2005

The Effects of Footshock on the Reinforcing Efficacy of Cocaine in Male Long-Evans Rats

Elizabeth S. Hendrick
Virginia Commonwealth University

THE EFFECTS OF FOOTSHOCK ON THE REINFORCING EFFICACY OF COCAINE IN MALE LONG-EVANS RATS

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University

by

Elizabeth S. Hendrick
B.A., University of Virginia, 1997

Director: Keith L. Shelton, Ph.D.
Assistant Professor, Department of Pharmacology and Toxicology

Virginia Commonwealth University
Richmond, Virginia
December, 2005

Acknowledgment

I would like to thank several people for their contributions to the completion of this thesis. Dr. Keith Shelton, my advisor and employer, has been particularly helpful by providing useful criticisms which have guided the learning and writing processes. I am grateful to Keith and to Dr. Patrick Beardsley for allowing me to work in their laboratory. The flexibility and educational opportunities which the job affords have been invaluable.

I am grateful to Drs. Jenny Wiley and Joseph Porter for agreeing to read this and, a bigger burden, to hear me defend it. I thank them for their suggestions and their cooperation as I finished the process.

My family was extremely supportive through this time in my life and I appreciate that they understood why they never got to see me. My mother's frequent pleas for cross-country travel made me feel missed and I return the sentiment.

Special appreciation is in order to Lee for loving friendship, excellent food and for fixing my computer day after day (and always right before a deadline). I couldn't have done this without her to lean on.

I also appreciate the friendship of (Drs.) Laura and John Betz who provided blunt yet sensitive and positive advice about graduate school and the writing process. I thank them for acting as a surrogate family to me and am grateful that our friendship has survived the time and distance after college.

Angela Batman and Jenn Newman both lent me copies of their theses so that I could see true genius and aspire to it. Angela also picked up my slack at work so that I could finally put this thesis to rest. I appreciate Jenn's suggestion of a few articles and our discussions over beer.

Members of the Virginia Boat Club, particularly the members of our "Solidowski" 4+, provided a valuable social outlet, as well as a mostly harmless way to work out frustrations on the river.

I would have gone mad(der) with stress if it weren't for Isabel's constant entertainment and tail-wagging.

Lastly, I thank coffee for making me a fully-functional nervous wreck.

Table of Contents

List of Tables

List of Figures

Figure Page

List of Abbreviations and Symbols

FR	fixed ratio
PR	progressive ratio
CNS	central nervous system
HPA	hypothalamic-pituitary-adrenal
DA	dopamine
CRH	corticotrophin-releasing hormone
POMC	proopiomelanocortin
ACTH	adrenocorticotropic hormone
HCl	hydrochloride
U	unit
w/v	weight per volume
%	percent
X	times
±	plus or minus
n	sample size
S.E.M.	standard error of the mean
df	degrees of freedom

mA	milliamp
Hz	hertz
W	watt
dB	decibel
μm	micrometer
mm	millimeter
cm	centimeter
g	gram
kg	kilogram
mg	milligram
ml	milliliter
i.p.	intraperitoneal
s.c.	subcutaneous
LED	light-emitting diode
M-F	Monday through Friday
VCU	Virginia Commonwealth University
NIH	National Institutes of Health

Abstract

THE EFFECTS OF FOOTSHOCK ON THE REINFORCING EFFICACY OF COCAINE IN MALE LONG-EVANS RATS

Elizabeth S. Hendrick, B.A.

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University

Virginia Commonwealth University, 2005

Director: Keith Shelton, Ph.D., Assistant Professor,
Department of Pharmacology and Toxicology

Many links exist between cocaine abuse and stress. The literature and laboratory studies in rats suggest that this could be because stress increases the reinforcing efficacy of cocaine. Using male Long-Evans rats, experiments in this thesis tested effects of footshock on the reinforcing efficacy of cocaine using a progressive ratio schedule of reinforcement. They also examined effects of footshock on the reinforcing efficacy of a half-maximal dose of cocaine. Finally, they tested the effects of footshock on cocaine self-administration in rats initially resistant to acquisition of cocaine self-administration. Footshock did not increase reinforcing efficacy of cocaine on a PR schedule of reinforcement, nor did it enhance sensitivity to a half-maximal dose of cocaine. Footshock did, however, cause acquisition of cocaine self-administration in acquisition-resistant rats. Therefore, while footshock stress may be capable of sensitizing

acquisition-resistant rats to the reinforcing efficacy of cocaine, it does not appear that it significantly increases the reinforcing efficacy of cocaine in rats with a history of cocaine self-administration.

I Introduction

Cocaine: History, routes of administration and pharmacokinetics

Cocaine (benzoylmethylecgonine) is a refined derivative of the South American plant *Erythroxylon coca* (Buttner et al., 2003; Warner, 1993; White and Lambe, 2003). These plants have been grown in Central and South America since pre-Columbian times (Calatayud and Gonzalez, 2003) and have been used by humans for more than 5000 years (VanDyke and Byck, 1982). A 16th century letter from Friar Vicente de Valverde to Emperor Charles V describes constant chewing of coca leaves for sustenance and refreshment by Peruvian natives (Calatayud and Gonzalez, 2003).

The most common routes of administration of cocaine are intranasal insufflation (i.e. "snorting") followed by smoking and then intravenous injection (Warner, 1993). Although the preferred method of cocaine administration has varied over the years, cocaine can be absorbed through any mucous membrane. Intranasal use of cocaine hydrochloride begins to produce effects within three to five minutes, peaking within 30 – 60 minutes. This route of administration leads to relatively low bioavailability of cocaine (roughly 20%) (Warner, 1993). Cocaine can be smoked after conversion to either the "freebase" or "crack" form, producing effects extremely rapidly, within six to eight seconds. Intravenous injection of cocaine hydrochloride also rapidly produces effects

within 12 – 16 seconds and results in the greatest bioavailability of any route (Warner, 1993).

Once cocaine enters circulation, its half-life is approximately one hour. There are two major inactive metabolites of cocaine. Plasma and liver esterases hydrolyze cocaine into ecgonine methyl ester while benzoylecgonine results from spontaneous hydrolysis of cocaine in the blood. These two processes account for 80-90 % of cocaine's metabolism (Wansaw et al., 2005). Norcocaine, a minor metabolite, is also produced by N-demethylation of cocaine in the liver (Buttner et al., 2003; Warner, 1993; White and Lambe, 2003).

Due to its lipophilicity, cocaine rapidly crosses the blood-brain barrier to affect the central nervous system (Buttner et al., 2003). Cocaine acts on the CNS by blocking the dopamine transporter, which results in increased synaptic dopamine levels (Wise, 1998). Increased norepinephrine and serotonin concentrations, as well as increased dopamine concentrations, result from the blockage of re-uptake of these neurotransmitters presynaptically by cocaine. The inhibition of monoamine oxidase (which degrades dopamine, norepinephrine and epinephrine) by cocaine also contributes to the elevation of concentrations of these neurotransmitters in the synapse as does activation of the enzyme tyrosine hydroxylase (Buttner et al., 2003). As well as having CNS effects, cocaine also acts as a local anesthetic by blocking neuronal sodium channels (Warner, 1993; White and Lambe, 2003). Sympathomimetic effects of cocaine include tachycardia, vasoconstriction, dysrhythmias, hyperthermia, pupil dilation and

hyperglycemia. These latter effects are due to the increased presence of norepinephrine at sympathetic nerve terminals.

Cocaine administration results in euphoria as well as subjective effects of increased alertness, greater energy and self-confidence, loquaciousness, suppression of appetite, and enhanced performance of repetitive behaviors (Buttner et al., 2003; Warner, 1993). The euphoric and anxiolytic effects of cocaine confer high abuse potential. The 2003 National Survey on Drug Use & Health, conducted by SAMHSA, Office of Applied Studies, estimated that 2.3 million Americans (1% of the population) aged 12 or older were current cocaine users. Lifetime users of cocaine within this same population were estimated to include almost 35 million people (or 14.7% of the population). 1.5 million Americans aged 12 or older (0.6% of this population) were estimated to be dependent on or abusing cocaine. The euphoria which occurs with cocaine use is thought to be mediated by increased concentrations of dopamine, norepinephrine and epinephrine, particularly by increased dopamine in mesocorticolimbic brain areas (Andrews and Lucki, 2001; Warner, 1993; White and Lambe, 2003).

Cocaine use and stress

Drug abuse has been linked to stress in several ways. There is documented comorbidity between stress, anxiety and cocaine abuse (Kulka et al., 1990). Cocaine has been reported to relieve effects of stress and both stress and cocaine can activate common brain areas and endocrine pathways. In laboratory animals, stress increases responding for cocaine in several models which measure the reinforcing efficacy of cocaine

(Covington and Miczek, 2001; Falck et al., 2004; Gawin and Ellinwood, 1988; Goeders and Guerin, 1994; Gordon, 2002; Haney et al., 1995; Karlsgodt et al., 2003; McMahon, 2001; Miczek and Mutschler, 1996; Najavits et al., 2003; Prakash and Das, 1993; Ramsey and Van Ree, 1993; Sinha et al., 2000).

Sinha et al. report that craving for cocaine by cocaine abusers was significantly and consistently increased in human test subjects undergoing acute psychological stress. In male human subjects, stress as measured on intake assessment was positively correlated with both relapse to cocaine abuse and high cocaine abuse severity (Sinha et al., 2000). Additionally, subjects classified as "high-stress" according to the Profile of Mood States (POMS) and Speilberger State-Trait Anxiety Inventory exhibited significantly longer duration of cocaine use than did "low-stress" individuals (Karlsgodt et al., 2003). The most consistent and extensive correlation between stress and cocaine abuse can be found in the comorbidity of post-traumatic stress disorder (PTSD) and cocaine abuse, with PTSD present in a large percentage of patients seeking treatment for cocaine dependence (Kulka et al., 1990; Najavits et al., 2003). In fact, in one study 11.8% comorbidity was shown to exist between crack cocaine use and PTSD (Falck et al., 2004).

The high comorbidity of cocaine abuse and stress may be explained in part by either cocaine's ability to relieve stress or alternatively the stress associated with abstinence from cocaine promoting its continued use. Cocaine abusers report feelings of well-being and decreased anxiety as a result of use of the drug (Gawin and Ellinwood, 1988) and cocaine has been commonly reported to produce an intense, orgasmic euphoria

(Warner, 1993). This euphoria is thought to be the result of cocaine's blockage of dopamine re-uptake, leading to increased dopamine in the nucleus accumbens, one of the brain's reward centers (Wise, 1998). Another proposed neurobiological action of cocaine is to decrease activity in the pontine nucleus and locus coeruleus, which may produce anxiolysis (Prakash and Das, 1993). Severe anxiety often results during withdrawal from cocaine abuse (Gawin and Ellinwood, 1988), suggesting relief of anxiety with recurrence of cocaine use.

However, while cocaine can act as a reinforcer, and may be an anxiolytic under certain conditions, it has also been proposed to be an anxiogenic in rats (Fontana et al., 1989; Rademacher et al., 2000). This is evidenced by the fact that cocaine increased latency of entry into and decreased time spent in the open arms of elevated plus mazes, a behavior that has been proposed to model human anxiety (Roserio & Takahashi, 1992; Yang et al., 1992). Additionally, in humans, panic attacks have been precipitated by cocaine use. Similar discriminative stimulus effects have also been produced by cocaine and stressors in rodents (Goeders, 2002). So while cocaine can relieve anxiety in some instances, it could exacerbate anxiety in others. There is, therefore, some question as to why people experiencing stress would continue to abuse cocaine if cocaine simply enhances stress responses.

One possible explanation is that stress alters the neurobiological basis of reinforcing properties of drugs of abuse. In fact, stress could increase responsiveness in motivation and reward systems in the brain by increasing their activity (Piazza and LeMoal, 1998). One example is the previously-mentioned long-lasting increase in

dopamine release in the nucleus accumbens in response to stress or to administration of stress-like levels of glucocorticoids, a reliable biological indicator of stress (Kant et al., 1988), a change which has been proposed to increase self-administration of drugs (Wise, 1998; Piazza and LeMoal, 1998). Administration of glucocorticoids at levels which mimic those produced by experimental stressors has been shown to increase self-administration of amphetamine (Piazza and LeMoal, 1998). Piazza and LeMoal have proposed a specific mechanism for durable increases in drug self-administration as a result of acute and continued stress. It is their hypothesis that dopamine (DA) release by the nucleus accumbens is directly proportional to glucocorticoid concentrations. Stress increases glucocorticoid concentrations, increasing DA release, enhancing sensitivity to the reinforcing effects of drugs, which may increase their self-administration. But high levels of glucocorticoid bind to corticosteroid receptors in the hippocampus causing negative feedback which returns glucocorticoid release to normal levels within two hours. Continued stress leads to consistently high levels of glucocorticoids, causing hippocampal corticosteroid receptor down-regulation, decreasing the negative feedback mechanism, maintaining high glucocorticoid concentrations along with high levels of DA release from the nucleus accumbens, maintaining sensitivity to the reinforcing effects of drugs, which thereby maintains self-administration of these drugs (Piazza and LeMoal, 1998).

Stressful experiences and cocaine administration share several common effects on the central nervous system and the endocrine system, providing further evidence for a link between stress and cocaine use. For example, similar to the development of long-

term potentiation, both stress and single cocaine exposures have been demonstrated to enhance the strength of excitatory synapses on midbrain dopamine neurons (Saal et al., 2003). Stress and cocaine also have similar effects on the hypothalamic-pituitary-adrenal (HPA) axis. Specifically, corticotrophin-releasing hormone (CRH) is released from the hypothalamus into the anterior pituitary where it binds receptors, causing synthesis of pro-opiomelanocortin (POMC) which is cleaved into ACTH among other products. ACTH travels through the general circulation to the adrenal glands where it stimulates the synthesis of cortisol in humans or corticosterone in rats (Goeders, 2002).

Laboratory models of stress

There are several ways to model stress in the laboratory and many factors to take into consideration when doing so. Any model of stress in the laboratory should increase glucocorticoid hormone levels (cortisol in humans, corticosterone in rats), the principle biological response to stress (Goeders, 2002b). These models generally involve forced exposure of the subject to aversive situations or stimuli (Piazza and LeMoal, 1998).

Examples of laboratory models of stress include the imposition of physical stressors such as repeated tail pinch, food restriction, electric footshock, restraint and prenatal stress (mothers are subjected to restraint during the rats' third and fourth gestational week) (Rouge-Pont et al., 1998; Lin et al., 2002; Lu et al., 2003; Van den Hove et al., 2005) . Psychological stressors can also be used and produce similar effects on drug self-administration. Psychological stressors, including witnessing another rat receiving footshock, have been shown to facilitate acquisition of cocaine self-

administration by rats (Ramsey and VanRee, 1993). Introduction as an intruder rat into an aggressive rat's home cage increases cocaine self-administration (Miczek and Mutschler, 1996), as does raising male rats in mixed gender colonies, causing high social competition for access to females (Piazza and LeMoal, 1998). While all of these stressors have been shown to be effective, the studies conducted for this thesis used electric footshock. Footshock stress has been shown to increase glucocorticoid release (Kant et al., 1988). Footshock intensity is also quantifiable and controllable, perhaps producing less variable stress responses in subjects than would other less precise methods of imposing stress.

Factors modulating efficacy of footshock as a stressor

Stressors that are unpredictable are more efficacious than are predictable stressors at affecting drug self-administration. In fact, predictable footshocks had no effect on cocaine self-administration in one study (Goeders and Guerin, 1994). Therefore, in studies conducted for this thesis, electric footshocks were administered for half-second durations at randomized intervals, preventing the prediction of occurrence of shocks by the rats.

The intensity of the stressor is another important factor to consider when modeling stress in the laboratory. Stressors must be of adequate intensity without reaching intensities that suppress behavior (Piazza and LeMoal, 1998). The shock intensities used in experiments for this project are those that have been used successfully

in our lab to cause reinstatement to drug-seeking behavior in rats in which this behavior had been extinguished (Shelton et al., 2004; Beardsley et al., 2005).

Finally, for acute stressors such as footshock, the interval between the stressor and measurement of behavior must be short enough so that any effects on behavior due to the stressor can be measured (Piazza and LeMoal, 1998). For these studies, footshock segments immediately preceded self-administration sessions (which lasted two hours), ensuring that the effects of stress directly impacted self-administration and preventing dissipation of these effects.

