BEHAVIORAL MECHANISMS UNDERLYING THE EXTINCTION OF COCAINE SELF-ADMINISTRATION

A Dissertation

by

RODRIGO VALLES JR

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

December 2005

Major Subject: Psychology

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Approved by:

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ABSTRACT

Behavioral Mechanisms Underlying the Extinction of Cocaine Self-Administration.

(December 2005)

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Chair of Advisory Committee: Dr. Jack R. Nation

The aim of the present series of experiments was to outline the influence of different doses of cocaine during training, training schedule, training length and abstinence duration to modulate subsequent extinction and reinstatement patterns. Abram Amsel's general theory of persistence were used to both design and explain various aspects of these models.

For Experiment 1, rats self-administered cocaine (0.25, 0.50 or 1.00 mg/kg) intravenously and were then tested in an extinction preparation using saline infusions (5 days) and then only the stimulus light as the reinforcer (3 days). Experiment 2 examined schedules by magnitude interactions by training rats on two fixed-ratio (FR) schedules (FR-1 or FR-10 using either 0.25 or 1.00 mg/kg cocaine). Animals were tested in an extinction protocol (10 days; no stimulus light) and subsequently tested for reinstatement (1 day) that utilized presentations of the stimulus light. Experiment 3 addressed the effects of training length (15 or 30 days of training using either 0.25 or 1.00 mg/kg cocaine) using the same protocol as in Experiment 2. Experiment 4 examined the

modulation potential of two abstinence lengths (15 or 30 days using either 0.25 or 0.50 mg/kg cocaine) using the same conditions as Experiment 2.

Experiment 1 indicated the greatest resistance to extinction using the lowest training dose (0.25 mg/kg). The removal of saline caused an apparent extinction burst indicative of reward seeking. Experiment 2 showed that animals trained under partial reinforcement schedules persisted more during extinction. Furthermore, rats trained using 1.00 were more resistant than those trained with 0.25 mg/kg. Reinstatement of drug seeking was more pronounced in rats trained using an FR-10 schedule. Experiment 3 indicated greater resistance to extinction in rats trained for 15 versus 30 days. Rats trained on 0.50 mg/kg for 30 days showed less cue-induced reinstatement than those trained for 15 days. Experiment 4 showed increased resistance to extinction when rats were trained on 0.25 mg/kg and forced to abstain for 30 versus 15 days. Directionally opposite effects were apparent in groups trained with 0.50 mg/kg. Reinstatement data indicated greater responsivity to cues by animals abstaining for 30 versus 15 days.

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TABLE OF CONTENTS

Page

Page

LIST OF FIGURES

FIGURE Page

INTRODUCTION

Drug Abuse Statistics

The term addiction may be applicable to any number of incessant acts that have developed over the course of chronic engagement of the behavior (i.e. sex, gambling or drugs). Individuals are incapable of curbing the need to carry out these acts to the point that it may become the sole focus of their lives. However, it is the intake of foreign substances into the body, compounds that produce biochemical and physiological alterations, that sets drug addiction in a class all its own. The problem of drug addiction is therefore both a behavioral and neurochemical entrapment with consequences more profound than that of many other addictive behaviors.

Drug addiction is presently one of the worlds leading social, economic and health related problems. Figures from drug related negative social consequences are staggering to say the least as shown by the following statistics from the Office of National Drug Control Policy (2001). Drug use in the United States in 1998 alone was involved in or responsible for, over a 98.5 billion dollar loss in productivity. Programs for drug rehabilitation and other medical consequences cost the government 12.9 billion in 1998 and this figure is still rising. Crime related expenses for 1998 exceeded 88 billion dollars alone. In total, costs due to drug use in the United States were placed at around 143.4 billion dollars with trends suggesting that future years will yield higher dollar costs.

Reports from the Substance and Mental Health Services Administration's

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This dissertation follows the style and format of Psychopharmacology

(SAMHSA) National Survey on Drug Use and Health (2003) underscore the severity of drug use in 2003. Approximately 20.8 million Americans over the age of 12 (8.8% of the population) reported having non-medically used prescription-type stimulants (methamphetamine [12.3 million], diet pills [8.7 million] and Ritalin [4.2 million]) at least once in their lifetime. An estimated 378,000 of those were medically dependent. Among dependents the greatest incidents of use were by Whites (10.7%), Native Americans/ Alaska Natives (10.2%) and Hawaiians/ Pacific islanders (8.3%) while the lowest reported rates were among Hispanics (5%) and African-Americans (2.7%).

Other drugs reported in the National Survey on Drug Use and Health (2003) include heroin which is reported as having 144,000 incidents of first use (3.7% under the age of 25). Hallucinogens were used by 1.6 million users for the first time (36.7% under the age of 25). Marijuana use was the highest reported with 2.6 million people using the drug for the first time, the overwhelming majority of which were under the age of 25. One of the more recent trends in drug use is that of 3,4-methylenedioxymethamphetamine (MDMA) or "ecstasy" use. Extensive use seems to have surfaced in the mid-eighties and has steadily climbed with incidents of first use rising from 98,000 in 1983 to 1.7 million. Of those 57.5% were under the age of 25. Lastly, there has been a resurgence in inhalant use with approximately 1.1 million users (44.1% under the age of 25).

One of the more potent stimulants abused by humans is cocaine in all its various forms (cocaine, crack cocaine etc.). Reports show approximately 1.1 million people having used cocaine for the first time in their life (National Survey on Drug Use and Health, 2003). Of those, an estimated 36.9% were under the age of 25. Approximately

369,000 people first used crack cocaine in 2003. Of those, 7.8% were under the age of 25. An estimated 14% of drug related treatment admissions into hospitals in 2000 were accounted for by some form of cocaine use, particularly crack cocaine. Smoked cocaine emergency patients were overwhelmingly African-American (59%) and 41% of the total users admitted to daily use. These data outline the severity of the drug abuse problem in the US and in particular the abuse of cocaine. Without major breakthroughs in basic research, the problem of cocaine, and drugs of abuse in general, will never be efficiently combated on therapeutic or preventative level.

Cocaine History and Overview

The severity of cocaine abuse can be attributed to its dramatic abuse liability and subsequent ability to induce behavioral and physiological addiction. A complete examination of the effects of cocaine should begin with a description of its history, structure and function.

Historically, the coca leaf was used by the Incan Empire of Peru for medicinal, local anesthetic, and religious purposes. Eventually, the use and popularity of cocaine would spread to Europe and North America, where it would be touted as a cure for all manner of ailments. Among its many advocates, Sigmund Freud and druggist John Sith Pemberton of Coca-Cola fame would herald the miraculous qualities of cocaine. While the local anesthetic properties of cocaine would stand the test of time; the world would eventually come to realize the extent to which cocaine held dramatic abuse potential. By the late 1920's America was undergoing its first cocaine epidemic which led to

government regulation and eventually prohibition of the drug (Das, 1993). The U.S. would subsequently experience a second, more profound, increase in cocaine abuse in the 1980s. This second epidemic played a large role in the exponential increase in cocaine research. The scientific community has since placed increasing efforts into uncovering the mechanisms that underlie cocaine abuse; however much work remains.

Cocaine has various methods that are typically employed to introduce it into the body. One of the most common routes of administration is intranasal where it is readily absorbed across the mucous membranes. Other methods of self-administering cocaine include chewing coca leaves, intravascular (IV) injection and the inhalation of crack cocaine, or the free base form of cocaine, created by heating it and adding baking soda.

A common dose used intranasally is about 25-30 mg. Using 1-2 g falls in the range of overdose amounts for most users. Peak plasma concentration after IV administration will typically occur at about 5 minutes corresponding to reported peak euphoric, or "high," effects (Javaid et al., 1978; Smith et al., 2001). Freebase (or crack) use is characterized by equally rapid actions. The "high" typically lasts on the order of 5-10 minutes and is extremely addictive due to its raw form. The short duration of its effects is a prominent reason for its addictive potential. Users must continually seek out the drug in order to maintain prolonged periods of euphoria or alleviation of withdrawal. Cocaine plasma half-life is on the order of about 40 minutes (Javaid et al., 1983). Cocaine is metabolized into the major inactive metabolites benzoylecognine and ecognine methyl ester. This is accomplished by liver and serum carboxylesterase, liver esterase, and serum cholinesterase. The active metabolite, norcocaine, is created by hepatic mixed function oxidases (Dean et al., 1991; Jatlow, 1998). The short term

physiological effects of all cocaine use include vasoconstriction, local anesthesia (if applied topically), increased heart rate, elevated blood pressure, increases in core body temperature, increased mental alertness, motoric alterations and feelings of euphoria. Long term effects include, tolerance, sensitization, irritability, paranoia, anxiety, depression, irregular heart beat, weight loss, heart failure, mucosal membrane ulceration and, of course addiction, incarceration or death.

In addition to using cocaine, users will often combine it with other drugs. Combinations of cocaine and heroin are known as "speedball." Mixing cocaine with alcohol leads to the formation of cocaethylene during metabolism and is a leading cause of fatal and near-fatal overdoses. Polydrug use is a common occurrence and, as in the case with alcohol, can completely change the structure and effects of cocaine on the body. For the purposes of this paper, and simplicity, only cocaine use will be examined.

Human Data

The persistent use of cocaine by humans is one of the key characteristics of addiction, particularly in the face of negative consequences. Beginning with the initial exposure, users will engage in behaviors that may lead to cycles that arrive at clinical definitions of addiction. Feelings of euphoria become associated with a variety of cues including context and paraphernalia as continued use leads to tolerance and ingestion of greater amounts to attain similar internal states. Greater resources are spent on finding and using the drug until it may become the centerpiece for existence. Numerous negative consequences become evident, yet the addict persists in seeking and using the drug.

These characteristics can be generalized to a cycle of addiction that includes acquisition or initial use of the drug, maintenance of the behavior or regular use, cessation by some means (voluntary or otherwise) and finally relapse.

The definition of the term addiction, as discussed within this paper, is an inability to cease use in the face of negative consequences. It is precisely within the abstinence portion of the cycle of addiction that some of the most crucial investigations of cocaine use may lie. The eventual goal of many researchers is not only the mapping of the mechanisms of cocaine addictions but the implementation of an effective therapy. Human studies however, have not elucidated much in the way of therapeutic strategies for addicts. Examinations in humans may follow correlational methodologies to determine risk factors in an addicted population but are not necessarily causational. Behavioral studies aim at outlining and altering habitual behaviors that manifest themselves during addiction. However, examinations of human addicts take place in individuals who are already addicted and therefore biased. Voucher studies (Donny et al., 2004) and questionnaire based studies (Weiss et al., 2003) have shown some limited success in curbing immediate use (minutes-hours) and predicting craving respectively. The least amount of progress has been made in the area of pharmacological interventions.

A review of over 1000 available pharmacotherapies showed no effects in almost all cases examined (Silva de Lima et al., 2002). The glutamate enhancing drug monafidil has received some attention due to emerging evidence of glutamate-mediated modulation of cocaine reward. Preliminary results using monafidil indicate some decreases in relapse to drug use and decreased reactivity to cocaine-paired cues (Dackis and O'Brien, 2003). Finally, cocaine "vaccinations" also have received attention recently (Kantank,

2003). The reality of this particular therapy is that the absorption is slowed and the immediate effects are thereby diminished. However, there are no effects on cocaine craving and the effects of the vaccine are overcome with increasing amounts of cocaine.

To reiterate, investigations are limited to people who are, typically already either addicts or users with diverse drug histories that have not been controlled for. In addition, the limitations of human research do not allow for ethical manipulations to determine the brain structures and processes in psychostimulant addiction. As such, the bulk of the studies in cocaine addiction must be conducted in animal models of human addiction.

Cocaine Neuropharmacology

Upon introduction into to the central nervous system (CNS), cocaine will target the transport reuptake mechanisms of 3 neurotransmitter systems: dopamine (DA), serotonin (5-HT) and norepinephrine (NE). This occurs in various loci throughout the brain. Of these, the most pertinent to this series of investigations are those associated with reward and reinforcement. In addition, motor areas and nuclei associated with impulsivity may be affected. A basic overview of known neuronal mechanisms and brain systems that are integral to cocaine reward/reinforcement follows.

Brain Nuclei

Once cocaine has been taken into the body, the effects are not localized to the above mentioned targets. Cascades ensue that affect numerous transmitter systems and travel

along projections associated with reward/reinforcement, impulsivity and motoric function. This section will outline general areas that are known to be critical in the effects of cocaine.

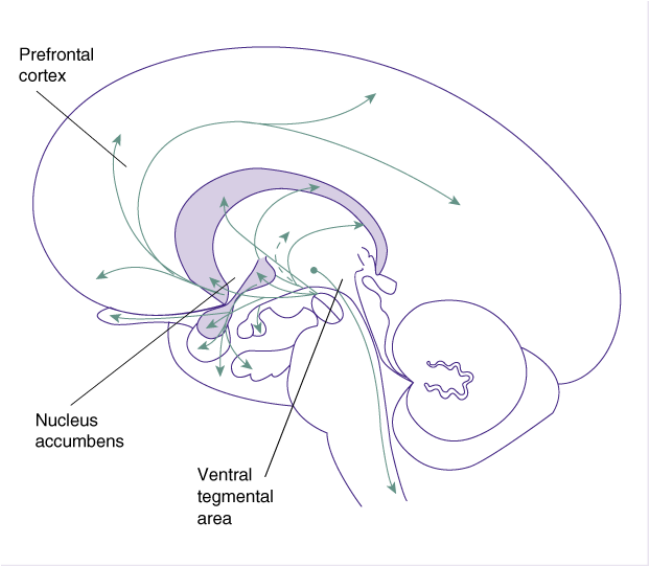

Fig. 1. General representation of the mesocorticolimbic reward/reinforcement pathway. Cell bodies originating in the VTA project to the NAcc and finally the PFC. Various other afferents and efferents may modulate this system (not detailed).

Without question, the most studied nucleus pertaining to cocaine reward, and reward in general, is the nucleus accumbens (NAcc). Numerous studies have implicated this structure, and associated increases in DA after cocaine administration, as critical mediators of reward/reinforcement (Carboni et al., 1989; Hemby et al., 1997; Pettit and Justice, 1989, 1991; Roberts et al., 1980). The origins of dopaminergic neurons lie in midbrain ventral tegmental area (VTA) cell bodies (Ungerstedt, 1971). From this nucleus, the mesocorticolimbic system projects to the NAcc and then the prefrontal cortex (PFC). Nuclei within the PFC are thought to be involved in decision making and

thereby may influence escalations of drug intake when choices are presented (Butter, 1969; Kolb et al., 1974).

Furthermore, the NAcc can be divided into 2 distinct compartments, the shell and core. Projections from the NAcc core have more direct connections to the substantia nigra (SN). The NAcc shell region comprises part of the extended amygdala, an area consisting of the bed nucleus of the stria terminalis (BNST) and the central and medial amygdala. These areas project strongly to the VTA and hypothalamus (Zahm and Brog, 1992). D_2 receptors seem to be more prevalent in the shell whereas D_3 receptors are more abundant in the core (Larson and Ariano, 1995). As such the two areas are thought to play distinct roles in the effects of cocaine. NAcc core areas are thought to play more critical roles in the motor-activating effects of cocaine (increased distance traveled, stereotypy). The NAcc shell area, including the extended amygdala, may have a more prevalent role in the reinforcement, anxiety inducing and associative learning areas resulting from cocaine. Data from Koob et al. (2004) propose that the extended amygdala plays a key role in dysregulation of homeostasis. This is accomplished via the hypothalamic-pituitary-adrenal (HPA) axis connections of the extended amygdala and its involvement in reward and reinforcement. Work from the Goeders laboratory also has pinpointed the HPA-axis and corticotropin releasing hormone (CRH) as modulators of cocaine reward values (Goeders, 2003). Data from this laboratory indicate an enhancement of functional reward values when animals are undergoing, or have recently been exposed to, anxiogenic conditions. This adds to Koob's notion of anxiety as a cyclical event that becomes a negative reinforcer and thereby entraps users into escalating, uncontrollable intake.

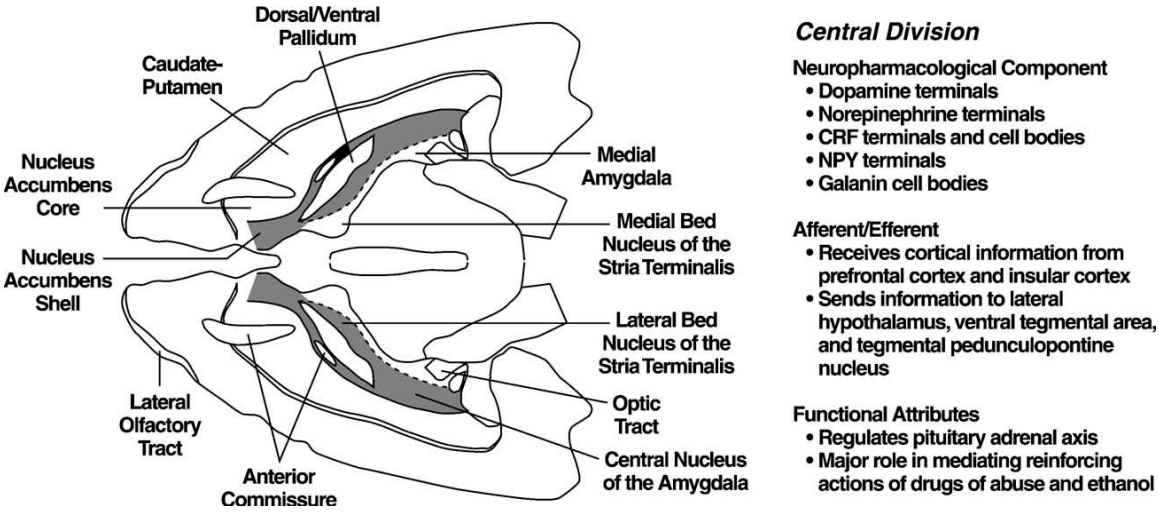

Fig. 2. Diagram illustrating the central division of the extended amygdala, its neuropharmacological components, afferent and efferent connections, and functional attributes. From Koob et al. (2004).

As detailed above, the effects of cocaine involve more than the dopaminergic system. Connections to limbic and cortical areas allow for this modulation of the primary effects of cocaine. Glutamatergic efferents from the PFC innervate both the NAcc and the VTA (Christie et al., 1985) and are believed to operate in a modulatory manner on dopaminergic neurons (Duvauchelle et al., 1998). In addition, the medial (m) PFC receives glutamatergic afferents from the hippocampus, amygdala, mediodorsal thalamus and contralateral mPFC (Groenewegen et al., 1990; Pirot et al., 1994; Tzschentke et al., 2001). Also, GABAergic projections from the ventral pallidum (VP) innervate the mPFC (Bigl et al., 1982; Johnston et al., 1981).

