 20 minute after cocaethylene administration, statistical significance was seen (F = 4.823, p = 0.021) with a mean dopamine level of 171.7 $\pm$ 25.17%. However, administration of isopropyl cocaine resulted in no significant increase in nucleus accumbens dopamine levels (F =0.088, p = 0.9168).

TABLE 4: ABSOLUTE MICRODIALYSIS BASAL LEVELS		
drug	*mean $\pm$ S.E.M. (fmol/μL)*	*n*
cocaine	0.54 $\pm$ 0.06	11
cocaethylene	0.65 $\pm$ 0.06	10
isopropyl cocaine	0.36 $\pm$ 0.05	6
Mean values measured in femtomoles per microliter (fmol/μL)		
Basal values were not significantly different between groups ($p \geq 0.14$)		

In order to determine the impact of the cocaine challenge *following* the bolus/infusion pretreatment, repeated measures ANOVA of the data—corresponding to 40 minutes before cocaine challenge until 20 minutes after the challenge—was performed. There was no significant effect in the group receiving a constant infusion of cocaine (F = 2.191, p = 0.1379) or in the cocaethylene group (F = 2.444, p = 0.1151), but the change was statistically significant in the isopropyl cocaine group (F = 8.349, p = 0.0185) yielding a mean value of 168 $\pm$ 17.7% of the immediately preceding baseline (one hour before challenge) (Please refer to *Figure 6*). The baseline immediately preceding challenge was used rather than the one from times –60 to 0 minutes because of differences in steady-state

dopamine levels between the two; the interest was in the change to a cocaine challenge from the immediate pre-challenge state. Cocaine animals had a stable, elevated dopamine level during the three hour drug exposure; cocaethylene animals had a decreasing and then increasing level. Though the trend was an increase in dopamine levels in all three groups after cocaine challenge, the only significant increase was seen with the isopropyl cocaine group. A statistically significantly response to the challenge is expected in animals that do not have tolerance to cocaine, as seen in drug naïve animals and rats pretreated with isopropyl cocaine.

The effects of the pretreatment study drugs (cocaine, cocaethylene, and isopropyl cocaine) on the dopamine levels over time is different in each case. As seen in *Figure 7*, a bolus and constant infusion of cocaine causes a stable elevation of dopamine concentration throughout the maintenance phase of the experiment. Cocaethylene causes an immediate peak in dopamine levels after the loading dose is given, but then drops to baseline levels by two hours of constant infusion at which time the dopamine levels begin to rise again. Isopropyl cocaine at this low concentration, much like an inactive control, has virtually no effect on dopamine levels during the course of the experiment.

For behavioral data, *Figures 8–10* are labeled *Cocaine Behavior, Cocaethylene Behavior,* and *Isopropyl Cocaine Behavior* in a fashion analogous to that used in the microdialysis charts. The *y*-axis denotes the mean number of non-consecutive beam breaks made in a five minute period as described in section 2.4, *Behavioral Studies for Acute Tolerance Studies.* Standard error bars are included. A combined graph of all three data sets combined without error bars follows in *Figure 11*.

Statistically significant increases in activity were seen following the initial bolus of drug in the cocaine and cocaethylene groups but not in the isopropyl cocaine group. Increased activity from a mean of 1.1 ± 0.1 counts per five minute time bin 60 minutes prior to bolus 1 to 7.5 ± 0.8 non-consecutive beam breaks per time bin in the 20 minutes following the first pre-infusion bolus (0 minutes post-injection) was statistically significant ($F = 11.005$, $p = 0.0001$) in the cocaine group using repeated measures ANOVA. For the group receiving cocaethylene as bolus 1, the change in behavior from a baseline of 2.3 ± 0.3

to a value of 6.2 ± 1.1 counts per five minutes was also significant ($F = 2.587$, $p = 0.0012$). There was no significant increase in activity in the isopropyl cocaine groups over the same time period ($F = 0.899$, $p = 0.5672$).

By repeated measures ANOVA all study animals demonstrated significant increases in activity to cocaine challenge after a three hour maintenance phase on their respective drug (see *Figure 12*). The activity in the cocaine group increased to a maximum of 19.4 ± 3.5 counts per five minutes following cocaine challenge compared to the preceding 60 minute mean of 11.5 ± 0.9 counts per five minutes; the trend over this period of time was statistically significant by repeated measures ANOVA ($F = 5.402$, $p = 0.0001$). Increased activity with cocaine challenge at the same time intervals was also statistically significant in both the cocaethylene group from 2.8 ± 0.4 to 8.4 ± 1.7 counts per five minutes ($F = 2.259$, $p = 0.0101$) and in the isopropyl cocaine group from 0.7 ± 0.2 to 6.0 ± 1.2 counts per five minutes ($F = 2.218$, $p = 0.0155$).

With two way repeated measures ANOVA, there were no significant differences in activity when comparing between groups from 15 minutes before the cocaine challenge (165 minutes post-injection of bolus 1) to 15 minutes after cocaine challenge. However, when comparing the maximal locomotor response of rats in five minute time periods after cocaine challenge using an unpaired, two-tailed t-test, there was a statistically significant difference between cocaine and cocaethylene groups ($p = 0.0095$) and between cocaine and isopropyl cocaine groups ($p = 0.0372$), but not between cocaethylene and isopropyl cocaine groups ($p = 0.5024$). The absolute activity of each groups is shown in *Figure 12*. *Figure 13* shows the amount of locomotor change seen in each group after a cocaine challenge.

3.2 Results from Isopropyl Cocaine Dosing Studies

The purpose of these experiments was to determine the dose of isopropyl cocaine that would elicit an equivalent dopamine response in the nucleus accumbens as seen with 3μmol/kg cocaine in the anesthetized rat. This has never been studied previously.

One group of rats received cocaine at 3μmol/kg, another group received isopropyl cocaine at 6μmol/kg, and a third was treated with isopropyl cocaine at 12μmol/kg. The results from these three groups are shown in *Figure 14*. The dopamine increases in the

nucleus accumbens when compared to baseline values were statistically significant in the 3 μmol/kg cocaine group (F = 17.916, p = 0.0133); the mean change in this group was 441.68 $\pm$ 93.55%. Dopamine levels after a bolus of isopropyl cocaine at 6 μmol/kg peaked at 185.10 $\pm$ 27.66%; however, this was not statistically significant (F = 3.992, p = 0.1164). A bolus of 12 μmol/kg resulted in a mean dopamine response of 236.06 $\pm$ 13.55% which was significant (F = 28.344, p = 0.006). In another group of three animals given a bolus of 24 μmol/kg isopropyl cocaine, two died immediately. Thus this dosage was abandoned, and the only surviving rat showed a nucleus accumbens dopamine response of 362.07% baseline after injection. A challenge of both 3 μmol/kg cocaine and 12 μmol/kg isopropyl cocaine together resulted in a response of 497.98 $\pm$ 39.51% of baseline in two rats. Thus it did not appear that the concurrent presence of isopropyl cocaine diminished the effect of cocaine.

3.3 Results from Chronic Tolerance Studies

Figures 15–22 in this section depict the behavior of rats that have undergone a seven day continuous infusion of control (water), cocaine, cocaethylene, or isopropyl cocaine. *Figures 15–18* document activity of the animals on the night before the experiments, and *Figures 19–22* show the experimental session which included the cocaine challenge: the continuous infusion was stopped 120 minutes before the cocaine challenge to allow sufficient time for clearance of drug, and a bolus of cocaine was given at time zero.

There was a slight basal activation at night from cocaine and cocaethylene in the chronically treated animals that was not observed in the isopropyl cocaine or control groups. When using two way repeated measures ANOVA to analyze activity between groups at night beginning approximately one hour prior to darkness, there was no statistically significant difference between cocaine and isopropyl cocaine animals, cocaine and cocaethylene rats, cocaethylene and isopropyl cocaine subjects, or isopropyl cocaine versus control animals. There was a significant difference between cocaine and control animals at night (F = 1.622, p = 0.0012) and between cocaethylene and control groups (F = 1.313, p = 0.0472). On *Figure 23*, the differences in night activity between the groups are shown.

There was tolerance to subsequent cocaine challenge following chronic cocaine and cocaethylene treatments which was not seen with rats maintained chronically on isopropyl cocaine or water. A bar graph comparing the total activity per rat in the 5–20 minutes after cocaine challenge for cocaine-, cocaethylene-, and water-maintained animals is found in *Figure 24.* This period corresponds to the time of drug action. One way repeated measures analysis of variance of the data from 30 minutes pre- to 30 minutes post-challenge showed a significant increase in activity in the control group (F = 3.464, p = 0.0003). There was no significant increase in behavior following challenge in the group receiving seven days of continuous cocaine infusion (F = 0.735, p = 0.7129). The cocaethylene group also showed no statistically significant increase in activity (F = 0.769, p = 0.6792). In contrast to the cocaine and cocaethylene groups, the increase in activity was statistically significant in the isopropyl cocaine group (F = 3.023, p = 0.0023). Multifactorial ANOVA examining the total activity from 5–20 minutes per rat (see *Figure 24*) shows significant differences between the cocaine and control groups as well as between the cocaethylene and control groups.

4. DISCUSSION

4.1 Discussion Introduction

The prevalence of cocaine abuse and its impact on society has necessitated the search for an effective therapy to cocaine addiction, a disease for which there are currently no good treatments. As methadone is able to blunt the effects of subsequent heroin use by inducing tolerance, an analogous compound aimed at producing cross-tolerance to cocaine may be useful in treating cocaine addiction. The search for a pharmacological treatment of cocaine addiction is the basis of this present study.

These experiments examined the impact of acute and chronic treatment with cocaine, cocaethylene, and isopropyl cocaine on dopamine levels in the nucleus accumbens (Acute Tolerance Studies only) and also on locomotor behavior in the rat, and in particular, compared the effects of a subsequent cocaine challenge. It was hypothesized that continuous infusion of cocaine, cocaethylene, or isopropyl cocaine would blunt response to a

later bolus of cocaine; if this hypothesis were proved correct, then some or all of these compounds could potentially lead the way to a pharmacological management of cocaine addiction. As mentioned in section 1, *Introduction*, various studies have demonstrated tolerance with continuous cocaine administration (Emmett-Oglesby *et al.*, 1993; Frank *et al.*, 1992; Katz *et al.*, 1992; Masserano *et al.*, 1994). It appears from our results that we have demonstrated tolerance to some of the dosing regimens utilizing intravenous administration of drug as discussed below.

Chronic treatment with cocaine and cocaethylene produced behavioral tolerance. Acute (three hours) maintenance of cocaine and cocaethylene produced a suggestion of neurochemical tolerance but no behavioral tolerance. Isopropyl cocaine produced little effect on the rats neurochemically or behaviorally though may be due to a dose used that was too small.

4.2   Discussion for Acute Tolerance Studies

Cocaine and cocaethylene are approximately equal in their ability to elevate extracellular dopamine levels with an acute bolus infusion as seen in *Figure 7*. The mean increase in dopamine levels in response to the initial cocaethylene bolus was similar to that elicited from the cocaine in the Acute Tolerance studies which is consistent with an earlier study using anesthetized rats. (Bradberry *et al.*, 1993). The initial isopropyl cocaine bolus resulted in no change in extracellular dopamine concentrations. Though isopropyl cocaine is approximately 50 times more selective for dopamine than for serotonin transporters, it is known to be 15–25 times less active at both sites (Elsworth *et al.*, 1993; Jatlow and Bradberry personal communication). Thus the lack of dopamine effect from isopropyl cocaine administration may be a dose effect.