Self-administration and measurement of reinforcing efficacy

Self-administration occurs when a subject emits a behavior that leads to drug delivery (such as pressing a lever for a drug infusion). If a drug is self-administered at a greater rate than is its vehicle, that drug is considered to be a reinforcer. "Reinforcing efficacy" of a drug refers to that drug's ability to support self-administration. There are several methods commonly used to measure reinforcing efficacy in the laboratory, many of which center on the self-administration of drugs of abuse (Meisch, 1987). Rate of acquisition of self-administration, continued self-administration despite increased work requirements and self-administration of drug doses that would normally not support behavior are all ways by which reinforcing efficacy can be measured.

Effects of stress on acquisition of self-administration

Many studies have assessed the effects of stressors on acquisition of self-administration of various drugs of abuse, including cocaine. For example, Goeders and Guerin (1994) found that non-contingent electric footshock facilitated acquisition of self-administration of cocaine; lower doses were required for self-administration to occur in shocked versus non-shocked rats, perhaps indicating that stress caused by footshock increased the reinforcing efficacy of those lower doses of cocaine. Other studies using models of social stress have found enhanced acquisition of cocaine self-administration over a wide range of doses in rats exposed as an intruder in an aggressive rat's cage (Haney et al., 1995; Miczek and Mutschler, 1996). Ramsey and VanRee (1993) observed that rats which had been in the presence of other rats experiencing footshock acquired self-administration of a low dose of cocaine not self-administered by rats which had not been in the presence of other rats being shocked. Neonatal rats which experienced one hour per day of isolation exhibited long-lasting effects on acquisition of cocaine self-administration; these rats learned to self-administer intravenous cocaine at lower doses and acquired self-administration of a standard dose of cocaine more quickly than did non-isolated rats (Gordon, 2002). Covington and Miczek (2001) examined the effects of behavioral sensitization brought about by social-defeat stress on intravenous cocaine self-administration. These measures included rates of acquisition, sensitivity to various cocaine doses, break points on a progressive ratio schedule of reinforcement and drug intake during 24-hour cocaine binges. They found only that stress-sensitized rats exhibited increased cocaine intake during the 24-hour binges.

These studies clearly indicate that stress can enhance the rate of acquisition of cocaine self-administration as well as facilitate acquisition of lower cocaine doses. They do not, however, address the possibility that stressors may promote cocaine self-administration in animals that would not have otherwise self-injected cocaine. To anthropomorphize, they do not examine whether stress will make someone take cocaine who would not have taken the drug anyway if given the opportunity. Answering this question has some unique technical problems. Cocaine is so efficacious as a reinforcer that most laboratory rats readily acquire cocaine self-administration. In a representative sample of 44 rats from our laboratory, 38 had acquired cocaine self-administration within ten days, with most of these rats acquiring self-administration within the first few days of cocaine availability (unpublished observation). Approximately 95% of rats which have the opportunity to self-administer cocaine eventually acquire self-administration. Ordinarily this would make studies in rats that do not initially self-administer cocaine impossible. However, due to the large number of animals used in the laboratory for other projects, it was possible to obtain sufficient acquisition-resistant rats to examine if stress can promote self-administration in rats that would not, without a stress experience, self-inject cocaine.

Effects of stress on response requirement for drug self-administration

Altering response requirement is another means of determining the reinforcing efficacy of a drug. There are a number of possible methods by which the work requirement to receive a drug injection can be manipulated. One of the most well

characterized and utilized of these methods is the progressive ratio procedure (Stafford et al., 1998). Under a progressive ratio schedule of reinforcement, each successive drug infusion requires a greater number of responses (the work requirement for infusions increases according to a formula throughout the session). At some point in the session, responding maintained by infusions will cease. This point is defined as the "breaking point" and is considered to be an indicator of the reinforcing efficacy of a drug. Higher breaking points are used to infer greater reinforcing efficacy (Stafford et al., 1998).

Progressive ratio schedules of reinforcement can also be used to compare reinforcing efficacy of a given drug under different circumstances (e.g. during stress versus the absence of stress). For example, Shaham and Stewart (1994) found that footshock increased breaking points attained by rats responding for heroin on a progressive ratio schedule of reinforcement. Footshock also increased rats' breaking points when responding was maintained by oral fentanyl on a progressive ratio schedule of reinforcement (Shaham et al., 1993). Aside from these studies, most studies of the effects of stressors on the reinforcing efficacy of drugs have focused on other schedules of reinforcement besides progressive ratios. Experiments conducted for this thesis were performed in part to increase the volume of data available regarding the effects of footshock on reinforcing efficacy of cocaine during responding maintained by a progressive ratio schedule of reinforcement.

Study goals

Given that there are correlations between stress and cocaine abuse in humans and that cocaine produces subjective effects which could mitigate the effects of stress, there is reason to suspect that stress makes cocaine more desirable. The literature suggests that many types of stressors can increase self-administration of cocaine by rats using a variety of experimental methods; therefore, it seems likely that stress increases the reinforcing efficacy of cocaine in rats. The studies described in the following chapter were designed to determine if stressful stimuli would, in fact, increase the reinforcing efficacy of cocaine in rats. The methods used are ones which have not yet been widely used to examine the effects of footshock stress on the reinforcing efficacy of cocaine in rats. Experiments in this thesis tested effects of footshock on the reinforcing efficacy of cocaine using a progressive ratio schedule of reinforcement. They also examined the effects of footshock on the reinforcing efficacy of a dose of cocaine which produced half-maximal responding. Finally, they tested the effects of footshock on cocaine self-administration in rats initially resistant to acquisition of cocaine self-administration. The literature concerning effects of stress on human cocaine abusers and laboratory animals suggested that footshock stress could be hypothesized to increase the reinforcing efficacy of cocaine in rats in all of these experiments.

II Materials and Methods

Subjects

All experiments used experimentally-naïve male Long-Evans rats (Harlan, Indianapolis, IN). Rats were housed individually in standard hanging plastic rodent cages in a temperature and humidity controlled 12 hour:12 hour reversed light/dark cycle colony room with unlimited access to water. Rats were food restricted to maintain an average weight of 320 g (rats weighed 305 – 335 g during cocaine self-administration and testing). To reach and maintain the target weight of 320 g, rats were fed as follows: rats weighing 305 – 315 g were fed 20 g of rat chow per day, rats weighing 315 – 325 g were fed 15 g of rat chow per day and rats weighing 325 – 335 g were fed 12 g of rat chow per day. Animal care adhered to standards set forth by the university's Institutional Animal Care and Use Committee and was in keeping with NIH Guidelines for Care and Use of Laboratory Animals.

Surgery

Catheters for intrajugular implantation were constructed from 3.5 French polyurethane catheter tubing (Access Technologies; Skokie, IL). Sesame oil (Acros Organics, New Jersey) was heated and the tip of the tubing was inserted into the hot oil. The tubing was then stretched to create a tapered tip which was then trimmed so that the

end of the catheter had an inner diameter of approximately 0.75 mm. A small cuff was formed from a 2 mm length of the same tubing used to make the catheters and was pulled over the tapered end of the catheter and glued in place with Loctite QuickTite super glue (Manco, Inc.; Avon, Ohio) 3.2 cm from the tapered tip. A second larger cuff was formed from a 2 mm length of micro-renathane tubing (Braintree Scientific, Inc.; Braintree, MA) with an inner diameter of 0.8 mm; this cuff was pulled over the non-tapered end of the catheter and glued in place 3.6 cm from the tapered tip. After the glue had dried, these catheters were disinfected with iodine surgical scrub (The Purdue Frederick Company; Norwalk, CT) diluted 1:4 with sterile water for 30 minutes. They were then rinsed with and stored in sterile heparinized normal saline until they were used.

All rats receiving intravenous drug infusions were equipped with indwelling intrajugular vein catheters. Rats were pre-treated with 0.04 mg glycopyrrolate (American Regent, Inc; Shirley, NY) to decrease bronchial secretions and then anesthetized with a combination of 50mg/kg ketamine, 1 mg/kg acepromazine s.c. (Phoenix Pharmaceuticals, Inc; St. Joseph, MO), 2 mg/kg morphine s.c. and 15 mg/kg pentobarbital i.p. Rats were shaved and prepared with iodine surgical scrub (The Purdue Frederick Company; Norwalk, CT) mid-scapularly and at the anterior neck on the side to be catheterized. A 1.5 cm incision was made on the anterior right side of the neck. The right jugular vein was then dissected from the surrounding tissue. The vein was ligated with 4-0 silk suture (Surgical Specialties Corporation; Reading, PA) rostral to the intended site of incision. Ball-tipped vein-cutting scissors were used to cut a 0.5 mm hole in the right jugular vein. The catheter was implanted in the right jugular vein such that the smaller cuff affixed

closest to the tapered tip of the catheter was passed into the vein and the other larger cuff affixed to the catheter remained outside of the jugular vein rostral to the incision into which the catheter was inserted. A second piece of silk suture was tied around the vein and catheter between the two cuffs to secure the catheter into the vein. The first piece of silk suture that had been used to ligate the vein was tied around the catheter tubing rostral to the larger cuff. The tails of the suture were attached to the fascia on either side of the jugular vein using a curved suture needle. The tails of the silk were then sutured together to cover the vein and anchor the catheter. The tip of the catheter terminated just prior to the right atrium. The distal end was passed subcutaneously to the mid-scapular region where a 15 mm radius dacron mesh cannula connector pedestal with a riveted-plastic-encased 22-gauge surgical steel "L" (Plastics One; Roanoke, VA) was implanted subcutaneously. The distal end of the catheter was connected and glued to the pedestal and the rat's incisions were treated with a topical spray of 0.57 mg/ml gentamicin and 0.284 mg/ml betamethasone valerate (Med-Pharmex, Inc; Pomona, CA) and the incisions were stapled closed. Rats received 75,000 units of penicillin (Hanford's U.S. Vet; Syracuse, NY) s.c. immediately after surgery and 12.5 mg amoxicillin oral antibiotic (BioServ; Frenchtown, NJ) for the next three days after surgery as prophylaxis. Rats were given at least five days of recovery and post-surgical observation before beginning any self-administration procedures.

Apparatus

Experiments were conducted in operant chambers equipped with two levers, each with LED cue lights mounted above them, a 2.8 W overhead lamp, a 2900-Hz tone generator, and electrified floor grid enclosed in ventilated, sound-attenuating chambers (Med Associates, Inc.; St. Albans, VT). The floor of each operant chamber was made from 19 4 mm cylindrical metal rods spaced 11 mm apart which were wired to a microprocessor- controlled feedback-regulated DC shocker designed and constructed by VCU's custom design and fabrication shop. The intensity of the footshocks delivered by the metal bars of the floor grid could be adjusted in 0.5 mA increments, using the shocker mounted outside of the chambers. Drug infusions were supplied from syringe pumps (Med Associates, Inc.; St. Albans, VT) that delivered 0.18 ml infusions over the course of six seconds via Tygon tubing (Small Parts, Inc.; Miami Lakes, FL). The tubing was connected by way of a swivel suspended above the operant chamber (which allowed full range of motion of the rat within the chamber) to a spring steel tether which protected the internal infusion tubing and connected to the rat's back-mounted cannula connector pedestal. All stimuli and schedule parameters were controlled using Med-Associates interfacing and MED-PC IV software.

Drugs

Cocaine HCl (NIDA; Bethesda, MD) was diluted with sterile heparinized normal saline and then sterile filtered through 0.2 μm acrodiscs (Pall Corporation; Ann Arbor,

MI) to make stock solutions. This was further diluted with sterile heparinized (5 U/ml) normal saline to make infusion solutions.

Experiments

Experiment 1: Effects of footshock on the reinforcing efficacy of cocaine or saccharin on a progressive ratio schedule of reinforcement

Group 1

Six rats implanted with indwelling intrajugular vein catheters were initially trained to self-administer 0.5 mg/kg cocaine infusions on a fixed ratio 1 (FR1) schedule of reinforcement (each response on the active lever resulted in one cocaine infusion) during daily (M-F) two-hour training sessions. Completion of the FR resulted in a 6-second intravenous cocaine infusion along with 6 seconds of 3-Hz flashing stimulus lights and a 6-second, 72-dB, 2900-Hz tone. The houselight was extinguished for 6 seconds following completion of each FR, during which responses on the active lever did not count toward completion of the next FR. Responses on the inactive lever had no scheduled consequences. The FR value was increased to 2 (every two responses on the active lever resulted in one cocaine infusion) after the first session during which the number of active-lever responses was greater than or equal to 15. From FR2, the FR value was increased one response after every two consecutive sessions during which the number of infusions was greater than or equal to 15 until a FR5 was reached. Rats remained at FR5 until they had self-administered greater than or equal to 15 infusions

during each of four consecutive sessions at FR5. At this point, rats were considered to have met testing criteria.

Rats were then placed on a within-session progressive ratio (PR) schedule of reinforcement. Under this schedule, the first response on the active lever resulted in one infusion of cocaine. The work requirement for each of the next infusions was increased so that the rats were required to respond twice for the second infusion, four times for the third infusion, etc. (the number of responses required for each infusion = $[5e^{(\text{infusion number X } 0.2)}] - 5$ as shown in the table on the next page). Each session terminated after 30 minutes had elapsed with no responses on the active lever. To allow responding on this schedule to stabilize, the rats were tested on one PR session per day for each of 12 consecutive days. The break point was defined as the last completed ratio within each session.

To test the effects of footshock stress on break points, on test days 13 - 16, each PR session was preceded by a 15-minute shock component during which rats received 1.02 mA intermittent footshocks (an average of 23 shocks lasting 0.5-seconds each with a mean intershock interval of 40 seconds). Baseline responding in the absence of footshock was re-established on days 17 – 21 (sessions were identical to those preceded by footshock except that they were preceded by a 15 minute timeout in the operant chambers with the levers retracted and the houselight off). On test days 22 – 25, sessions were again preceded by a 15 minute footshock segment, but with the shock intensity decreased to 0.39 mA (the lowest value reliably delivered by our system). Baseline responding in the absence of footshock was again established on test days 26 – 30. Five extinction sessions were then conducted; these sessions were identical to non-footshock

PR sessions except that responses on the active lever resulted in saline, not cocaine, infusions.

Table 1: Progression of response requirements

Infusion number	Number of responses required for infusion	Cumulative responses
1	1	1
2	2	3
3	4	7
4	6	13
5	9	22
6	12	34
7	15	49
8	20	69
9	25	94
10	32	126
11	40	166
12	50	216
13	62	278
14	77	355
15	95	450
16	118	568
17	145	713
18	178	891
19	219	1110
20	268	1378
21	328	1706
22	402	2108
23	492	2600
24	603	3203
25	737	3940

When using a progressive ratio schedule of reinforcement, there were several procedural factors to consider. These included the choice of an algorithm for the progression of response requirements for individual drug infusions and the establishment

of break point criteria. It was important that the progression chosen insured a reasonable session length, so that the effects of any pre-treatment were less likely to dissipate during testing sessions. Limiting drug intake during the session was another concern. Using progressions with rapidly escalating work requirements ensured that the break point was reached before the subject had self-administered enough drug to become satiated or to develop tolerance or sensitization to the drug's effects (Stafford et al., 1998). An algorithm that addressed these concerns has been developed by Richardson and Roberts (1996) for cocaine self-administration on a progressive ratio schedule of reinforcement: ratio requirement = $[5e^{(\text{infusion number X } 0.2)}] - 5$. This was the algorithm chosen for experiments conducted for this thesis.

The break point criterion used for this thesis was 30 minutes without a response on the cocaine-reinforced lever. This criterion was selected because it is a long enough time period to account for any post-reinforcement pause during which no responding normally occurs following individual cocaine infusions (Richardson and Roberts, 1996; Stafford et al., 1998). A longer time period would have been unnecessary because of the typically rapid cessation of responding for cocaine on a progressive ratio schedule (Richardson and Roberts, 1996). In fact, a longer period to allow for responding could have led to nearly indefinite session lengths due to the likelihood of random responses occurring on the reinforced lever.

Group 2

Three experimentally-naïve rats without intrajugular vein catheters were trained to orally self-administer a 0.3% w/v saccharin solution during two-hour training sessions. During these sessions, responses on the active lever resulted in six second availability of the saccharin solution. Completion of the FR activated a motor which raised a 0.02 ml dipper cup attached to a lever arm into an alcove in the chamber. Completion of the FR also resulted in 6 seconds of 3-Hz flashing stimulus lights and a 6-second, 72-dB, 2900-Hz tone. The houselight was turned off for 6 seconds following completion of each FR and responses on the active lever during this period did not count toward completion of the next FR. Responses on the inactive lever had no scheduled consequences. As with Group 1 (rats responding for cocaine), these rats were initially trained to self-administer saccharin at FR1 and were increased to a FR5 schedule of reinforcement over successive sessions. Criteria for testing were the same as for the cocaine self-administering rats, as were the sequence and procedures for establishing baseline responding on a PR schedule and for testing the effects of footshock on break points in responding for saccharin. During extinction, water instead of saccharin was made available by responding on the active lever.