Neurotransmitters

Dopamine

Cocaine ($C_{17}H_{21}NO_4$), or benzoylmethyl-ecognine, is a derivative of the erythroxylon coca plant. The chemical structure of cocaine allows it to preferentially block Na^+/Cl^- dependent plasma membrane transporters for DA, NE and 5-HT (O'Brien, 1996). Monoamine transporters are the primary means of DA, NE and 5-HT removal from the synaptic space (Deutch and Roth, 1999). The resultant accumulation of neurotransmitters will stimulate various receptors both pre- and postsynaptically (Ritz et al., 1997). Among the most widely studied is the DA receptor, which is implicated as one of the primary mediators of reward and reinforcement. There are currently two classes of DA receptor families; the D_1-like and the D_2-like. The D_1 family includes D_1 and D_5 receptors. The primary actions are G-protein linked. Postsynaptic stimulation of these receptors stimulates adenylyl cyclase activity. The D_2 family includes the D_2, D_3 and D_4. D_2-like receptors have opposing effects in that they decrease adenylyl cyclase activity. D_3 receptors, thought to be located pre- and postsynaptically, are believed to function as autoreceptors that control the rate of action potential-induced DA release via a phosphoinositide second-messenger system. D_2 dopamine receptors also are believed to function as autoreceptors and are thought to be located pre and postsynaptically as well. D_2 and D_3 receptors are located on both cell bodies and terminals and are therefore of particular interest in cocaine pharmacology.

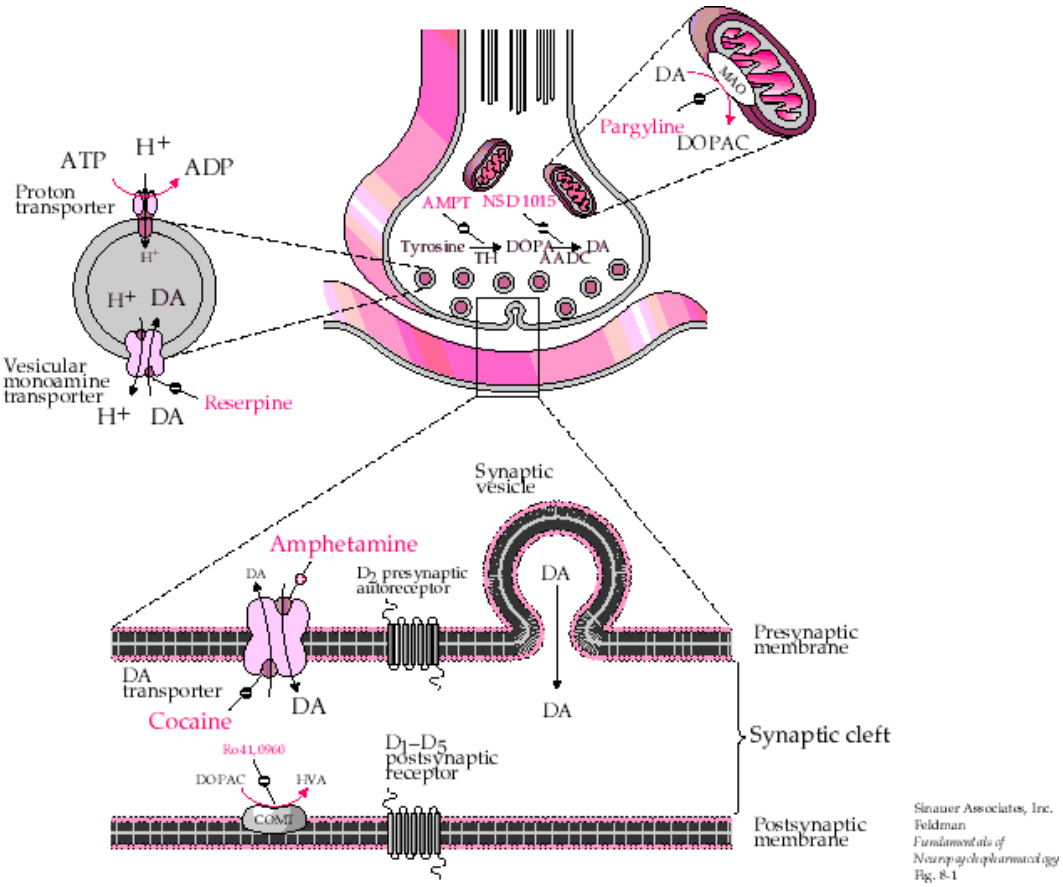

Fig. 3. DAergic synapse showing key proteins involved in DA-mediated neurotransmission. Synthesis, uptake, release and reuptake of DA are shown. Cocaine is active at the DAT where it causes an accumulation of DA in the synapse.

The activation of DAergic terminals begins with the synthesis of DA within the presynaptic neuron. DA is a catecholamine which is enzymatically synthesized from tyrosine hydroxylase which is converted to DOPA and subsequently DA. One additional step will produce NE. DA is synthesized in the nerve terminal where it is taken up into vesicles and transported to the outer membrane for release into the synapse. Subsequent reuptake by the DA reuptake transporter (DAT) will allow for its degradation by monoamine oxidase (MAO) within the mitochondria or repackaging into vesicles and subsequently being released in the synapse again. DA also can be inactivated by

catechol-O-methyltransferase (COMT), a postsynaptically located enzyme. Cocaine will bind preferentially to the DAT and prevent reuptake into the presynaptic terminal, particularly within areas of the brain associated with reward and movement.

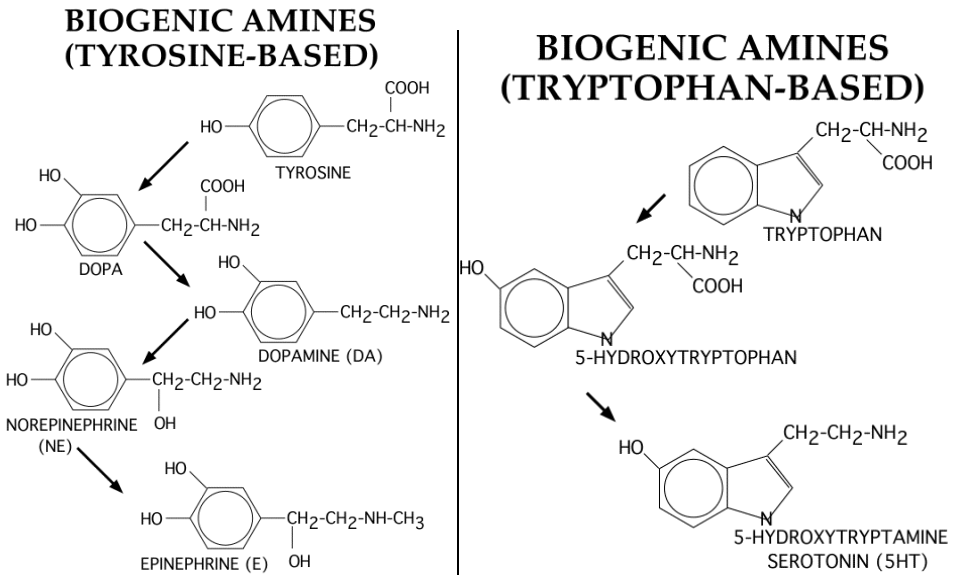

Fig. 4. Synthesis and structure of the major neurotransmitters (DA, NE and 5-HT), indirectly affected by cocaine, are shown.

Dopaminergic activation resulting from psychostimulant intake is predominantly manifested via the mesocorticolimbic dopaminergic pathway (Nestler, 2004). Projections arising from the VTA innervate the NAcc which then projects to the PFC. Of recent interest, are the distinctions between the NAcc core and shell. The NAcc shell appears to be predominantly involved with the reinforcing effects and of cocaine, projecting to the VTA, while the core seems to be a modulator of motoric effects with projections to the subthalamic nucleus and SN (Deutch and Cameron, 1992). However, there is functional

overlap. Also, this circuit is not solely responsible for reward and reinforcement and is known to be modulated by various other neurotransmitter systems.

The primary action of cocaine in the central nervous system (CNS) is the blockade of the DAT. As will be explained in the following sections, cocaine will also bind to the 5-HT reuptake transporter (SERT), the NE reuptake transporter (NET) and other voltage and ligand gated ion channels (which will not be discussed) [Kuhar et al., 1991]. Still, there is no doubt that the primary reinforcing and locomotor stimulant actions of cocaine arise from DAT blockade.

Blockade of the DAT will result in associated increases in DA bioavailability, particularly throughout limbic areas that are known to be associated with reward and reinforcement (Koob and Bloom, 1988). Further, DAT blockade by cocaine will result in upregualtion of DAT receptor sites that may then be partially responsible for withdrawal mechanisms resulting from subsequent under-stimulation of DAergic neurons (Kahlig and Galli, 2003). In terms of reward, DAT knockout mice still show conditioned place preference (CPP) to cocaine but combinations of DAT/SERT knockouts will eliminate CPP and self-administration (Uhl et al., 2002). However, the locomotor stimulatory effects of cocaine appear to be exclusively mediated by DAT blockade as knockouts of SERT or NET show no changes in total distance traveled (Sora et al., 2001; Xu et al., 2000).

Serotonin

Equally as affected by the presence of cocaine, the serotonergic (5-HT; 5-hydroxytryptamine) system plays a prominent role in mediating the reinforcing, and perhaps aversive, effects of cocaine. 5-HT is a biogenic amine synthesized from the precursor tryptophan, which is converted to 5-hydroxytryptophan, which is then decarboxylated into 5-HT. The 5-HT receptor is made up of 7 sub-classes. Cocaine appears to target 5-HT_3 receptors, the only ligand-gated ion channels; however, there is minimal information on its complete effects at the neuronal level. The efficacy of cocaine for the SERT is about equal to that of the DAT. Reuptake via the SERT allows for either repackaging into synaptic vesicles for transport to the synaptic membrane or degradation by MAO within the terminal bouton. Removal of 5-HT from the synaptic cleft is similar to that of DA therefore accumulation of 5-HT will result in a general stimulation of 5-HT receptors similar to that of selective serotonin reuptake inhibitors (SSRIs) although to what degree is generally unknown. SSRIs are known to be used as "mood enhancers" therefore to suggest that cocaine may modulate euphoria or reinforcement is not entirely illogical.

Various areas within the brain are dense in 5-HT and coincide with brain nuclei known to be critical in drug rewarding properties. Stimulation of serotonergic pathways arising from the raphe nuclei will result in the accumulation of dopamine in the VTA (Chen et al., 1994; Kelland et al., 1993), NAcc (Parsons and Justice, 1993) and dorsal striatum (De Deurwaerdere et al., 1996). Direct cocaine administration will result in elevated levels of 5-HT in the NAcc (Parsons and Justice, 1993; Reith et al., 1997),

dorsal striatum (Bradberry et al., 1993), hippocampus (Muller et al., 2002), thalamus (Rutter et al., 1998), and VTA (Chen et al., 1994; Parsons and Justice, 1993; Reith et al., 1997).

The subsequent effects of synaptic accumulation of 5-HT, in regards to cocaine, are less understood than dopamine. However, it is becoming increasingly clear that the reinforcing and rewarding properties of 5-HT, associated with other drugs of abuse like MDMA, are at least partially responsible for the reinforcing values assigned to cocaine and its reported euphoric effects. A recent study from Hall et al. (2004) showed compensation by the SERT and NET in DAT knockout mice placed in a CPP study. Conversely, DAT/NET knockouts do not show comparable losses in conditioned reinforcement. The overriding issue here is the involvement of other neurotransmitter systems in cocaine reinforcement. In addition, it has been proposed that the less than clear role of 5-HT in cocaine reward/reinforcement may be explained by dual aversive and reinforcing properties (Uhl et al., 2002).

SERT knockouts have shown enhancements (Sora et al., 1998) while DAT/SERT knockout mice show almost a complete loss of cocaine reward (Sora et al., 2001). DAT and NET knockouts show enhanced fluoxetine (an SSRI) reward (Hall et al., 2002) plausibly arguing for dual roles in cocaine reinforcement. Additional evidence from human data may support opposing effects that functionally cancel each other in that little abuse liability is seen in fluoxetine and other SSRIs (Tella, 1995; Yen and Fuller, 1992; Zawerailo et al, 1995).

Norepinephrine

Cocaine also targets the NET however; the involvement of this transporter in cocaine reward and reinforcement is less clear than is the case for cocaine/DAT interactions. NE is synthesized from DA and is similarly affected by the presence of cocaine. Reuptake from the synaptic cleft is blocked and vesicular repackaging and degradation by MAO is prevented leading to accumulation. The actions of NE primarily take place at one of four receptor subtypes: α_1, α_2, β_1 and β_2. The α_1 receptor is primarily linked to phospholipidase activity in the peripheral nervous system, while α_2 activity results primarily in inhibition of adenylyl cyclase activity in presynaptic nerve terminals throughout the brain. The β_1 and β_2 receptors are primarily located in the cerebellum and cerebral cortex and stimulate adenylyl cyclase activity. Noradrenergic projections from the locus ceruleus project to areas that may have indirect connections to known reward pathways such as the amygdala and hippocampus. Activation of this system is typically involved in responses to anxiety-provoking or arousing stimuli (as per the "fight or flight" response). However, the available literature does not present a clear picture of the behavioral effects of cocaine blockade of the NET. As such, there is a case to be made for its involvement in the anxiety and reinforcement effects of cocaine.

As previously mentioned, the blockade of the NET leads to similar accumulation of the neurotransmitter in the synapse and thereby is involved in cocaine biochemical consequences. However, as per the Hall et al. (2004) study, combined DAT/SERT knockouts do not display a CPP whereas DAT/NET and SERT/NET knockouts do. The inability to compensate with an intact noradrenergic system might indicate a lesser role in

cocaine reinforcement mechanisms. Alternatively, a case may be made for aversive modulation of cocaine effects by NET blockade. Data from both human (Grabowski et al., 1997) and animal (DeVries and Pert, 1998; Ettenburg et al., 1999) studies may suggest some involvement in reports of "jitteriness" and CRH-mediated anxiogenic mechanisms respectively. This is conceivable given the direct connections between NE and sympathetic activation in the presence of arousing or anxiety provoking stimuli.

Glutamate and γ–aminobutyric acid

While the primary, indirect, effects of cocaine are mediated by the above 3 neurotransmitters systems both glutamate and γ–aminobutyric acid (GABA) appear to play a modulatory role. Specifically, behaviors such as cue- or anxiety-induced activation of drug seeking recruit these specific transmitter systems. For instance, the glutamatergic system is thought to play a pivotal role in the response to cocaine associated cues. Recent evidence suggests that glutamate and not DA receptors control response-dependent presentations of CSs paired with cocaine (Di Ciano and Everitt, 2001; Hotsenpiller and Wolf, 2003). In addition, withdrawal related decreases in *N*-methyl-*D*-aspartate (NMDA) receptor subunit 1 are evident across a 2 week cocaine-free period in several forebrain areas (Crespo et al., 2002). Subsequent acute presentations of cocaine after periods of abstinence appear to result in glutamate overflows in areas such as the NAcc, SN, caudate putamen and VTA (Zhang et al., 2001). Further, glutamate transmission from the PFC to the NAcc is thought to be involved in motivation and impulse control deficits that contribute to addiction patterns (Kalivas et al., 2005).

Finally, GABA and glutamate manipulation has been shown to modulate cocaine self-administration and reinstatement (Cornish and Kalivas, 2000; Cornish et al., 1999; De Vries et al., 1998; Di Ciano and Everitt, 2003; Roberts and Brebner, 2000; Smith et al., 2003; Valles et al., 2005). Specifically regarding GABA modulation, interneurons between the VTA and NAcc are strong inhibitors of DAerigc neurons. Disinhibition of these neurons will result in elevated DA cell activation and are indirectly linked to anxiety-related increases in functional reward values of cocaine.

In summary, while current research has developed numerous methods for addressing the persisting problem of drug addiction, there are still factors within the discipline that need reevaluation. Data from the past 40-50 years has revealed many of the major players in cocaine reward/reinforcement, namely DA and the NAcc. Various modulations by other neurotransmitter systems and the involvement of afferents and efferents to and from numerous brain nuclei also have been identified, further revealing the complex nature of the disease of addiction. However, it is increasingly clear that there is still much to be discovered in the area of drug addiction. The integration of these very specific biochemical and behavioral phenomena is yet to be accomplished. The creation of an accurate animal representation of drug addiction still escapes us. The following section addresses the shortcomings of current methodologies and proposes a re-examination of basic animal models.

Animal Models of Addiction

The formulation of accurate animal models of drug addiction is, likely, the greatest obstacle in the development of potential therapies for human addicts. As detailed in the previous section, the complexity of human drug addiction is readily apparent in light of the lack of applicable scientific breakthroughs. Systematic investigations of drug addiction cannot be conducted in human subjects for numerous reasons. Of course, ethical dilemmas prevent the administration of cocaine to naïve subjects. Therefore, human studies, by default, employ individuals with histories of prior use. This introduces a bias for several reasons. Chronic drug users are often polydrug abusers therefore complex interactions likely exist prior to any sort of testing. People who participate in studies will typically have a motivation for doing so. Monetary rewards or a desire to stop using may be factors for enrolling in studies. This may cause the subject pool to be unrepresentative of the typical addict or user. While epidemiological studies may uncover interesting correlational data, manipulations that aid in testing hypotheses are, of course, not possible. Accordingly, the manipulation of physiological, neurochemical and behavioral changes induced by addictive drugs must take place in animal models that approximate human patterns of use and abuse.

Drug addiction research in animals has employed a wide variety of paradigms in order to study the effects of use and abuse. Models such as CPP examine the associative value of reinforcing, or aversive stimuli, by pairing them with a distinct context. Locomotion studies examine the acute motor effects of drugs and many other models test the nuances of various drug intake effects. However, no model more closely

approximates human drug use/abuse than self-administration (Carroll, 1993; Deroche-Gamonet et al., 2004; Fitch and Roberts, 1993; Vanderschuren and Everitt, 2004).

Self-administration studies provide the experimenter with a tool that measures voluntary intake of the drug being tested. The versatility of this model, also, allows for numerous traits of drug addiction to be systematically tested. Specifically, four key stages of addiction (acquisition, maintenance, abstinence/extinction, relapse/reinstatement) have become the focus of a large portion of current drug addiction, specifically self-administration, literature (Gardner, 2000; Lynch and Carroll, 2001; Rowlett, 2000; Shaham et al., 2003; Shalev et al., 2003). Of the phases of addiction modeled in animals, extinction or forced abstinence has received the least scrutiny. To that end, the data from acquisition, maintenance and reinstatement, although studied in depth for the past 40 years (Weeks, 1962), have likely suffered to varying degrees from a lack of emphasis on uniform methodologies present across studies. For instance, the definition of an extinguished behavior varies widely between studies. Also, models have classically focused on behavioral endpoints (i.e. lever responses, CPP, DA increase, etc.) and have neglected to address the complex nature of drug histories that lead to these changes. Very few humans use only one drug, at one concentration, consistently, throughout the transition from use to abuse. The steps that lead to an inability to cease are marked with numerous interactions arising from other drugs (polydrug abuse), powerful contextual stimuli and persisting cycles that continue in the face of severe, negative consequences. Therein lies the nature of drug addiction that few labs have endeavored to model.