These microdialysis data are consistent with what is known about these three compounds as it relates to the rat. Cocaine and cocaethylene both elicit large extracellular dopamine responses; both were approximately equipotent in ability to increase dopamine levels from baseline. Isopropyl cocaine is clearly the least active of the three compounds in terms of extracellular dopamine response.

The time course of altered dopamine resulting from the three drugs appears to differ during the maintenance phase of the study. Cocaine causes a dopamine increase which is maintained at a stable level throughout the constant infusion. Cocaethylene causes a peak in the extracellular dopamine concentration 20 minutes after the loading dose, but this level drops constantly for 100 minutes. This may suggest that cocaethylene demonstrates tolerance to itself over time with respect to dopamine elevation. After 100 minutes, the extracellular dopamine concentration rises until the cocaine challenge. The exact cause for this increase in dopamine concentration after 100 minutes is currently unclear. Extracellular dopamine levels after isopropyl cocaine administration remains at baseline levels throughout the constant infusion which is expected since the initial bolus of isopropyl cocaine at 3 μmol/kg resulted in no effect on dopamine.

No conclusions can be drawn regarding the effects of an acute maintenance of drug on neurochemical tolerance to subsequent cocaine because of the difficulties in interpreting the steady-state dopamine levels: dopamine levels are high throughout the experiment in the cocaine group, and dopamine levels are steadily increasing in the cocaethylene group—even before the cocaine challenge. Extracellular dopamine levels increased after the cocaine challenge in all groups. Using one way repeated measures ANOVA, the cocaine and cocaethylene groups showed no statistical change when comparing dopamine levels before and after cocaine challenge. This may be a result of acute neurochemical tolerance since the effect of the cocaine bolus resulted in minimal dopamine elevation, or in the case of cocaine, it may be due to the fact that all cocaine receptors were saturated which would imply that any additional cocaine administered (such as the challenge) would have no effect on the animals.

When examining behavioral response to the initial bolus of drug, there was a statistically significant increase in behavior with cocaine and cocaethylene groups (see *Figure 11*). This behavioral activation was not seen with isopropyl cocaine suggesting again that this compound is less potent than cocaine and cocaethylene when examining nucleus accumbens dopamine activation or locomotor activation at these dosages. Also seen is that the behavioral and neurochemical effects are consistent—or parallel—within this

experimental paradigm: cocaine animals exhibited increasing behavioral activation during the maintenance phase while during that same time dopamine was constantly elevated above pretreatment levels; cocaethylene animals had falling dopamine levels during early maintenance and showed locomotor activity during that same period that fell to pretreatment levels.

The behavioral trends in cocaine, cocaethylene, and isopropyl cocaine groups were similar after a cocaine challenge in the Acute Tolerance studies. The cocaine infusion group showed greater absolute behavioral activation with a cocaine challenge than the other two groups. While cocaine and cocaethylene bind the dopamine transporter equipotently, their main difference is a relatively greater affinity of cocaine for the serotonin transporter than cocaethylene and isopropyl cocaine. This may result in serotonin-related augmentation of behavior from cocaine as described in previous studies (Cameron and Williams, 1994; Parsons *et al.*, 1996). The differences in dopamine and serotonin transporter selectivity could account for the increased behavioral activation seen in the cocaine bolus/infusion group compared to animals receiving cocaethylene or isopropyl cocaine bolus/infusion.

The analysis of data from the Acute Tolerance studies yielded no definite evidence for the development of tolerance in a three hour period. These data were difficult to interpret because of the unstable locomotor activity levels immediately prior to challenge in the cocaine group. Even during the maintenance phase of the experiment, the cocaine-treated rats showed continually increasing behavioral activation. This phenomenon was not seen with the cocaethylene-treated rats; their activity returned to baseline during the three hour infusion, and they responded no differently to a cocaine challenge than the isopropyl cocaine rats (who were treated with such a small dose of isopropyl cocaine that it was presumed to have been equivalent to placebo). Thus, acutely, it can be concluded that cocaethylene causes less behavioral activation than cocaine.

4.3 Discussion for Isopropyl Cocaine Dosing Studies in the Anesthetized Rat

In order to compare the potency of isopropyl cocaine with cocaine, the experiments on chloral hydrate anesthetized rats were conducted (see *Figure 14*). While a dose of

isopropyl cocaine at 6 μmol/kg resulted in an extracellular dopamine concentration increase that was not statistically significant, 12 μmol/kg yielded a statistically significant response. However, even at this dosage, the maximal response was a mean of 236.06 $\pm$13.55% baseline for the group—much less than the 441.68 $\pm$ 93.55% of baseline response elicited by a cocaine challenge. When the administered dose was doubled to 24 μmol/kg, two of the three rats died immediately after injection. Thus, this line of pursuit was abandoned with the conclusion that the lethal dose of isopropyl cocaine was less than the dose required to elicit a dopamine response similar to that found after cocaine infusion in chloral hydrate anesthetized rats. Cocaethylene has also been previously described as having a lower lethal dose than cocaine (Hearn *et al.*, 1991). It is currently unclear why the cocaine analogs appear to be more toxic than the parent compound.

Peak response to cocaine was significantly greater for the chloral hydrate anesthetized rats than for the awake animal. Our data show that a bolus of 3 μmol/kg cocaine via jugular catheter in a previously drug-naïve animal increases dopamine levels in the nucleus accumbens to a statistically significant degree from baseline. It is interesting to note that the previous studies using chloral hydrate anesthetized rats at the same challenge doses (though with only a bolus and no infusion) showed a statistically significant increase in nucleus accumbens dopamine levels ranging from 304% to 400% of baseline while the present study with awake animals responded with an increase to only 163.5 $\pm$ 19.0% of baseline (Bradberry *et al.*, 1993; Bradberry and Roth, 1989; Jatlow *et al.*, 1991). The difference in dopamine elevation when comparing awake and anesthetized rats receiving the same doses of drug was also demonstrated in these studies and might be a consequence of a drug interaction between cocaine and chloral hydrate though the exact mechanism is currently unclear. The presence of chloral hydrate seems to augment the extracellular dopamine response in the nucleus accumbens.

4.4 Discussion for Chronic Tolerance Studies

Doses for the seven day constant infusion studies were much less than that given in the acute trials (*Table 3: Minipump Study Drugs* in *Section 2.8: Chronic Tolerance Studies*). Estimated plasma concentrations were between 85 and 90 ng/ml for cocaine and

cocaethylene animals; plasma concentrations were estimated to be approximately 47 ng/ml for the isopropyl cocaine rats—the isopropyl cocaine animals were given a dose half that of animals in the other treatment groups due to limited availability of this specially synthesized compound—though these target plasma concentrations may be underestimates as described in section 3.1, *Results from Acute Tolerance Studies*. Plasma concentrations were less but sustained for a greater amount of time when the seven day constant infusion study is compared to the three hour study. There was no statistically significant increase in activity subsequent to cocaine challenge in the chronically maintained cocaine and cocaethylene groups. Thus it appears that behavioral tolerance was achieved in these two groups. On the other hand, behavioral activation by a subsequent cocaine challenge was seen with a statistically significant increase in activity for the animals that were subjected to the control or to isopropyl cocaine for a week. This suggested that no behavioral tolerance was achieved for either of these groups at the maintenance levels provided.

While it is clear that there is little difference in the behavior after challenge between control and isopropyl cocaine groups using these dosages, it is unclear whether the amount of isopropyl cocaine given was enough to produce a physiologically significant alteration in response to a cocaine challenge. It is possible that the plasma levels of the isopropyl cocaine during the week of infusion were too low to be able to effect a subsequently change in behavior. Possibly a higher dose of isopropyl cocaine than we had employed—but a less than lethal one—might produce tolerance. A compound like isopropyl cocaine that has more activity at the dopamine axis and relatively even less at the serotonin one than cocaethylene may have better promise as a drug used to treat cocaine addiction since theoretically it would still decrease the cocaine-induced euphoria but be even less behaviorally activating than cocaethylene.

4.5 General Discussion

From these studies it appears that neurochemical and behavioral tolerance to cocaine develops at different times with respect to length of continuous cocaine or cocaethylene infusion required. With cocaine and cocaethylene pretreatment, the ability of a cocaine challenge to elevate extracellular dopamine levels was reduced within three hours

of constant infusion (though it is not clear whether true tolerance was achieved as described in section 4.2, *Discussion for Acute Tolerance Studies*). By giving rats a constant infusion of drug—cocaine or cocaethylene—for seven days, behavioral tolerance was observed. Throughout these experiments, isopropyl cocaine at the dosages used did not display an ability to produce cross-tolerance to subsequent cocaine administration neurochemically or behaviorally though this may be a result of the low potency of this compound (as measured by extracellular dopamine concentrations and locomotion) compared to the other two and the relatively low doses used. In fact, isopropyl cocaine-treated rats displayed the same behavior in the seven day continuous infusion experiments as the control group.

The schedule of drug administration as well as the dosage play a significant role in the effect of a subsequent cocaine challenge. When rats were given the pretreatment of 120 μmol/kg cocaine daily by osmotic minipump and subject to the same trials after one or seven days of withdrawal, tolerance was observed (King *et al.*, 1992). A pretreatment schedule of cocaine given at 60 μmol/kg three times a day was found to produce tolerance by day two which remained at 60% of baseline from days four through 10 (Emmett-Oglesby *et al.*, 1993). Acute tolerance to cocaine has also been reported to occur in humans (Foltin and Fischman, 1991; Foltin *et al.*, 1990). While tolerance has been observed in animal trials, the exact parameters of drug administration to achieve either state successfully are still under study. In general, it is believed that a constant infusion of a pre-challenge drug, in this case cocaine or cocaethylene, causes tolerance.

While previous experiments have confined themselves to higher doses of the study medications, possibly because drug administration was not intravenous and thus resulted in lower bioavailability of drug, this present study used 3 μmol/kg for the first bolus—the loading dose—followed by 3 μmol/hour for three hours in the acute studies. The seven day continuous drug infusion and behavior studies used 32.6 μmol/day for the cocaine and cocaethylene studies; the pretreatment was 16.3 μmol/day for isopropyl cocaine. Drug doses for present chronic administration studies were only slightly less than used in previously reported trials. Even with these lower doses, tolerance was still observed using these dosing schedules.

Infusion rates in the present experiments were based on drug clearance which is equal to the product of the target steady state drug concentration and the total body clearance of compound. Clearance rates for intravenously administered cocaine and cocaethylene have been previously described as 73.5 ± 9.5 ml/min and 82.8 ± 7.4 ml/min respectively (Nobiletti *et al.*, 1994). Isopropyl cocaine clearance rates are currently unknown and could only be presumed at present.

Human states of euphoria derived from these drugs clearly cannot necessarily be equated with the behavioral activation seen in rats. At present, behavioral measurements such as the amount of grooming, locomotor activity, or other stereotypical movements are a popular method for determining the level of response achieved by drug administration. Microdialysis is a method to quantify the neurochemical drug response. However, it is uncertain how well these measurements might correlate with euphoric states. The importance of examining dopamine concentrations at the nucleus accumbens is suggested by studies showing that dopaminergic projections to the nucleus accumbens are required for the development of cocaine self-administration (Roberts and Koob, 1982). Self-administration animal paradigms may better reflect the ability a certain drug has in causing euphoria in people and may be the next step in future studies. And of course, this is one of the goals of current drug studies. We want to understand how compounds such as cocaine cause euphoria and tolerance because we wish to discover methods by which we can modulate these responses in humans. In this way, we may be able to find better modalities to treat cocaine abuse. Rat drug self-administration has been utilized with some success. One study suggested that rats titrate the amount of cocaine during self-administration to maintain a fairly constant, elevated dopamine level in the nucleus accumbens (Pettit and Justice, 1989); this leads us to believe that the reinforcing properties of cocaine are specifically related to extracellular dopamine levels in the nucleus accumbens. Another study using self-administration was shown to demonstrate no difference in the development of tolerance compared to continuous or intermittent drug administration schedules (Emmett-Oglesby *et al.*, 1993). The Emmett-Oglesby *et al.* study simply explored another schedule of drug administration and its relationship to the development

of tolerance; it did not elucidate the relationship between the state of drug tolerance, once achieved, and the subsequent effects it had on behavior. It may be interesting to ask the question: how different are the rates of self-administration when comparing a group of rats that has developed neurochemical or behavioral tolerance to a control group. Then we begin to examine the concept of tolerance as it relates to craving and possibly to euphoria.