Experiment 2: Effects of footshock on cocaine self-administration in rats initially resistant to cocaine self-administration

Eight rats with jugular vein catheters were used in this experiment. In order to be used in this study, rats had to exhibit a number of criteria which defined them as being

resistant to cocaine acquisition. First, the rat had been allowed to self-administer cocaine during two-hour daily training sessions for at least 10 –15 days. Second, in order to insure that the rat had received some cocaine, it had to have self-administered at least 10 infusions of cocaine over the course of these 10 – 15 days. Third, the rat had self-administered less than 15 cocaine infusions on any self-administration day. Fourth, the rat had been given 12 hours of overnight access to intravenous cocaine self-administration on days 4 and 8. Fifth, the active lever had been baited with peanut butter or with jelly on days 1-7 to increase the likelihood of active lever responding. Lastly, the rat's catheter was patent as demonstrated by rapid and transient anesthesia induced by an i.v. infusion of 0.2 mg ketamine. These rats were considered acquisition-resistant because they had been allowed access to cocaine for more than one standard deviation beyond the mean number of days required for a representative sample of 44 rats in this lab to acquire self-administration of cocaine yet they had failed to acquire cocaine self-administration.

The effects of footshock on acquisition of cocaine self-administration in these acquisition-resistant rats were then examined. During 16 subsequent test days, each response on the active lever resulted in a 6-second, 0.5 mg/kg, cocaine infusion along with 6 seconds of 3-Hz flashing stimulus lights paired with a 72- dB, 2900-Hz tone. The houselight was extinguished for 6 seconds following completion of each FR. Responses on the active lever during this 6-second period did not count toward completion of the next FR. Responses on the inactive lever had no scheduled consequences. Test days 1 – 4 began with a 15 minute shock component during which rats received 1.02 mA

intermittent footshocks (an average of 23 shocks lasting 0.5-seconds each with a randomized mean intershock interval of 40 seconds), followed by a two-hour cocaine self-administration session. Levers were retracted and the houselight was extinguished during the shock component. Test days 5 – 8 began with a 15 minute timeout in the operant chamber instead of a shock component, followed by a two-hour cocaine self-administration session. Days 9 – 12 began with a 15 minute 1.02 mA shock component followed by a two-hour cocaine self-administration session. Test sessions 13 – 16 again began with a timeout followed by a two-hour cocaine self-administration session. For half of the rats in the study, the order of shock vs. no shock presentation was reversed to control for order effects. Table 2 below illustrates conditions for each test day.

Table 2: Summary of testing procedure for Experiment 2

Test days	Conditions
1 – 4	**15 minute 1.02 mA shock segment followed by cocaine self-administration**
5 – 8	**15 minute timeout followed by cocaine self-administration**
9 – 12	**15 minute 1.02 mA shock segment followed by cocaine self-administration**
13 - 16	**15 minute timeout followed by cocaine self-administration**

Experiment 3: Effects of footshock on sensitivity to the reinforcing efficacy of low doses of cocaine

Six rats were trained to self-administer 0.5 mg/kg/infusion i.v. cocaine during daily (M-F), two-hour sessions. Responses on the active lever resulted in 6-second intravenous cocaine infusions along with 6 seconds of 3-Hz flashing stimulus lights and a

6-second, 72-dB, 2900-Hz tone. The houselight was extinguished during the 6 seconds following completion of each FR. Responses on the active lever during this 6-second period did not count toward completion of the next FR. Responses on the inactive lever had no scheduled consequences. The work requirement for each infusion was increased in the same manner as described for Experiment 1, until the rats responded on the active lever greater than or equal to 15 times during each of four consecutive self-administration sessions at FR5. The cocaine self-administration dose was then decreased to 0.25 mg/kg/infusion. Rats responded on a FR5 schedule of reinforcement for this dose for four consecutive days. The cocaine infusion dose was subsequently halved every 4 days. This procedure was continued until responding decreased to less than 50% of that generated by the dose of cocaine that produced the highest mean response rates. This dose was defined as the half-maximal dose and was used for subsequent test sessions. For an additional four days, a 15 minute 1.02 mA intermittent footshock component (an average of 23 shocks lasting 0.5-seconds each with a randomized average intershock interval of 40 seconds) preceded two-hour self-administration sessions during which rats responded for the half-maximal dose of cocaine. During four additional test sessions, rats again were allowed to respond for the half-maximal dose of cocaine during two-hour self-administration sessions but without a shock component preceding the self-administration sessions.

III Results

Experiment 1: Effects of footshock on the reinforcing efficacy of cocaine or saccharin on a progressive ratio schedule of reinforcement

When responding for cocaine was measured using a progressive ratio schedule of reinforcement, footshock stress did not increase mean number of responses per session. The higher intensity (1.02 mA) in fact tended to decrease responding. Figure 1 shows mean reinforced lever responses for the group of six rats for each session after self-administration had been established. Responding for 0.5 mg/kg/infusion cocaine on a progressive ratio schedule of reinforcement increased each day, reached a maximum of 1474 (±310.7) responses per session on day 8 and then stabilized at a level of approximately 1200-1300 responses per session on days 10-12. On the first day that the rats received 1.02 mA intermittent footshock before the self-administration session (day 13), mean responding was virtually unchanged from pre-shock levels. However, responding on the subsequent three days of 1.02 mA shock pre-treatment was suppressed, reaching as low as 695 (±235.2) responses per session on day 15. Responding on the reinforced lever recovered to at, or even above, baseline levels for each of the next five sessions, although between-day variability was very high compared to pre-shock levels. Mean responding again decreased during the first three sessions in which the rats received the lower intensity (0.39 mA) footshock before sessions. However, on the last

day on which rats received 0.39mA footshock, average responding was again back to pre-shock baseline levels. Responding on the reinforced lever then stabilized back to baseline levels during the next five sessions (days 26-30) in which the rats did not undergo footshock pre-treatment. On the first day of saline extinction, reinforced-lever responding decreased to 277 (±69.8) responses per session and stayed near that level, showing little variability between rats. However, when these rats responded for cocaine, there was a great degree of variability in numbers of reinforced-lever responses per session between rats.

Since individual animal responding varied so widely, figure 2 shows the effects of 1.02 mA and 0.39 mA footshock on mean reinforced lever responding averaged across all test sessions as a percent of their 0.5 mg/kg/infusion cocaine baseline responding on a progressive ratio schedule of reinforcement (where "baseline" refers to mean responding during the last four self-administration days before shock began and was calculated for each rat). Mean responding decreased to 68 percent (±13%) of baseline responding on the test sessions preceded by 1.02 mA footshock. Mean responding then increased to above baseline levels (106 percent (±25%)) during the subsequent control sessions in which the rats were not shocked prior to the self-administration sessions. When rats were given15 minutes of intermittent 0.39 mA footshock before self-administration sessions, mean reinforced-lever responding again slightly decreased to 79 percent (±12.1%) of baseline reinforced-lever responding. During the second no-shock condition mean reinforced-lever responding again increased to baseline levels. Under extinction conditions, during which rats responded for saline instead of cocaine on a progressive

ratio schedule of reinforcement, mean reinforced-lever responding decreased to 28 percent (±7.1%) of baseline mean reinforced-lever responding. There was a statistically significant ($p=0.0433$, $t=2.078$, $df=46$) decrease in responding expressed as a percentage of baseline under 1.02 mA but not 0.39 mA footshock conditions.

Figures 3 and 4 show the effects of 1.02 mA and 0.39 mA footshock on responding on a progressive ratio schedule of reinforcement for 0.5 mg/kg/infusion cocaine by three individual rats. Reinforced-lever responses by subject 1 (figure 3, top panel) increased across progressive ratio sessions, peaking at 2934 responses on day 10 (a break point of 492). During all four days on which subject 1 received 1.02 mA footshock before the progressive ratio session, reinforced-lever responses and break points were lower than pre-shock levels, decreasing to 1267 responses (a break point of 219) on the first day of shock. During the five no-shock days that followed, responding and break points rebounded back to baseline levels, reaching as high as 2794 reinforced-lever responses (a break point of 492) on day 20. During the sessions which were preceded by 0.39 mA footshock, reinforced-lever responding was more variable, sometimes being higher and other times lower than the pre-shock baseline. On the subsequent no-shock test days, responding remained similar to the first pre-shock baseline period.

Reinforced-lever responses by subject 2 (figure 3, middle panel) were between 700-1319 responses per session during the pre-shock baseline period with break points of between one and two hundred. During three of the four days on which subject 2 received 1.02 mA footshock before the progressive ratio session, reinforced-lever responses and

break points remained virtually unchanged, with 1060 responses (a break point of 145) on the first day of shock. Responding decreased only on the second shock day, with 43 responses (a break point of 12) that day. During the five shock-free days that followed, responding and break points trended lower than those during the first pre-shock baseline. During the sessions which were preceded by 0.39 mA footshock, responding was quite variable with the highest responding occurring on day 25 (1050 reinforced-lever responses, a break point of 178). On the subsequent no-shock control days responding was very unstable, with 1375 responses on day 26 (a break point of 219), the highest level of responding by subject 2 throughout the experiment, and 614 responses on day 29 (a break point of 118). Reinforced-lever responses decreased to 248 on the first day of extinction (a break point of 50) and remained low during the extinction period.

Reinforced-lever responses by subject 3 (figure 3, bottom panel) were the lowest of the 6 rats tested. Responding reached its highest levels of the experiment on day 2 (546 responses, a break point of 95) then decreased to somewhat lower levels on subsequent baseline test days. During three of the four days on which subject 3 received 1.02 mA footshock before the progressive ratio session, reinforced-lever responses and break points were almost completely abolished with as few as 60 responses (a break point of 15) on the third day of shock. Responding increased only on the second shock day, with 353 responses (a break point of 62) that day. During the five no-shock control days that followed, responding and break points increased to higher-than-baseline levels, with 364 reinforced-lever responses (a break point of 77) on day 19. During the sessions which were preceded by 0.39 mA footshock, greater variability was again observed.

Reinforced-lever responses increased to 405, a break point of 77, on the first day then decreased to 39, a break point of 12, on the second day. On the final no-shock control days responding again increased above baseline levels, with 507 responses on day 29 (a break point of 95). When saline was substituted for cocaine, reinforced-lever responses decreased to a low of 20 by the third day of extinction (a break point of 6).

Figure 4 shows the effects of 1.02 mA and 0.39 mA footshock on responding on a progressive ratio schedule of reinforcement for 0.5 mg/kg/infusion cocaine by rats 4-6. Reinforced-lever responding by subject 4 (figure 4, top panel) during the baseline period never showed any degree of stability. For instance, responding increased to 2347 (a break point of 402) by the second progressive ratio session and then decreased to 978 responses (a break point of 178) on day 12. Responding during the four sessions preceded by 1.02 mA footshock was also quite variable, peaking at 1865 responses on day 13 (a break point of 328). During the subsequent five baseline sessions, during which there were no footshock segments, responding by subject 4 increased to its highest levels of the experiment, reaching 3804 reinforced-lever responses on day 18 (a break point of 603). The 0.39 mA footshock pretreatment had no discernable effect on responding compared to baseline. Only extinction produced any clear effect on reinforced-lever responses in rat 4, with responding rapidly decreasing to 318 (a break point of 62) on the first day of extinction and remaining low for the duration of the extinction period.

Responding by subject 5 (figure 4, middle panel), was initially extremely stable at near 500 responses per session during baseline days 1-5. On days 6-7 behavior transitioned to much higher levels and again became very stable with identical break points and responding only varying between 1800 and 2000 responses on baseline days 8-12. While responding remained high on the first day of sessions preceded by 1.02 mA footshock, it decreased precipitously during the last three days, with 8 reinforced-lever responses on day 15 (a break point of 4). Responding increased above shocked levels and was much more variable than the initial baseline period during the five no-shock baseline days which followed the 1.02 mA footshock sessions, reaching 1860 reinforced-lever responses (a break point of 328) on day 18. The 0.39 mA footshock had no substantial effect on responding with break points varying between 145 and 328 during the four test sessions. Reinforced-lever responses showed a general downward trend on the five no-shock control days which followed the 0.39 mA footshock test sessions, reaching 794 reinforced-lever responses (a break point of 145) on day 30. Reinforced-lever responding decreased to 198 (a break point of 40) on the first day of extinction and remained low for the remaining extinction test sessions.

During the first twelve progressive ratio sessions, reinforced-lever responding by subject 6 (figure 4, bottom panel) peaked on day 3 at 1515 responses (a break point of 268), and remained very stable for the remainder of the baseline period. Among all six rats, the responding by rat 6 was the most consistently affected by footshock. Reinforced-lever responses decreased for all four sessions which were preceded by a 1.02 mA footshock segment, reaching as low as 429 responses (a break point of 77) on the

fourth shock day. Reinforced-lever responses then increased but not quite to baseline levels during the following no-shock control sessions. Reinforced-lever responses again decreased on all four days during which sessions were preceded by 0.39 mA footshock segments, reaching as low as 213 responses (a break point of 40) on day 22. Reinforced-lever responding increased back to baseline levels over the next five sessions which were not preceded by footshock segments, reaching 1330 responses (a break point of 219) on day 29. During extinction, reinforced-lever responses decreased over test sessions to a low of 5 responses (a break point of 2) on day 34.

Figure 5 shows the effects of 1.02 mA and 0.39 mA footshock on responding by three individual rats for 0.3% weight/volume saccharin by dipper presentation on a progressive ratio schedule of reinforcement. In general, saccharin produced break points far lower than those produced by cocaine, with even larger between-session variability. Reinforced-lever responding by subject SA1 (top panel) did not stabilize during the first twelve progressive ratio sessions. It peaked at 367 responses (a break point of 77) on day 1 then decreased to 5 responses (a break point of 2) on day 3 and continued to vary widely through day 12. On three of the four 1.02 mA footshock days, reinforced-lever responses were low (44 on days 15 and 16) while responding on the second shock day was high (310 reinforced-lever responses, a break point of 62). Peak responding during the five no-shock days which followed the 1.02 mA footshock occurred on day 21 (200 reinforced-lever responses, a break point of 40). Administration of 0.39 mA footshock segments before sessions 22-25 had little effect on responding compared to the no-shock baseline. During the subsequent no-shock baseline, responding was again quite variable

peaking at 245 responses (a break point of 50) on day 29. Unlike when the rat responded for cocaine, when the rat responded for water by dipper presentation, responding initially decreased, but remained higher than that seen on some days of responding for saccharin.

Reinforced-lever responding by subject SA2 (figure 5, middle panel) was also variable across all twelve baseline days and never stabilized. It peaked at 325 responses (a break point of 62) on day 1 then continued to vary widely and decreased to 1 response (a break point of 0) on day 10. On three of the four 1.02 mA footshock days, reinforced-lever responses were low (57 on day 13) while responding on the second shock day was quite high (439 reinforced-lever responses, a break point of 77). Peak responding during the five no-shock control days which followed the 1.02 mA footshock tests occurred on day 20 (241 reinforced-lever responses, a break point of 50). During the subsequent four test sessions preceded by 0.39 mA footshock, responding varied non-systematically as it also did during the next five no-shock control days. During extinction, when the rat responded for water by dipper presentation, responding appeared to decrease but on subsequent extinction sessions was actually greater than that when saccharin was available. As was the case with the other two subjects, saccharin-reinforced lever responding by subject SA3 (figure 5, bottom panel) also did not stabilize during the first twelve progressive ratio baseline sessions. It peaked at 506 responses (a break point of 95) on day 1 then continued to vary widely for the next eleven days. During the four 1.02 mA footshock days, reinforced-lever responses showed no trends and varied from a high of 276 (a break point of 50) on the first shock day to a low of 0 on the second shock day. During the test sessions preceded by 0.39 mA footshock, responding varied from a high

of 231 reinforced-lever responses (a break point of 50) on the first shock day to a low of 87 reinforced-lever responses (a break point of 20) on the third 0.39 mA footshock day. During extinction, when the rat responded for dipper presentation of water, responding varied, but remained higher than that seen on some days of responding for saccharin.