Without regard to interactions, labs will often train animals on a high dose of cocaine in order to engender a quick acquisition of the instrumental response (Carroll and Lac, 1997; Gerrits and van Ree, 1995). Cocaine doses on the descending limb of the dose-effect curve are very often used for training animals up to stable responding. Many studies use the fast acquisition latencies of cocaine in order to transition animals to other drugs, such as heroin and MDMA, that are more difficult to train on. These studies do not examine possible dose-related differences or interaction effects stemming from the inclusion of a drug with a very different chemical profile. Without a detailed model of addiction that approximates the dynamic nature of addiction in humans, the results from drug-seeking and drug-taking studies will be interesting but consistently difficult to interpret within the larger scope of "addiction."

Highlighting the shortcomings of drug abuse research to the present day, two recent Science articles have developed a novel method of targeting "addicted" populations within animal groups that more closely approximate the characteristics of humans who are clinically classified as addicts. Vanderschuren and Everitt (2004) used a novel paradigm that included prolonged training and drug access periods that distinguished between seeking and taking behaviors using two sequential lever pressing requirements. In addition, the use of negative consequences was used to uncover persistent sub-groups of self-administering rats. Specifically, animals that had stably acquired self-administration were given shocks as a result of receiving, or seeking, cocaine. This is critical in that it attempts to parallel the numerous negative side effects of chronic drug intake. Humans who are compulsive users will often experience social, economic and health detriments as a result of continued use. Results from a study by

Deroche-Gamonet et al. (2004) showed that less than 20% of self-administering animals persisted in spite of recurring negative consequences; a figure much closer to that of humans that are considered to be addicted (around 17%) [Anthony et al., 1994]. This study employed similar procedures as the previous in that training employed complex taking procedures (3 progressive ratio sessions per day). In addition, shock punishment also was used to assess motivation in the face of negative consequences. These findings are compelling for addiction researchers because they shed light on the inaccuracy of definitions of "addicted" animals.

In addition to the problems in methodologies addressed by the previous studies there are several aspects of typical self-administration protocols that remain to be thoroughly examined. Specifically, a lack of attention to extinction or abstinence phases of animal self-administration is evident throughout drug abuse literature. This phase of drug addiction is seldom systematically analyzed or manipulated yet is the most directly relevant as a model of behavioral cessation. Also, there are few instances in which dose-related differences during training or testing are addressed. Typical studies employ a single, high dose of a drug and neglect to ascertain the effects of examining other concentrations. It is increasingly clear that the nuances of drug-induced phenomena will not be examined efficiently until more meticulous attention to the design of experiments is given.

Along these lines, the lack of research projects that exhaustively address prior drug histories is particularly evident in extinction, or forced abstinence procedures. This area of the addiction cycle has not been adequately investigated. Usually, extinction results are embedded within a reinstatement protocol and are given little importance.

Experimenters will typically extinguish across 7-10 days and then move animals to reinstatement testing. The definition of an extinguished response varies across studies but is usually defined as lever presses below a pre-determined number per hour. What is not evident, are the differences that occur during these sessions and whether drug seeking will resume again given enough time. The focus, then, is to show a depressed amount of active lever responding in order to begin reinstatement testing where effects are more readily apparent. However, it is the opinion of the author, that this area holds the most potential for possible cessation therapies.

Animals will voluntarily engage in abstinence periods after prolonged exposure to psychostimulants much like humans (Bozarth and Wise, 1985; Johanson et al., 1976). It is critical to mention at this point, that animal studies of extinction do not precisely model human abstinence. While animal will temporarily cease responding of their own volition, as cited above, rats are "forced" to quit in the majority of examinations. The drug is removed and replaced with saline, or nothing at all, and the animal is returned to the operant chamber to engage in typical seeking behaviors such as bar pressing or nose pokes. While not accurate as a model of abstinence, these tests do hold potential as therapies that aid cessation of responding (or quitting) because they actively dissociate the instrumental response and the reward.

While the effects during extinction are likely subtle it cannot be said with certainty that they do not exist. Moreover, few studies have purposefully included manipulations that may show comparative differences across groups. Most extinction phases within a study will report results from groups that were all similarly trained (a single dose or single combination of drugs or doses) and subsequently extinguished

before reinstatement testing. Without differences in drug history and careful attention to extinction session conditions, an accurate characterization of forced abstinence cannot be attained.

General Rationale

Consequent to the data mentioned above, the purpose of these 4 experiments was to address methodological issues that may aid in developing more precise models of drug addiction. Issues of drug history must be taken into account when monitoring behavioral tests, neurotransmitter turnover and physiological changes in neuronal organization or structure. In other words, differences in prior drug histories may have consequences that affect behaviors as well as physiology and brain chemistry. This is critical given that the purpose of animal models are to provide pre-clinical data that leads to viable clinical therapies to addiction.

To illustrate, it is known that self-administration behaviors have different biochemical outcomes in yoked versus self-administering animals (Schenk and Partridge, 1997; Smith et al., 2003). Voluntary administration of a drug obviously produces different outcomes that likely lend to emerging cycles of addiction. Yet, there are numerous instances where forced administrations of drugs produce results that are later equated or compared to those of self-administration studies. Animals with different histories of non-drug reinforcers produce different results in terms of behavioral and biochemical measures (Nader et al., 1992; Nader and Bowen 1995; Nader and Reboussin, 1994). This is another example of the influence that drug history, or reward history, may

play in the results observed in subsequent, often different, measures. While these examples are limited to maintenance phases of self-administration, this could certainly apply to extinction research as well. Food and water reward literature has shown that training methods will influence behavioral outcomes during extinction. As follows, interactions stemming from drug, training or reward history are very possible during extinction phases and may hold particular relevance as models of cessation. If realistic interpretations of human addiction are to be created, they must attempt to address the wide range of variables that may affect addiction. Just as important, this must be done for every phase of addiction that is modeled including extinction.

Therefore the specific aim of these studies was to create tests that manipulated drug histories prior to testing under extinction, or forced abstinence, conditions. The issue of drug training dose is of particular importance and is addressed in each experiment. Further, the designs included manipulations aimed at uncovering possible differences induced by schedules of reinforcement, acquisition training duration, and abstinence duration. The specific conditions during extinction training, specifically the involvement of extra-drug cues, also were examined. Finally, reinstatement tests were included in 3 of the 4 experiments in order to detail the relative persistence of any effects both temporally and across testing procedures, or rather phases of the addiction cycle.

EXPERIMENT 1: DOSE-EFFECT

Rationale

Research methods for animal models of drug abuse have likely suffered, to varying degrees, from the absence of consistently uniform methods of examining drug self-administration. Systematic cocaine research has classically characterized the dose/intake profiles of drugs by outlining dose-effect curves using self-administration preparations. While this method has been employed in models of acquisition (Carroll and Lac, 1997; Gerrits and van Ree, 1995) and maintenance (Lynch and Carroll, 2001; Nation et al., 2004), the effects of training dose on the extinction portion of the addiction cycle has not been characterized. Of course, any effects would arise from differences in drug history being that no drug is given during extinction training. However, the premise is very relevant given the known influence of prior drug histories on subsequent behaviors in animal models (Le Sage and Glowa, 1999). Interestingly, this area (extinction and prior drug histories) of self-administration research has remained relatively overlooked. Only recently has attention been given to the importance of the abstinence phase and the interaction of drug histories (see Lu et al., 2004 for a review). However, the most logical first step upon entering this arena of research would have been a basic dose response study solely examining modulation of behavioral responding under extinction conditions but this has not been done. To that end, the first experiment was designed in order to fill this gap in the cocaine addiction literature.

Specifically, the aim of Experiment 1 was to investigate the modulation potential of 3 doses of cocaine (0.25, 0.50, and 1.00 mg/kg/infusion), typically used during

acquisition and maintenance, on subsequent behaviors during forced abstinence periods (extinction). Different cocaine concentrations available during a self-administration study are known to produce differences in instrumental responding depending on their location on the biphasic dose-effect curve (Lynch and Carroll, 2001). These different doses will engender different levels (number of total infusions) of active drug intake. Therefore each group will be subject to different amounts of experience building response-reinforcer associations within an operant setting. In addition, the reward magnitude differences will occasion different amounts of total cocaine intake. Specifically, higher doses produce greater amounts of cumulative intake (Rowlett et al., 1996). Therefore, differences in both learning phenomena and neurochemical alterations may exist when animals are trained on varying doses of cocaine.

In terms of methodological issues used as a rationale for this study, discrepancies in drug histories will likely prevent a clean comparison of results from one study to the next. If an accurate representation of the effects of training histories in animal models can be made in general, then the interpretation of behavioral results may be made clearer. It is likely that the variation in training methods, between studies, before abstinence periods and any subsequent testing may be, in part, responsible for observed dependent measures. Therefore the obvious initial experiment for this dissertation appeared to be a characterization of extinction responding after different histories of drug training. Specifically, animals were trained on a single dose of cocaine having no exposure to other concentrations. Furthermore, 3 concentrations of cocaine were used in order to determine if effects were consistent across doses.

Studies of extinction are necessarily studies of cue-responsivity. Associated cues are clearly influential in the absence of the primary reward. Therefore, this experiment was divided into two batteries of extinction tests: the first included saline and the onset of the stimulus light as the outcome to a right (active) lever response during extinction, the second only resulted in a stimulus light presentation after a right lever press. The rationale was that the removal of a conditioned stimulus (CS) [Goldberg et al., 1979] would accelerate extinction of the learned response unless the reinforcement value was of a sufficient magnitude that an extinction burst, or no change at all, would occur. An extinction burst is typically observed after the removal of the primary reinforcer. It is believed to reflect an increased amount of effort in searching for the expected reward outcome.

Materials and Methods

Animals

The research design and conduct of the experiment were approved by the Texas A&M University Laboratory Animal Care Committee, and all aspects of the research followed the guidelines outlined in Principles of Laboratory Animal Care (NIH publication No.85-23). Twenty male Sprague-Dawley (Harlan; Houston, Texas, USA) rats weighing between 300-325 g were used for Experiment 1. Rats were single housed in hanging polycarbonate cages for the entire study. All animals were kept on a 12H/12H light/dark light cycle with lights on at 8:00 AM and were given tap water and standard rat chow *ad*

libitum. Training and testing was conducted only during the light portion of the cycle between the hours of 9:00 AM-7:00 PM.

Apparatus

Self-administration and extinction tests were conducted in 16 standard operant chambers (Med-Associates, ENV-001; St. Albans, VT, USA). Each chamber was equipped with 2 levers (right and left) and a stimulus light positioned over each lever. Depression of the active (right) lever resulted in the presentation of a 0.1 ml intravenous infusion of cocaine HCL dissolved in heparinized saline. Each infusion was a total of 12 seconds in length and was accompanied by the simultaneous illumination of the stimulus light positioned above the lever. During this infusion period, further responding on either lever was recorded but had no programmed consequences. Responding on the inactive (left) lever was recorded but had no programmed consequences at anytime. Drug deliveries were made using mechanical pumps (Razel Model A; St. Albans, VT, USA) equipped with 1 rpm motors and 20 ml syringes. Programs were controlled and data recorded using two IBM computers using OPN software (Spencer and Emmett-Oglesby, 1985).

Surgeries

Rats underwent stereotaxic surgery 1 week after arrival at Texas A&M University. Animals were given atropine (1.4 mg/ml) intraperitoneally (IP) and subsequently anesthetized using a mixture of ketamine (100 mg/ml) and xylazine (100 mg/ml) given

intramuscularly (IM). Intrajugular catheterizations were performed using 0.01 ID Silastic tubing (Dow Corning; Midland, MI, USA) of approximately 6 inches in length. A silicon ball was secured around the catheter at the proximal (side entering vein) end approximately 2 inches from the end of the catheter. The left jugular vein was isolated and tied off in order to prevent hemorrhage. A small incision was made below the knot on the dorsal part of the vein. The catheter was then threaded into the jugular vein until the silicon ball was flush with the vein opening and then anchored using size 000 silk suture string (Harvard Apparatus; Holliston, MA, USA) on both sides of the silicon ball. The distal end of the catheter, having a blunted and bent 22 gauge L-shaped needle fitted onto it, was then passed subcutaneously around the left ear to the top of the animal's skull. There it was anchored onto the skull of the animal where an incision of approximately one inch had been made running along the midline of the head of the animal. The skull was cleaned and then cauterized using blood stop. Four jewelers screws (Small Parts Inc.; Miami Lakes, FL, USA) were screwed into the skull (one in each of the quadrants surrounding bregma) in order to provide an anchor for the needle and dental cement (Plastics One; Roanoke, VA, USA). The cement was then poured onto the skull and allowed to harden with approximately 13 mm of the needle left exposed. The tip of the needle was capped using a plastic obturator to prevent blood loss. Nitrofurazone ointment was applied to the head and neck incisions daily for approximately 1 week. Each day, the catheters were infused with 0.1 ml of a sterile saline solution containing heparin (1.25 U/ml), penicillin G Potassium (250,000 U/ml) and streptokinase (8000 IU/ml) to prevent infection and the formation of clots and fibroids.

Drugs

The Research Technology Branch of the National Institute of Drug Abuse generously supplied the cocaine HCl.

Testing

Animals were allowed to recover for 1 week after surgeries. The following day, animals were transported to the testing chambers from the home cage using individual plastic boxes. Before being placed into the testing chambers, each animal was flushed with 0.1 ml of sterile saline to ensure catheter patency. Rats were then placed in pre-determined testing chambers and connected via the exposed end of the blunted 22 ga needle. This was attached to a length of 0.02 ID Tygon tubing housed within a spring leash attached to the chamber. This was directed to another length of 0.02 ID tubing via a single channel fluid swivel (Harvard Apparatus; Holliston, MA, USA). This connection then formed a closed system that would receive infusions directly from the syringe on the mechanical pump.

Three doses of cocaine HCL were used for this experiment: 0.25 (N=6), 0.50 (N=7) and 1.00 mg/kg/infusion (N=7). Each group of animals was trained initially on an FR-1 schedule of reinforcement, where one lever press resulted in one infusion of cocaine. Animals were allowed to self-administer once daily for a period of 2 hours. Onset of sessions was signaled by an experimenter-delivered prime. Animals received consecutive daily sessions until responding was stable (< 20% variability for three

consecutive sessions). Subsequently, animals were moved to an FR-2 until responding was stable, then an FR-5 schedule, again until responding was stable. The following day, animals were placed into the chambers as usual, however, cocaine syringes were replaced with heparinized saline instead. Animals were allowed to self-administer at will for saline and this was termed "extinction conditions" for this experiment. Every 5th response resulted in the presentation of the stimulus light and a saline infusion. Responding was allowed to continue for daily 1 hour sessions for a period of 5 days.

Following the 5th extinction day, animals were tested under similar conditions without the availability of saline as the reward outcome. Active lever responses only resulted in the presentation of the stimulus light and the activation of the pump (without an infusion of any kind) for a period of 12 seconds. All other testing conditions remained in place. This continued for a period of 3 days.

Data Analysis

Terminal acquisition (the final day of acquisition training) was analyzed using two-way ANOVA tests (Training Dose x Levers). Extinction data were analyzed using three-way repeated measures ANOVA (Training Dose x Levers x Days). Tukey's post hocs were used when appropriate. In all cases statistical significance levels were set at $p<0.05$.

Results

Terminal Acquisition

The last day of baseline responding (terminal acquisition) data were first analyzed to determine if significant differences existed between Active and Inactive Lever responding. Two-way ANOVA (Training Dose [0.25 mg/kg, 0.50 mg/kg, 1.00 mg/kg] x Levers [Active, Inactive]) results from the terminal acquisition showed significant differences between Levers [$F(1,17)=256.00$, $p<0.001$]. Visually (see Figure 5), it is apparent that Inactive Lever responding was at near zero levels, which prompted the exclusion of Inactive Lever responding from any further analyses. A subsequent one-way ANOVA on Active Lever responding alone showed no significant differences between the 3 training doses of cocaine [$F(1,17)=0.78$, $p>0.05$]. This is of particular interest in light of the results obtained during the first day of extinction. Carryover effects were likely not responsible for the differences observed. In terms of the lack of trends resembling a dose-effect curve, the limited experience with the FR-5 schedule was manifested as a cost/benefit interaction that diminished responding at the lower doses.

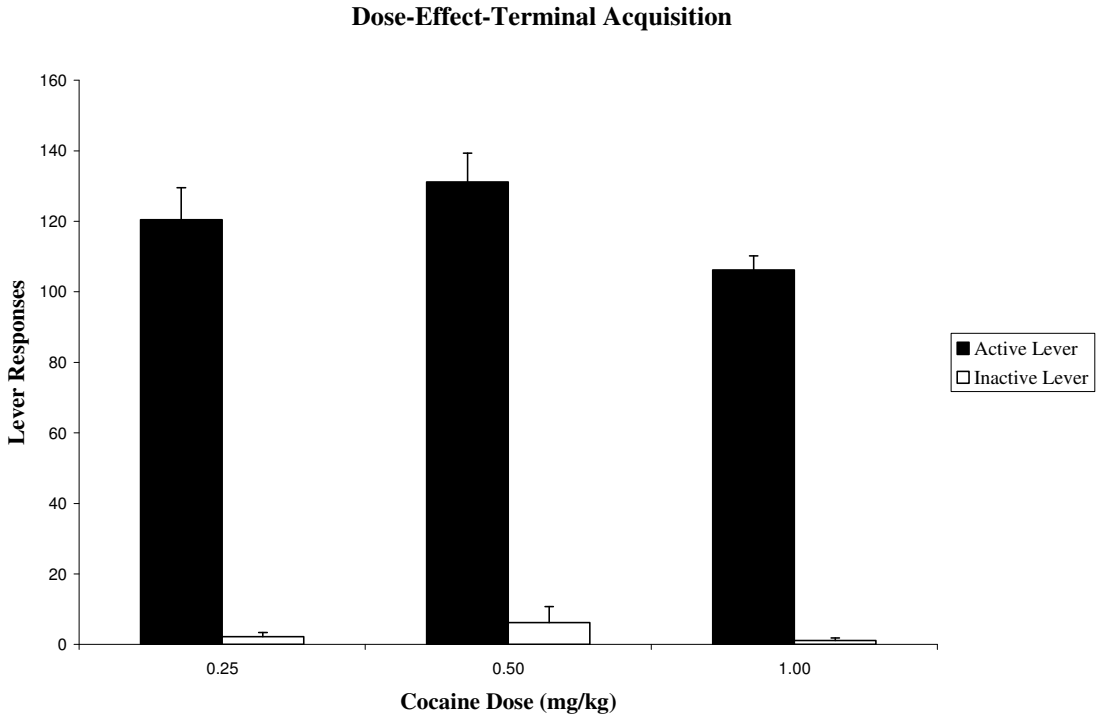

Fig. 5. Experiment 1, mean number of active and inactive lever responses on the last day of baseline responding (terminal acquisition).