4.6 Conclusion

In summary, the Chronic Tolerance studies showed that behavioral tolerance was seen in rats pretreated with cocaine or cocaethylene when challenged with cocaine; the behavior of the isopropyl cocaine group appeared to be no different than that of the control. Rats pretreated with three hours of cocaine or cocaethylene in the Acute Tolerance studies showed a decreased change in dopamine levels due to a cocaine challenge compared to isopropyl cocaine animals; because these data are difficult to interpret it cannot be definitively stated that tolerance was observed though tolerance is certainly suggested. There was no behavioral tolerance seen to a cocaine challenge during the Acute Tolerance study. However, during the Acute Tolerance study, it was observed that cocaethylene maintained animals displayed activation that was not sustained (both neurochemically and behaviorally) suggesting that cocaethylene may produce acute tolerance to itself. In the Isopropyl Cocaine Dosing Studies in the anesthetized rat, the extracellular dopamine response seen was found to be about four times that seen in awake rats when given a cocaine challenge. Isopropyl cocaine was much less potent than cocaine on eliciting a neurochemical change: the amount of isopropyl cocaine required to increase dopamine concentrations in the nucleus accumbens the same amount as seen when an anesthetized rat was given a challenge of 3 μmol/kg cocaine appears to be greater than the lethal dose. The dopamine response to isopropyl cocaine at 12 μmol/kg was still only 236 $\pm$ 13.55% of baseline in contrast to the 441.68 $\pm$ 93.55% of the baseline value seen with 3 μmol/kg cocaine.

Behavioral tolerance to cocaine can be achieved with a chronic cocaethylene pre-treatment. The characteristics of cocaethylene show some promise as a model for possible pharmacological therapies for cocaine addiction: as known previously, cocaethylene is
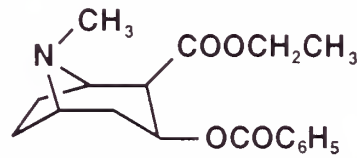
more selective for dopamine than serotonin when compared to cocaine; this cocaine analog is cleared more slowly and has a longer half-life than cocaine; and as seen with the Acute Tolerance studies, it is less activating than cocaine acutely. An examination of cocaine and the two cocaine analogs at different doses or drug administration schedules may bring out more of the unique qualities that each compound possesses. Further studies, perhaps utilizing a self-administration paradigm, may answer some of these interesting and important questions.

5. FIGURES AND GRAPHS

Figure 1: Cocaine, Cocaethylene and Isopropyl Cocaine Structures
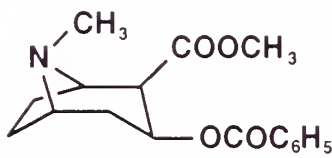
1A: Structure of cocaine   1B: Structure of cocaethylene   1C: Structure of isopropyl cocaine
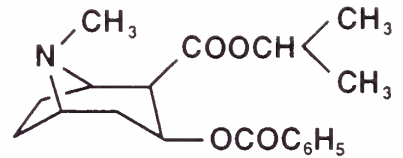
Figure 2: Microdialysis Probe
(not drawn to scale)

Outlet

Inlet

Epoxy   Dialysis Tubing   23 Gauge Tubing   Fused Silica

Cocaine Microdialysis (n=11)

Time post-injection (min)
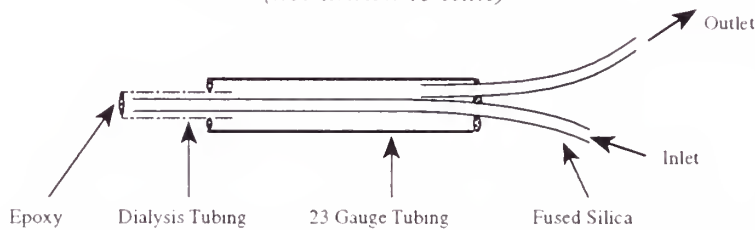
Constant infusion of cocaine
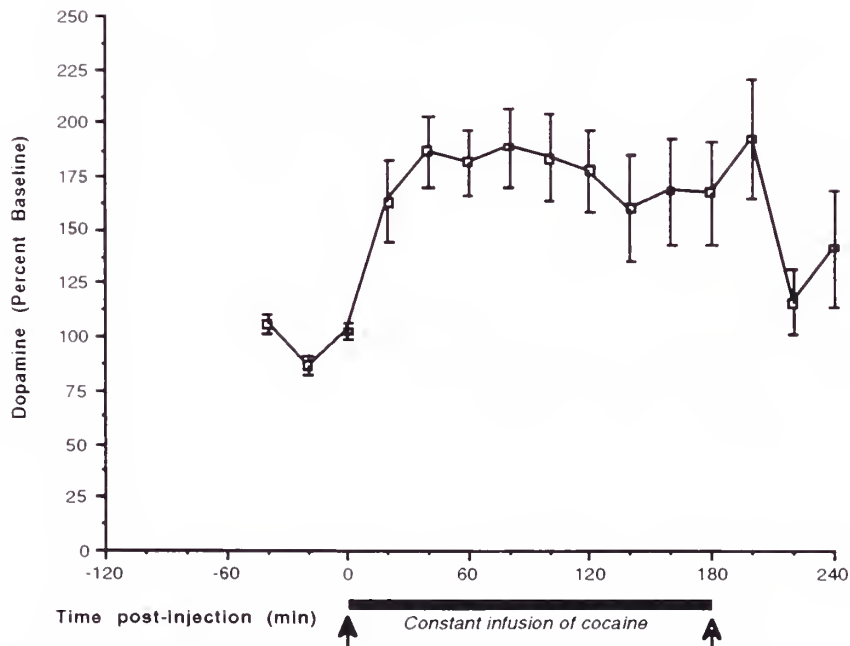
Figure 3: Mean values ($\pm$ SEM) of extracellular dopamine response to IV boluses of cocaine (3 μmol/kg) at times 0 and 180 with an intervening constant infusion of cocaine (51 nmol/min) from times 0–180 minutes ($n = 11$).

Cocaethylene Microdialysis (n=10)
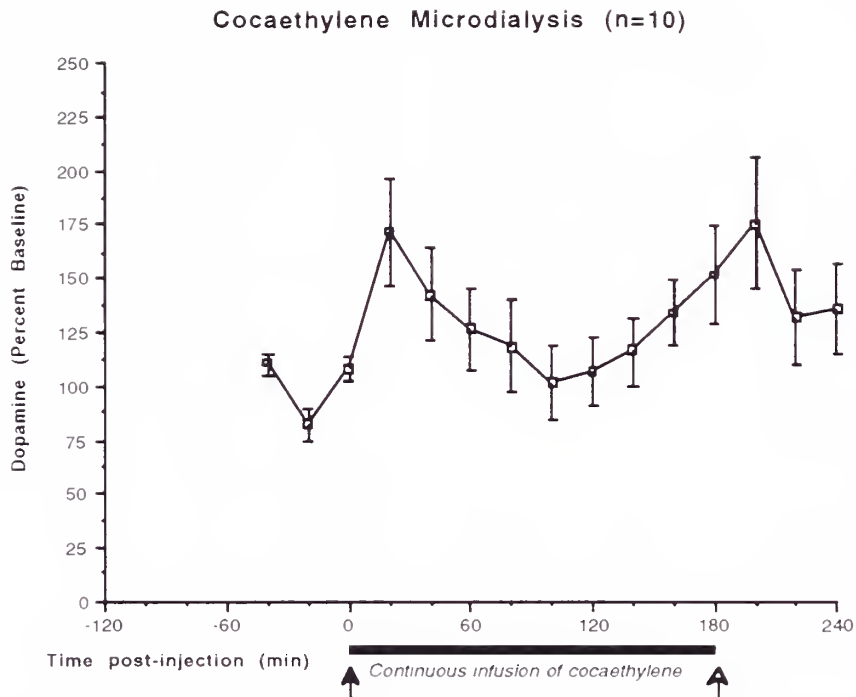
Figure 4: Mean values (± SEM) of extracellular dopamine response to IV bolus of cocaethylene (3 μmol/kg) at time 0 followed by a constant infusion of cocaethylene (51 nmol/min) until time 180 minutes and challenged with a bolus of cocaine (3 μmol/kg) at time 180 minutes (*n* = 10).

Isopropyl Cocaine Microdialysis (n=6)
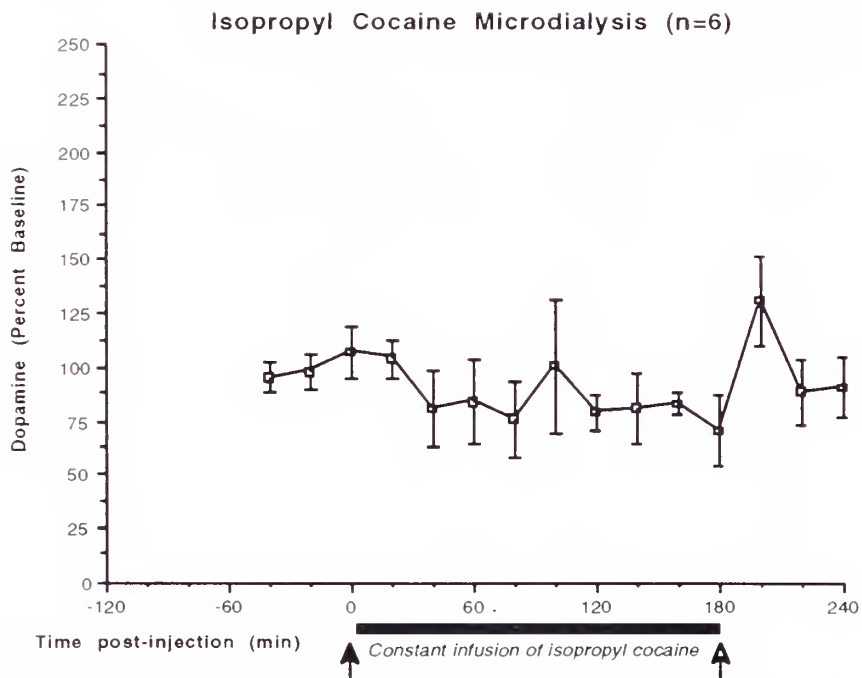
Figure 5: Mean values (± SEM) of extracellular dopamine response to IV bolus of isopropyl cocaine (3 μmol/kg) at time 0 followed by a constant infusion of isopropyl cocaine (51 nmol/min) until time 180 minutes and challenged with a bolus of cocaine (3 μmol/kg) at time 180 minutes (*n* = 6).