Experiment 2: Effects of footshock on cocaine self-administration in rats initially resistant to cocaine self-administration

Figure 6 shows the effects of footshock on cocaine self-administration in eight rats which were initially resistant to acquisition of cocaine self-administration. Prior to receiving footshock, reinforced-lever responses were 10 or less during the first 10 days of cocaine self-administration (last 4 days shown). The mean of the reinforced-lever responses was 1.6 (±0.7) on day 10, while none of the 8 rats emitted any responses on the inactive lever. When a 15-minute, 1.02 mA, intermittent footshock component was introduced before self-administration sessions on days 11 – 14, mean reinforced-lever responding dramatically increased for all four days, peaking at 57.8 (±31.2) on day 12. Mean non-reinforced-lever responding also increased when the shock segments were introduced, but to a lesser degree. Mean non-reinforced-lever responding remained significantly lower than reinforced-lever responding, returning to near zero levels for the remainder of the experiment. Peak reinforced-lever responses during the next set of four no-shock control days initially decreased somewhat compared to responses during the session preceded by footshock, but was still much higher than that prior to the footshock test sessions. During the second set of four days on which self-administration sessions

were preceded by 1.02 mA footshock segments, mean reinforced-lever responding again increased, peaking at 74.4 (±49.4) responses on day 19 (the greatest mean response value for the experiment). During the subsequent four no-shock control days (days 23 – 26), reinforced-lever responding again fell but remained elevated above initial baseline levels prior to the first shock session. Because of the high degree of variability between rats in reinforced-lever responses on each day, individual graphs are shown in Figures 8 – 11.

Figure 7 shows the effects of 1.02 mA footshock on group mean cocaine-lever responding under each of five conditions. This is the same data as in Figure 6 except that for each rat, responses in each condition were collapsed into a mean value for that animal. A group mean was then calculated for each condition. Mean reinforced-lever responses under pre-shock baseline conditions were 2.2 (±0.7) responses per session. There were no inactive lever responses for any of the 8 rats. When self-administration sessions were preceded by 1.02mA footshock for the first set of four days, mean reinforced-lever responses increased to 38.6 (±12.7) responses while mean non-reinforced-lever responses also increased to 10.2 responses. Under the first post-shock baseline conditions, mean reinforced-lever responding decreased but remained above baseline levels at 18.9 (±5.6) responses while mean non-reinforced-lever responses decreased to 1.8 (±0.6) responses. When self-administration sessions were preceded by footshock for the second set of four days, mean reinforced-lever responses increased to their highest level, 56.4 (±18.2) responses, while mean non-reinforced-lever responses only increased to 3.3 (±1.5). Finally, under the second no-shock baseline condition,

mean reinforced-lever responding again decreased to 23.6 (±6.9) responses while mean non-reinforced-lever responding stopped almost entirely (0.2 responses (±0.06)).

Figures 8-11 show the effect of 1.02 mA footshock on four-day mean responding under each of five conditions on a fixed ratio 1 schedule of reinforcement for 0.5 mg/kg/infusion cocaine in individual rats. The most striking feature of the data is that, with the exception of rat 302224 (figure 11, bottom panel) all of the rats showed enhanced responding both during footshock as well as on the intervening baseline control sessions. The degree of increase differed widely across animals with some subjects such as 203177 and 302180 (figure 8, top and bottom panel) showing pronounced and stable increases in cocaine-lever responding with only small or no increases in inactive lever responding. The effect of footshock in other rats was more variable. For instance, rats 102273, and 102225 showed large increases in responding during sessions preceded by footshock, but these effects largely dissipated on the intervening no-shock control sessions (figure 10, top panel and figure 9, top panel respectively). Finally, rat 102274 showed much smaller, but consistent, increases in active but not inactive lever responding following footshock that were more transient, decreasing to almost zero levels immediately following the end of the second four-session footshock test period.

Experiment 3: Effects of footshock on sensitivity to the reinforcing efficacy of low doses of cocaine

Figure 12 shows the effects of 1.02 mA footshock on mean responding by a group of six rats for a dose of cocaine producing half-maximal responding under three

conditions on an FR5 schedule of reinforcement. Under baseline conditions of responding for the half-maximal dose of cocaine, the mean of the reinforced-lever responses per session was 124 (±42.2) but varied widely between rats. Mean non-reinforced-lever responses per session were extremely low. When a 15-minute intermittent footshock component preceded self-administration of the same half-maximal cocaine dose, mean reinforced-lever responding increased slightly to 125 (±58.2) responses per session, again varying widely (in large measure due to the fact that one rat failed to make any active-lever responses during any of the sessions preceded by footshock). Mean non-reinforced-lever responses per session increased to 9.5 (±3.5) during the shock condition. During the post-shock baseline condition, mean responding decreased to 53 (±28.5) responses per session while mean non-reinforced-lever responding decreased to 5.7 (±2.6) responses per session.

Figures 14 and 15 show daily responding for decreasing doses of cocaine by all 6 rats on an FR5 schedule of reinforcement; they also show the effects of 1.02 mA footshock on responding for the half-maximal dose of cocaine. Data from the same half-maximal dose of cocaine for an additional four sessions without footshock is also depicted. Rat DR1 emitted a mean of 253 (±13.6) responses on the reinforced lever per session for the 0.5 mg/kg/infusion acquisition dose of cocaine (figure 14, top panel). When the cocaine dose was lowered to 0.25 mg/kg/infusion, responding increased on the first day to 550 and then decreased to levels only slightly higher than those produced by the 0.5 mg/kg/infusion cocaine dose. Mean responses per session further increased to 454.75 (±78) when the cocaine dose was halved again to 0.125 mg/kg/infusion and then

decreased to 351 (±107.6) when the cocaine dose was halved to 0.0625 mg/kg/infusion (again, responding from day to day for this dose showed a general downward trend). Reinforced-lever responses decreased each day to a mean of 65 (±16.1) (less than half of peak average responses) at the 0.0313 mg/kg/infusion dose of cocaine. When self-administration sessions at this dose were preceded by a 15-minute intermittent footshock component, mean reinforced-lever responses per session fell to 27 (±11.3), then further decreased to 17 (±8.7) reinforced-lever responses per session when self-administration sessions at this same cocaine dose were again not preceded by a footshock segment.

Rat DR4 emitted 218 (±16.5) mean responses on the reinforced lever per session for 0.5 mg/kg/infusion cocaine (figure 14, middle panel). Mean reinforced lever responding for progressively lower doses increased to a high of 714 (±65.9) per session at the 0.0625 mg/kg/infusion dose. Reinforced-lever responses decreased to less than 50% of peak at the 0.0313 mg/kg/infusion dose. When self-administration sessions at the 0.0313 mg/kg/infusion dose were preceded by a 15-minute intermittent footshock component, mean reinforced-lever responses per session decreased to 128 (±49.8). Mean responding further decreased to 35 (±11.5) responses per session when self-administration sessions at the 0.0313 mg/kg/infusion dose were not preceded by a footshock component.

Rat DR5 emitted a mean of 147 (±5.6) responses on the reinforced lever per session for 0.5 mg/kg/infusion cocaine (figure 14, bottom panel). Reinforced lever responding for progressively lower doses increased to a mean of 781 (±58.4) reinforced-

lever responses at the 0.0625 mg/kg/infusion dose. Mean reinforced-lever responses were 347 (±239.8)(less than half of peak mean responses) at the 0.0313 mg/kg/infusion dose. When self-administration sessions at the 0.0313 mg/kg/infusion dose were preceded by a 15-minute intermittent footshock component, mean active-lever responses per session decreased to 127 (±39.9). Mean responding further decreased to 9 (±3.5) responses per session when self-administration sessions at the 0.0313 mg/kg/infusion dose were not preceded by a footshock component.

Rat DR8 emitted a mean of 208 (±11.6) responses on the reinforced lever per session for 0.5 mg/kg/infusion cocaine (figure 15, top panel). Mean reinforced lever responding for progressively lower doses increased to a high of 330 (±66.8)at the 0.25 mg/kg/infusion dose. Mean reinforced-lever responses were 154 (±140.5) (less than half of peak mean responses) at the 0.125 mg/kg/infusion dose. When self-administration sessions at the 0.125 mg/kg/infusion dose were preceded by a 15-minute intermittent footshock component, mean active-lever responses per session increased to 397 (±92.8). Mean responding decreased to 186 (±113.9) responses per session when self-administration sessions at the 0.125 mg/kg/infusion dose were not preceded by a footshock component.

Rat DR9 emitted a mean of 225 (±8.5) responses on the reinforced lever per session for 0.5 mg/kg/infusion cocaine (figure 15, middle panel). Mean reinforced lever responding for progressively lower doses increased to a high of 738 (±37.6) reinforced-lever responses at the 0.125 mg/kg/infusion dose. Mean reinforced-lever responses were

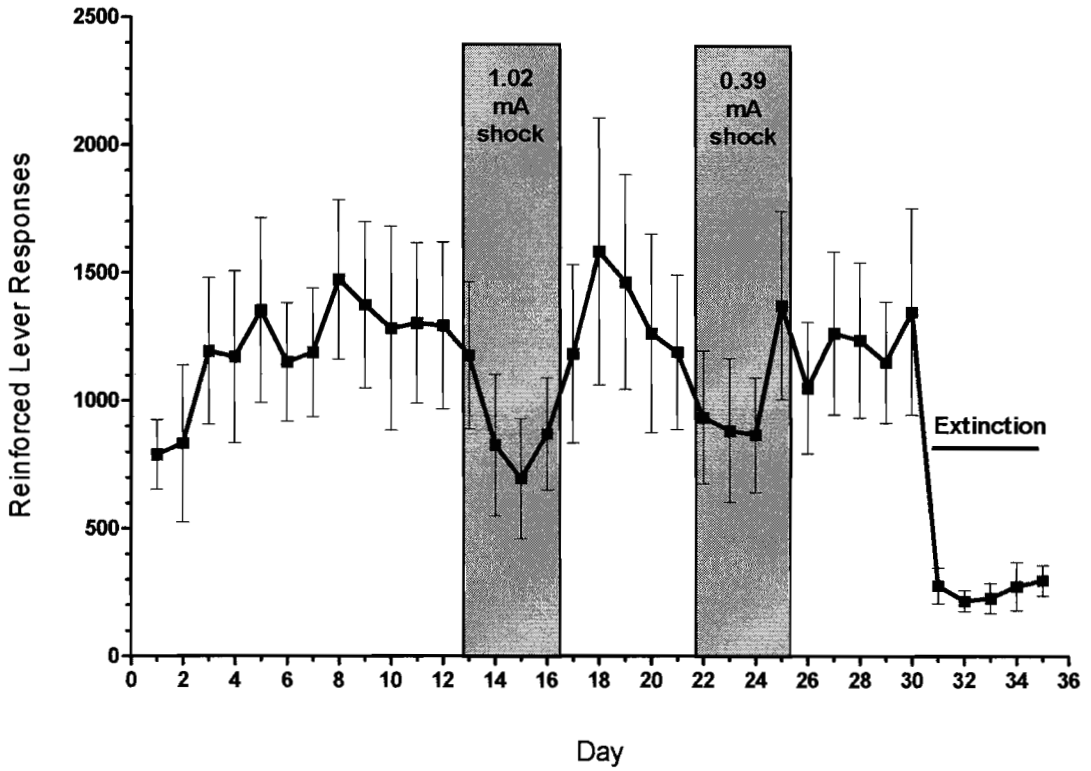

Figure 1. Effects of footshock at two intensities on responding for 0.5 mg/kg/infusion cocaine on a progressive ratio schedule of reinforcement

Mean (±S.E.M.) reinforced lever responses are shown for each day for all rats (n=6). Boxed areas (▒) show days on which rats underwent a 15-minute intermittent footshock segment before each session. Five days of responding for saline are indicated by the line labeled "Extinction" above the values for those days.

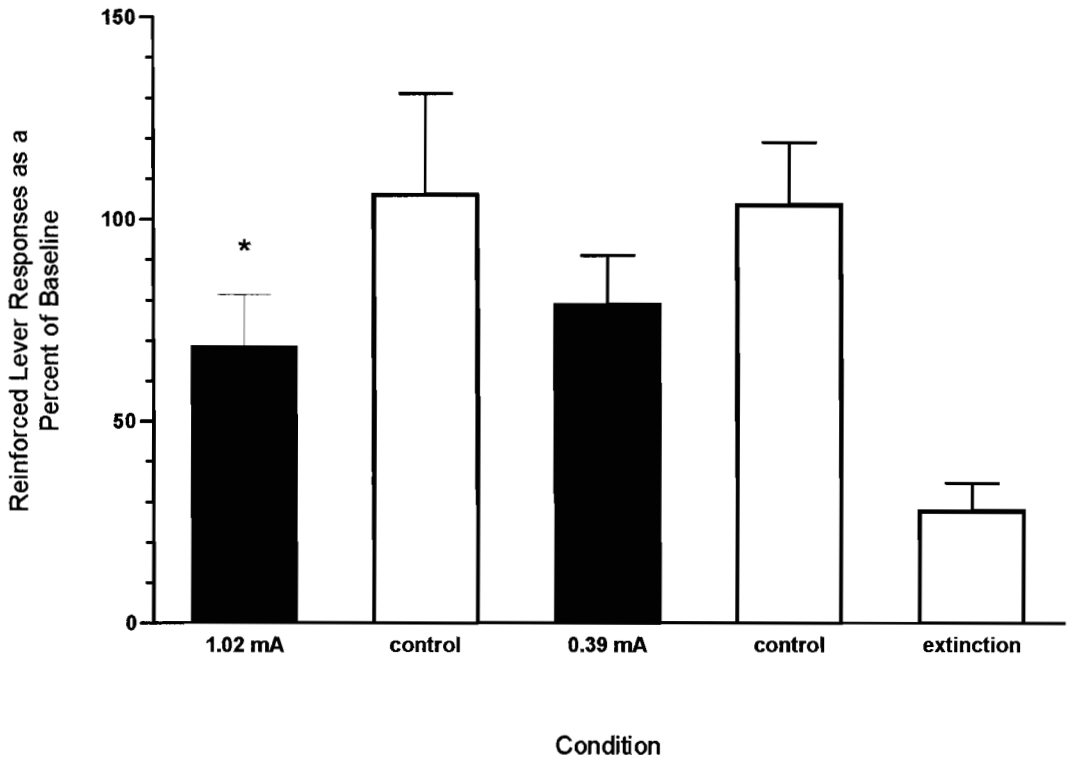

Figure 2. Effects of footshock at two intensities on responding for 0.5 mg/kg/infusion cocaine on a progressive ratio schedule of reinforcement

Mean (±S.E.M.) reinforced lever responses expressed as a percent of baseline ("baseline" refers to the mean responses on the last four days of cocaine self-administration before shock segment days began) for all six rats is shown for each condition. Filled bars (■) indicate shock conditions while open bars (□) indicate responding in the absence of shock.

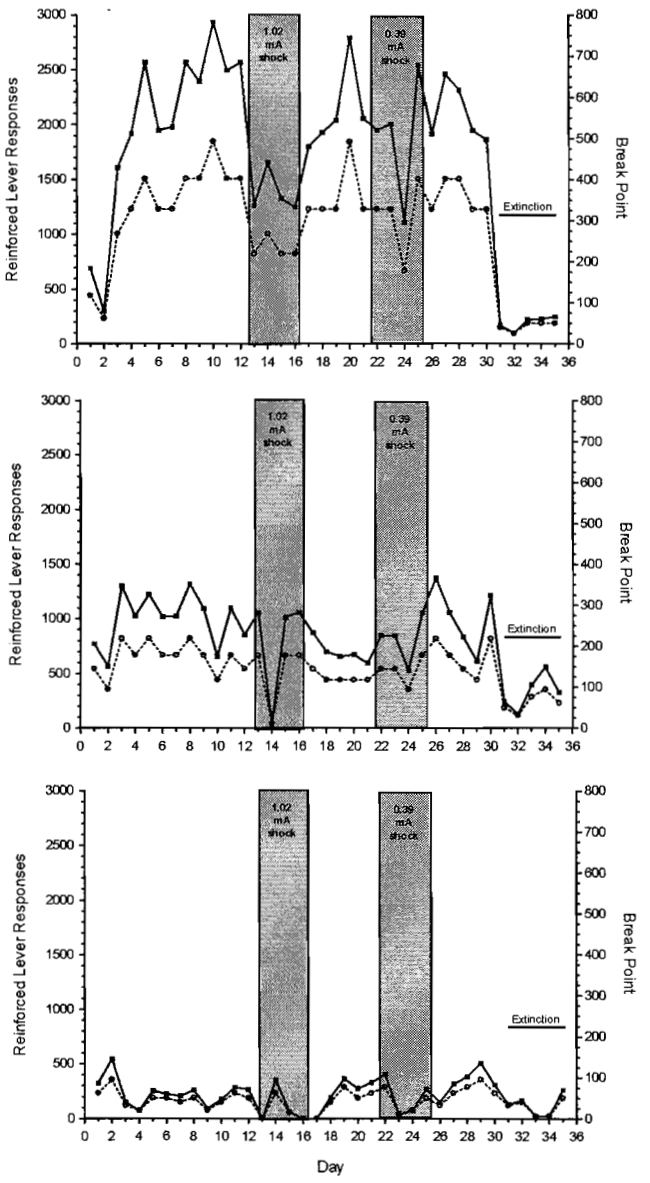

Figure 3. Effects of two footshock intensities on responding for 0.5 mg/kg/infusion cocaine on a progressive ratio schedule of reinforcement by subjects 1 (top), 2 (middle) and 3 (bottom)

Total reinforced lever responses for each day are shown on the left y-axis and are indicated by closed squares (■) and a solid line (—) while break points in responding are shown on the right y-axis and are indicated by open circles (○) and a dashed line (▪▪▪). Boxed areas (▒) indicate sessions which were preceded by a footshock segment. The last five days, which are extinction days, are indicated by the line above values for those sessions.