For the purposes of comparing extinction responding to terminal acquisition throughout the first hour, an analysis using lever responses in 15 minute intervals was conducted. The four 15 minute, bins were used as the repeated measure. Subsequent extinction responding analyses for Experiment 1 included a similar analysis for Day 1 (i.e. ANOVA using 15 minute intervals). A two-way ANOVA (Training Dose [0.25 mg/kg, 0.50 mg/kg, 1.00 mg/kg] x Bin [1, 2, 3, 4]) was used to analyze behavioral differences. Main effects by Bin [$F(3,51)=9.86$, $p<0.001$] were observed but none by Training Dose [$F(2,17)=2.10$, $p>0.05$]. No interactions (Training Dose x Bins) were present [$F(6,51)=1.20$, $p>0.05$]. Differences by Bin were not surprising due to a slight amount of loading occurring at the onset of the session, during Bin 1 as is apparent by an

examination of Figure 6.

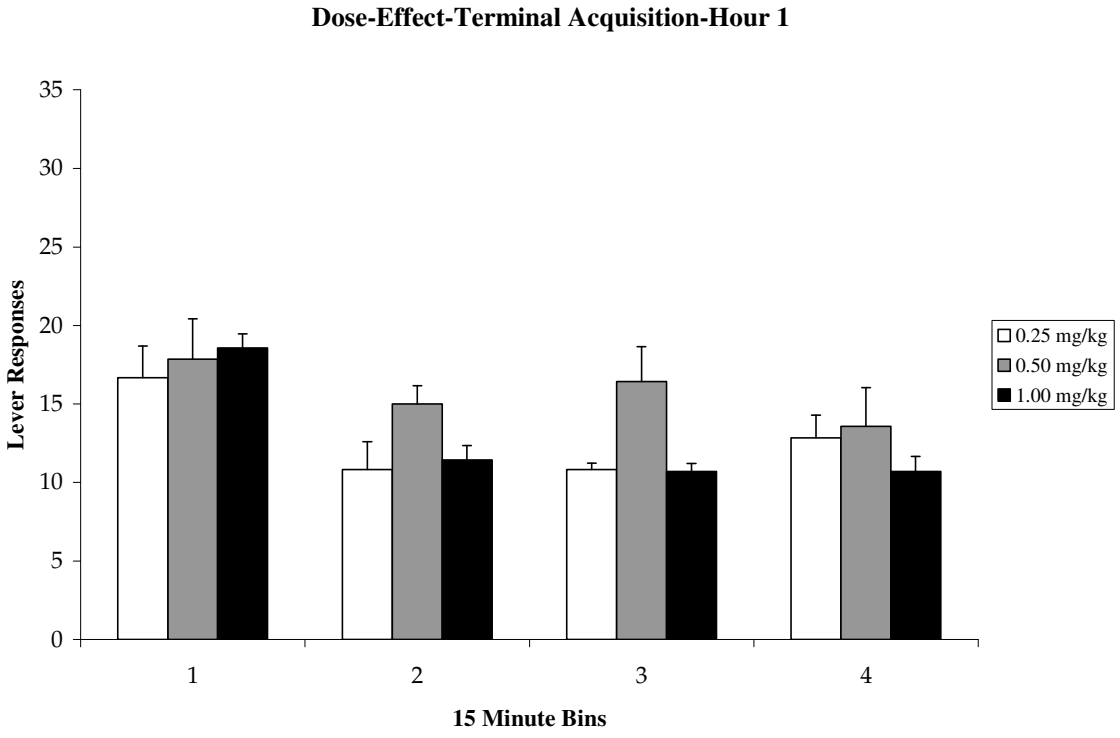

Fig. 6. Experiment 1, mean number of active lever responses during the first hour of terminal acquisition. Data is represented in intervals of 15 minutes for all groups.

Extinction

Saline

Responding under conditions where a lever response resulted in a saline infusion and stimulus light presentation was next analyzed. A three-way ANOVA (Training Dose [0.25 mg/kg, 0.50 mg/kg, 1.00 mg/kg] x Levers [Active, Inactive] x Days [1-5]) was first used to determine if there were any significant differences between Active and Inactive Lever responding. Results indicated significant main effects by Levers [$F(1,17)=391.87$, $p<0.001$]. Figure 7 shows the comparably minimal and steady amount of responding on

the left lever which prompted the exclusion of Inactive Lever responding from any further analyses.

Analysis of Active Lever responding provided a measure of active drug seeking and was conducted using a two-way ANOVA (Training Dose [0.25 mg/kg, 0.50 mg/kg, 1.00 mg/kg] x Days [1-5]). Results showed significant main effects by Days [$F(4,68)=4.66$, $p<0.01$] and by Training Dose [$F(1,17)=3.93$, $p<0.05$]. No interactions (Training Dose x Days) were present [$F(8,68)=1.70$, $p>0.05$].

Post hoc tests on Training Dose showed significant differences between Group 0.25 versus Group 1.00 mg/kg Training Dose ($p<0.05$). Overall, it appeared that the lower dose of 0.25 mg/kg engendered a greater amount of resistance to extinction and that lever responding decreased as testing progressed across days.

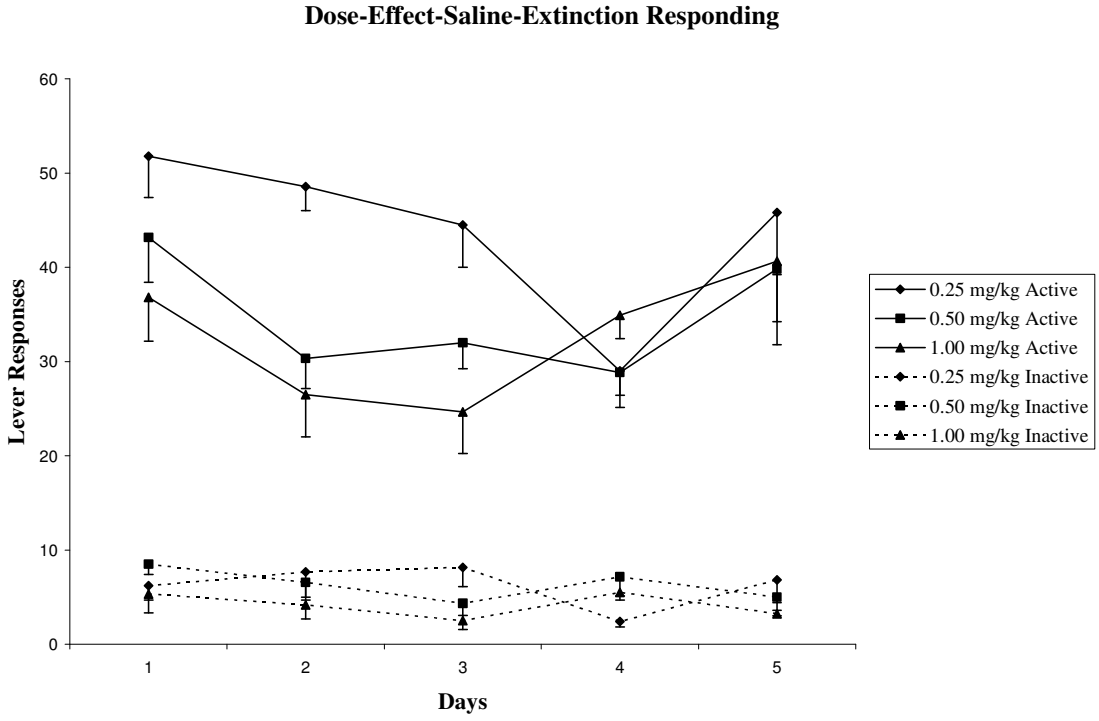

Fig. 7. Experiment 1, mean number of active and inactive lever responses during saline-available extinction, days 1-5. Group 0.25 mg/kg cocaine showed the greatest amount of resistance to extinction ($p<0.05$).

In addition to a standard analysis across Days, ANOVA tests were performed on lever responding during the first session (Day 1) using intervals of 15 minutes as the repeated measure. The purpose of this analysis was to reveal any potential differences in lever responding that could otherwise be averaged out throughout the course of the entire initial session. It was hypothesized that the onset of the session might show the most responding due to the initial drug-seeking in the absence of the reward, sometimes referred to as an "extinction burst."

Results from the two-way ANOVA (Training Dose [0.25 mg/kg, 0.50 mg/kg, 1.00 mg/kg] x Bin [1, 2, 3, 4]) showed significant main effects by Bin [$F(1,17)=81.23$, $p<0.001$] but not by Training Dose [$F(2,17)=1.16$, $p>0.05$]. Significant interactions (Training Dose x Bin) were present [$F(2,17)=5.82$, $p<0.05$].

Subsequent post hocs showed significantly greater responding during Bin 3 by Group 0.50 versus Group 0.25 ($p<0.05$) and Group 1.00 mg/kg Training Dose ($p<0.05$). Conversely, Group 1.00 mg/kg showed greater responding than Group 0.50 mg/kg Training Dose in Bin 4 ($p<0.05$). Significantly greater responding by Group 0.25 versus Group 1.00 mg/kg Training Dose was evident in Bin 1 ($p<0.05$). Differences were approached between Group 0.50 and Group 1.00 mg/kg Training Dose in Bin 1 ($p>0.05$).

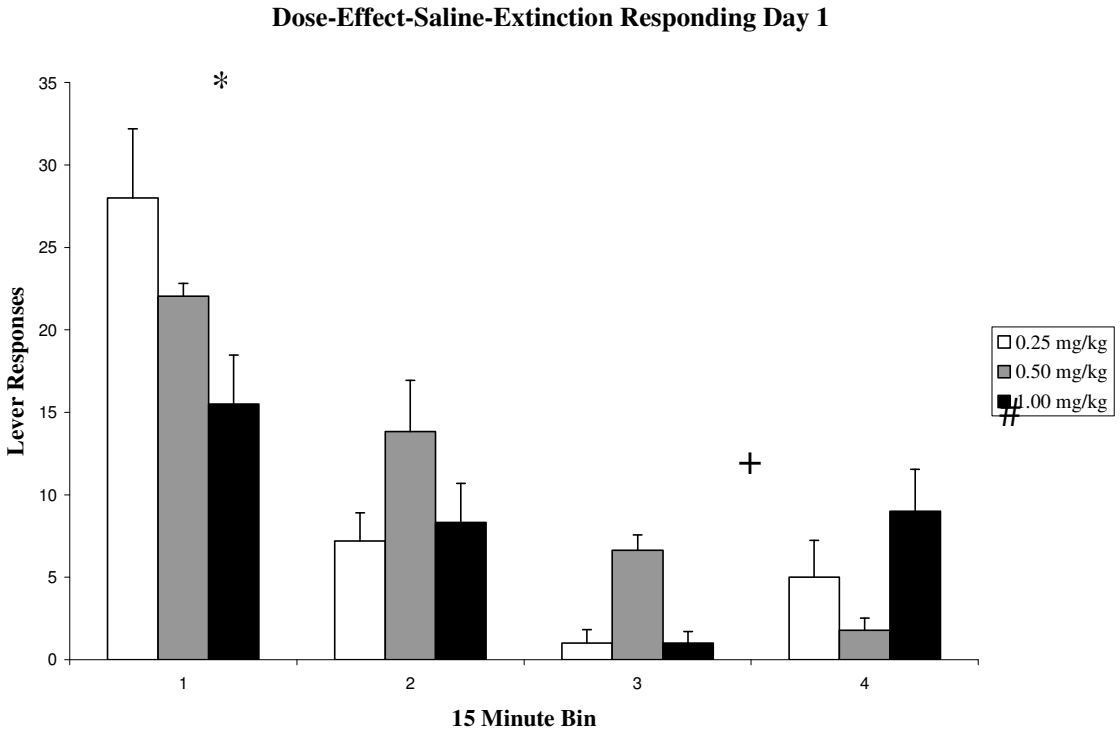

Fig. 8. Experiment 1, mean number of active lever responses during hour 1 of the first day of saline-available extinction. Significantly greater responding by group 0.25 mg/kg Training Dose during Bin 1 is denoted with an *. Greater responding by group 0.50 mg/kg Training Dose during Bin 3 is denoted with a +. Greater responding by group 1.00 mg/kg Training Dose during Bin 4 is denoted with #.

The data for Experiment 1 also were converted to reflect percentage baseline changes in responding from terminal acquisition to extinction training. This was done as an alternate method of analyzing extinction responding. The premise is that lever responding under extinction conditions is compared to previous responding under standard reward-available conditions. This places all the animals in the study on a common metric and allows for comparisons that take into account the response histories of each subject. In other words, the transformation controls for carryover effects due to differences in responding produced by different doses of cocaine (as shown in the familiar biphasic dose-effect curve).

Results from the two-way ANOVA (x Training Dose [0.25 mg/kg, 0.50 mg/kg, 1.00 mg/kg] x Days [1-5]) showed significant main effects by Days [$F(4,68)=3.18$, $p<0.05$] but none by Training Dose [$F(1,17)=1.45$, $p>0.05$]. No significant interaction effects were present (Training Dose x Days) [$F(8,68)=1.40$, $p>0.05$]. This is an important contrast to the significance found in the above analyses which did not take into consideration the differences in terminal acquisition. Also, visual examination of Figure 9 shows that, although significant by Days, relatively little change is apparent between Day 1 and Day 5 indicating the relative persistence of motivational factors.

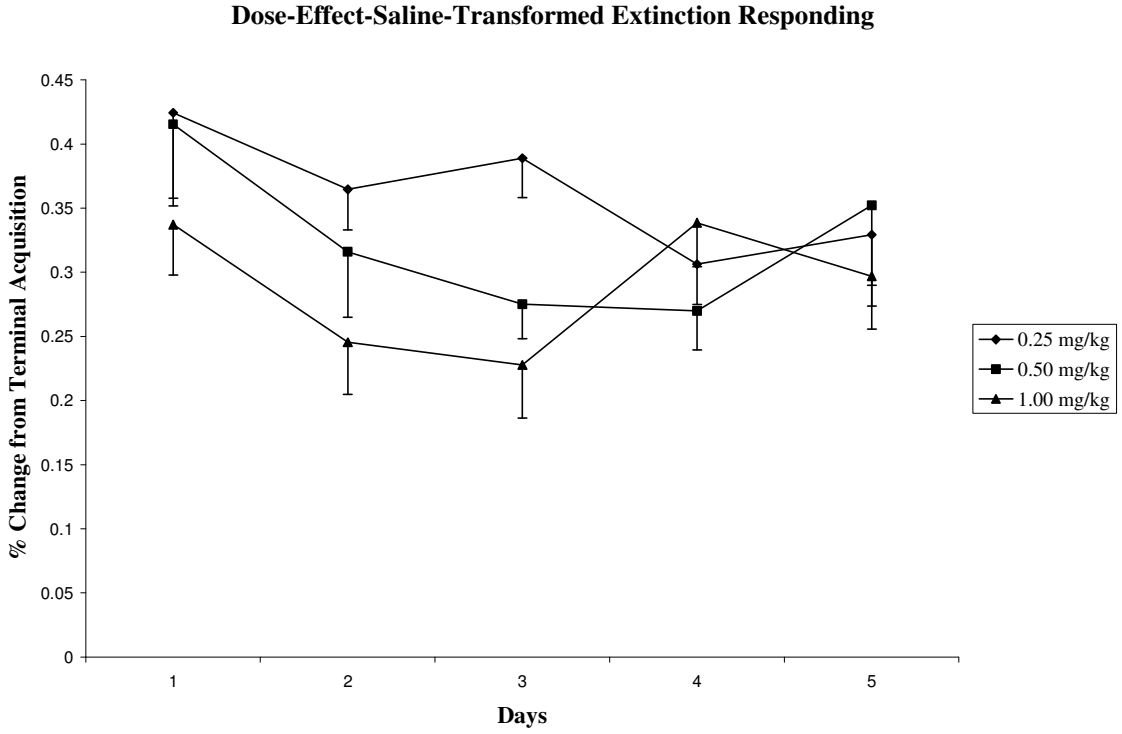

Fig. 9. Experiment 1, mean number of percentage changes in baseline responding from terminal acquisition compared to days 1-5 of extinction training.

Saline removed

All subjects were then tested for a period of 3 days using comparable extinction conditions that also excluded the presentation of saline as the reward outcome, however the stimulus light was still utilized. A three-way ANOVA (Training Dose [0.25 mg/kg, 0.50 mg/kg, 1.00 mg/kg] x Levers [Active, Inactive] x Days [1-3]) was first used to determine if there were any significant differences between Active and Inactive Lever responding. Results indicated significant main effects by Levers [$F(1,17)=150.95$, $p<0.001$]. Once again, it is visually apparent that left lever responding was at near zero levels throughout the 3 days of testing, which prompted the exclusion of Inactive Lever responding from any further analyses.

Results from the omnibus two-way ANOVA (Training Dose [0.25 mg/kg, 0.50 mg/kg, 1.00 mg/kg] x Days [1-3]) showed no main effects by Days [$F(2,34)=0.25$, $p>0.05$] or any interaction effects (Training Dose x Days) [$F(4,34)=0.70$, $p>0.05$). Significant main effects were present by Training Dose [$F(2,17)=3.94$, $p<0.05$].

Of particular interest are the results from post hocs on Training Dose indicating significantly elevated resistance to extinction by Group 0.25 versus Group 1.00 mg/kg Training Dose ($p<0.05$). Figure 10 shows essentially no change in responding throughout the 3 days of testing, particularly in Group 0.25 mg/kg Training Dose explaining the absence of a main effect for Days.