Dopamine Levels in Nucleus Accumbens
Percentage increase from pre-challenge baseline
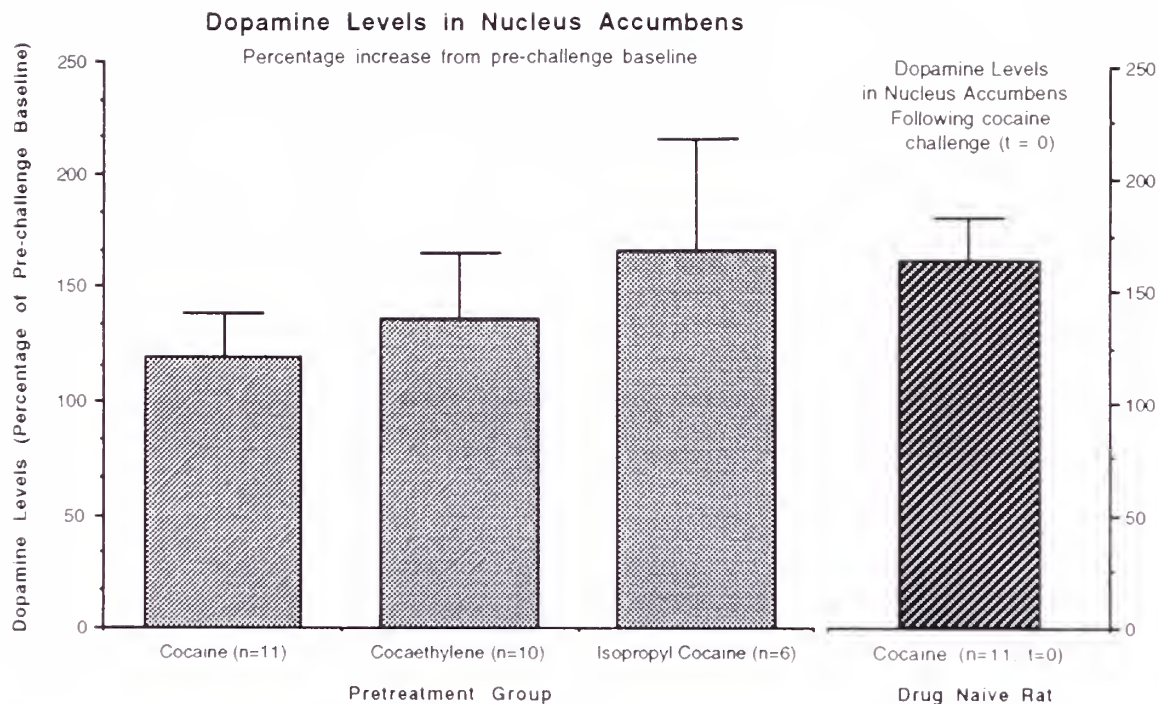
Figure 6: Mean (± SEM) extracellular dopamine concentrations after IV challenge of cocaine (3 μmol/kg) at time 200 minutes as a percentage of immediately preceding baseline (times 140 to 180) for rats pretreated with a 3 hour infusion of cocaine, cocaethylene, and isopropyl cocaine. Far right graph shows mean (± SEM) extracellular dopamine concentrations after IV challenge of cocaine (3 μmol/kg) at time 0 (drug naive rat) as a percentage of immediately preceding baseline. Cocaine- and cocaethylene-maintained rats show similar response trends while isopropyl cocaine- and drug-naive-rats exhibit similar responses to a cocaine challenge.
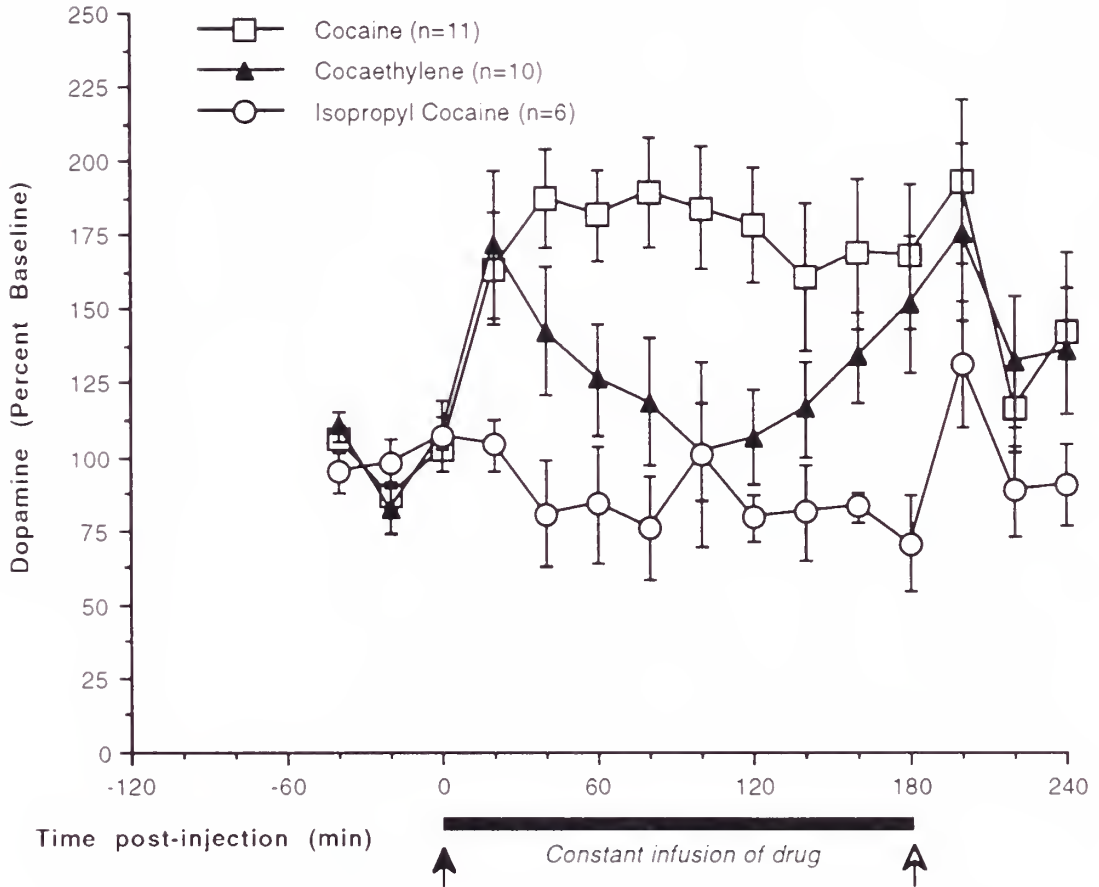
Figure 7: Mean values (± SEM) of extracellular dopamine response to IV bolus/infusion of cocaine, cocaethylene, or isopropyl cocaine (3 μmol/kg bolus and 51 nmol/min infusion) at time 0 until time 180 minutes and challenged with cocaine (3 μmol/kg) at time 180 minutes.
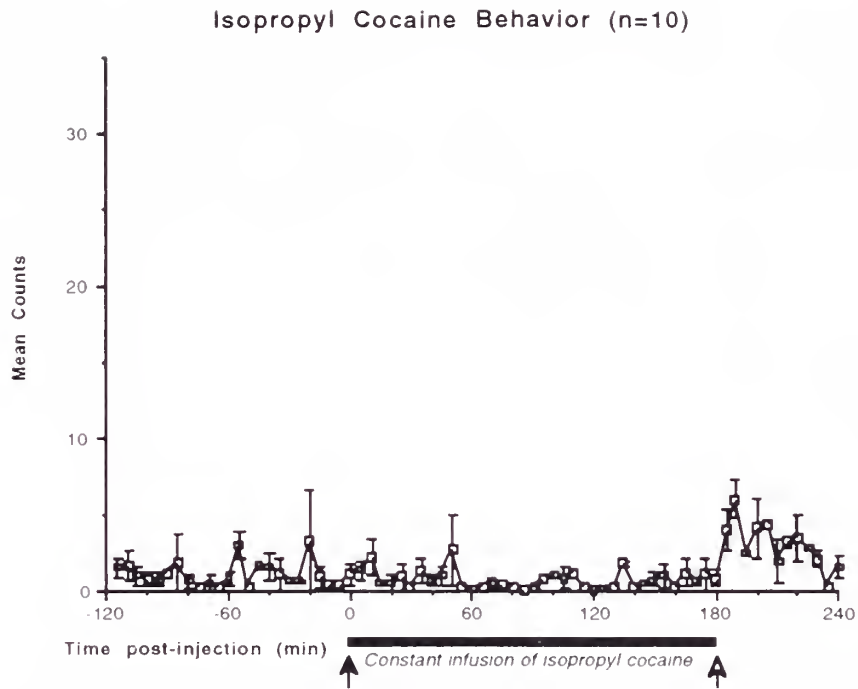
Cocaine Behavior (n=32)
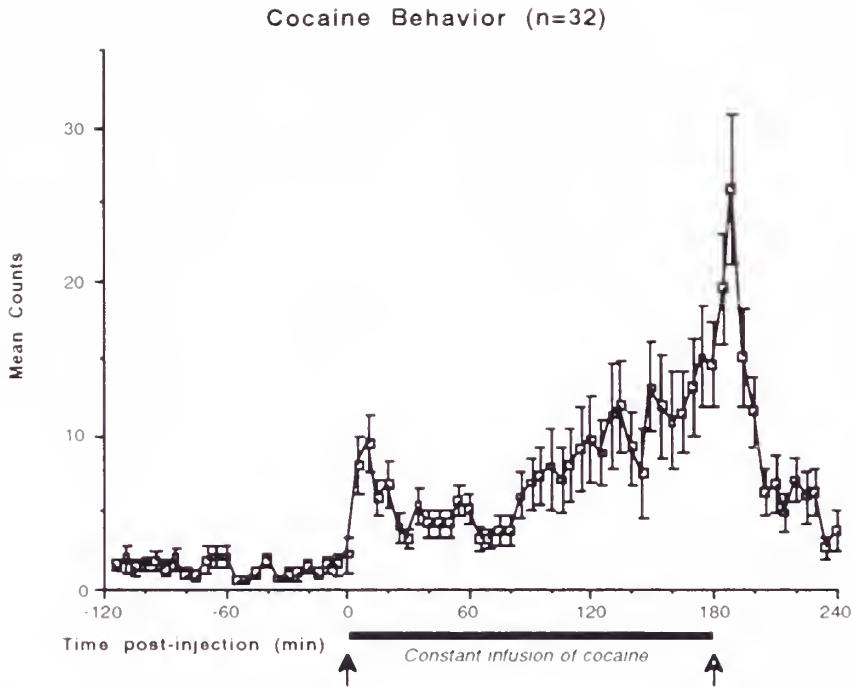
Figure 8: Mean values (± SEM) of non-consecutive beam breaks measuring locomotor activity subsequent to IV bolus of cocaine (3 μmol/kg) at time 0 and time 180 minutes with a constant cocaine infusion (51 nmol/min) from time 0 to time 180 minutes (*n* = 32).