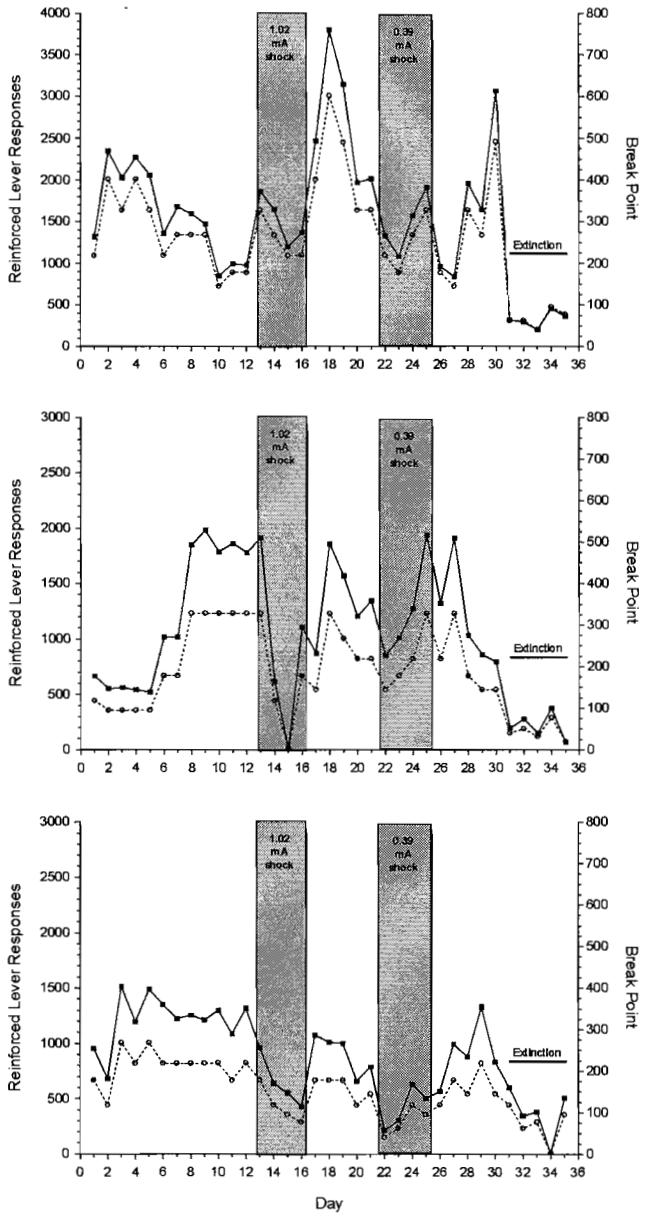

Figure 4. Effects of two footshock intensities on responding for 0.5 mg/kg/infusion cocaine on a progressive ratio schedule of reinforcement by subjects 4 (top), 5 (middle) and 6 (bottom)

Reinforced lever responses for each day are shown on the left y-axis and are indicated by closed squares (■) and a solid line (—). Break points in responding are shown on the right y-axis and are indicated by open circles (○) and a dashed line (▪▪▪) . Boxed areas (▒) indicate sessions which were preceded by a footshock segment. Extinction days are indicated by the line above values for those sessions.

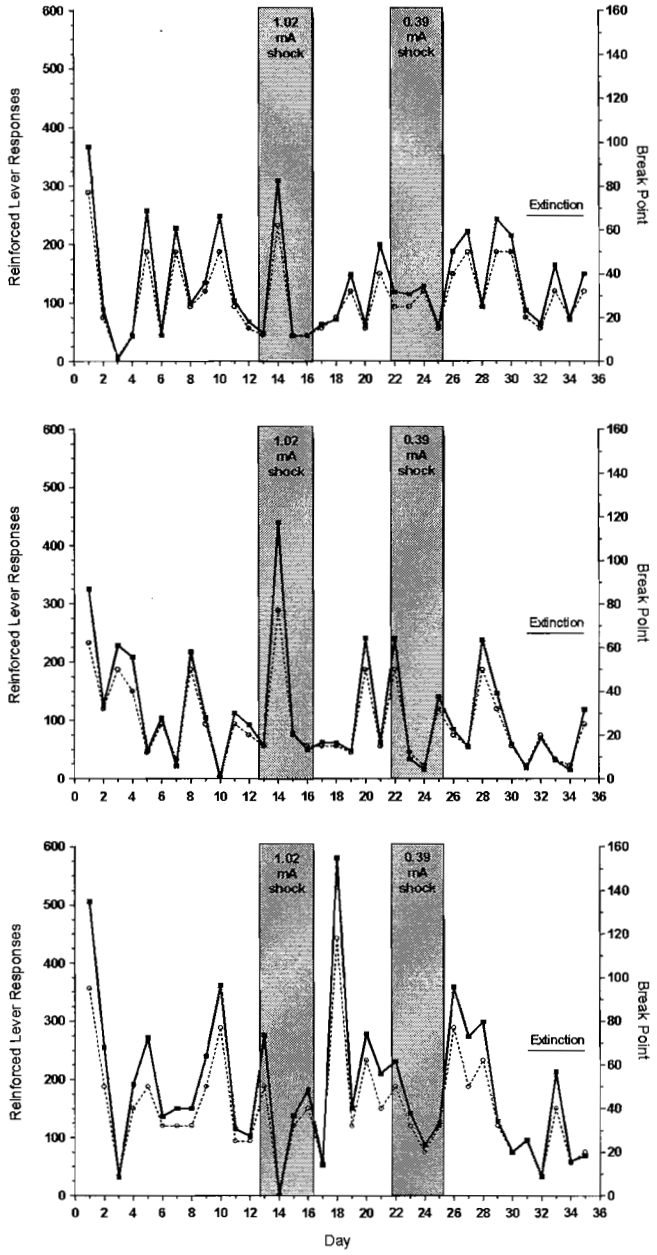

Figure 5. Effects of two footshock intensities on responding for 0.3% w/v saccharin by dipper presentation

Reinforced lever responses for subjects 1 (top), 2 (middle) and 3 (bottom) are shown on the left y-axis and are indicated by closed squares (■) and a solid line (—). Break points in responding are shown on the right y-axis and are indicated by open circles (○) and a dashed line (▪▪▪). Boxed areas (▒) indicate sessions which are preceded by a footshock segment. Extinction days are indicated by the line above values for those sessions.

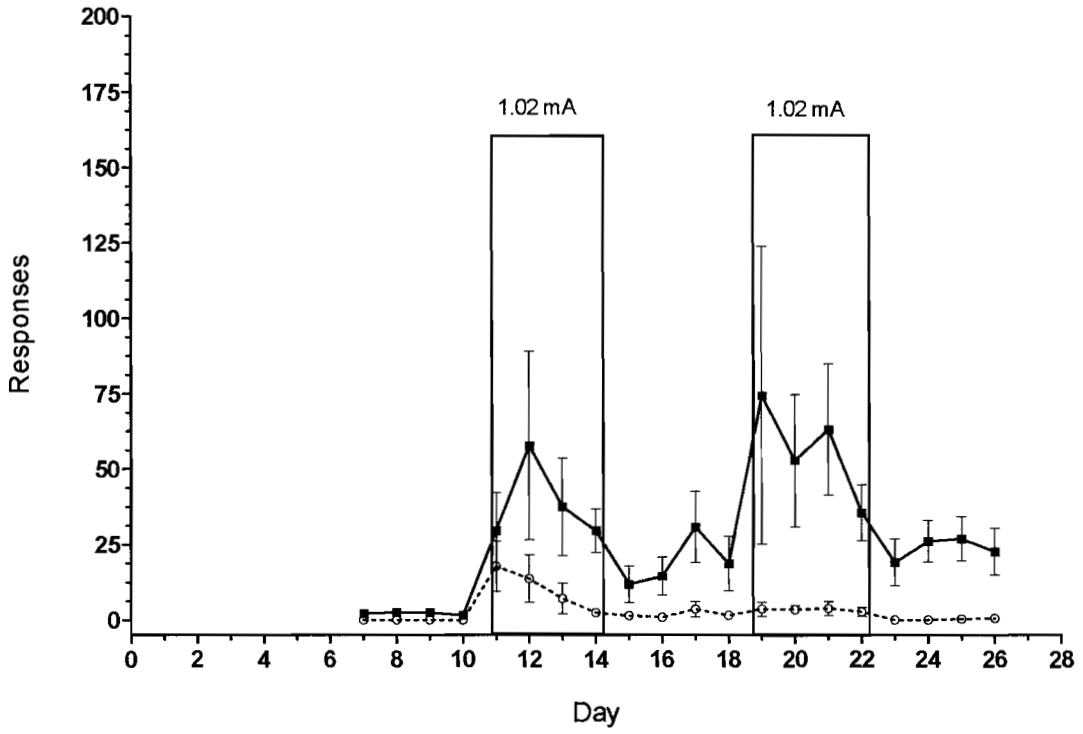

Figure 6. Effect of 1.02 mA footshock on responding for 0.5 mg/kg/infusion intravenous cocaine on a fixed ratio 1 schedule of reinforcement on days 7 – 26 of self-administration by acquisition-resistant rats

Mean (±S.E.M.) reinforced lever responses are shown for each day for all rats (n=8) and are indicated by filled squares (■) and a solid line (—). Mean (±S.E.M.) responses on the non-reinforced lever are indicated by open circles (○) and a dashed line (▪▪▪). Boxed-in values indicate days on which sessions were preceded by a 15 minute intermittent 1.02 mA footshock segment.

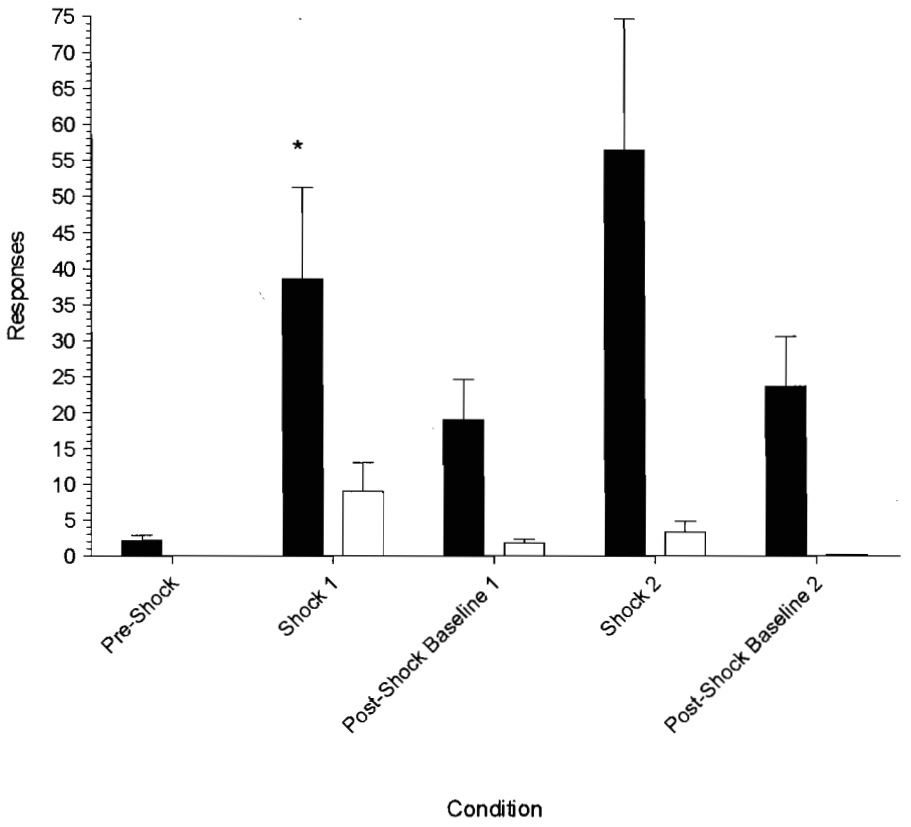

Figure 7. Effect of 1.02 mA footshock on four day mean responding for 0.5 mg/kg/infusion intravenous cocaine under each condition on a fixed ratio 1 schedule of reinforcement

Mean (±S.E.M.) responses are shown for each condition for all rats (n=8). Mean reinforced lever responses are indicated by filled bars (■) while mean responses on the non-reinforced lever are indicated by open bars (□). The "Pre-Shock" condition indicates responding which occurred during the last four days before rats experienced a footshock segment before sessions. "Shock 1" indicates responding on the first four days during which rats experienced a 15 minute intermittent 1.02 mA footshock segment before each session. "Post-Shock Baseline 1" indicates responding during the four days after the first days of shock pre-treatment (sessions under this condition were not preceded by footshock). "Shock 2" indicates responding on the second set of four days during which rats experienced a footshock segment before each session. "Post-Shock Baseline 2" indicates responding during the four days after "Shock 2." Sessions under this condition were not preceded by footshock segments.

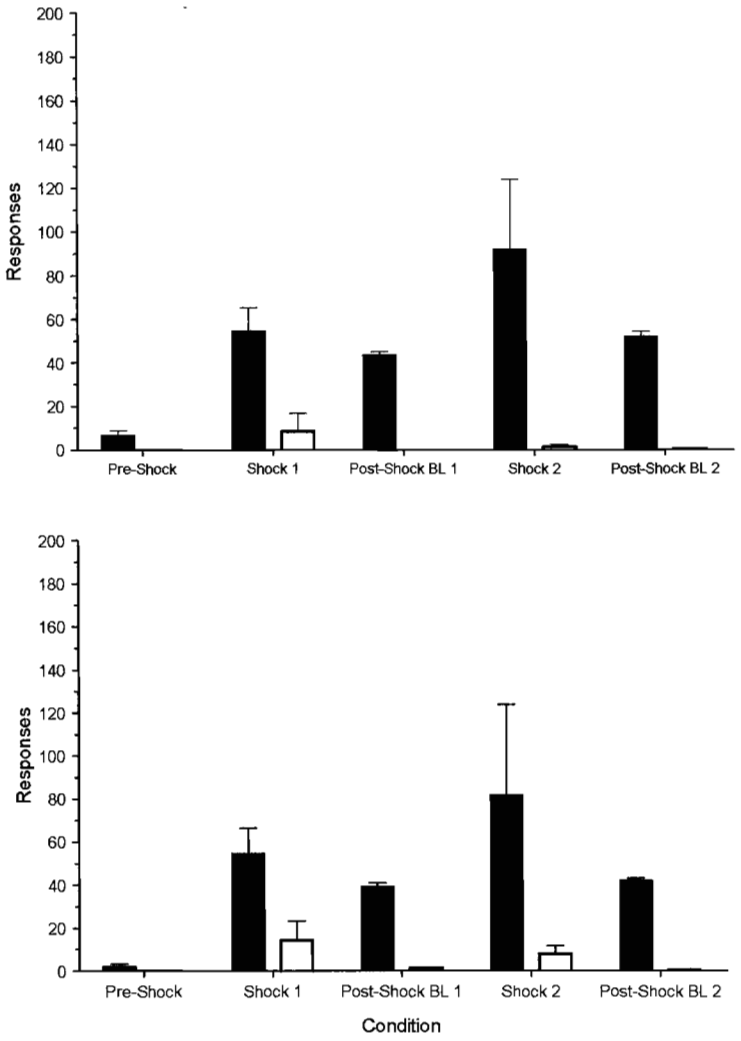

Figure 8. Effect of 1.02 mA footshock on four day mean responding for 0.5 mg/kg/infusion intravenous cocaine

Mean (±S.E.M.) responses are shown for each condition for rats 302177 (top) and 302180 (bottom). Mean reinforced lever responses are indicated by filled bars (■) while mean inactive-lever responses are indicated by open bars (□). The "Pre-Shock" condition indicates responding during the last four days before testing began. "Shock 1" indicates responding on the first four days on which rats experienced 1.02 mA footshock before each session. "Post-Shock Baseline 1" indicates responding during the four days after "Shock 1" days. "Shock 2" indicates responding on the second set of four days during which rats experienced footshock before each session. "Post-Shock Baseline 2" indicates responding during the four days after "Shock 2."