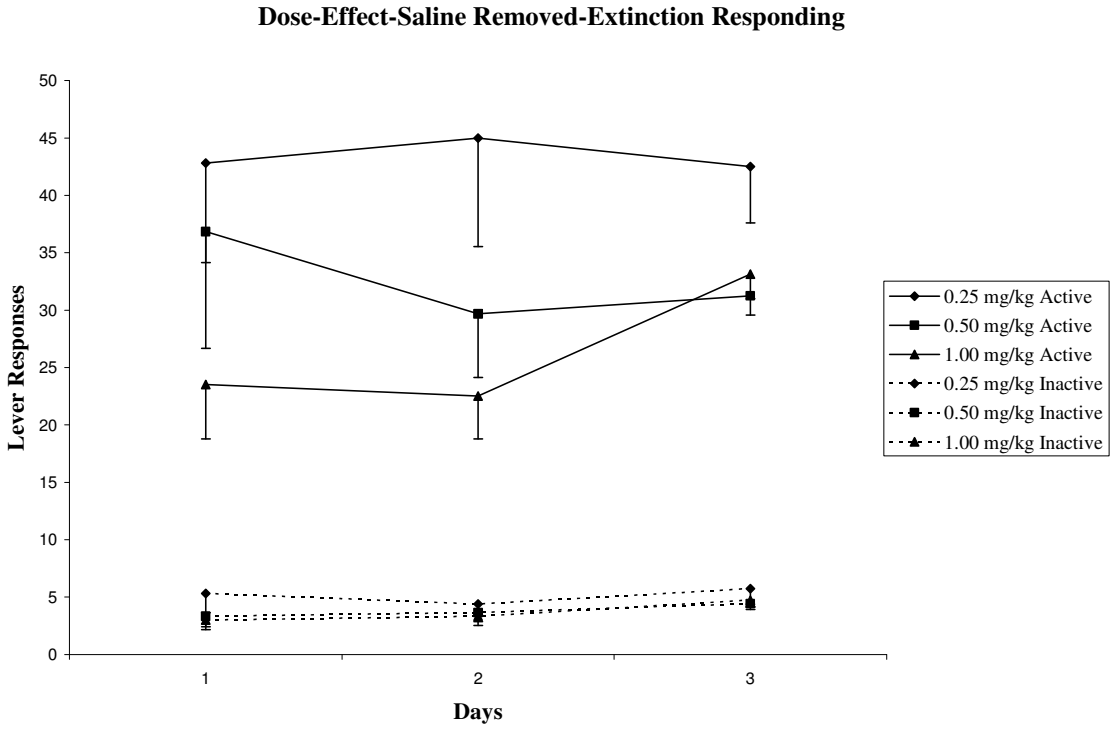

Fig. 10. Experiment 1, mean number of active and inactive lever responses during no saline-available extinction training from days 1-3. Significantly greater resistance to extinction was evident by group 0.25 mg/kg Training Dose compared to group 1.00 mg/kg Training Dose ($p<0.05$).

As with the saline portion of testing, data also were analyzed by separating responding during Day 1 into four 15 minute bins. These bins were used as the repeated measure for each animal. This was done in order to determine if any differences present between groups were averaged out in a standard analysis using the entire hour as a single data point.

A two-way ANOVA (Training Dose [0.25 mg/kg, 0.50 mg/kg, 1.00 mg/kg] x Bin [1, 2, 3, 4]) showed significant main effects by Bin [$F(3,51)=32.30$, $p<0.001$] and by Training Dose [$F(1,17)=4.42$, $p<0.05$]. No significant interactions (Training Dose x Bin) were present [$F(6,51)=1.99$, $p>0.05$].

Post hocs on Training Dose indicated overall significantly greater responding by Group 0.25 compared to Group 1.00 mg/kg Training Dose. Post hoc tests by Bin showed elevated responding during Bin 1 compared to Bins 2, 3 and 4 (all ps<0.05). A visual inspection of Figure 11 shows increased resistance for the lowest dose of cocaine. In particular, these effects appear greatest during Bins 1 and 3. This contrasts sharply with the steady decrease in responding by both of the higher doses of cocaine from Bins 1-4.

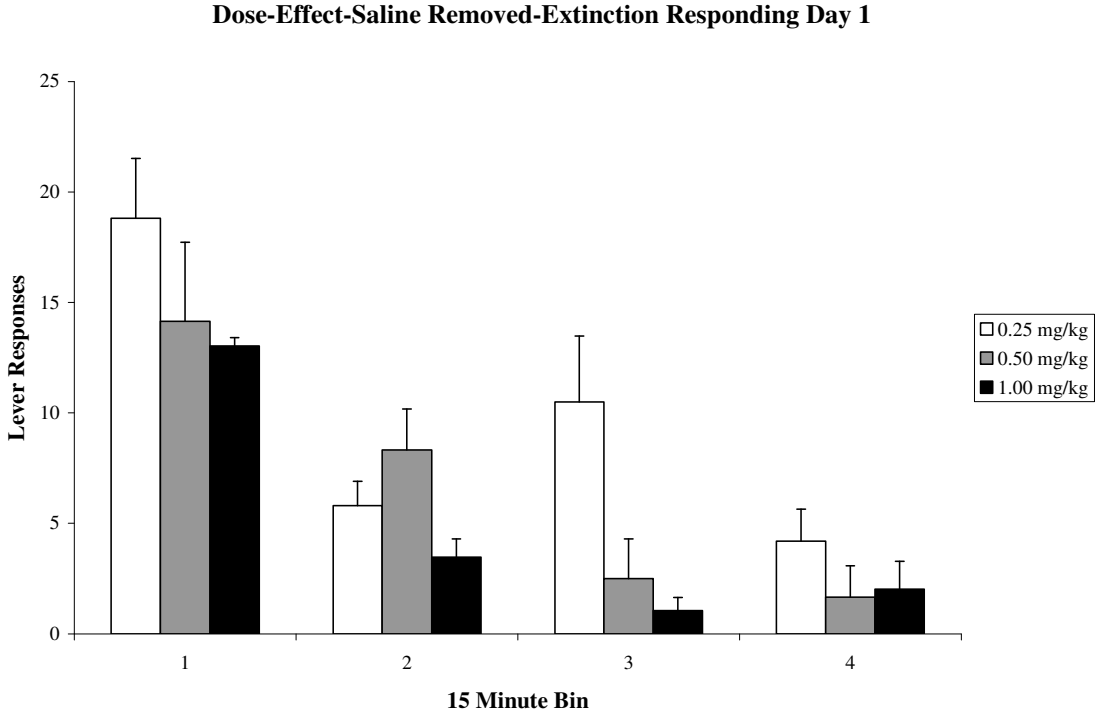

Fig. 11. Experiment 1, mean number of active lever responses during hour 1 of the first day of no saline-available extinction training. Elevated responding was evident during Bin 1. The lowest dose of cocaine showed the greatest resistance to extinction (ps<0.05).

As in the previous portion of this experiment, these data also were converted in order to examine percentage changes in daily responding as compared to last day of saline responding for each animal. Results from the two-way ANOVA (Training Dose [0.25 mg/kg, 0.50 mg/kg, 1.00 mg/kg] x Days [1-3]) showed no significant main effects

by Days [F(2,34)=0.53, p>0.05] but did show effects for Training Dose [F(2 17)=3.92, p<0.05]. No significant interaction effects were present (Training Dose x Days) [F(4,34)=0.48, p>0.05].

Post hocs showed significantly greater responding, in other words greater resistance to extinction, by Group 0.25 compared to Group 0.50 mg/kg Training Dose (p<0.05). Attention should be given to the relatively unchanging amounts of responding by the two highest doses of cocaine despite an absence of reward. Additionally, the presence of an extinction burst is readily apparent in the case of Group 0.25 mg/kg Training Dose, an event most often associated with the removal of a salient behavioral outcome (reward).

Fig. 12. Experiment 1, mean number of percentage changes in baseline responding from last day of saline-available extinction compared to no saline extinction responding from days 1-3. Greatest resistance to extinction was observed by group 0.25 mg/kg Training Dose (ps<0.05).

Discussion

The data from Experiment 1 showed that the issue of drug history effects on extinction training results is a valid concern. Not only were differences evident between groups trained on different doses of cocaine but these effects seemed to persist when saline was removed. Of particular interest, were the lack of substantial decreases and the presence of an extinction burst during the no-saline phase of extinction training.

Terminal Acquisition

The results from terminal acquisition are interesting given the lack of differences under the FR-5 schedule of reinforcement. Differences indicating a replication of the biphasic dose-effect curve (not pictured) were evident under the transitional FR-2 schedule. As mentioned earlier, a cost/benefit interaction was likely responsible for the comparably depreciated amount of responding by groups trained with the lower doses of cocaine. Perhaps greater amounts of training sessions under this schedule might have shown a recovery of responding. In any case, the results were stable according to our criteria and further the argument that the results during extinction were independent of lever response patterns during terminal acquisition.

Extinction

Saline

The results from experiment 1 indicate a dose sensitive outcome in behavioral tests outlining non-reward responding. Regarding the overall trends within the different sections of Experiment 1, it can be said that the lower doses of cocaine appeared to produce the greatest amounts of resistance to extinction. Second, the phenomenon called an "extinction burst" was only observed at the lowest training dose of cocaine. Third, differences in dose-dependent extinction responding are not as pronounced when examined as changes in percent baseline. Finally, the removal of saline in the final testing preparation, did not elicit behavioral decreases in drug seeking, as would be expected of a "typical" CS, indicating a more potent associative reinforcement value than that of CSs, such as the stimulus light.

The results from the initial experiment of this report support the hypothesis that drug training history, specifically training dose, affects subsequent extinction/forced abstinence drug seeking. Of the potential explanations for this set of data, Abram Amsel's general theory of persistence may hold some insight (Amsel, 1967; 1992). The incorporation of Amsel's theory of counterconditioning mediated approach versus avoidance in response to repeated experience with non-reward, may support Experiment 1 if the response schedule is considered to be a, functionally, continuous reinforcement (CRF) schedule, typically defined as an FR-1. As is detailed fully in Experiment 2, the use of a lower-density partial reinforcement (PRF) schedule (FR-10) will produce pharmacologically induced behaviors comparable to the robust learning principles

outlined in frustration theory. What is not certain in this data set, however, is the nature of an FR-5 schedule; i.e., is it a functionally CRF or a PRF? Assuming that the successive depressions of a lever on an FR-5 schedule are more in line with CRF, the data are in line with frustration theory. Specifically, animals trained under a CRF-Small (0.25 mg/kg) are more resistant to extinction than those trained under a CRF-Large (0.50 or 1.00 mg/kg). This occurs due to frustration-mediated avoidance behaviors. Frustration is associated with non-reward and is never counterconditioned to predict reward as is done in a PRF schedule. Given the opposite (FR-5 is akin to a PRF schedule), then they are in opposition. Since the nature of "partial" versus "continuous" could arguably be interpreted as a continuum rather than absolutes, it is possible that Amsel's principles are in alignment with these effects. Experiment 2 builds on these results and provides clear examples of Amsel's learning principles applied to behavioral pharmacology.

From a purely reinforcement focused interpretive angle, the results from extinction training using saline, and without it, do not parallel findings that higher doses of cocaine are more rewarding; i.e. results showing a greater willingness to work (Rowlett, 2000) and a greater overall amount of intake (Lynch and Carroll, 2001), as doses of cocaine are increased. In addition, the lack of differences evident at terminal acquisition (see Figure 5) likely discounts the possibility that extinction resistance was a manifestation of maintenance responding carryover effects. Further examination of extinction responding during the first saline-only trial revealed comparable effects within a session.

Results by 15 minute bins showed a significantly greater resistance to extinction by animals trained using 0.25 mg/kg cocaine. This within session effect was most evident during the initial 15 minutes. Of additional interest, are return to drug seeking trends observed during the last 15 minutes of the session (see Figure 8) hinting at possible, cyclic, patterns in searching for the drug. Finally, of the 3 doses employed only the median dose of 0.50 mg/kg produced steady patterns of decreased responding. Only this group of animals showed the expected response to the removal of a salient reward, that is, a consistently lowering amount of drug seeking throughout the session.

Results from the transformed data provide another method for quantifying the changes in response patterns when animals are faced with non-reward. When examined as changes in percent responding from the first condition (terminal acquisition) to the second (saline) the effects do not appear as robust. In addition it is further obvious that an extinction burst, often mentioned as response to the sudden absence of a salient reward outcome, is not present in any of the doses. This method of visualizing these data is, arguably, equally as relevant as the un-transformed response measure, which would normally be used in a self-administration/extinction study. Variability is increased when comparing across doses in the non-transformed data sets. This is of course expected due to known differences in responding according to the available dose of cocaine. However, comparing the animal's performance to itself may be an index of the relative strength of the independent variable to induce change in each group, or rat. Pertaining to methodological issues that formed the rationale for this experiment, multiple training doses are not often used within a given study. This point is critical because it may pose an obstacle when deriving methodologies from another laboratory. Findings could

potentially not agree, or be comparable, between laboratories because of differences in training doses that were not accounted for.

Saline removed

The final phase of Experiment 1 involved extinction conditions as previously described without the availability of saline. As suspected, the dose-dependent variance seen during saline extinction carried over to the no-saline portion of testing. Furthermore, differences within a session were comparable to that of saline extinction. Finally, an extinction burst was evident at the lowest training dose of cocaine (0.25 mg/kg) suggesting the removal of the "primary" reward.

Resistance to extinction was greatest in the lowest training dose of cocaine, replicating the effects seen during saline extinction. Of particular interest is the lack of a main effect by Days. As is readily apparent in Figure 10, there is essentially no change in responding from days 1-3, particularly in group 0.25 mg/kg. Surely, the infusion of saline is acting as a potent CS under extinction conditions. However, the removal of saline prompted, what can be interpreted, as seeking patterns rather than a sharp decline in responding. Data from Schenk and Partridge (2001) show evidence of dose-related differences related to the invigorating or sensitizing effects of a conditioned light stimulus on maintenance responding. These effects were more pronounced at lower doses and may aid in explaining the high resistance to extinction engendered by the lowest training doses in the absence of the CS. To further this argument, an extinction

burst was evident at the lowest dose of cocaine, again, a phenomenon associated with the removal of a salient (often the primary) reward outcome.

Bin analysis again revealed the greatest amounts of differences during the initial 15 minute period. Again there appeared to be a time-related return to seeking during the latter part of the hour (group 0.25 mg/kg Training Dose during Bin 3) of the first testing session. These subtle differences may further indicate cyclical patterns of active drug seeking that has become habitual.

The results from the transformed data partially reflect the results from the standard analysis. Group 0.25 mg/kg showed elevated resistance to extinction compared to group 0.50 mg/kg Training Dose. Furthermore, the extinction burst by the low dose of cocaine was evident. Additionally, the 2 higher doses of cocaine showed essentially no change compared to baseline measures of responding on the last day of saline responding.

In conclusion, the data from Experiment 1 are in line with the hypothesis that different training doses during acquisition and maintenance will engender varying levels of resistance to extinction with saline and no-saline as the reward outcomes. In addition, the associative reinforcement value of saline appears to be greater than often suspected. Experimenters should account for differences in training dose and extinction conditions and be aware of the possible effects on subsequent testing. Finally, the results from Experiment 1 served as the rationale for Experiment 2 which examined the interaction of reinforcement schedules and reward magnitude as possible modulators of extinction and reinstatement responding.

EXPERIMENT 2: SCHEDULES BY MAGNITUDE

Rationale

Based on the results of Experiment 1, an examination of dose/reinforcement schedule interactions was developed. The tenets of Amsel's frustration theory were elaborated on within the second experiment, specifically the schedules by magnitude interaction effect (Amsel, 1992; Hulse, 1958). The general theory of persistence, specifically frustration theory, makes clear predictions regarding this particular interaction. Approach/avoidance behaviors during sudden non-reward (i.e. extinction conditions in an operant setting) may be consequent to changes based on prior training histories. Previous experience with frustration during training will come to elicit approach rather than avoidance behaviors during extinction training. This occurs when the animal is trained on a PRF rather than a CRF reinforcement schedule. Every behavioral response that is not rewarded elicits frustration in the animal, until the animal reaches the rewarded response and is presented with the reinforcer. After repeated trials, the subject will come to associate the frustration of non-rewarded trials as a predictor of subsequent reward. This is later manifested during extinction as increased persistence. The frustration of non-reward becomes an approach cue rather a signal for cessation (avoidance). Animals trained under a CRF schedule will not have experienced non-reward induced frustration during acquisition. Consequently, the initial removal of the primary reward will result in faster rates of cessation, having experienced no counterconditioning, in terms of the learned instrumental response. Furthermore, the reward magnitude within each reinforcement schedule will profoundly affect the overall resistance to extinction.

Reward outcomes will incur commensurate levels of frustration when they are removed. In other words, the greater the reward value, the greater the frustration. Accordingly, the large magnitude reward will induce more frustration mediated approach in the PRF condition. Within the CRF condition, however, greater frustration induced by the absence of the large magnitude reward will elicit greater levels of avoidance.

Classical food reward literature has proven these effects to be one of the more robust learning principles (Amsel, 1992; Hulse, 1958). It is believed that these behavioral phenomena may apply to a pharmacological setting. To date, no one has attempted to extend the basic tenets of Amsel's theories to drug self-administration. Therefore, the purpose of Experiment 2 was to examine the possible reward magnitude/reinforcement schedule interactions that may exist, according to the explicit predictions of frustration theory. Specifically, two doses of cocaine (either 0.25 or 1.00 mg/kg) were used to train animals on either a CRF or PRF schedule. Extinction testing in this experiment did not employ either saline infusions or presentations of the stimulus light in order to minimize any cue-activated approaches to the active lever. Subsequently, all animals were tested in a cue-maintained reinstatement procedure.

Materials and Methods

Animals

The research design and conduct of the experiment were approved by the Texas A&M University Laboratory Animal Care Committee, and all aspects of the research followed the guidelines outlined in Principles of Laboratory Animal Care (NIH publication No.85-

23). Thirty male Sprague-Dawley (Harlan; Houston, Texas, USA) rats weighing between 300-325 g were used for Experiment 2. Rats were individually housed in hanging polycarbonate cages for the entire study. All animals were kept on a 12H/12H light/dark light cycle with lights on at 8:00 AM and were given tap water and standard rat chow *ad libitum*. Training and testing were conducted only during the light portion of the cycle between the hours of 9:00 AM-7:00 PM, and body weights were monitored daily.

Apparatus

The operant apparatus used for Experiment 2 was the same as in Experiment 1.

Surgeries

Surgeries were performed as described in Experiment 1.

Drugs

The Research Technology Branch of the National Institute of Drug Abuse generously supplied the cocaine HCl.

Testing

Animals were allowed to recover for 1 week after surgeries. The following day, animals were transported to the testing chambers from the home cage using individual plastic boxes. Before being placed into the testing chambers, each animal was flushed with sterile saline to ensure catheter patency. Rats were then placed in pre-determined testing chambers and connected via the exposed end of the blunted 22 ga needle. This was attached to a length of 0.02 ID Tygon tubing housed within a spring leash attached to the chamber. This was directed to another length of 0.02 ID tubing via a single channel fluid swivel. This connection then formed a closed system that would receive infusions directly from the syringe on the mechanical pump.