Cocaethylene Behavior (n=20)
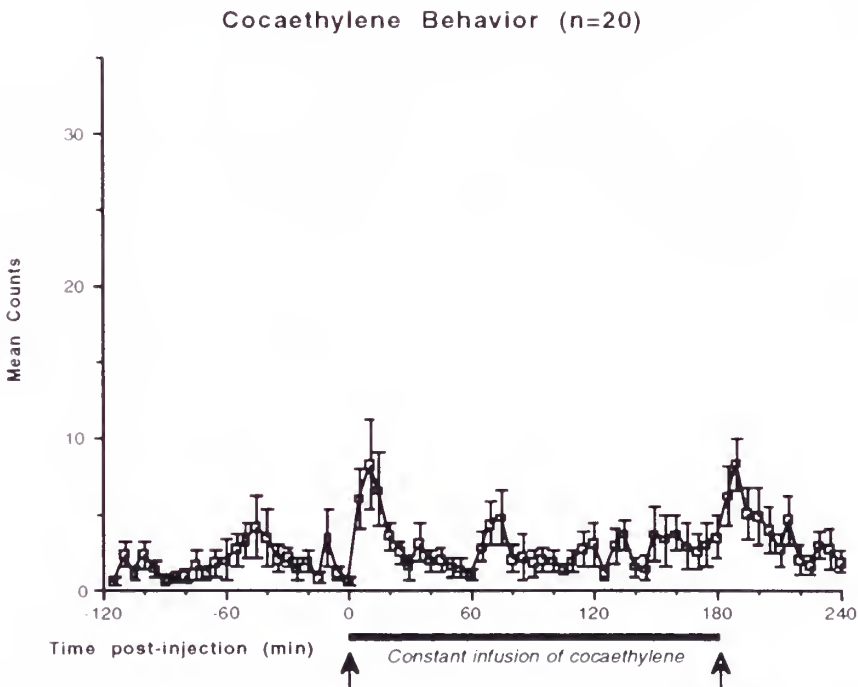
Figure 9: Mean values (± SEM) of non-consecutive beam breaks measuring locomotor activity subsequent to IV bolus of cocaethylene (3 μmol/kg) at time 0 minutes followed by infusion of cocaethylene (51 nmol/min) until time 180 minutes and challenged with cocaine (3 μmol/kg) at time 180 minutes (*n* = 20).

Isopropyl Cocaine Behavior (n=10)

Figure 10: Mean values (± SEM) of non-consecutive beam breaks measuring locomotor activity subsequent to IV bolus of isopropyl cocaine (3 μmol/kg) at time 0 minutes followed by infusion of isopropyl cocaine (51 nmol/min) until time 180 minutes and challenged with cocaine (3 μmol/kg) at time 180 minutes ($n = 10$).

Behavior Graphs Combined
Standard error bars omitted for clarity
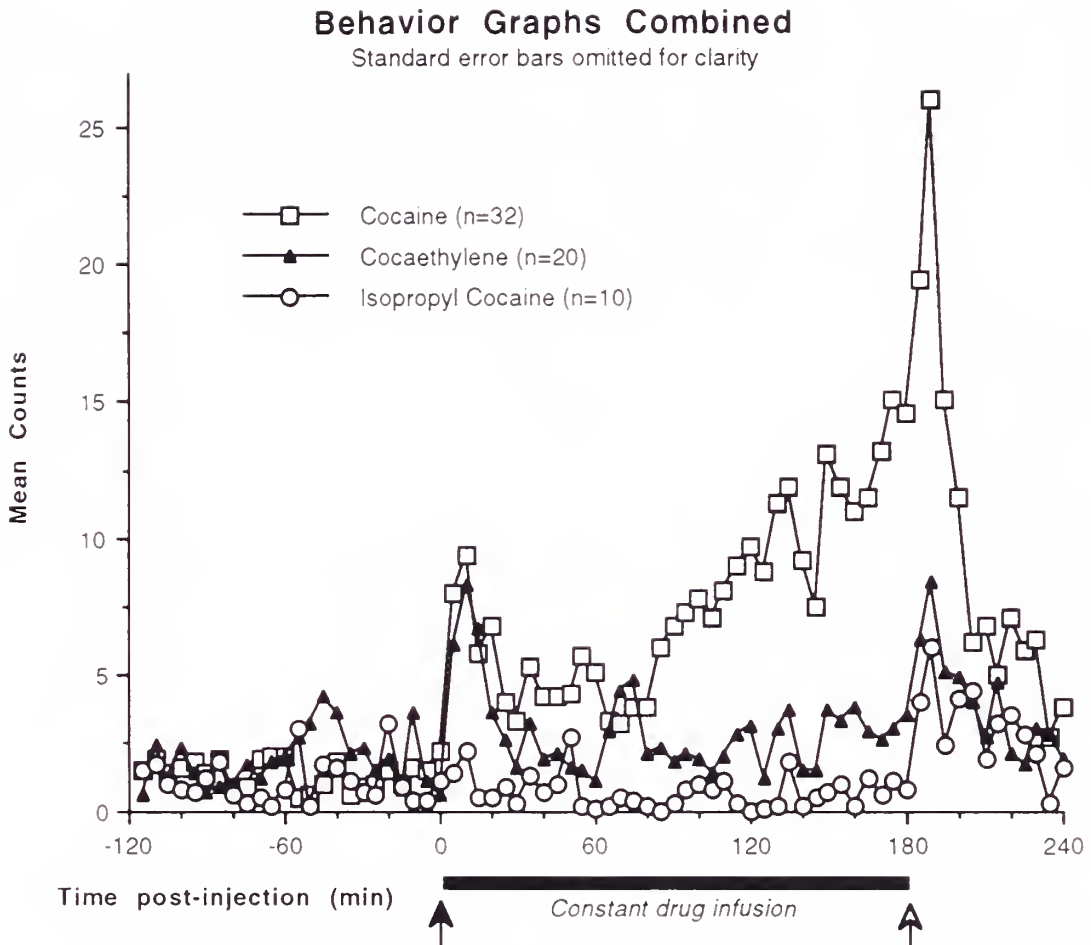
Figure 11: Mean values of non-consecutive beam breaks measuring locomotor activity subsequent to an IV bolus/infusion of cocaine, cocaethylene, or isopropyl cocaine (3 μmol/kg bolus and 51 nmol/min infusion) until time 180 minutes and challenged with cocaine (3 μmol/kg) at time 180 minutes. SEM bars were omitted for clarity.

Maximal Locomotor Activity

after Cocaine Challenge

Maximal Behavioral
Response Following Cocaine
Challenge (t = 0)
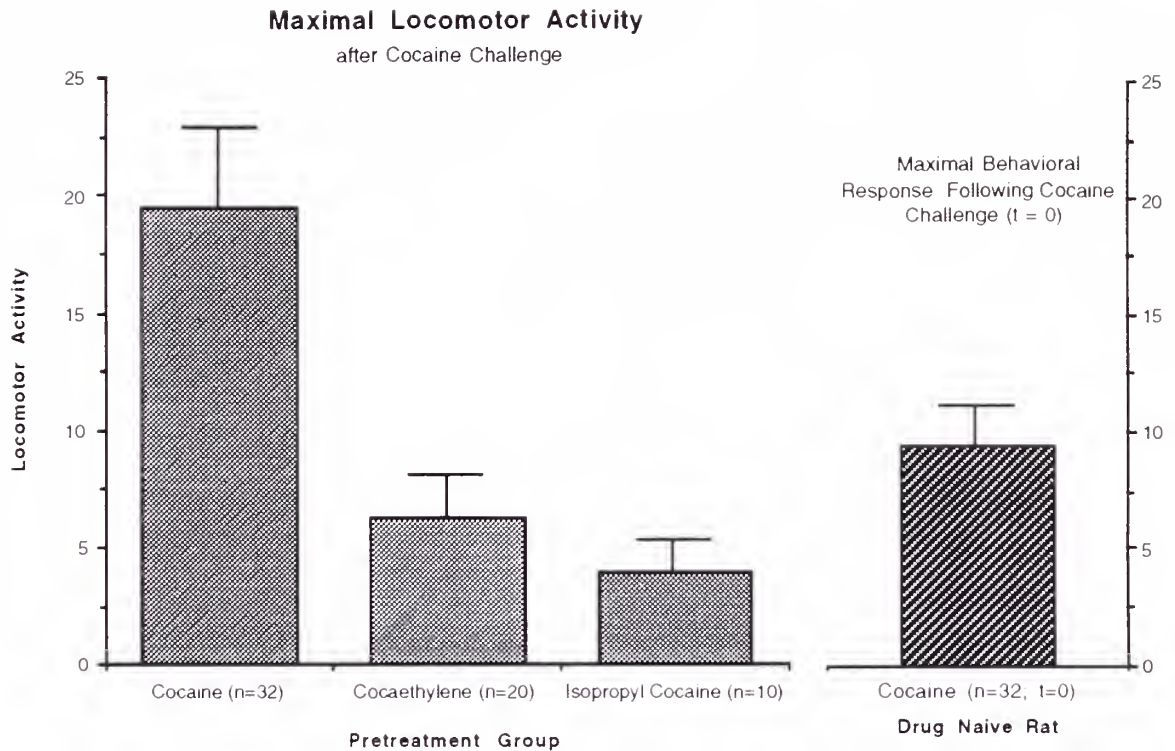
Figure 12: Left three graphs show mean (± SEM) locomotor activity as measured by nonconsecutive infrared beam breaks during a five minute period of greatest activity after IV challenge of cocaine (3 μmol/kg) at time 180 minute for rats pretreated with cocaine, cocaethylene, and isopropyl cocaine. Far right graph shows mean maximal locomotor activity (± SEM) during a five minute period immediately after IV challenge of cocaine (3 μmol/kg) at time 0 (drug naïve rats) preceding maintenance in the cocaine group.

Change in Locomotion

difference between peak value and mean of

three prior points (baseline) after Cocaine Challenge
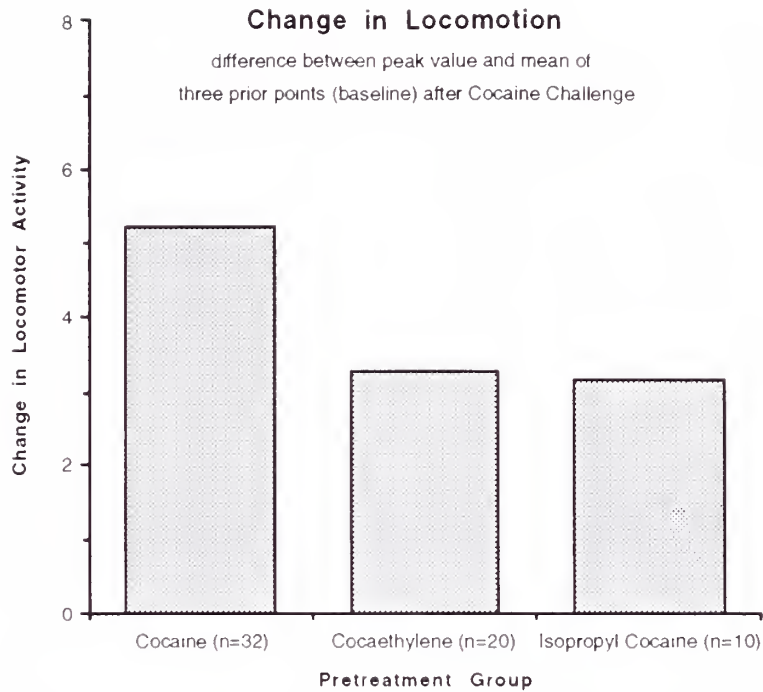
Figure 13: Mean change in locomotor activity from immediately preceding baseline (15 minute period prior to challenge) as measured by nonconsecutive infrared beam breaks during a five minute period during the period of greatest activity after IV challenge of cocaine (3 μmol/kg) at time 180 minute for rats pretreated with cocaine, cocaethylene, and isopropyl cocaine. Error bars omitted.