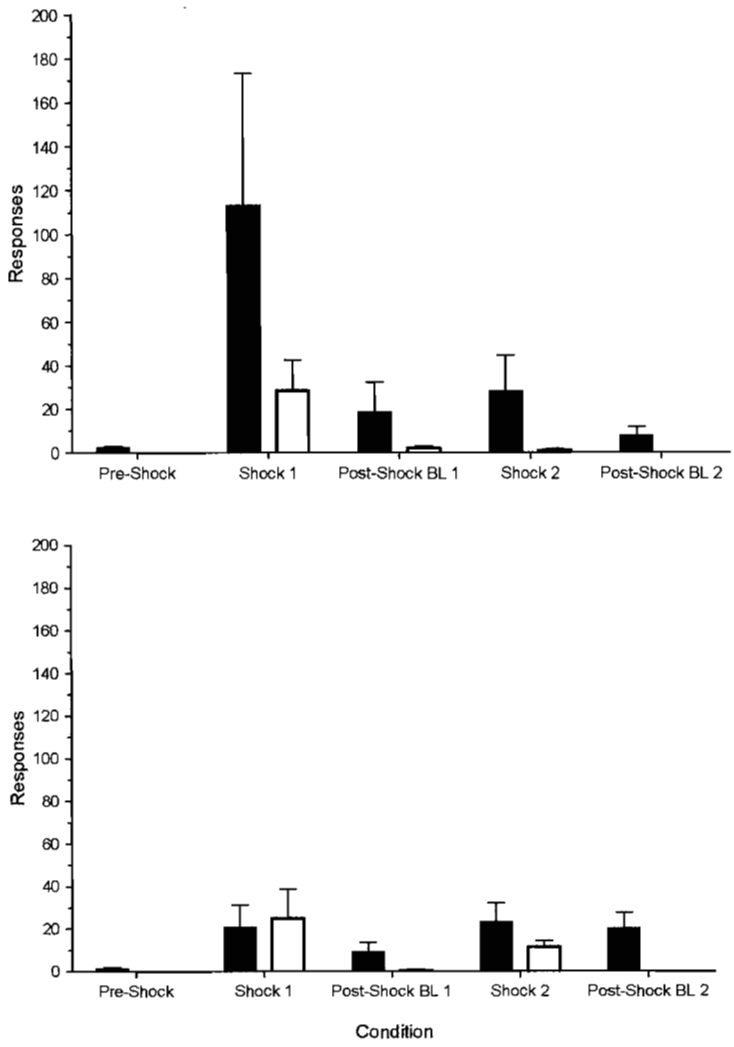

Figure 9. Effect of 1.02 mA footshock on four day mean responding for 0.5 mg/kg/infusion intravenous cocaine

Mean (±S.E.M.) responses are shown for each condition for rats 102225 (top) and 202212 (bottom). Mean reinforced lever responses are indicated by filled bars (■) while mean inactive-lever responses are indicated by open bars (□). The "Pre-Shock" condition indicates responding during the last four days before testing began. "Shock 1" indicates responding on the first four days on which rats experienced 1.02 mA footshock before each session. "Post-Shock Baseline 1" indicates responding during the four days after "Shock 1" days. "Shock 2" indicates responding on the second set of four days during which rats experienced footshock before each session. "Post-Shock Baseline 2" indicates responding during the four days after "Shock 2."

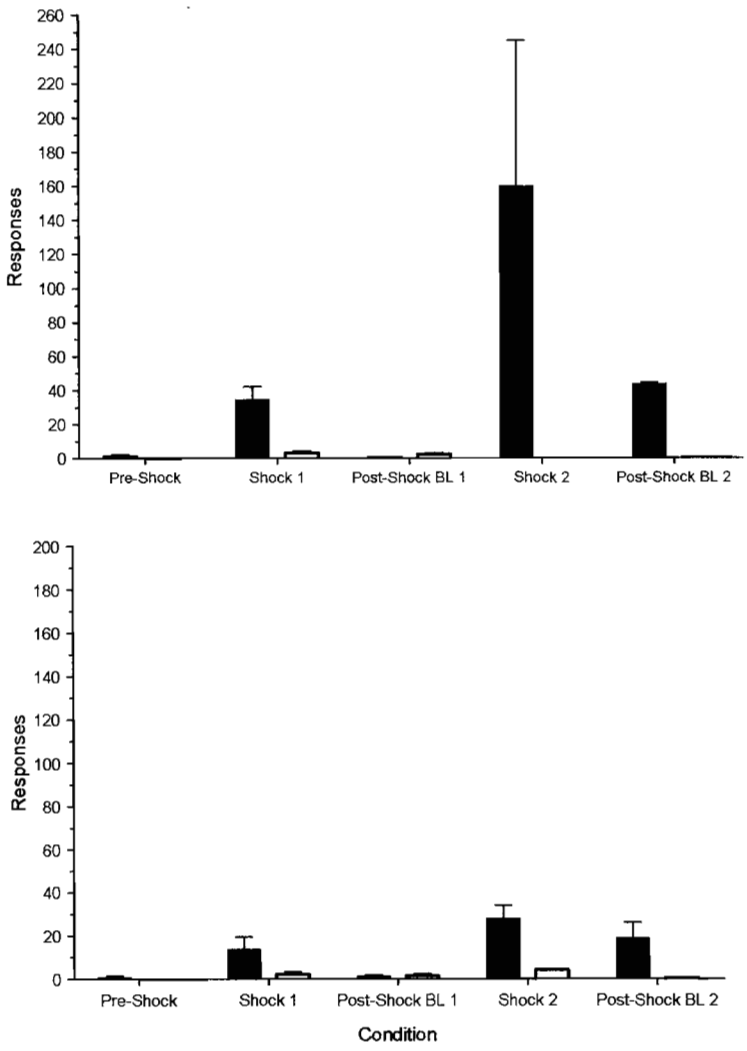

Figure 10. Effect of 1.02 mA footshock on four day mean responding for 0.5 mg/kg/infusion intravenous cocaine

Mean (±S.E.M.) responses are shown for each condition for rats 102273 (top) and 202235 (bottom). Mean reinforced lever responses are indicated by filled bars (■) while mean inactive lever responses are indicated by open bars (□). The "Pre-Shock" condition indicates responding during the last four days before testing began. "Shock 1" indicates responding on the first four days on which rats experienced 1.02 mA footshock before each session. "Post-Shock Baseline 1" indicates responding during the four days after "Shock 1" days. "Shock 2" indicates responding on the second set of four days during which rats experienced footshock before each session. "Post-Shock Baseline 2" indicates responding during the four days after "Shock 2."

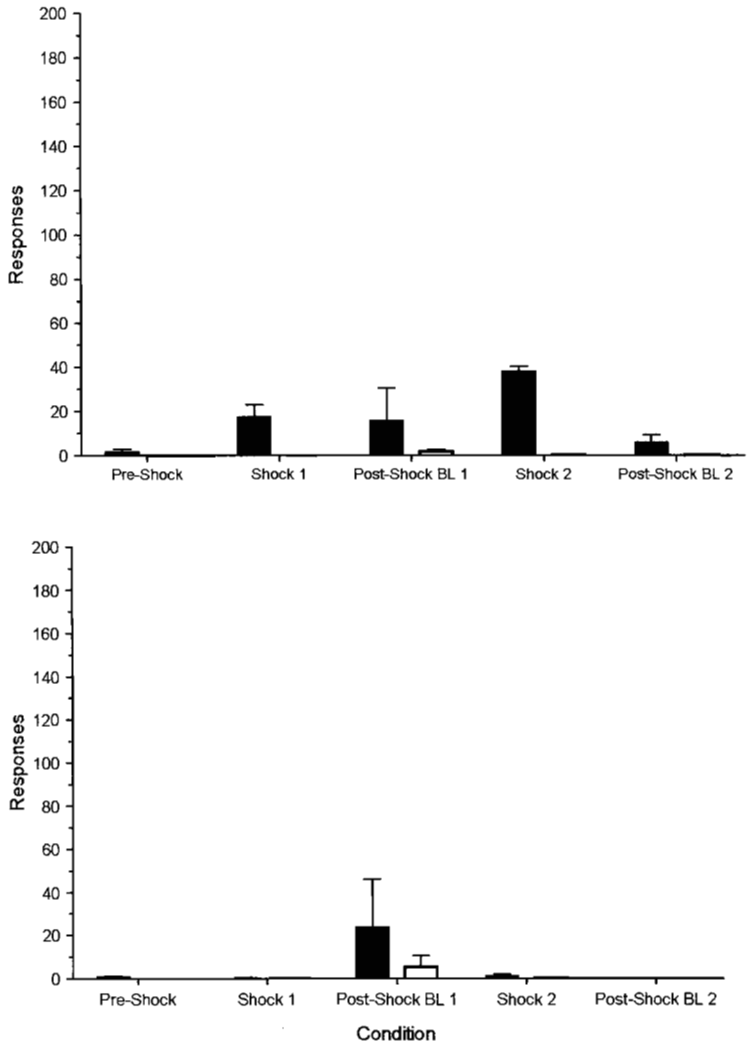

Figure 11. Effect of 1.02 mA footshock on four day mean responding for 0.5 mg/kg/infusion intravenous cocaine

Mean (±S.E.M.) responses are shown for each condition for rats 102274 (top) and 302224 (bottom). Mean reinforced lever responses are indicated by filled bars (■) while mean inactive lever responses are indicated by open bars (□). The "Pre-Shock" condition indicates responding during the last four days before testing began. "Shock 1" indicates responding on the first four days on which rats experienced 1.02 mA footshock before each session. "Post-Shock Baseline 1" indicates responding during the four days after "Shock 1" days. "Shock 2" indicates responding on the second set of four days during which rats experienced footshock before each session. "Post-Shock Baseline 2" indicates responding during the four days after "Shock 2."

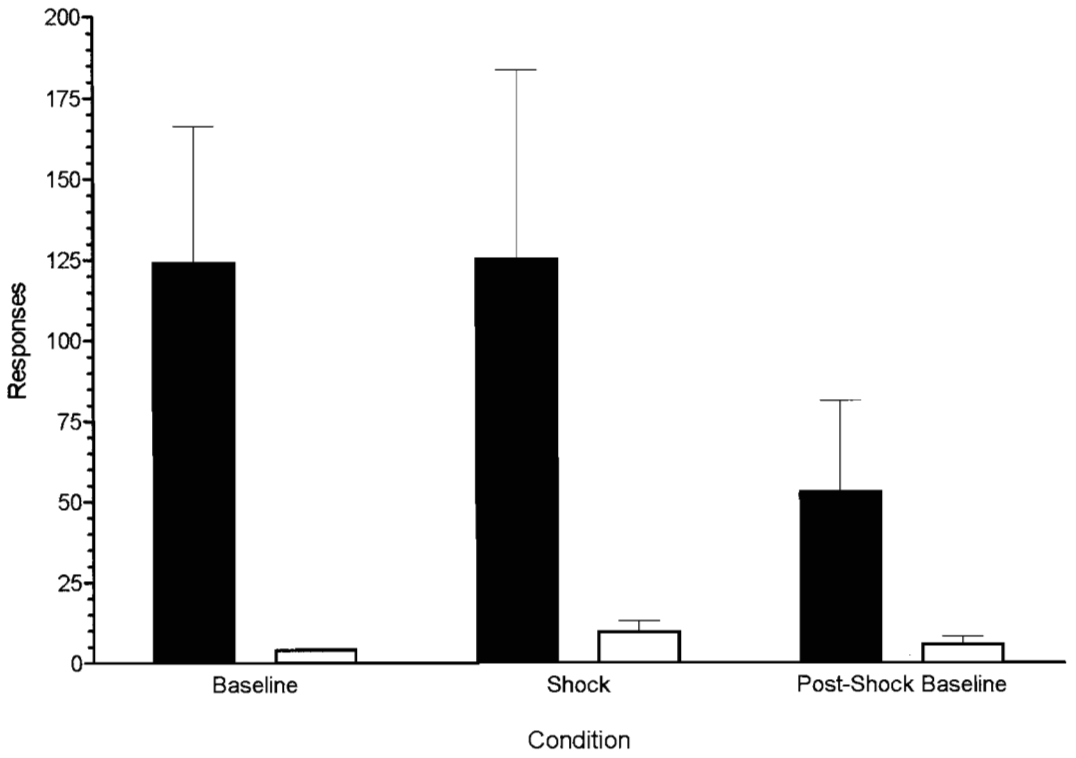

Figure 12. Effects of 1.02mA footshock on responding for a low dose of cocaine on an FR5 schedule of reinforcement

Mean (±S.E.M.) responses for all six rats are shown for each of three conditions. Reinforced lever responses are indicated by filled bars (■) while inactive lever responses are indicated by open bars (□). The "Baseline" condition indicates the mean responses during the last two of four days of self-administration of the lowest dose of cocaine. The "Shock" condition is mean responding during four days of self-administration of that same low dose of cocaine with a 15-minute intermittent footshock segment preceding each session. "Post-Shock Baseline" indicates four-day mean responding for the same low cocaine dose with no footshock segment preceding the sessions.

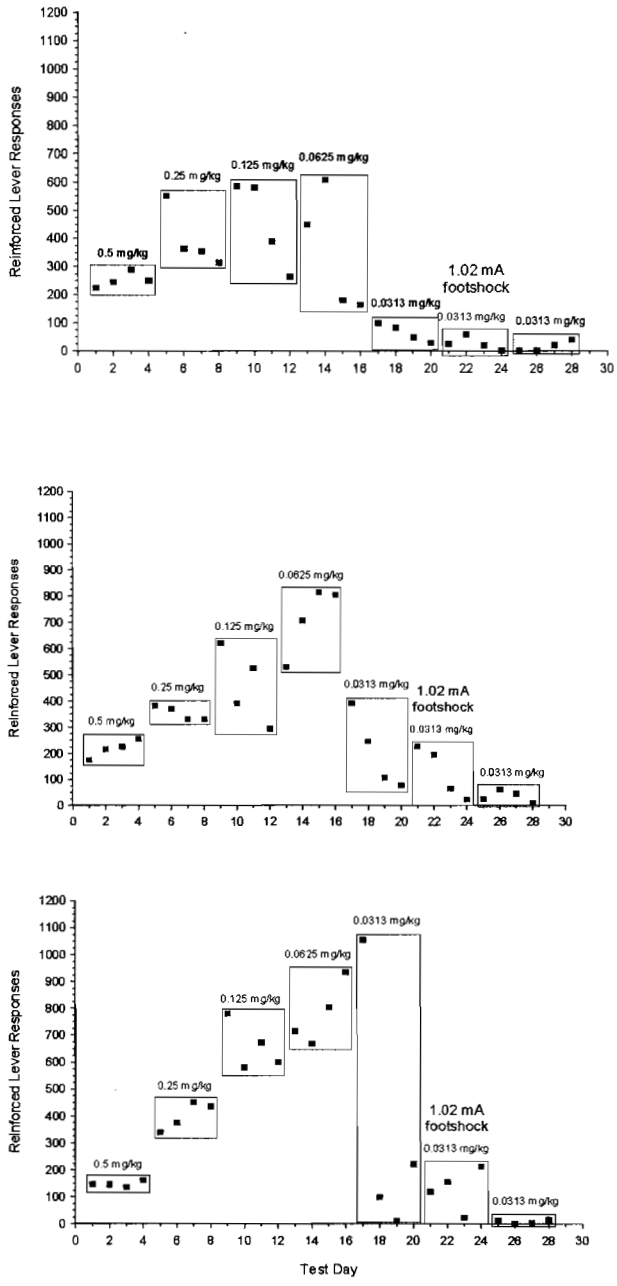

Figure 13. Effects of 1.02 mA footshock on responding for a low dose of cocaine on an FR5 schedule of reinforcement

Reinforced lever responses by rats DR1 (top), DR4 (middle) and DR5 (bottom) are shown for each day after self-administration of cocaine was established. Labeled boxes delineate responses for each dose of cocaine. The label "1.02 mA footshock" indicates days on which rats experienced footshock before self-administration.