Initial training for all subjects began using an FR-1 schedule of reinforcement. Daily 2-hour sessions were began following an experimenter delivered prime. Animals were allowed unlimited access to cocaine throughout this period. After stable responding was observed for two consecutive days (< 20% variability) animals were moved to either an FR-5 or remained on an FR-1 (CRF) schedule of reinforcement. Once responding under an FR-5 was again stable, PRF animals were moved to an FR-10 and stable responding was reestablished. Each group was progressively trained and maintained on their respective dose (expressed as the salt) and schedule: 0.25 mg/kg/infusion-FR-1 [Group Continuous Reinforcement-Low Dose; CRF-L] (N=8), Group 0.25 mg/kg-FR-10 [Group Partial Reinforcement-Low Dose; PRF-L] (N=8), Group 1.00 mg/kg-FR-1 [Group Continuous Reinforcement-High Dose; CRF-H] (N=8) and Group 1.00 mg/kg-FR-10 [Group Partial Reinforcement-High Dose; PRF-H] (N=6). Animals under an FR-1

were allowed to self-administer for an average of 15 days in order to have a comparable number of sessions as the PRF groups.

After stable responding had been established, animals subsequently underwent extinction sessions consisting of consecutive 1 hour sessions over a period of 10 days. These sessions resulted in no saline or stimulus light presentations following an active lever press. Animals were allowed to press the lever freely with no overt consequences. Extinction was defined as < 20% responding, for two consecutive days, as compared to responding on the first day of extinction. Animals were only allowed 10 total days of extinction responding. Upon reaching 10 days of extinction animals were tested in a single reinstatement session.

Reinstatement testing consisted of 1 hour of extinction responding under the same conditions as described above. Subsequently, during hours 2-3 animals were permitted to respond freely under extinction conditions with the stimulus light being presented for a period of 12 seconds after every active lever response (FR-1). Animals were then allowed to respond freely under the extinction conditions described above. The following day, animals were tested again for reinstatement behaviors (return to drug-seeking manifested as renewed active lever responding). Upon completion of testing, all animals were tested for catheter patency using sodium pentobarbital (7.5 mg/kg) given IV as a test for the onset of brief anesthesia.

Data Analysis

Terminal acquisition (the final day of acquisition training) and reinstatement data were analyzed using three-way ANOVA tests (Training Schedule x Training Dose x Levers). Extinction data were analyzed using four-way repeated measures ANOVAs (Training Schedule x Training Dose x Levers x Days). Tukey's post hocs were used when appropriate. In all cases statistical significance levels were set at $p<0.05$.

Results

Terminal Acquisition

Analysis of baseline responding on the last day (terminal acquisition) using a repeated measures three-way (Training Schedule [FR-1, FR-10] x Training Dose [0.25 mg/kg, 1.00 mg/kg] x Levers [Active, Inactive]) ANOVA showed significant effects by Levers [$F(1,26)=191.61$, $p<0.001$]. The near zero levels of left lever responding prompted the exclusion of Inactive Lever from any further analyses.

Subsequent analysis of Active Lever baseline responding using a two-way (Training Schedule [FR-1, FR-10] x Training Dose [0.25 mg/kg, 1.00 mg/kg]) ANOVA showed significant main effects for Training Schedule [$F(1,26)=84.99$, $p<0.001$]. Significant differences by Training Doses [$F(1,26)=1.01$, $p>0.05$] were not present. Significant interactions were present (Training Schedule x Training Dose) [$F(1,26)=11.04$, $p<0.01$].

Tukey's post hocs showed significantly elevated responding in PRF-L versus CRF-L animals ($p<0.05$). Significantly elevated responding was evident in PRF-H versus CRF-H animals ($p<0.05$), as predicted. Finally, PRF-H animals were pressing significantly more at terminal acquisition than PRF-L animals ($p<0.05$) but the reverse was true in the CRF groups ($p<0.05$) where the low dose animals responded in greater amounts than the high dose. The pattern observed in the PRF conditions is not in line with that of the biphasic dose-effect curve produced by cocaine (see Lynch and Carroll, 2001). This is intriguing because it suggests a schedule-mediated sensitivity affecting behavioral responding, and thereby cumulative drug intake on the descending limb of the dose-effect curve.

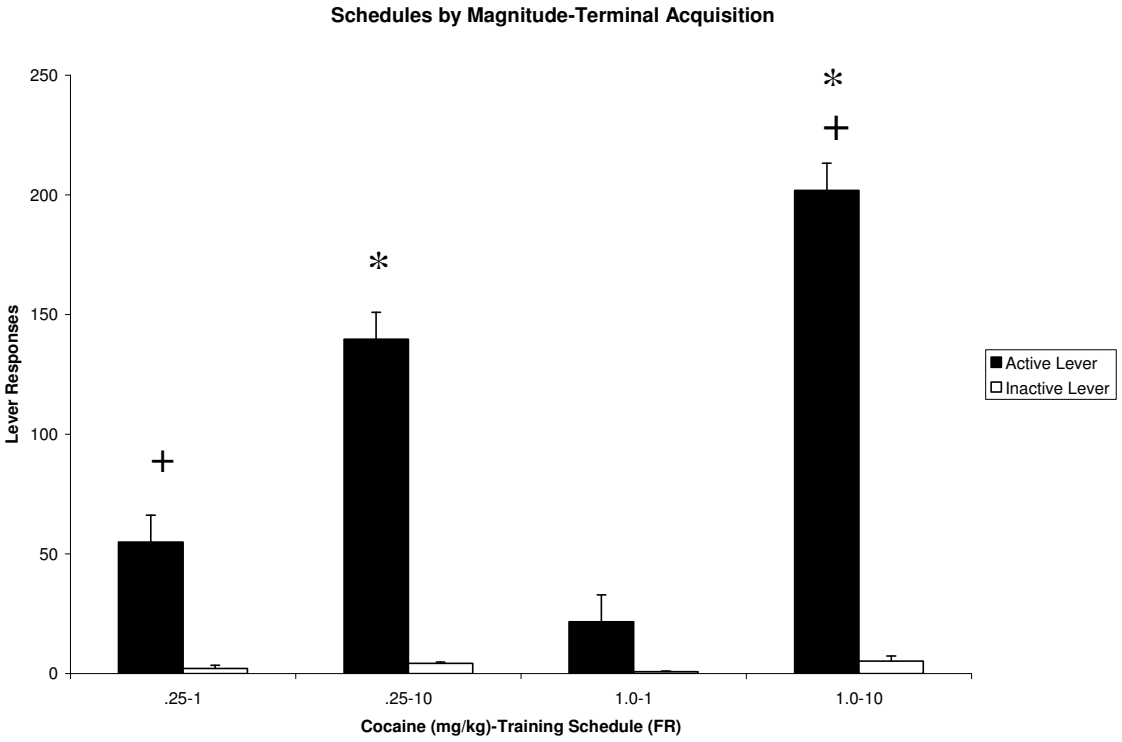

Fig. 13. Experiment 2, mean number of active and inactive lever responses during terminal acquisition. Significantly greater responding by animals trained using PRF versus CRF schedules are denoted with an *. Significantly greater responding according to training dose, within either PRF or CRF trained groups, is denoted with a +.

Extinction

Extinction responding was first analyzed to determine if Active and Inactive Lever responding was significantly different. Four-way repeated measures (Training Schedule [FR-1, FR-10] x Training Dose [0.25 mg/kg, 1.00 mg/kg] x Levers [Active, Inactive] x Days [1-10]) ANOVA tests revealed significant effects by Levers [$F(1,234)=234.03$, $p<0.001$]. A visual examination of Figure 14 shows greater responding on the left lever than is typical during drug availability, therefore it was believed to be pertinent to conduct a separate analysis of Inactive Lever responding.

Inactive lever responses may indicate drug seeking after the discontinuation of a formerly present drug reward. Animals may be actively searching for the drug and thereby generalizing from the Active to the Inactive Lever after the reward has been removed. A three-way (Training Schedule [FR-1, FR-10] x Training Dose [0.25 mg/kg, 1.00 mg/kg] x Days [1-10]) ANOVA showed main effects by Days [$F(9,234)=12.21$, $p<0.001$] but no significant effects by Training Dose [$F(1,26)=4.16$, $p>0.05$], Training Schedule [$F(1,26)=1.23$, $p>0.05$] or a significant interaction effect (Training Schedule x Training Dose x Days) [$F(9,234)=0.67$, $p>0.05$]. The sole main effect was due to the steady decrease in responding from Day 1-10. However, it is critical to note the more pronounced, although statistically insignificant, responding on the left lever compared to Experiment 1 where the stimulus light was available as a reward outcome. As will be detailed, this is a trend throughout the remaining experiments that may indicate a greater amount of drug seeking as a result to the presence of less drug-associated stimuli.

Three-way (Training Schedule [FR-1, FR-10] x Training Dose [0.25 mg/kg, 1.00 mg/kg] x Days [1-10]) ANOVA tests on Active Lever responding revealed significant effects by Days [$F(9,234)=46.25$, $p<0.001$]. Significant main effects also were present by Training Dose [$F(1,26)=5.23$, $p<0.05$] and by Training Schedule [$F(1,26)=14.81$, $p=0.001$]. No interaction effects were present (Training Dose x Training Schedule x Days) [$F(9,234)=1.14$, $p>0.05$].

Post hocs showed significant differences between CRF-L and PRF-L and also CRF-H and PRF-H (all $ps<0.05$). Specifically, both PRF groups were more resistant to extinction than the CRF groups trained on the same dose. In addition, Group PRF-H showed more resistance to extinction than Group CRF-L ($p<0.05$).

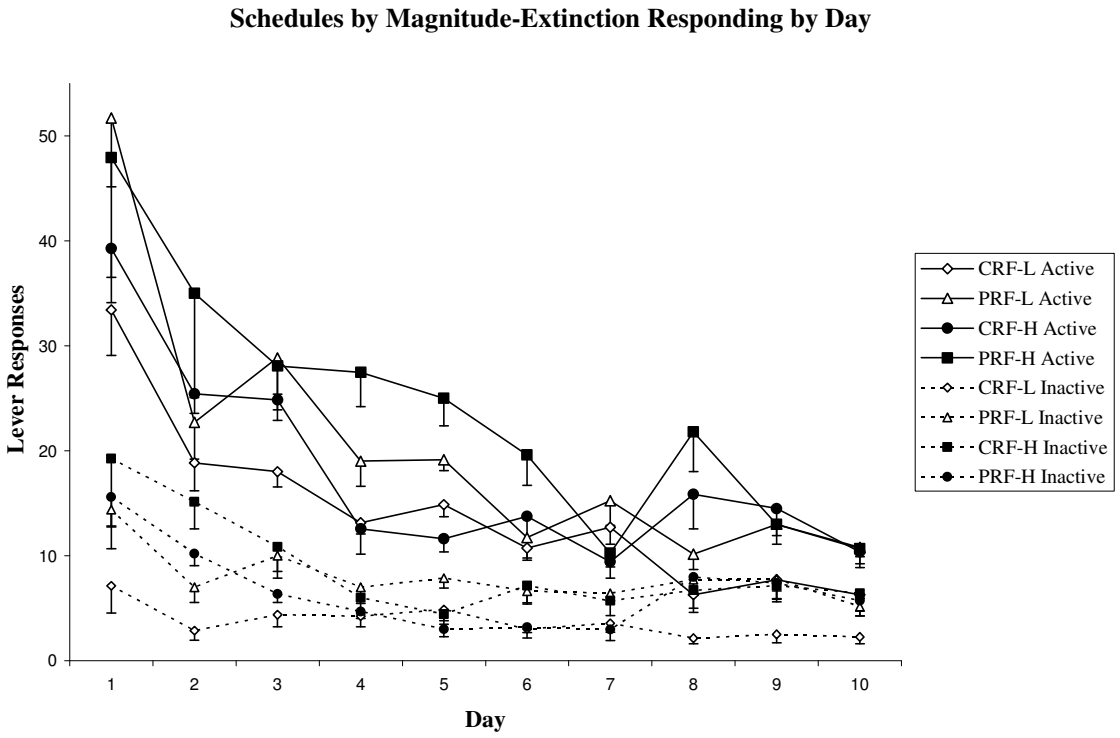

Fig. 14. Experiment 2, mean number of active and inactive lever responses during extinction training on days 1-10. Both PRF trained groups showed increased resistance to extinction. ($ps<0.05$).

In addition to a standard ANOVA analysis on lever presses, the extinction data were transformed in order to reflect changes in responding compared to terminal acquisition. This is particularly relevant to Experiment 2 due to the differences in baseline response schedules. Three-way (Training Schedule [FR-1, FR-10] x Training Dose [0.25 mg/kg, 1.00 mg/kg] x Days [1-10]) ANOVA results on Active Lever responding during extinction showed significant effects by Days [$F(9,234)=27.26$, $p<0.001$]. In addition, significant effects were present by Training Schedule [$F(1,26)=119.34$, $p<0.001$], Training Dose [$F(1,26)= 46.42$, $p<0.001$] and Training Schedule x Training Dose x Days [$F(9,234)=7.03$, $p<0.001$].

Post hocs indicated no differences between group PRF-H and PRF-L ($p>0.05$). Both showed the lowest amount of resistance to extinction followed by CRF-L then CRF-H (all $ps<0.05$). Daily mean comparisons using Tukey's post hocs resulted in Group 0.25 mg/kg Training Dose showing significantly greater percentage changes in responding by animals trained using CRF schedules during extinction on Days 2, 4, 5, 6 and 7 (all $ps<0.05$). Post hocs on animals trained on 1.00 mg/kg cocaine showed significantly greater percentage changes in responding by animals trained using CRF schedules during extinction on Days 1-10 (all $ps<0.05$). Post hocs on animals trained on CRF schedules showed significantly greater percentage changes by 1.00 mg/kg trained animals on Days 1-10 (all $ps<0.05$). Finally, post hocs on animals trained on PRF schedules showed significant differences only on Day 10 ($p<0.05$).

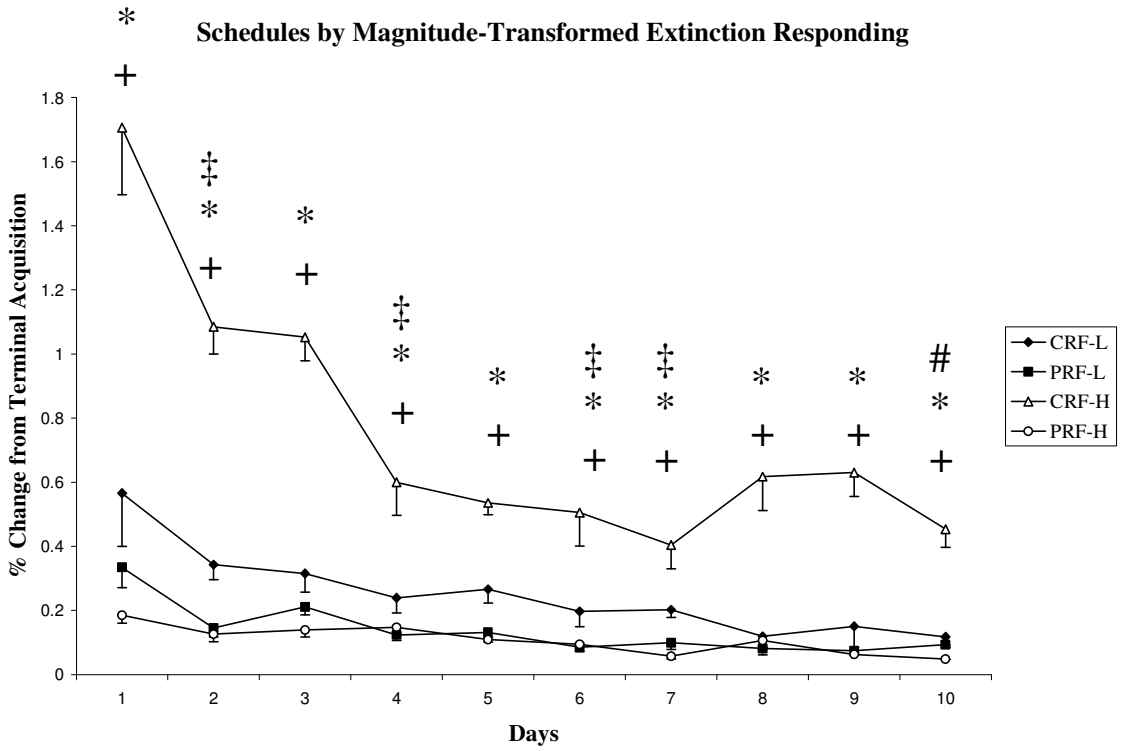

Fig. 15. Experiment 2, mean number of percent changes in baseline responding from terminal acquisition compared to extinction training days 1-10. Significantly lower responding by PRF animals within group 0.25 mg/kg Training Dose are denoted using a ‡. Significantly lower responding by PRF animals within group 1.00 mg/kg Training Dose are denoted using a *. Significantly lower responding by group 0.25 mg/kg Training Dose animals within the CRF training group are denoted using a +. Significantly lower responding by group 1.00 mg/kg Training Dose within the PRF training group is denoted by a #.

Reinstatement

Three-way repeated measures (Training Schedule [FR-1, FR-10] x Training Dose [0.25 mg/kg, 1.00 mg/kg] x Levers [Active, Inactive]) ANOVA results showed significant effects by Levers [$F(1, 26)=188.87$, $p<0.001$]. As is readily seen in Figure 16, the majority of responding occured on the right lever. In addition, the near zero levels of left lever responses prompted the exclusion of Inactive Lever responding from any further analyses.

A two-way (Training Schedule [FR-1, FR-10] x Training Dose [0.25 mg/kg, 1.00 mg/kg]) ANOVA on reinstatement responding showed no significant effects by Training Dose [$F(1,26)=2.236$, $p>0.05$]. Significant effects by Training Schedule were present [$F(1,26)=11.274$, $p<0.01$]. No interaction (Training Schedule x Training Dose) effects were present [$F(1,26)=0.173$, $p>0.05$].

Examination of Figure 16 clearly shows an increased likelihood of returning to active drug seeking by animals formerly under the PRF schedule ($p<0.05$) compared to those trained under CRF conditions. This parallels the principles of Amsel's frustration theory and further supports the data presented during extinction training where PRF training was more likely to incur persistence during non-reward and further, extends the effects to include cue-induced drug seeking.