Microdialysis for Anesthetized Rat Dosing Studies
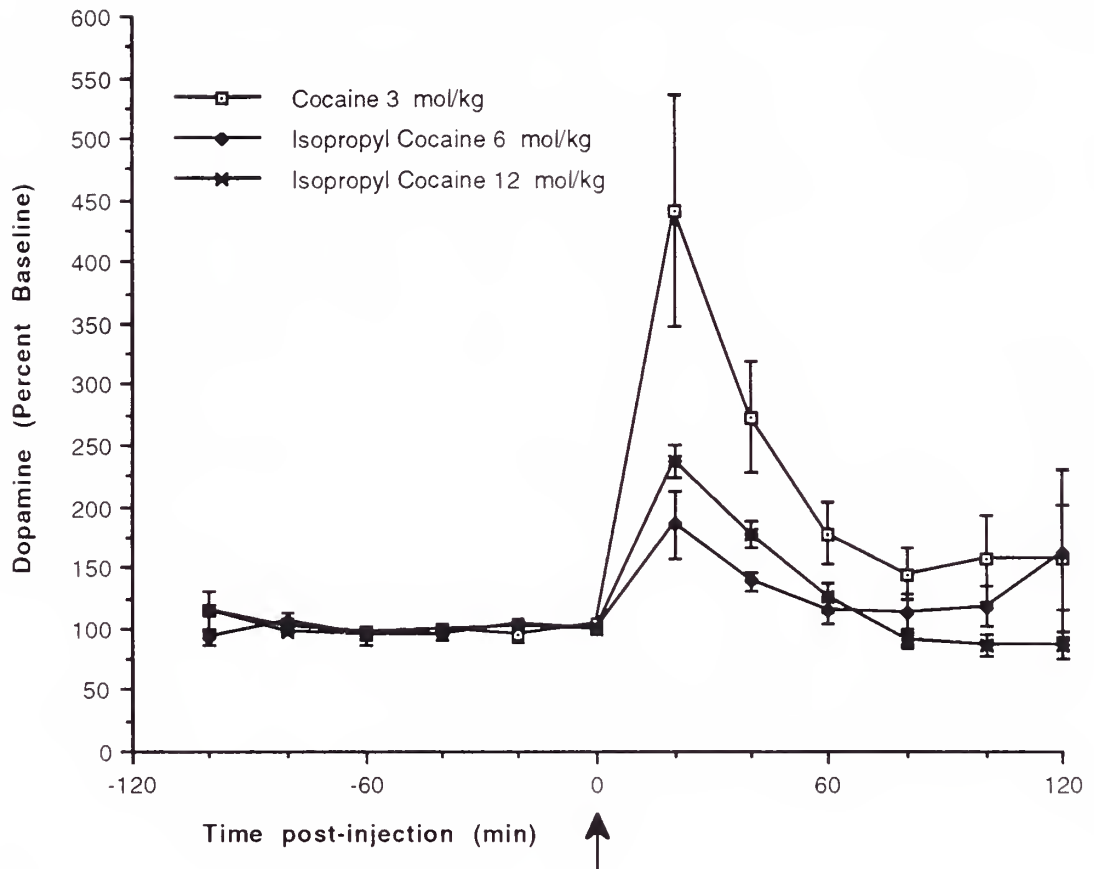
Figure 14: Mean values (± SEM) of extracellular dopamine response to cocaine at 3 μmol/kg (n = 5) compared to isopropyl cocaine at 6 μmol/kg (n = 5) and 12 μmol/kg (n = 5) doses in anesthetized rats.
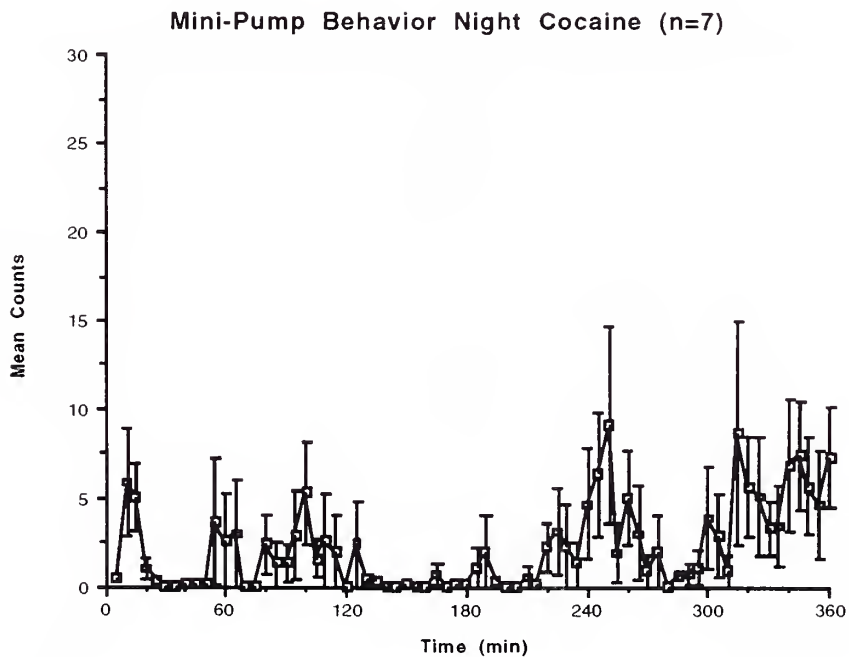
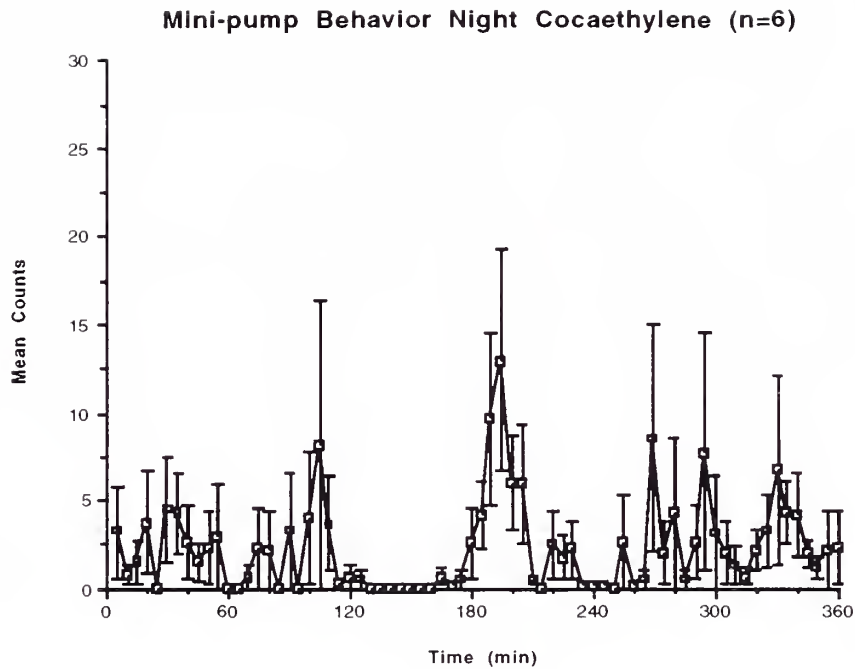## Mini-pump Behavior Night Control (n=9)
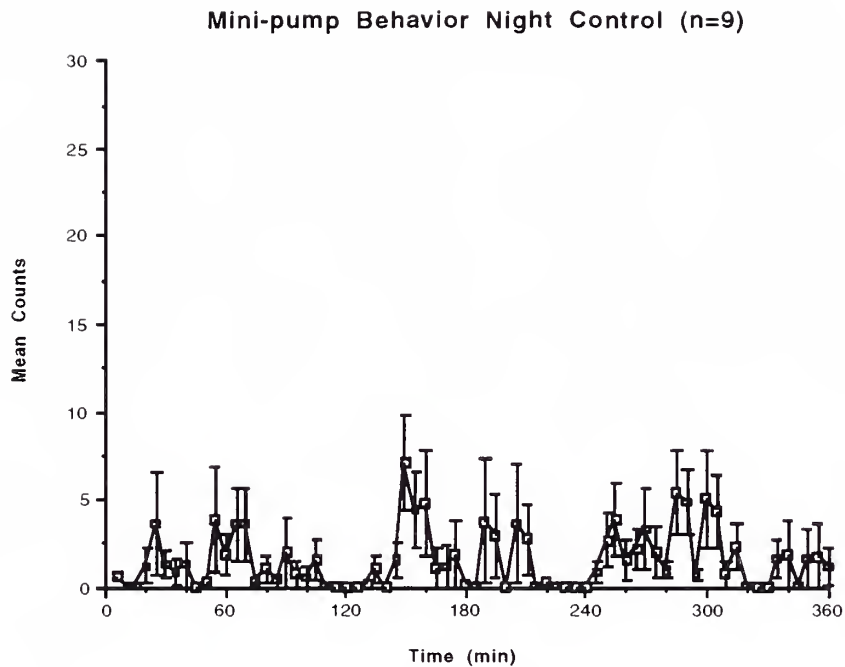
Figure 15: Mean values (± SEM) of non-consecutive beam breaks in five minute time bins measuring rat locomotor activity starting approximately one hour before darkness on day six of a seven day control (water) infusion by mini-pump ($n = 9$).
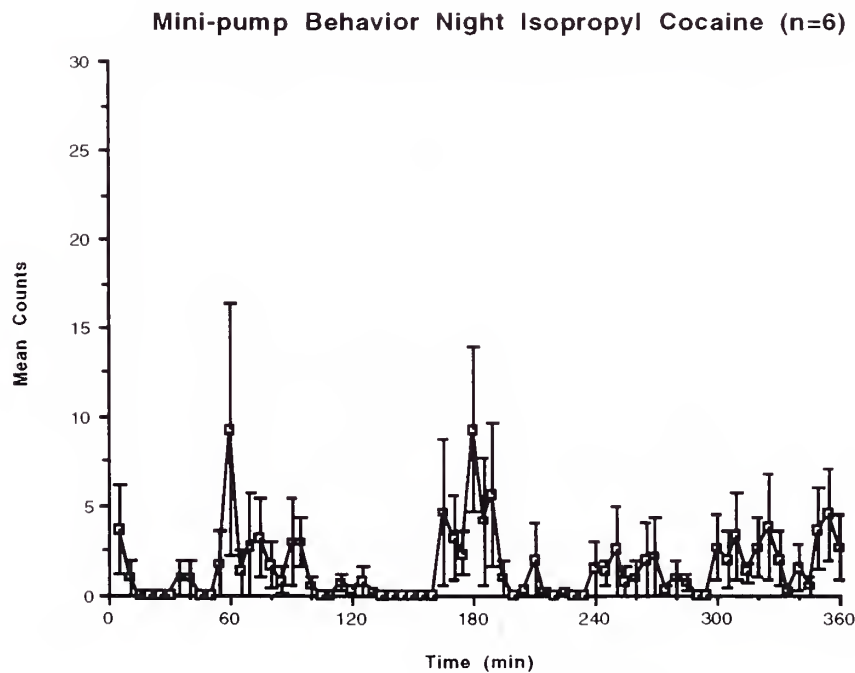
Mini-Pump Behavior Night Cocaine (n=7)

Figure 16: Mean values (± SEM) of non-consecutive beam breaks in five minute time bins measuring rat locomotor activity starting approximately one hour before darkness on day six of a seven day cocaine (7.7 μg/min; 22.6 nmol/min) infusion by mini-pump ($n = 7$).

Mini-pump Behavior Night Cocaethylene (n=6)

Figure 17: Mean values (± SEM) of non-consecutive beam breaks in five minute time bins measuring rat locomotor activity starting approximately one hour before darkness on day six of a seven day cocaethylene (11.1 μg/min; 22.6 nmol/min) mini-pump infusion (n = 6).