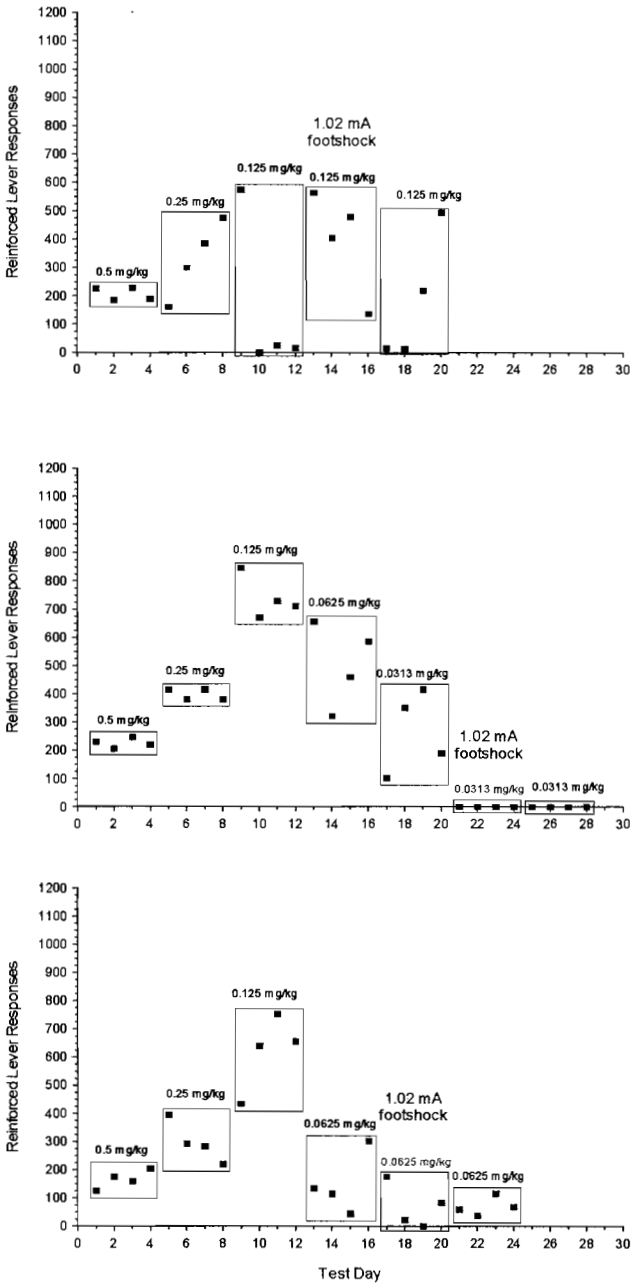

Figure 14. Effects of 1.02 mA footshock on responding for a low dose of cocaine on an FR5 schedule of reinforcement

Reinforced lever responses by rats DR8 (top), DR9 (middle) and DR10 (bottom) are shown for each day after self-administration of cocaine was established. Labeled boxes delineate responses for each dose of cocaine. The label "1.02 mA footshock" indicates days on which rats experienced footshock before self-administration.

264 (±71.8) (less than half of peak mean responses) at the 0.0313 mg/kg/infusion dose. When self-administration sessions at the 0.0313 mg/kg/infusion dose were preceded by a 15-minute intermittent footshock component, mean active-lever responses were completely abolished for all four shock test sessions. When the pre-session footshock component was terminated for the subsequent four test sessions at the 0.0313 mg/kg/infusion dose, responding remained completely suppressed.

Rat DR10 emitted a mean of 167 (±16.6) responses on the reinforced lever per session for 0.5 mg/kg/infusion cocaine (figure 15, bottom panel). Mean reinforced lever responding for progressively lower doses increased to a high of 621 (±66.6) reinforced-lever responses at the 0.125 mg/kg/infusion dose. Mean reinforced-lever responses were 149 (±54.4) (less than half of peak mean responses) at the 0.0625 mg/kg/infusion dose. When self-administration sessions at the 0.0625 mg/kg/infusion dose were preceded by a 15-minute intermittent footshock component, mean active-lever responses per session decreased to 71 (±39.4). When the pre-session footshock component was terminated for the subsequent four test sessions at the 0.0625 mg/kg/infusion dose, responding remained essentially unchanged at 71 (±16.7) responses per session.

IV Discussion

A number of anecdotal reports and clinical studies suggest that stress may increase the reinforcing effects of drugs in general and cocaine in particular. For instance, a high degree of comorbidity exists between post-traumatic stress disorder and cocaine abuse (Falck et al., 2004; Kulka et al., 1990; Najavits et al., 2003). Positive correlations have also been found between cocaine abuse and stress as measured by psychological testing (Karlsgodt et al., 2003; McMahon, 2001; Sinha et al., 2000). Cocaine users have reported increased well-being and decreased anxiety as a result of cocaine use as well as relief of withdrawal-associated anxiety with recurrence of cocaine use (Gawin and Ellinwood, 1988). Cocaine has been found specifically to produce anxiolysis by decreasing activity in the pontine nucleus and locus coeruleus (Prakash and Das, 1993).

Unfortunately, there have been only a handful of studies conducted to address whether these human reports can be reproduced in animals models of cocaine self-administration. Stressed rats have been shown to respond for drugs of abuse other than cocaine under greater work requirements than do non-stressed rats (Shaham and Stewart, 1994; Shaham et al., 1993) although similar studies have not been conducted with cocaine. A few experiments have noted that stress enhances acquisition of cocaine self-

administration (Covington and Miczek, 2001; Goeders and Guerin, 1994; Gordon, 2002; Haney et al., 1995; Miczek and Mutschler, 1996; Ramsey and VanRee, 1993). While these latter studies are consistent in their findings of enhanced acquisition by stress, they do not address the more general question of whether stress actually enhances the reinforcing efficacy of cocaine or acts by another mechanism to speed acquisition. This series of studies was designed to examine that more general hypothesis that stress increases the reinforcing efficacy of cocaine. Several different procedures which each might be expected to be sensitive to measuring reinforcing efficacy were employed.

Experiment 1: Effects of footshock on the reinforcing efficacy of cocaine or saccharin on a progressive ratio schedule of reinforcement

While footshock stress increases break points in responding for drugs of abuse such as heroin (Shaham and Stewart, 1994), few studies have examined effects of footshock on responding for cocaine on a progressive ratio schedule of reinforcement. This experiment attempted to determine if footshock stress increased break points in responding for cocaine on a PR schedule of reinforcement. This would have suggested that the reinforcing efficacy of cocaine increases as a result of stress. Contrary to the hypothesis, intermittent 1.02 mA footshock did not increase reinforcing efficacy of cocaine over that measured in the absence of footshock. When compared to baseline conditions, reinforced-lever responding decreased significantly ($p=0.0433$, $t=2.078$, $df=46$) when 1.02 mA footshock segments preceded sessions. This finding would suggest that rather than increasing the reinforcing efficacy of cocaine, footshock stress

may decrease the reinforcing efficacy of cocaine in rats that have already acquired self-administration.

In addition to failure of footshock stress to increase reinforcing efficacy of cocaine, there are other possible reasons why increased reinforced-lever responding was not observed, including the possibility that 1.02 mA footshock was not a stressor. This is unlikely for at least two reasons. First, footshock reliably causes increases in glucocorticoid levels, a biological indicator of stress (Kant et al., 1988). Second, the higher intensity of footshock used in experiments for this thesis has been used successfully in the lab in which these experiments were conducted (Beardsley et al., 2005; Shelton et al., 2004) to cause reinstatement of cocaine-seeking behavior in rats. Therefore, it is likely that footshock at the intensity employed was an adequate stressor.

Another possible reason that reinforcing efficacy did not increase is that the procedures used in this experiment did not successfully measure changes in the reinforcing efficacy of cocaine. It is thought, however, that PR schedules of reinforcement effectively measure reinforcing efficacy of self-administered drugs (Giordano et al., 2001; Richardson and Roberts, 1996; Stafford et al., 1998). Accordingly, the most likely explanation for the results of Experiment 1 is that footshock stress did not increase the reinforcing efficacy of cocaine.

The lower intensity (0.39 mA) footshock caused inconsistent, non-significant changes in responding for cocaine. For most rats, responding decreased below baseline on some days while increasing above baseline on other days. It is possible that this shock intensity may have been too low to cause a noticeable level of stress in most rats and

may, therefore, not have affected the reinforcing efficacy of cocaine in these rats. It is also possible, although unlikely, that the within-subject procedure used in this study may have been a factor. Specifically it could have been the case that 1.02 mA footshock may have desensitized the rats to the much lower 0.39 mA intensity used subsequently. In any case, this lower intensity of footshock and probable minimal level of stress did not increase the reinforcing efficacy of cocaine as measured with a PR schedule of reinforcement in this experiment.

It was initially the goal to compare the effects of footshock on a drug and a non-drug reinforcer to determine if there was an interaction between cocaine and footshock, or simply a non-selective effect of footshock on reinforcers across classes. Oral saccharin has been used as a reinforcer in self-administration experiments (Campbell and Carroll, 2000; Cosgrove and Carroll, 2003). Therefore, a group of rats were trained to respond for 0.3% w/v saccharin by dipper presentation on a PR schedule of reinforcement. Unfortunately, despite attempts to optimize saccharin concentration, responding for saccharin was quite low compared to cocaine and varied so widely even during the twelve baseline days that no comparison could be made between responding for cocaine and responding for saccharin. This data would suggest that saccharin, even at a highly preferred concentration is less reinforcing than is cocaine. In retrospect, it might have been advantageous to have chosen another drug reinforcer, such as i.v. heroin, as a comparison reinforcer, even though this would have not completely answered the specificity question.

Experiment 2: Effects of footshock on cocaine self-administration in rats initially resistant to cocaine self-administration

Many studies have demonstrated enhanced acquisition of cocaine self-administration resulting from stress (Covington and Miczek, 2001; Goeders and Guerin, 1994; Gordon, 2002; Haney et al., 1995; Miczek and Mutschler, 1996; Ramsey and VanRee, 1993). Experiment 1 failed to show that footshock stress increases the reinforcing efficacy of cocaine in a population of rats which already reliably self-administered cocaine. Over the course of testing literally hundreds of cocaine self-administration rats in the laboratory, there have been a small number of rats which fail to acquire cocaine self-administration when given the opportunity. Only five percent of the rats given the opportunity to self-administer cocaine in the lab in which these experiments were conducted (unpublished observation) fail to acquire self-administration using the standard laboratory acquisition procedure. These animals present a unique opportunity to determine if stress might enhance the reinforcing efficacy of cocaine in animals that appeared to be resistant to cocaine's reinforcing effects. Catheterizing sufficient rats to conduct a study in which only one of twenty animals could be used would ordinarily be impossible. However, the large volume of rats tested in cocaine self-administration protocols in the lab in which experiments for this thesis were conducted made it possible to acquire sufficient acquisition-resistant animals to determine if footshock stress could facilitate cocaine acquisition in subjects that had demonstrated no propensity for self-administration. All of the acquisition-resistant rats which received 1.02 mA intermittent footshocks before self-administration sessions acquired and maintained cocaine self-administration. Reinforced-lever responding increased

significantly ($p=0.0002$, $t=3.929$, $df=62$) and consistently above baseline responding (which was near zero). Inactive lever responding also initially increased above baseline but returned to low levels by the fourth day of shock pre-treatment. When a timeout rather than a shock segment preceded self-administration sessions (for half of the rats, this followed shock pre-treatment days, but for the other half, this followed baseline days), cocaine self-administration decreased but settled at levels typical of non-acquisition-resistant rats self-administering cocaine in the lab in which these experiments were conducted. When 1.02 mA intermittent footshock segments again preceded cocaine self-administration sessions, reinforced-lever responding again increased significantly ($p=0.0143$, $t=2.52$, $df=62$) over reinforced-lever responding during self-administration sessions preceded by a timeout rather than a footshock component.

Reinforced-lever responding during self-administration sessions preceded by the second series of four days of 1.02 mA intermittent footshock was not significantly higher ($p>0.05$) than reinforced-lever responding during self-administration sessions preceded by the first series of 1.02 mA intermittent footshock. In these two shock series, footshock effectively was imposed on two different populations of rats: the first series of footshock occurred in rats which had self-administered negligible amounts of cocaine (acquisition-resistant rats) while the second round of footshock occurred in the same rats, but now with a history of cocaine self-administration (at typical levels of self-administration).

It is possible that stress-induced increases in locomotor activity led to increased responding on both reinforced and non-reinforced levers. Most rats did, in fact, respond more on both levers during their first cocaine self-administration session preceded by

footshock than they did during baseline conditions. However, the fact that non-reinforced-lever responding subsequently decreased to baseline levels indicates that increased locomotor activity alone does not explain the persistent increases in reinforced-lever responding following footshock. Increased reinforced-lever responding produced by footshock resulted in the acquisition resistant rats receiving substantial cumulative cocaine doses. Exposure to cocaine decreases brain reward thresholds in general (Kenny et al., 2003). Additionally, pre-exposure to cocaine decreases latency to acquisition of cocaine self-administration, priming sensitized rats to cocaine's reinforcing effects (Horger et al., 1990). Repeated exposure to cocaine (such as what could happen when stress-induced increases in locomotor activity lead to reinforced lever presses which result in cocaine infusions) also sensitizes rats to its reinforcing effects (Schenk and Partridge, 2000). This pre-exposure of the acquisition-resistant rats may sensitize them to the reinforcing effects of cocaine, leading to acquisition of cocaine self-administration (Carey et al., 1998; Deroche et al., 1999; Lett, 1989).

Increased cocaine self-administration exhibited when self-administration sessions were preceded by the second series of intermittent footshock was less pronounced than that produced by the first series of footshocks. This may have been because by the second series of footshocks, the rats were already sensitized to the reinforcing effects of cocaine. It is also possible that the effects were due solely to increased locomotor activity.

One other factor which could have contributed to increased responding on the reinforced lever revolves around the relationship between stress, the HPA axis and the

reinforcing effects of cocaine. The onset of cocaine self-administration following footshock by initially acquisition-resistant rats could be due to alterations in levels of stress hormones. Goeders & Guerin (1994) posit that a threshold level of HPA axis activation may be required for sensitivity to the reinforcing effects of cocaine. It is possible that initially acquisition-resistant rats have low basal levels of stress hormones circulating and that the experience of stress increases these hormones to the levels necessary for sensitivity to the reinforcing effects of cocaine. This could explain the increased cocaine self-administration seen in rats during the first set of self-administration sessions preceded by footshock, which might have then been maintained by sensitization to the reinforcing effects of the drug. In order to fully address this hypothesis, additional studies would be necessary examining basal and post-shock corticosterone levels in normal and acquisition-resistant rats both before and after footshock.

To summarize results for this experiment, footshock stress appears to have significantly increased reinforcing efficacy of cocaine in rats which were initially resistant to acquisition of cocaine self-administration. Footshock stress led to a non-significant ($p>0.05$) trend towards increased reinforced-lever responding in rats which had acquired cocaine self-administration.

Experiment 3: Effects of footshock on sensitivity to the reinforcing efficacy of low doses of cocaine

The first experiment found that footshock preceding cocaine self-administration on a PR schedule of reinforcement caused significantly decreased reinforced-lever

responding, suggesting that stress actually decreased reinforcing efficacy of cocaine. The second experiment found that footshock significantly increased reinforced-lever responding in rats which had not yet acquired cocaine self-administration, which would lead one to the conclusion that stress might increase the reinforcing efficacy of cocaine under a different set of conditions. Further clarification of the stress/reinforcing efficacy relationship was therefore needed.

The third experiment was conducted to determine if footshock stress would increase the reinforcing efficacy of a dose of cocaine which produced response rates of less than half of peak response rates. It was hypothesized that unit doses of cocaine that were too low to be reliably self-administered might begin to promote reliable self-administration behavior following stress. A half-maximal dose of cocaine was chosen by measuring reinforced-lever responding for progressively lower doses of cocaine until one was reached for which responding fell to less than 50 percent of that seen for the dose which produced the highest responding by each rat. Responding for the half-maximal dose was compared to responding for that same dose during self-administration sessions preceded by 1.02 mA intermittent footshock components. Increased self-administration of this low dose as a result of footshock would strongly suggest that footshock stress increased the reinforcing efficacy of a dose which, under non-shocked conditions, was not an effective reinforcer. When reinforced-lever responding for the half-maximal dose of cocaine after footshock was calculated as a percent of each rat's own pre-shock baseline, there was a non-significant ($p>0.05$) trend towards decreased reinforced-lever responding under shocked conditions. However, in the post-shock baseline sessions

which followed the footshock sessions, behavior continued to exhibit a decreasing trend, suggesting that behavior may have decreased even had no footshock been delivered. It is possible that footshock was tested before behavior had stabilized at a final floor level of responding. For individual rats, reinforced-lever responding varied more widely as the cocaine dose self-administered decreased. When responding for the lowest dose, most rats' reinforced-lever responding generally decreased across the first four days of self-administration of that dose. When footshock preceded self-administration sessions at that dose, responding generally increased for at least one of the four footshock days. Responding for the half-maximal dose in the subsequent four sessions not preceded by footshock was generally quite low.