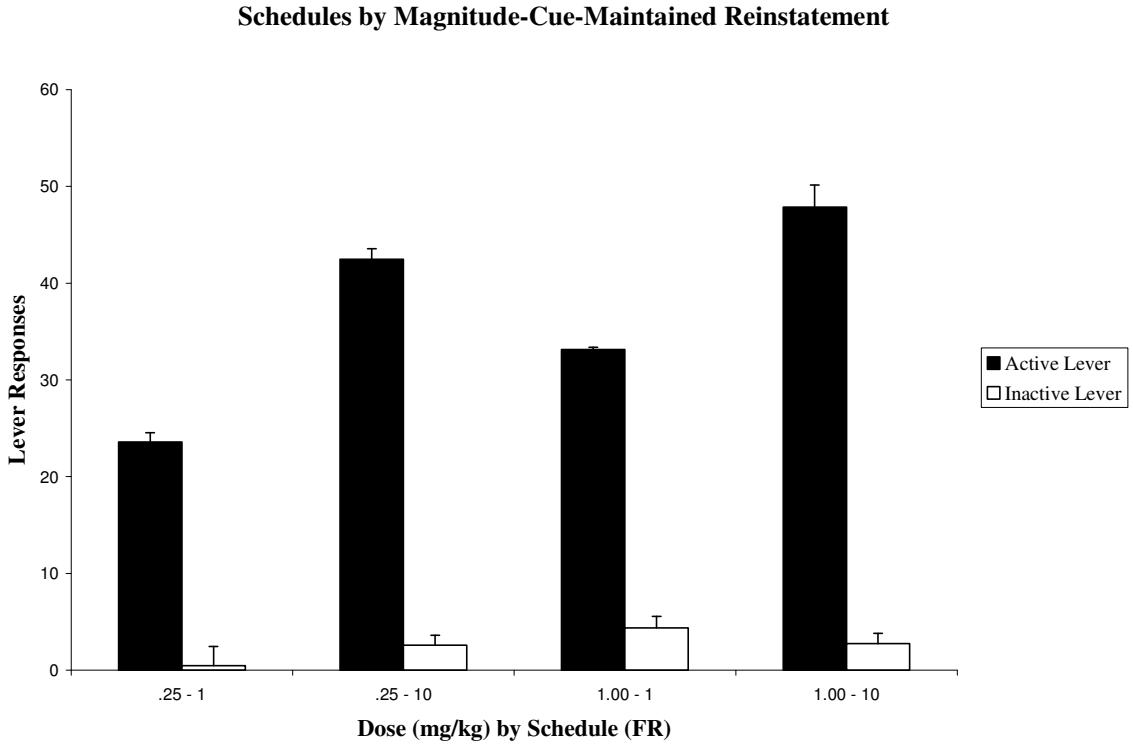

Fig. 16. Experiment 2, mean number of active and inactive lever responses during reinstatement. Significantly greater responding was observed by animals trained using a PRF schedule ($p<0.05$).

Discussion

The data from Experiment 2 generally support the applicability of Amsel's theory of relative persistence to a behavioral pharmacological setting. The effects of frustration-induced approach behaviors were evident in PRF trained animals. Furthermore, reinstatement data support the notion that drug histories affect subsequent drug-seeking patterns and that these interactions are relatively uniform over time.

Terminal Acquisition

Terminal acquisition under the CRF schedule showed normal distributions of active lever responses between groups. Animals self-administering the low dose of cocaine pressed more than those using the high dose. These levels of responding mimic response patterns on a standard dose-effect curve. Lynch and Carroll (2001) argue that optimal levels of internalized drug are regulated by overall amounts of lever responding. In other words, higher concentrations of the drug require fewer infusions to maintain this state as seen in a biphasic dose-effect curve. What is of interest during terminal acquisition, are the non-parallel increases in responding seen in the PRF condition. Animals trained with the high dose responded more than animals using the low dose. This may suggest a schedule sensitivity that modulates overall drug intake (infusion) amounts. Behavioral economic theories may suggest cost/reward relationship in the group training on 0.25 mg/kg cocaine that is responsible for the, comparably, lower amount of responding in the PRF condition (Hursh et al., 1988). Perhaps the low dose (0.25 mg/kg cocaine) did not

possess an incentive motivational value (reward magnitude) as high as that of 1.00 mg/kg. This would certainly agree with progressive ratio data which indicate an increased willingness to work (lever press) as the cocaine concentration is increased (Rowlett et al., 1996).

Extinction

Frustration theory clearly has some applicability to the pharmacological manipulations within Experiment 2. Most pertinent to the rationale behind this study, animals under PRF conditions showed increased resistance to extinction compared to those only exposed to CRF throughout training. This supports the basic tenets of frustration theory and furthermore, is critical in outlining the sensitive nature of extinction responding after modulation of training history. Animals undergoing PRF training experience counterconditioning to the usual avoidance-mediating effects of frustration. As followed, PRF groups showed greater amounts of drug seeking during extinction due to approach behaviors signaled by frustration. This was not evident in CRF groups as animals were more likely to cease drug-seeking behaviors in response to non-reward induced frustration. In addition, there seemed to be a dose-sensitive mechanism interacting with the effects of reinforcement schedule. Overall, Group PRF-H animals showed the most resistance to extinction followed by Group PRF-L. Groups CRF-L and CRF-H showed the least, further supporting frustration theory.

While not significant, it is important to note the differences in inactive lever responding in Experiment 2 compared to Experiment 1. As will become evident

throughout Experiments 2-4, there is an elevated amount of inactive lever responding indicative of increased drug seeking. The discrepancies from Experiment 1 to Experiments 3-4 may be due to the lack of CSs in the latter. Experiment 1 employed both a stimulus light and saline infusions, in place of cocaine, throughout extinction training. Drug-paired cues are known to increase lever responding during self-administration (Schenk and Partridge, 2001). The associative value of the cues in Experiment1 might have been sufficient to contain seeking behaviors to the right lever. Therefore, the lack of salient, drug-associated cues in Experiment 2 may have been responsible for the increased searching on the inactive lever after the removal of the primary reward.

Data transformed to reflect percent changes showed an interesting, albeit opposing, distribution of responding among groups. Percentage changes in responding were greatest in the CRF-H condition. This group also produced a very pronounced extinction burst. The remaining conditions showed decreases in responding compared to terminal acquisition. However, among the low cocaine dose condition, CRF trained animals still showed greater resistance to extinction. No differences were present between the PRF conditions. This is again very relevant within the scope of interpreting extinction data. Percentage changes are more applicable to the performance of an individual group and may prove to be a more accurate measure of the effectiveness of potential cessation therapies. However, the aim is not to undermine the importance of un-transformed data which does provide accurate measures of between group differences and generalization potential to other tests of drug addiction (acquisition, maintenance and reinstatement).

The application of learning principles to self-administration studies lends to the growing list of vectors that influence behavioral responding. With respect to animal studies, controlling for, and systematically examining, prior drug histories is essential in developing accurate applicable results for drug addiction. In terms of human therapies, extinction investigations are an excellent candidate for developing cessation therapies and merit closer scrutiny.

Reinstatement

Animals trained under PRF schedules showed a greater likelihood of returning to drug seeking than CRF-trained animals in both cocaine groups. These data lend further evidence for the presence of frustration theory principles within drug self-administration. Non-significant trends were also evident within CRF and PRF conditions. Animals trained on 1.00 mg/kg cocaine showed greater amounts of responding in both cases indicating a greater associative reward value assigned to the CS (stimulus light). Moreover, these data suggest that the resultant effects have some temporal stability and are responsive to associative cues even after a relative dissociation between the instrumental response and the former presence of a reward outcome has been achieved.

EXPERIMENT 3: OVERTRAINING EXTINCTION EFFECT

Rationale

For the third experiment, a design was created in order to investigate the overtraining extinction effect (OEE); another well known behavioral phenomenon of the theory of general persistence (Amsel 1967; 1992). The OEE addresses the effects of extended acquisition training periods during instrumental training on subsequent responding under conditions of recurrent non-reward (extinction). Once again, no incorporation of this learning principle into behavioral drug research has been attempted. Any elucidation of behavioral mechanisms that may explain drug intake, particularly an inability to cease, would prove invaluable in designing efficient models of drug addiction. Classical reward literature has shown that training for longer periods of time will increase the strength of the instrumental response and any associative cues. In the case of a CRF schedule, extended training will elicit a greater emotional response, after brief training, when the reward is removed during extinction sessions. Consequently, since no counterconditioning occurs using CRF schedules, animals will cease performing the instrumental response faster than those trained for shorter periods of time.

Regarding drug self-administration, the prediction, at least under conditions of CRF training, is that animals trained to self administer for longer periods of time in a standard operant procedure, will show less resistance to extinction when tested under extinction conditions. The emotional frustration induced by non-reward, also, will vary depending on the training dose used. Rats trained using a low dose will experience frustration parallel to the reward value of the drug. In other words, typically, the larger

the concentration of cocaine, the greater the frustration when it is not available. Therefore, animals trained with a low dose will show less persistence during extinction than those trained using the high dose. This should be manifest similarly within both training length groups.

Therefore, Experiment 3 trained animals for either 15 or 30 days, using CRF conditions, in order to examine OEE effects. Further, both a low and high dose of cocaine were used within each training condition in order to measure dose-related changes in instrumental responding. Following extinction training, animals were tested in a single cue-maintained reinstatement preparation.

Materials and Methods

Animals

Thirty adult male Sprague-Dawley (Harlan; Houston, Texas, USA) rats weighing between 300-325 g were used for Experiment 3. Rats were individually housed, and all other animal maintenance of Experiment 3 was as described for Experiment 1.

Apparatus

The operant apparatus used for Experiment 3 was the same as in Experiment 1.

Surgeries

Surgeries were performed as described in Experiment 1.

Drugs

The Research Technology Branch of the National Institute of Drug Abuse generously supplied the cocaine HCl.

Testing

Animals were allowed to recover for 1 week after surgeries. The following day, animals were transported to the testing chambers from the home cage using individual plastic boxes. Before being placed into the testing chambers, each animal was flushed with sterile saline to ensure catheter patency. Rats were then placed in pre-determined testing chambers and connected via the exposed end of the blunted 22 ga needle. This was attached to a length of 0.02 ID Tygon tubing housed within a spring leash attached to the chamber. This was directed to another length of 0.02 ID tubing via a single channel fluid swivel. This connection then formed a closed system that would receive infusions directly from the syringe on the mechanical pump.

After recovery from surgery, all rats were trained using an FR-1 (CRF) schedule of reinforcement in order to acquire the instrumental active lever press response resulting in drug administration. The onset of the daily sessions was signaled by an experimenter

delivered prime. Animals were then allowed to self-administer freely for 2-hours. Upon reaching stable responding (< 20% variability across two consecutive days) subjects were trained in their respective groups, either for a total of 15 days at 0.25 mg/kg (N=8) [Group Abbreviated Training-Low Dose; Abb-L] or 0.50 mg/kg (N=7) [Group Abbreviated Training-High Dose; Abb-H] or for 30 days at 0.25 mg/kg (N=8) [Group Extended Training-Low Dose; Ext-L], or 0.50 mg/kg (N=7) [Group Extended Training-High Dose; Ext-H] cocaine. Once animals completed training, each was tested the following day under extinction conditions as described in Experiment 1, i.e., no saline or stimulus light presentations following active lever responding. Procedures also were the same as in Experiment 2 for reinstatement testing as well. Upon completion of testing, all animals were tested for catheter patency using sodium pentobarbital (7.50 mg/kg) given IV as a test for the onset of brief anesthesia.

Data Analysis

Terminal acquisition (last day of acquisition training) and reinstatement data were analyzed using three-way ANOVA tests (Training Length x Training Dose x Levers). Extinction data were analyzed using four-way repeated measures ANOVA (Training Length x Training Dosex Levers x Days). Tukey's post hocs were used when appropriate. In all cases statistical significance levels were set at $p<0.05$.

Results

Terminal Acquisition

Analysis of baseline responding on the last day (terminal acquisition) using a repeated measures three-way (Training Length [15-Days, 30-Days] x Training Dose [0.25 mg/kg, 1.00 mg/kg] x Levers [Active, Inactive]) ANOVA showed significant effects by Levers [$F(1,26)=163.05$, $p<0.001$]. The relatively low amount of responding on the left lever permitted the exclusion of Inactive Lever responding from any further analyses.

Analysis of Active Lever responding during terminal acquisition using a two-way (Training Length [15-Days, 30-Days] x Training Dose [0.25 mg/kg, 1.00 mg/kg]) ANOVA showed significant differences by Training Length [$F(1,26)=10.49$, $p<0.01$] and Training Dose [$F(1,26)=7.56$, $p<0.05$]. Significant interactions however, were not present [$F(1,26)=0.22$), $p>0.05$].

Post hoc comparisons revealed that animals undergoing extended training are likely to respond at a higher level than those undergoing brief training ($p<0.05$). This phenomenon is known as tolerance and is a common consequence of chronic psychostimulant use (Schenk and Partridge, 1997). In addition, there was more Active Lever responding when animals were trained with the lower dose of 0.25 mg/kg cocaine compared to the high dose of 0.50 mg/kg ($p<0.05$). This effectively replicates the typical amount of allocated responses to increasing doses of cocaine seen in a typical biphasic dose-response curve.

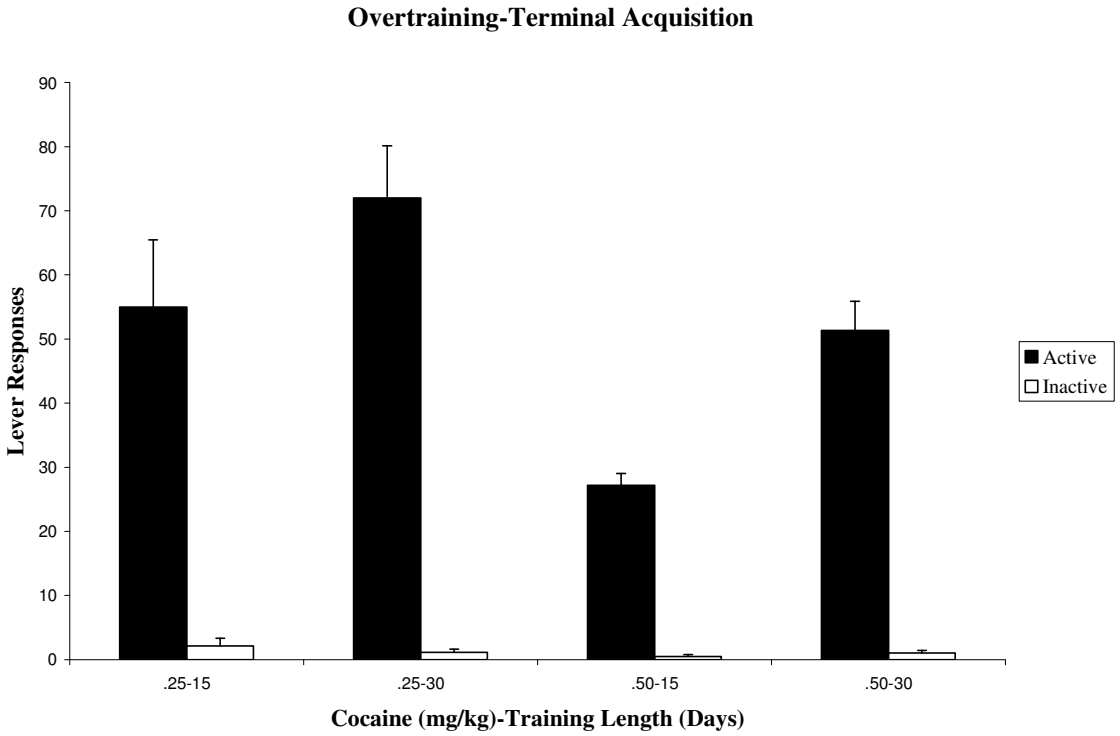

Fig. 17. Experiment 3, mean number of active and inactive lever responses for terminal acquisition. Animals trained for longer periods of time showed the effects of tolerance highlighted by an increased amount of lever responses. Animals on the low showed greater responding than those on the high dose (ps<0.05).

Extinction

Extinction responding was first analyzed to determine if Active and Inactive Lever responding was significantly different. Four-way repeated measures ANOVA (Training Length [15-Days, 30-Days] x Training Dose [0.25 mg/kg, 0.50 mg/kg] x Levers [Active, Inactive] x Days [1-10]) showed significant differences between Levers [$F(1,26)=152.53$, $p<0.001$]. Although it is visibly apparent that Inactive Lever responding was significantly lower. The near zero levels typically produce during standard self-

administration were not present. Due to its potential as a measure of active drug-seeking, it was deemed appropriate to conduct a separate analysis of left lever responses to uncover any potential effects.

Inactive Lever responding was analyzed using a three-way (Training Length [15-Days, 30-Days] x Training Dose [0.25 mg/kg, 0.50 mg/kg] x Days [1-10]) repeated measures ANOVA. Results revealed significant effects by Days [$F(9,234)=14.31$, $p<0.001$] but none for Training Dose [$F(1,26)=1.58$, $p>0.05$] or Training Length [$F(1,26)=1.41$, $p>0.05$]. Significant interactions by Training Length x Training Dose x Days [$F(9,234)=3.43$, $p=0.001$] were present.

Subsequent post hocs on Inactive Lever responding showed significantly greater responding by Group Ext-L compared to Group Abb-L on Days 2, 4, 6, 8 and 9 (all $ps<0.05$). Significantly greater responding was evident by Group Abb-H compared to Group Ext-H on Days 4, 9 and 10 (all $ps<0.05$). Greater responding by the Abb condition is in line with our predictions. Elevated responding by Group Ext-L parallels behaviors on the active lever and therefore, is of interest. Post hocs further showed significantly greater responding by Group Abb-H compared to Group Abb-L on Days 2, 4, 8, 9 and 10 (all $ps<0.05$). Significantly greater responding by Group Ext-L compared to Group Ext-H on Day 9 ($p<0.05$) also was evident. While the results are mixed, it is important to note that increased drug seeking was occurring at numerous time points and, moreover, showed dose and training length related differences.