Mini-pump Behavior Night Isopropyl Cocaine (n=6)

Figure 18: Mean values (± SEM) of non-consecutive beam breaks in five minute time bins measuring rat locomotor activity starting approximately one hour before darkness on day six of a seven day isopropyl cocaine (4.6 μg/min; 11.3 nmol/min) infusion by mini-pump (n = 6).
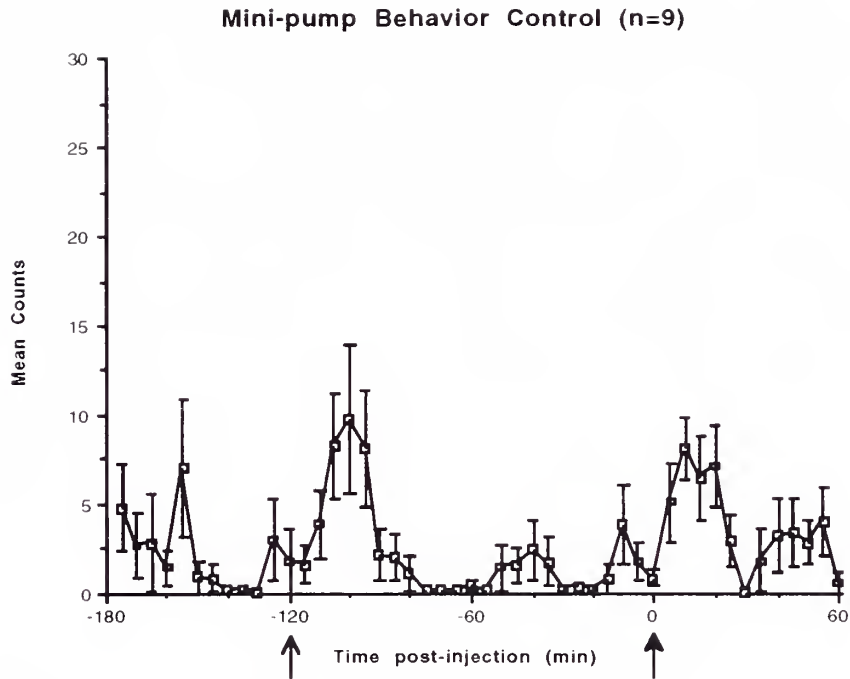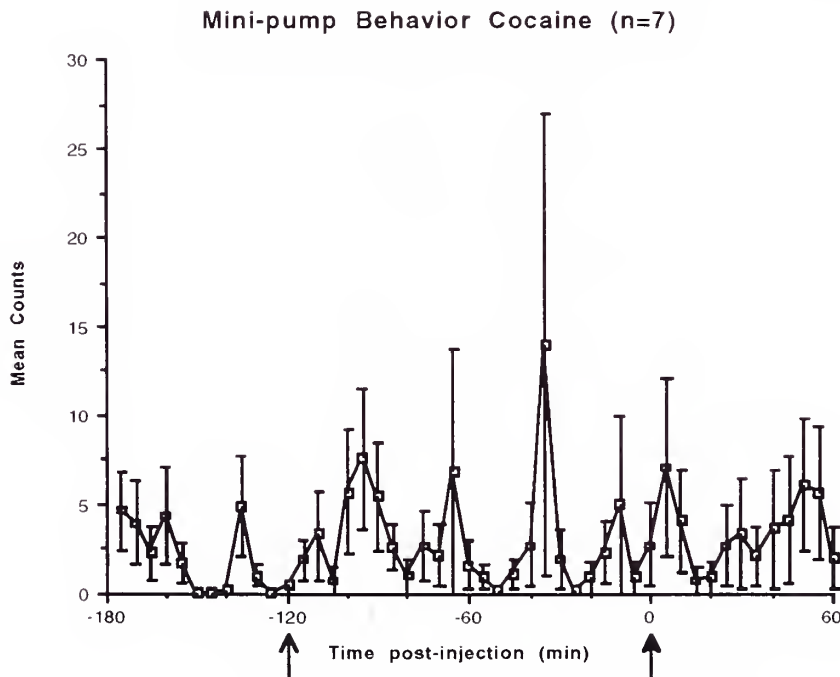
Mini-pump Behavior Control (n=9)

Figure 19: Mean values (± SEM) of non-consecutive beam breaks in five minute time bins measuring rat locomotor activity when a seven day infusion of control (water) was stopped at -120 minutes and a bolus of cocaine (3 μmol/kg) was given at time 0 minutes (n = 9).
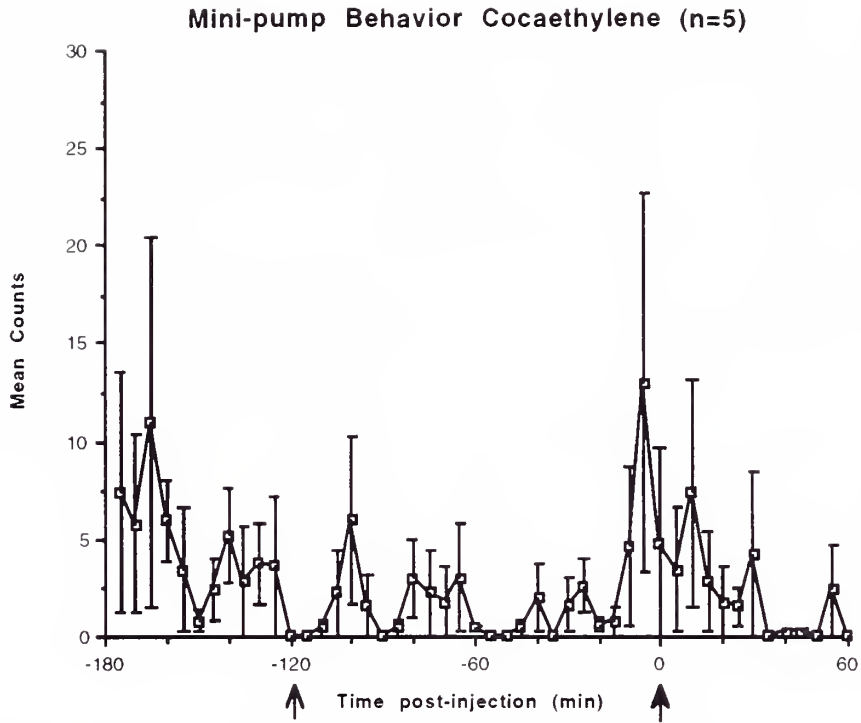
Mini-pump Behavior Cocaine (n=7)

Figure 20: Mean values (± SEM) of non-consecutive beam breaks in five minute time bins measuring rat locomotor activity when a seven day infusion of cocaine (7.7 μg/min; 22.6 nmol/min) was stopped at -120 minutes and a bolus of cocaine (3 μmol/kg) was given at time 0 minutes (n = 7).

Mini-pump Behavior Cocaethylene (n=5)

Figure 21: Mean values (± SEM) of non-consecutive beam breaks in five minute time bins measuring rat locomotor activity when a seven day infusion of cocaethylene (11.1 µg/min; 22.6 nmol/min) was stopped at -120 minutes and a bolus of cocaine (3 µmol/kg) was given at time 0 minutes (*n* = 5).

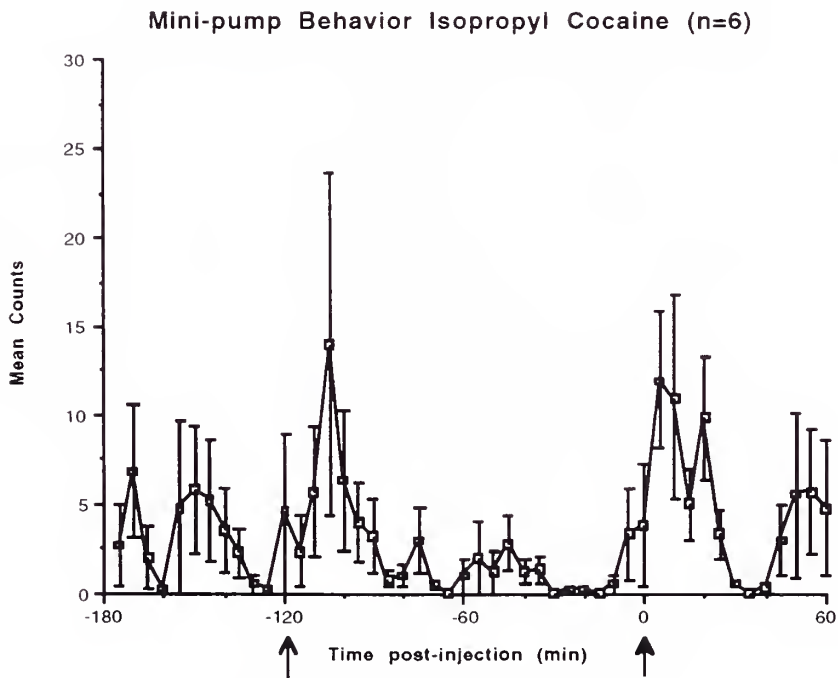### Mini-pump Behavior Isopropyl Cocaine (n=6)