A number of technical reasons might have been responsible for the failure of this experiment to produce reliable data. Some researchers believe that responding for doses on the ascending limb of the dose-response curve represents extinction or that there is no ascending limb at all and that responding for low doses fluctuates wildly (Norman and Tsibulsky, 2001; Sizemore and Martin, 2000; Flory and Woods, 2003). It is possible that the lowest doses chosen for each rat were too low to have any reinforcing efficacy and that reinforced-lever responding for these doses represented nothing more than extinction responding. The transient increases in responding seen during footshock in some animals could therefore be shock-induced reinstatement of responding for cocaine (Shaham et al., 2000; Shaham et al., 2003). It would be useful to conduct a study that includes responding for vehicle (saline) as a comparison to determine if reinforced-lever responding for the lowest doses represented extinction. Unfortunately, in the absence of

proof that the half-maximal dose of cocaine had at least some marginal reinforcing efficacy, it isn't possible to determine if footshock stress enhanced sensitivity to the reinforcing efficacy of these low doses.

These experiments did not generally support the hypothesis that footshock stress increases the reinforcing efficacy of cocaine in rats in the broader sense. Decreased reinforcing efficacy of cocaine as measured with a progressive ratio schedule of reinforcement was observed in rats with a history of cocaine self-administration. Footshock failed to enhance sensitivity to a half-maximal dose of cocaine and only non-significantly increased reinforced-lever responding in initially acquisition-resistant rats which had subsequently acquired cocaine self-administration. Footshock did, however, cause acquisition of cocaine self-administration in acquisition-resistant rats. Therefore, while footshock stress may be capable of sensitizing acquisition-resistant rats to the reinforcing effects of cocaine, it does not appear that it significantly increases the reinforcing efficacy of cocaine in rats with a history of cocaine self-administration.

References

Altman J, Everitt BJ, Glautier S, Markou A, Nutt D, Oretti R, Phillips GD, Robbins TW (1996) The biological, social and clinical bases of drug addiction: commentary and debate. Psychopharmacology 125:285-345

Andrews CM, Lucki I (2001) Effects of cocaine on extracellular dopamine and serotonin levels in the nucleus accumbens. Psychopharmacology 155:221-229

Arnold JM, Roberts DCS (1997) A critiques of fixed and progressive ratio schedules used to examine the neural substrates of drug reinforcement. Pharmacol biochem Behav 57:441-447

Beardsley PM, Howard JL, Shelton KL, Carroll FI (2005) Differential effects of the novel kappa opioid receptor antagonist, JDTic, on reinstatement of cocaine-seeking induced by footshock stressors vs cocaine primes and its antidepressant-like effects in rats. Psychopharmacology 183:118-26

Buttner A, Mall G, Penning R, Sachs H, Weis S (2003) The neuropathology of cocaine abuse. Legal Medicine 5:S240-S242

Calatayud J, Gonzalez A (2003) History of the development and evolution of local anesthesia since the coca leaf. Anesthesiology 98:1503-8

Campbell UC, Carroll ME (2000a) Acquisition of drug self-administration: environmental and pharmacological interventions. Exp Clin Psychopharmacol 8:312-325

Campbell UC, Carroll ME (2000b) Reduction of drug self-administration by an alternative non-drug reinforcer in rhesus monkeys. Psychopharmacology 147:418-25

Carey RJ, Gui J (1998) Cocaine conditioning and cocaine sensitization: what is the relationship? Behav Brain Res 92:67-76

Collier G (1962) Some properties of saccharin as a reinforcer. Journal of Experimental Psychology 64:184-191

Cone EJ (1995) Pharmacokinetics and pharmacodynamics of cocaine. J Anal Toxicol 19:459-78

Cosgrove KP, Carroll ME (2003) Effects of a non-drug reinforcer, saccharin, on oral self-administration of phencyclidine in male and female rhesus monkeys. Psychopharmacology 170:9-16

Covington HE III, Miczek KA (2001) Repeated social-defeat stress, cocaine or morphine: effects on behavioral sensitization and intravenous cocaine self-administration "binges." Psychopharmacology 158:388-398

Davidson C, Lee TH, Xiong Z, Ellinwood EH (2002) Ondansetron given in the acute withdrawal from a repeated cocaine sensitization dosing regimen reverses

the expression of sensitization and inhibits self-administration. Neuropsychopharmacology 27:542-553

Depoortere RY, Li DH, Lane JD, Emmett-Oglesby MW (1993) Parameters of self-administration of cocaine in rats under a progressive-ratio schedule. Pharmacol Biochem Behav 45:539-548

Deroche V, Le Moal M, Piazza PV (1999) Cocaine self-administration increases the incentive motivational properties of the drug in rats. Eur J Neurosci 11:2731-2736

Di Chiara G (1995) The role of dopamine in drug abuse viewed from the perspective of its role in motivation. Drug Alcohol Depend 38:95-137

Erb S, Shaham Y, Stewart J (1996) Stress reinstates cocaine-seeking behavior after prolonged extinction and a drug-free period. Psychopharmacology 128:408-412

Falck RS, Wang J, Siegal HA, Carlson RG (2004) The prevalence of psychiatric disorder among a community sample of crack cocaine users: an exploratory study with practical implications. J Nerv Ment Dis 192:503-7

Fibiger HC, Phillips AG, Brown EE (1992) The neurobiology of cocaine-induced reinforcement. Ciba Found Symp 166:96-124

Flory GS, Woods JH (2003) The ascending limb of the cocaine dose-response curve for reinforcing effect in rhesus monkeys. Psychopharmacology 166:91-94

Fontana DJ, Commissaris RJ (1989) Effects of cocaine on conflict behavior in the rat. Life Sci 45:819-27

Gawin FH, Ellinwood EH Jr. (1988) Cocaine and other stimulants. Actions, abuse and treatment. N Engl J Med 318:1173-82

Giordano LA, Bickel WK, Shahan TA, Badger GJ (2001) Behavioral economics of human drug self-administration: progressive ratio versus random sequences of response requirements. Behav Pharmacol 12:343-7

Goeders NE (1997) A neuroendocrine role in cocaine reinforcement. Psychoneuroendocrinology 22:237-259

Goeders NE (2002a) The HPA axis and cocaine reinforcement. Psychoneuroendocrinology 27:13-33

Goeders NE (2002b) Stress and cocaine addiction. J Pharmacol Exp Ther 301:785-789

Goeders NE, Guerin GF (1994) Non-contingent electric footshock facilitates the acquisition of intravenous cocaine self-administration in rats. Psychopharmacology 114:63-70

Goeders NE, Guerin GF (1996) Effects of surgical and pharmacological adrenalectomy on the initiation and maintenance of intravenous cocaine self-administration in rats. Brain Research 722:145-152

Gordon HW (2002) Early environmental stress and biological vulnerability to drug abuse. Psychoneuroendocrinology 27:115-126

Haney M, Maccari S, Le Moal M, Simon H, Piazza PV (1995) Social stress increases the acquisition of cocaine self-administration in male and female rats. Brain Res 698:46-52

Horger BA, Shelton K, Schenk S (1990) Preexposure sensitizes rats to the rewarding effects of cocaine. Pharmacol Biochem Behav 37:707-11

Kabbaj M, Norton CS, Kollack-Walker S, Watson SJ, Robinson TE, Ak H (2001) Social defeat alters the acquisition of cocaine self-administration in rats: role of individual differences in cocaine-taking behavior. Psychopharmacology 158:382-7

Kant GJ, Anderson SM, Dhillon GS, Mougey EH (1988) Neuroendocrine correlates of sustained stress: the activity-stress paradigm. Brain Res Bull 20:407-14

Karch SB (1999) Cocaine: history, use, abuse. J R Soc Med 92:393-7

Karlsgodt KH, Lukas SE, Elman I (2003) Psychosocial stress and the duration of cocaine use in non-treatment seeking individuals with cocaine dependence. Am J Drug Alcohol Abuse 29:539-51

Kenny PJ, Koob GF, Markou A (2003) Conditioned facilitation of brain reward function after repeated cocaine administration. Behav Neurosci 117:1103-7

Kosten TA, Miserendino MJ, Kehoe P (2000) Enhanced acquisition of cocaine self-administration in adult rats with neonatal isolation stress experience. Brain Res 875:44-50

Kulka RA, Schlenger WE, Fairbank JA, Hough RL, Jordan BK, Marmar CR, Weiss DS (1990) Trauma and Vietnam War generation: Report of findings from the National Vietnam Veterans Readjustment Study. New York, Brunner/Mazel

Lett BT (1989) Repeated exposures intensify rather than diminish the rewarding effects of amphetamine, morphine, and cocaine. Psychopharmacology 98:357-62

Lin D, Bruijnzeel AW, Schmidt P, Markou A (2002) Exposure to chronic mild stress alters thresholds for lateral hypothalamic stimulation reward and subsequent responsiveness to amphetamine. Neuroscience 114:925-33

Lu L, Shepard JD, Hall FS, Shaham Y (2003) Effect of environmental stressors on opiate and psychostimulant reinforcement, reinstatement and discrimination in rats: a review. Neuroscience and Biobehavioral Reviews 27:457-491

Majewska MD (2002) HPA axis and stimulant dependence: an enigmatic relationship. Psychoneuroendocrinology 27:5-12

Mantsch JR, Goeders NE (1999) Ketoconazole blocks the stress-induced reinstatement of cocaine-seeking behavior in rats: relationship to the discriminative stimulus effects of cocaine. Psychopharmacology 142:399-407

McMahon RC (2001) Personality, stress, and social support in cocaine relapse prediction. J Subst Abuse Treat 21:77-87

Meisch RA (1987) Factors controlling drug reinforced behavior. Pharmacol Biochem Behav 27:367-371

Miczek KA, Mutschler NH (1996) Activational effects of social stress on IV cocaine self-administration in rats. Psychopharmacology 128:256-264

Najavits LM, Runkel R, Neuner C, Frank AF, Thase ME, Crits-Christoph P, Blaine J (2003) Rates and symptoms of PTSD among cocaine-dependent patients. J Stud Alcohol 64:601-6

Nestler EJ (2004) Historical review: molecular and cellular mechanisms of opiate and cocaine addiction. TRENDS in Pharmacological Sciences 25:210-218

Norman AB, Tsibulsky VL (2001) Satiety threshold regulates maintained self-administration: comment on Lynch and Carroll. Exp Clin Psychopharmacol 9:151-4; discussion 160-2

Panlilio LV, Katz JL, Pickens RW, Schindler CW (2003) Variability of drug self-administration in rats. Psychopharmacology 167:9-19

Piazza PV, Le Moal M (1996) Pathophysiological basis of vulnerability to drug abuse: role of an interaction between stress, glucocorticoids, and dopaminergic neurons. Annu Rev Pharmacol Toxicol 36:359-78

Piazza PV, Le Moal M (1998) The role of stress in drug self-administration. Trends Pharmacol Sci 19:67-74

Prakash A, Das G (1993) Cocaine and the nervous system. Pharmacol Ther Toxicol 31:575-581

Rademacher DJ, Anders KA, Thompson KJ, Steinpreis RE (2000) The failure of some rats to acquire intravenous cocaine self-administration is attributable to conditioned place aversion. Behav Brain Res 117:13-19

Ramsey NF, Van Ree JM (1993) Emotional but not physical stress enhances intravenous cocaine self-administration in drug-naïve rats. Brain Res 608:216-22

Reilly S (1999) Reinforcement value of gustatory stimuli determined by progressive ratio performance. Pharmacol Biochem Behav 63:301-311

Richardson NR, Roberts DCS (1996) Progressive ratio schedules in drug self-administration studies in rats: a method to evaluate reinforcing efficacy. J Neuroscience Methods 66:1-11

Roserio R, Takahashi RN (1992) Anxiogenic properties of cocaine in the rat evaluated with the elevated plus-maze. Pharmacol Biochem Beh 43:631-633

Rouge-Pont F, Deroche V, Le Moal M, Piazza PV (1998) Individual differences in stress-induced dopamine release in the nucleus accumbens are influenced by corticosterone. Eur J Neurosci 10:3903-7

Rowlett JK (2000) A labor supply analysis of cocaine self-administration under progressive-ratio schedules: antecedents, methodologies, and perspectives. Psychopharmacology 153:1-16

Saal D, Dong Y, Bonci A, Malenka RC (2003) Drugs of abuse and stress trigger a common synaptic adaptation in dopamine neurons. Neuron 37:577-582

Sarnyai Z (1998) Neurobiology of stress and cocaine addiction: studies on corticotrophin-releasing factor in rats, monkeys, and humans. Ann N Y Acad Sci 851:371-87

Schenk S, Partridge B (1997) Sensitization and tolerance in psychostimulant self-administration. Pharmacol Biochem Behav 57:543-550

Schenk S, Partridge B (2000) Sensitization to cocaine's reinforcing effects produced by various cocaine pretreatment regimens in rats. Pharmacol Biochem Behav 66:765-770

Shaham Y, Erb S, Stewart J (2000) Stress-induced relapse to heroin and cocaine-seeking in rats: a review. Brain Res Brain Res Rev 33:13-33

Shaham Y, Klein LC, Alvares K, Grunberg NE (1993) Effect of stress on oral fentanyl consumption in rats in an operant self-administration paradigm. Pharmacol Biochem Behav 46:315-22

Shaham Y, Shalev U, Lu L, De Wit H, Stewart J (2003) The reinstatement model of drug relapse: history, methodology and major findings. Psychopharmacology 168:3-20

Shaham Y, Stewart J (1994) Exposure to mild stress enhances the reinforcing efficacy of intravenous heroin self-administration in rats. Psychopharmacology 114:523-527

Shelton KL, Hendrick E, Beardsley PM (2004) Interaction of noncontingent cocaine and contingent drug-paired stimuli on cocaine reinstatement. Eur J Pharmacol 497:35-40

Shippenberg TS, Heidbreder C (1995) Sensitization to the conditioned rewarding effects of cocaine: pharmacological and temporal characteristics. J Pharmacol Exp Ther 273:808-15

Sinha R, Fuse T, Aubin LR, O'Malley SS (2000) Psychological stress, drug-related cues, and cocaine craving. Psychopharmacology 152:140-148

Sizemore GM, Martin TJ (2000) Toward a mathematical description of dose-effect functions for self-administered drugs in laboratory animal models. Psychopharmacology 153:57-66

Smith BJ, Jones HE, Griffiths RR (2001) Physiological, subjective and reinforcing effects of oral and intravenous cocaine in humans. Psychopharmacology 156:435-444

Stafford D, Le Sage MG, Glowa JR (1998) Progressive-ratio schedules of drug delivery in the analysis of drug self-administration: a review. Psychopharmacology 139:169-184

Tidey JW, Miczek KA (1997) Acquisition of cocaine self-administration after social stress: role of accumbens dopamine. Psychopharmacology 130:203-212

Van den Hove DL, Blanco CE, Aendekerk B, Desbonnet L, Bruschettini M, Steinbusch HP, Prickaerts J, Steinbusch HW (2005) Prenatal restraint stress and long-term affective consequences. Dev Neurosci 27:313-20

Van Dyke C, Byck R (1982) Cocaine. Scientific American 246:128-41

Wansaw MP, Lin S, Morrell JI (2005) Plasma cocaine levels, metabolites, and locomotor activity after subcutaneous cocaine injection are stable across the postpartum period in rats. Pharmacology, Biochemistry and Behavior 82:55-66

Warner EA (1993) Cocaine abuse. Annals of Internal Medicine 119:226-235

White SM, Lambe CJT (2003) The pathophysiology of cocaine use. Journal of Clinical Forensic Medicine 10:27-39

Wise RA (1998) Drug-activation of brain reward pathways. Drug Alcohol Depend 51:13-22

Yang X, Gorman AL, Dunn AJ, Goeders NE (1992) Anxiogenic effects of acute and chronic cocaine administration:Neurochemical and behavioral studies. Pharmacol Biochem Beh 41:643-650

Vita

Elizabeth Stewart Hendrick was born on April 2, 1975 in Lawton, Oklahoma and is an American citizen. She graduated from Lawton High School in Lawton, Oklahoma in 1993. She received her Bachelor of Arts in biology from the University of Virginia, Charlottesville, Virginia in 1997 and subsequently worked as a lab technician in the lab of Madaline Harrison, M.D. in the Department of Neurology at the University of Virginia until 1999. She has worked as a lab technician for Patrick Beardsley, Ph.D. and Keith Shelton, Ph.D. in the Department of Pharmacology and Toxicology at Virginia Commonwealth University since 2001. She joined the department as a master's student in the fall semester of 2003.

Publications

Shelton, KL, Hendrick, E, Beardsley, PM (2004) Interaction of noncontingent cocaine and contingent drug-paired stimuli on cocaine reinstatement. Eur J Pharmacol 497:35-40