Figure 22: Mean values (± SEM) of non-consecutive beam breaks in five minute time bins measuring rat locomotor activity after seven day infusion of isopropyl cocaine (4.6 µg/min; 11.3 nmol/min) was stopped at -120 minutes and a bolus of cocaine (3 µmol/kg) was given at time 0 minutes (*n* = 6).
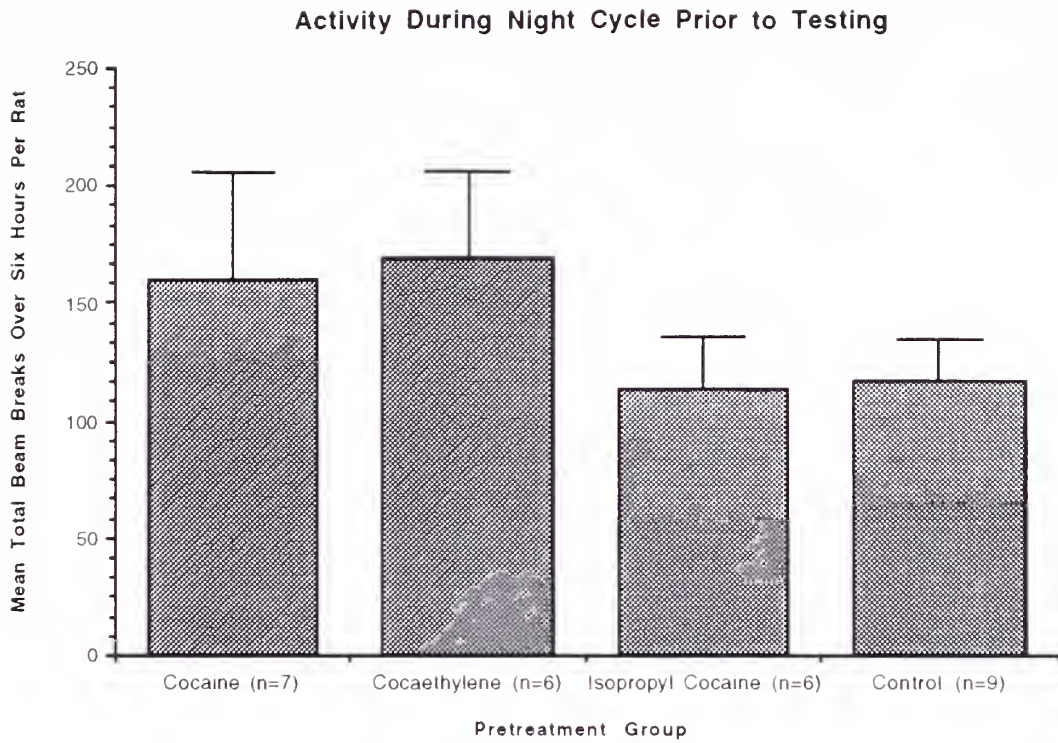
Figure 23: Mean total number of non-consecutive beam breaks (± SEM) over six hours of rats in various pretreatment groups (cocaine, cocaethylene, isopropyl cocaine, and control) one day prior to testing. Measurements started approximately one hour before darkness.
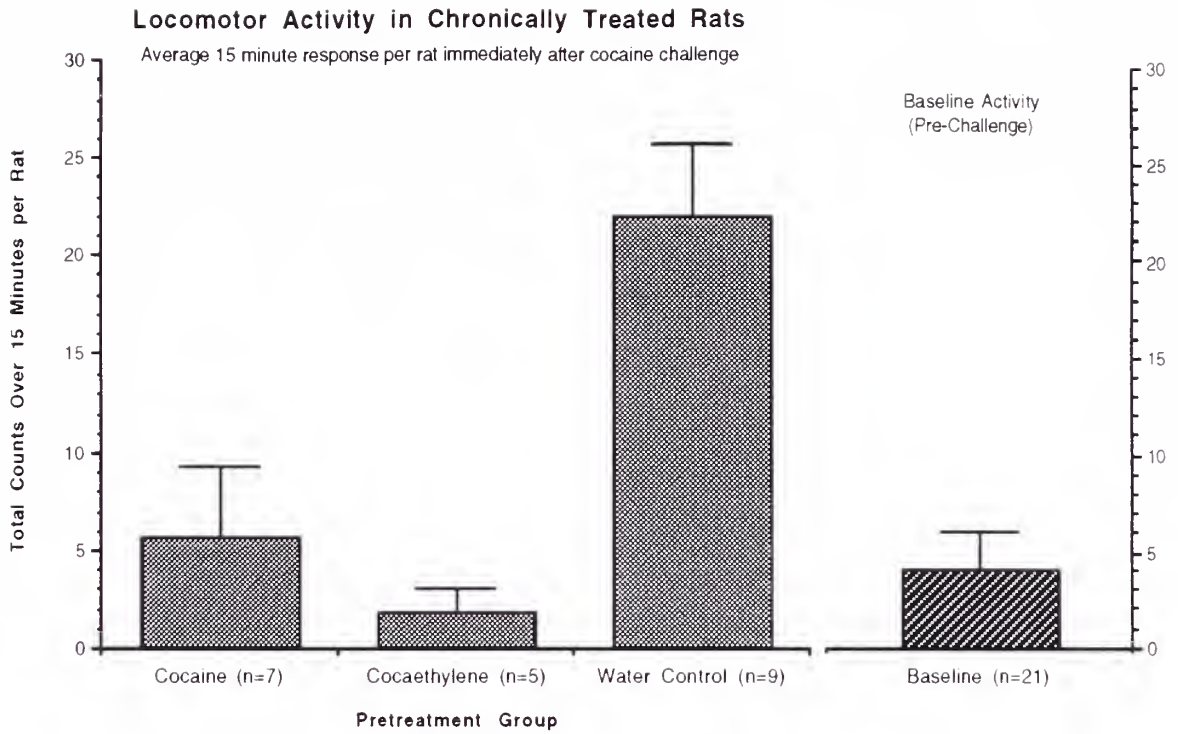
Locomotor Activity in Chronically Treated Rats

Average 15 minute response per rat immediately after cocaine challenge

Baseline Activity
(Pre-Challenge)

Figure 24: Total number of non-consecutive beam breaks (± SEM) from 5–20 minutes immediately following cocaine challenge (time 0). Rats are from various pretreatment groups (cocaine, cocaethylene, control, and baseline).

6. REFERENCES

Bailey, D.N. 1993. Plasma cocaethylene concentrations in patients treated in the emergency room or trauma unit. *Am J Clin Pathol* 99: 123-127.

Boyer, C.S. and D.R. Petersen. 1992. Enzymatic basis for the transesterification of cocaine in the presence of ethanol: evidence for the participation of microsomal carboxylesterases. *J Pharmacol Exp Ther* 260: 939-946.

Bradberry, C.W., J.B. Nobiletti, J.D. Elsworth, B. Murphy, P. Jatlow, and R.H. Roth. 1993. Cocaine and cocaethylene: microdialysis comparison of brain drug levels and effects on dopamine and serotonin. *J Neurochem* 60: 1429-1435.

Bradberry, C.W. and R.H. Roth. 1989. Cocaine increases extracellular dopamine in rat nucleus accumbens and ventral tegmental area as shown by in vivo microdialysis. *Neurosci Lett* 103: 97-102.

Cameron, D.L. and J.T. Williams. 1994. Cocaine inhibits GABA release in the VTA through endogenous 5-HT. *J Neurosci* 14: 6763-6767.

Dean, R.A., C.D. Christian, R.H. Sample, and W.F. Bosron. 1991. Human liver cocaine esterases: ethanol-mediated formation of ethylcocaine. *Faseb J* 5: 2735-2739.

Elsworth, J.D., J.R. Taylor, P. Jatlow, and R.H. Roth. 1993. Serotonin involvement in cocaine sensitization: clues from studies with cocaine analogs. *Drug Development Research* 30: 189-200.

Emmett-Oglesby, M.W., R.L. Peltier, R.Y. Depoortere, C.L. Pickering, M.L. Hooper, Y.H. Gong, and J.D. Lane. 1993. Tolerance to self-administration of cocaine in rats: time course and dose-response determination using a multi-dose method. *Drug Alcohol Depend* 32: 247-256.

Foltin, R.W. and M.W. Fischman. 1991. Smoked and intravenous cocaine in humans: acute tolerance, cardiovascular and subjective effects. *J Pharmacol Exp Ther* 257: 247-261.

Foltin, R.W., M.W. Fischman, G. Nestadt, H. Stromberger, E.E. Cornell, and G.D. Pearlson. 1990. Demonstration of naturalistic methods for cocaine smoking by human volunteers. *Drug Alcohol Depend* 26: 145-154.

Frank, R.A., P.Z. Manderscheid, S. Panicker, H.P. Williams, and D. Kokoris. 1992. Cocaine euphoria, dysphoria, and tolerance assessed using drug-induced changes in brain-stimulation reward. *Pharmacol Biochem Behav* 42: 771-779.

Grant, B.F. and T.C. Harford. 1990. Concurrent and simultaneous use of alcohol with cocaine: results of national survey. *Drug Alcohol Depend* 25: 97-104.

Hammer, R.P., Jr. and E.S. Cooke. 1994. Gradual tolerance of metabolic activity is produced in mesolimbic regions by chronic cocaine treatment, while subsequent cocaine challenge activates extrapyramidal regions of rat brain. *J Neurosci* 14: 4289-4298.

Hearn, W.L., D.D. Flynn, G.W. Hime, S. Rose, J.C. Cofino, E. Mantero-Atienza, C.V. Wetli, and D.C. Mash. 1991. Cocaethylene: a unique cocaine metabolite displays high affinity for the dopamine transporter. *J Neurochem* 56: 698-701.

Hearn, W.L., S. Rose, J. Wagner, A. Ciarleglio, and D.C. Mash. 1991. Cocaethylene is more potent than cocaine in mediating lethality. *Pharmacol Biochem Behav* 39: 531-533.

Jatlow, P. 1993. Cocaethylene: pharmacologic activity and clinical significance. [Review]. *Ther Drug Monit* 15: 533-536.

Jatlow, P., J.D. Elsworth, C.W. Bradberry, G. Winger, J.R. Taylor, R. Russell, and R.H. Roth 1991. Cocaethylene: a neuropharmacologically active metabolite associated with concurrent cocaine-ethanol ingestion. *Life Sciences* 48: 1787-1794.

Jatlow, P. and H. Nadim. 1990. Determination of cocaine concentrations in plasma by high-performance liquid chromatography [published erratum appears in Clin Chem 1991 Jun;37(6):886]. *Clin Chem* 36: 1436-1439.

Johnson, R.D. and J.B. Justice. 1983. Model studies for brain dialysis. *Brain Res Bull* 10: 567-571.

Katz, J.L., P. Terry, and J.M. Witkin. 1992. Comparative behavioral pharmacology and toxicology of cocaine and its ethanol-derived metabolite, cocaine ethyl-ester (cocaethylene). *Life Sci* 50: 1351-1361.

King, G.R., C. Joyner, T. Lee, C. Kuhn, and E.H. Ellinwood. 1992. Intermittent and continuous cocaine administration: residual behavior states during withdrawal. *Pharmacology Biochemistry and Behavior* 43: 243-248.

Koob, G.F. and F.E. Bloom. 1988. Cellular and molecular mechanisms of drug dependence. [Review]. *Science* **242**: 715-723.

Leshner, A.I. 1996. Molecular mechanisms of cocaine addiction. *The New England Journal of Medicine* **335**: 128-129.

Masserano, J.M., D. Venable, and R.J. Wyatt. 1994. Effects of chronic cocaine administration on [3H]dopamine uptake in the nucleus accumbens, striatum and frontal cortex of rats. *J Pharmacol Exp Ther* **270**: 133-141.

McCance, E.F., L.H. Price, T.R. Kosten, and P.I. Jatlow. 1995. Cocaethylene: pharmacology, physiology and behavioral effects in humans. *J Pharmacol Exp Ther* **274**: 215-223.

McCance-Katz, E.F., L.H. Price, C.J. McDougle, T.R. Kosten, J.E. Black, and P.I. Jatlow. 1993. Concurrent cocaine-ethanol ingestion in humans: pharmacology, physiology, behavior, and the role of cocaethylene. *Psychopharmacology (Berl)* **111**: 39-46.

National Household Survey on Drug Abuse, Main Findings 1985 (Rockville, MD: US Department of Health and Human Services, Public Health Service).

Nobiletti, J.B., P.I. Jatlow, and C.W. Bradberry. 1994. Differences in bioavailability between cocaine and cocaethylene and their implications for drug-reward studies. *Psychopharmacology (Berl)* **116**: 273-278.

Pan, H.T., S. Menacherry, and J.B. Justice, Jr. 1991. Differences in the pharmacokinetics of cocaine in naive and cocaine-experienced rats. *J Neurochem* **56**: 1299-1306.

Parsons, L.H., F. Weiss, and G.F. Koob. 1996. Serotonin1b receptor stimulation enhances dopamine-mediated reinforcement. *Psychopharmacology (Berl)* **128**: 150-160.

Paxinos, G. and C. Watson. 1982. The rat brain in stereotaxic coordinates. (New York: Academic Press).

Pettit, H.O. and J.B. Justice, Jr. 1989. Dopamine in the nucleus accumbens during cocaine self-administration as studied by in vivo microdialysis. *Pharmacol Biochem Behav* **34**: 899-904.

Ritz, M.C., R.J. Lamb, S.R. Goldberg, and M.J. Kuhar. 1987. Cocaine receptors on dopamine transporters are related to self-administration of cocaine. *Science* 237: 1219-1223.

Roberts, D.C. and G.F. Koob. 1982. Disruption of cocaine self-administration following 6-hydroxydopamine lesions of the ventral tegmental area in rats. *Pharmacol Biochem Behav* 17: 901-904.

Self, D.W., W.J. Barnhart, D.A. Lehman, and E.J. Nestler. 1996. Opposite modulation of cocaine-seeking behavior by D1- and D2-like dopamine receptor agonists [see comments]. *Science* 271: 1586-1589.