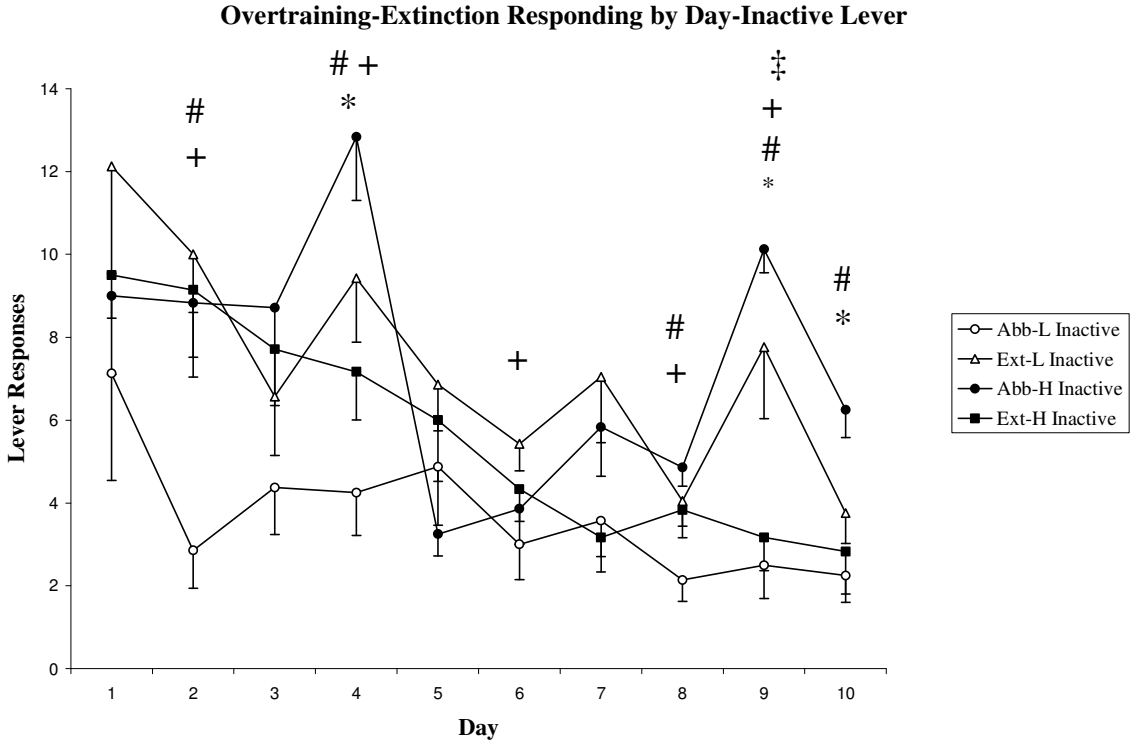

Fig. 18. Experiment 3, mean number of inactive lever responses during extinction training days 1-10. Significantly greater responding by group Ext-L compared to group Abb-L is denoted with a +. Greater responding by group Abb-H compared to group Ext-H is denoted using an *. Greater responding by group Abb-H compared to group Abb-L is denoted with a #. Greater responding by group Ext-L compared to group Ext-H is denoted with a ‡

Extinction responding was then analyzed using only Active Lever responding. A three-way (Training Length [15-Days, 30-Days] x Training Dose [0.25 mg/kg, 0.50 mg/kg] x Days [1-10]) repeated measures ANOVA revealed significant main effects by Days [$F(9,234)=42.43$, $p<0.001$]. No significant main effects were present for Training Dose [$F(1,26)=0.50$, $p>0.05$] or Training Length [$F(1,26)=0.09$, $p>0.05$]. However, significant interactions were present for Training Length x Training Dose x Days [$F(9,234)=5.32$, $p<0.001$].

Post hoc comparisons of Active Lever responses revealed significantly greater responding by Group Ext-L versus Group Abb-L on days 8, 9 and 10 (all ps<0.05), paralleling the behaviors observed on the Inactive Lever. Group Ext-H showed significantly less responding than Group Abb-H on days 1 and 8 but showed greater responding on days 3 and 4 (all ps<0.05). Significantly greater responding was observed in Group Abb-L versus Group Abb-H animals on day 1, but lower responding on days 4, 6 and 10 (all ps<0.05).

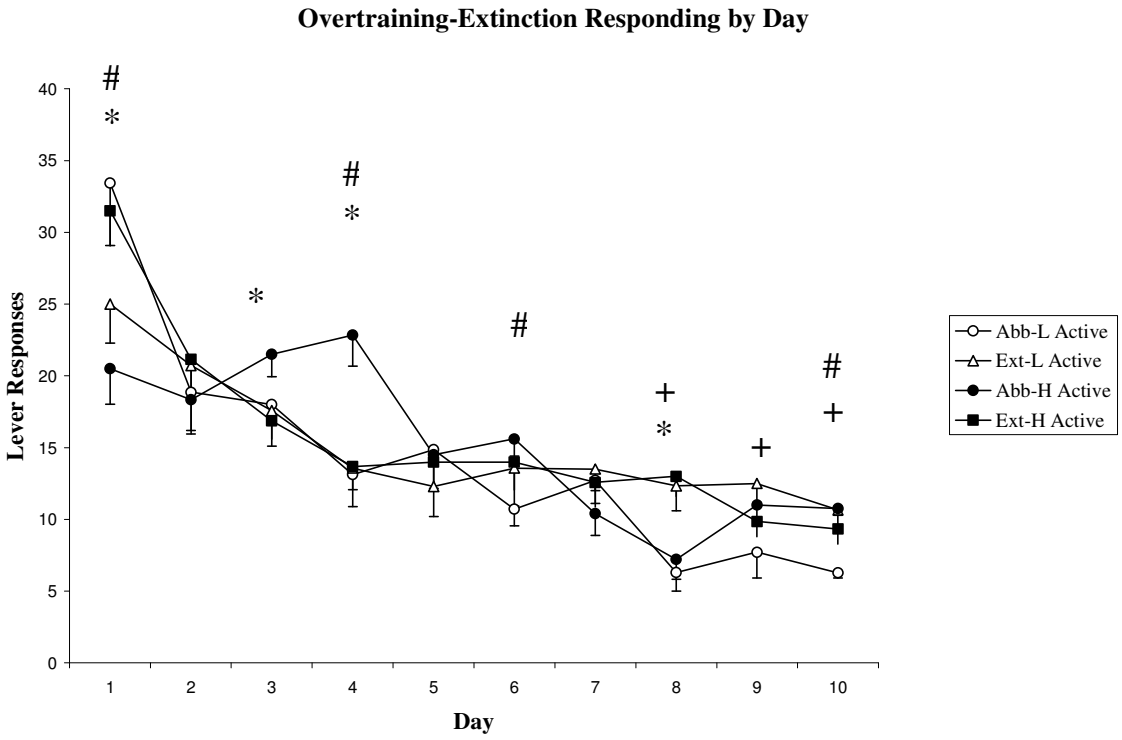

Fig. 19. Experiment 3, mean number of active and inactive lever responses during extinction training days 1-10. Significantly greater responding by group Ext-L compared to group Abb-L is denoted with a +. Significantly different responding between groups Abb-H and Ext-H is dentoed using an *. Significantly different responding between groups Abb-H and Abb-L is denoted with a #.

Transformed data were again used to examine differences in percentage changes compared to terminal acquisition. ANOVA tests (Training Length [15-Days, 30-Days] x Training Dose [0.25 mg/kg, 0.50 mg/kg] x Days [1-10]) on Active Lever responding showed pronounced main effects by Days [$F(9,234)=28.01$, $p<0.001$] and Training Dose [$F(1,26)=5.19$, $p<0.05$]. No effects were present for either Training Length [$F(1,26)=0.54$, $p>0.05$] or the pertinent interactions (Training Dose x Training Length x Days) [$F(9,234)=1.84$, $p>0.05$].

Subsequent post hocs revealed significantly greater persistence in the face of non-reward by Group Abb-H compared to both Groups Abb-L and Ext-L (all $ps<0.05$). These data support the dose related predictions in our hypothesis. In other words, the higher training dose engendered greater drug seeking during extinction than the low dose. However, the observed changes in responding are not enough to support the relationships that make up the OEE.

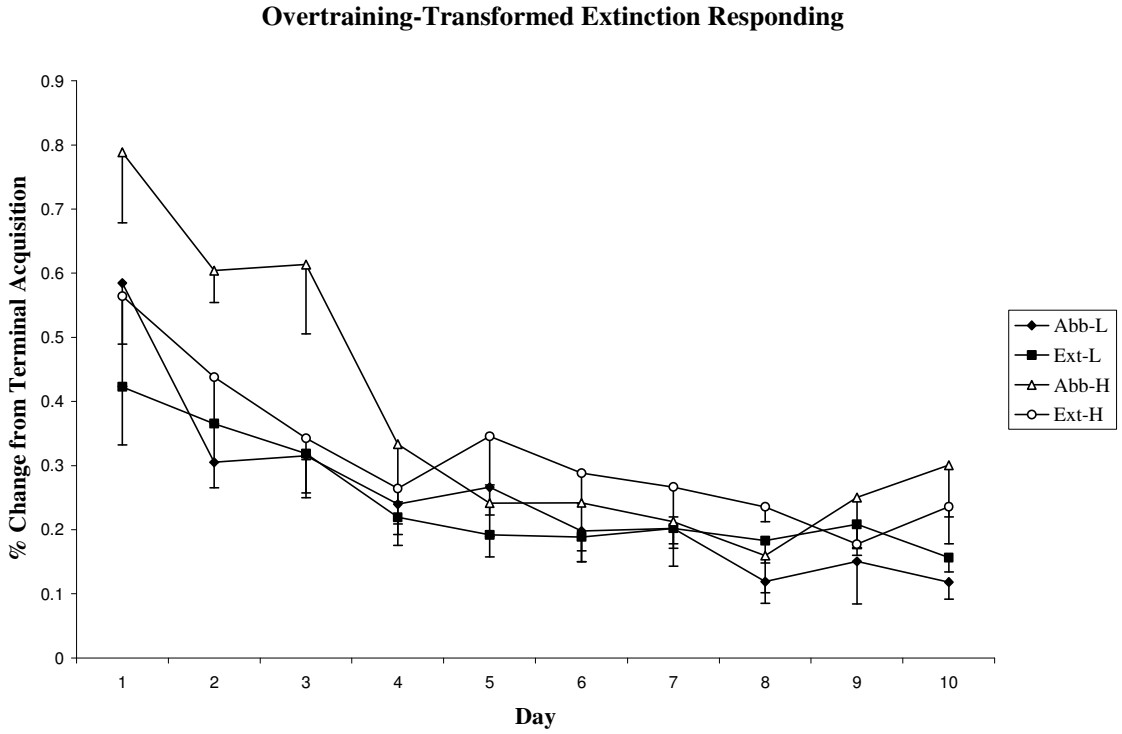

Fig. 20. Experiment 3, mean number of percentage changes in baseline responding from terminal acquisition compared to extinction days 1-10. Significantly greater persistence was evident by group Abb-H compared to Abb-L and Ext-L (ps<0.05).

Reinstatement

Reinstatement responding was again analyzed to determine differences between Active and Inactive Lever responding. A three-way repeated measures ANOVA (Training Length [15-Days, 30-Days] x Training Dose [0.25 mg/kg, 1.00 mg/kg] x Levers [Active, Inactive]) showed significant differences by Levers [$F(1,26)=99.36$, $p<0.001$]. The low levels of left lever responding permitted the exclusion of Inactive Lever responding from any further analyses.

Analysis of Active Lever responding during reinstatement using a two-way ANOVA (Training Length x Training Dose) showed no significant differences by

Training Dose [F(1,26)=0.16, p>0.05] or by Training Length [F(1,26)=3.816, p>0.05]. However, significant interactions (Training Length x Training Dose) were present [F(1,26)=7.563, p<0.05].

Highlighting some of the more interesting patterns of behavior associated with Amsel's frustration theory, post hocs of Active Lever responding revealed significantly less responding by Ext-H compared to Abb-H animals (p<0.05). Furthermore, Ext-H animals pressed in significantly lower amounts than Ext-L animals (p<0.05).

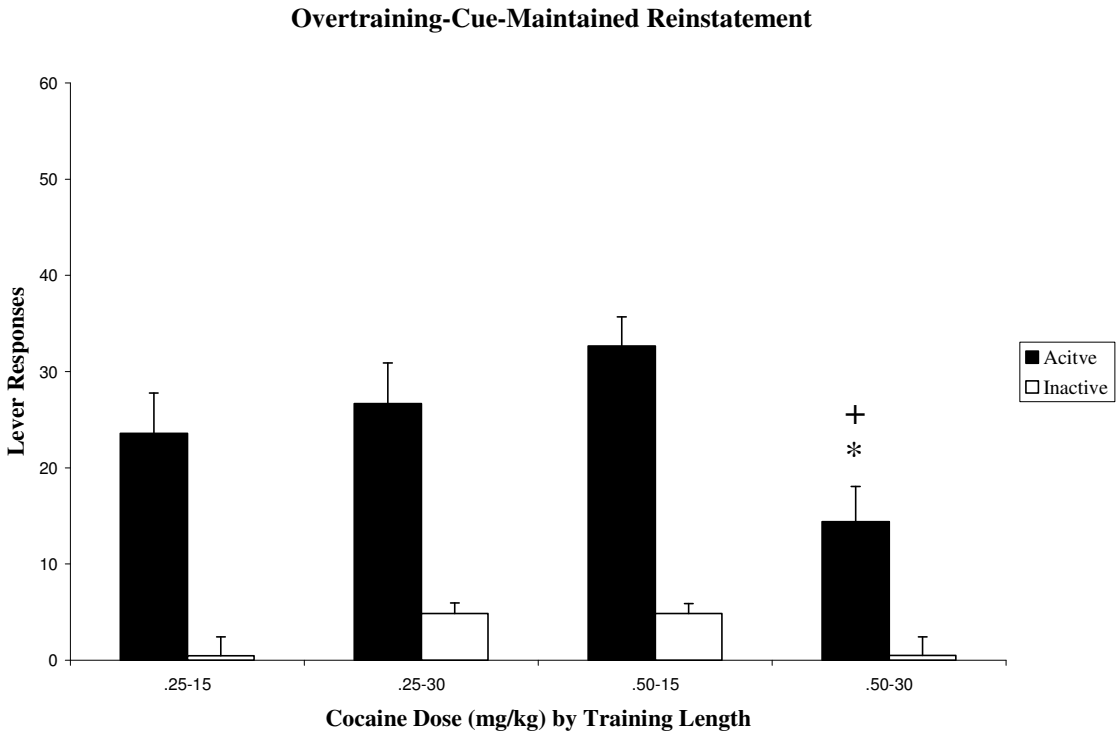

Fig. 21. Experiment 3, mean number of active and inactive lever responses during cue-maintained reinstatement. Lower amounts of responding compared to Ext-L are denoted with an *. Lower amounts of responding compared to Abb-H are denoted with a +.

Discussion

The results from Experiment 3 revealed an interesting pattern of dose-related effects that are partially explained by the OEE. Overall trends within this study showed significantly reduced resistance to extinction in animals trained for 30 days at the highest dose of cocaine. In addition, patterns of responding on the inactive lever and during reinstatement were very similar to the effects seen during extinction testing.

Terminal Acquisition

Responding during terminal acquisition, before extinction sessions commenced, produced significantly greater levels of responding in the extended training group trained on the high dose of 0.50 mg/kg cocaine. This is not surprising given the known side effects of chronic stimulant use, specifically tolerance. Given the lengthier training schedule, greater amounts of responding might have been necessary to overcome the neurochemical effects of tolerance. In addition, the increased amount of responding by low (0.25 mg/kg cocaine) versus high (0.50 mg/kg) dosed animals, again, replicates patterns from a dose-effect curve.

Extinction

The data from extinction training are partially explained by the OEE, however responding in the low dose condition showed novel effects. Specifically, the significant

effects observed in the analysis of left lever responding are worth mentioning in this instance. While left lever responding was higher than normal in all experiments during extinction, those of Experiment 3 showed interactions that supported results from extinction and reinstatement tests. While the graphical representation may seem sporadically distributed at first glance, significant trends are present. Specifically, animals trained on the low dose of 0.25 mg/kg cocaine showed more drug seeking (left lever presses) when trained for 30 days. This of course does not fit with the OEE however; it became apparent that this was a trend present throughout Experiment 3. Animals trained with 0.50 mg/kg cocaine showed decreased drug seeking when trained for 30 compared to 15 days. Lastly, greater responding was observed by animals trained for 15 days on the low dose compared to those trained for 15 days on the high dose of cocaine.

With respect to this experiment, the results from the inactive lever parallel the effects in the remaining analyses. This certainly argues for inactive lever responding as a measure of drug seeking in animal models of forced abstinence. Of course, the results from Experiment 3 are made all the more compelling in that the effects were robust enough to induce significant differences on an unpaired (with respect to cocaine deliveries) lever.

The results of the analyses on active lever responding during extinction yielded two trends. Most pertinent to the rationale behind Experiment 3, rats trained using abbreviated training lengths showed greater persistence early during extinction training. This was particularly evident in animals trained with the high dose of 0.50 mg/kg cocaine. Also, dose related differences were apparent in the abbreviated condition

indicating possible differential dose sensitivity depending on the training length used. However, these data are not entirely clear.

In addition, the data from the active lever follow the same general pattern of effects as those of the inactive lever. What is apparent from Figure 19, is that the two days of elevated responding by the abbreviated training group are robust effects that occur simultaneously to the two days of elevated inactive lever responding (days 3 and 4). This may be viewed as overall increases in activity or craving/frustration mediated drug searching. There is likely too little data to make a clear inference. Finally, the amount of bar pressing amongst the two 15 day training groups was higher in rats trained on the low dose of cocaine.

Results from the transformation data were not convincing. Indications were evident for the influence of the OEE. However, only greater levels of responding by animals trained for 15 days on the high dose of cocaine as compared to the 2 low dose conditions (15 and 30 days of training) were evident. This was certainly not enough to make a case for the overtraining effect as a modulator of extinction behaviors. While active lever results alone might not make a strong case for the presence of the OEE, it is made compelling when Experiment 3 is viewed in its entirety.

Reinstatement

Figure 21 shows a clear summary of the effects observed throughout this experiment. The OEE may be applicable to drug-related behaviors but only at certain concentrations. Of the various effects, the most directly relevant is the decreased propensity to return to

drug seeking behaviors by animals trained for 30 days on a high dose of 0.50 mg/kg cocaine. The reverse was the trend in animals trained with the lower dose of 0.25 mg/kg cocaine although an acceptable level of significance was not reached. Furthermore, decreased responding in animals trained for 15 days was evident with the low dose of cocaine. The reverse was true in the extended training groups. Together these data support the OEE in animals trained with a relatively high dose of 0.50 mg/kg cocaine. A characterization of other doses of cocaine would certainly determine whether OEEs are specific to higher doses and whether this effect appears elsewhere along the dose-effect curve.

EXPERIMENT 4: PROLONGED ABSTINENCE

Rationale

The final experiment in this report was designed to examine what is generally known as "incubation of cocaine craving (Grimm et al., 2001)." Data from numerous articles have indicated that the withdrawal related motivational factors that elicit renewals in drug seeking after periods of abstinence are, in part, temporally correlated. A recent review from Lu et al. (2003) outlines several of the seminal articles that examine this phenomenon. Interestingly, the effects of craving incubation appear to affect most drugs of abuse, and other positive reinforcers, to varying degrees.

Regarding the theme of this series of studies, Experiment 4 attempted to address yet another aspect of dose-related modulation of extinction responding that has failed to be systematically investigated. An alternate concern, then, is the methodological issues that arise when these basic differences are not uniform or accounted for across studies.

There is a very limited amount research concentrating exclusively on extinction, or withdrawal, related mechanisms. As previously mentioned, most drugs show temporally sensitive increases, and subsequent decreases, in drug-seeking behaviors. Animals in these studies are typically trained in an instrumental response that results in a positive reinforcer. Upon reaching predetermined response stability, they are removed from the operant settings and returned to engage in extinction testing after different lengths of "true" forced abstinence. Abstinence periods are spent in the homecage where they do not perform an operant response of any kind. Perhaps this portion of the model, abstinence away from the operant chamber, applies most directly to humans, however the

potential for innovative interventions lies in extinction training done within the testing apparatus. True extinction of a response that results in a reward in animal models is the first step to creating effective therapies in humans. The motivational factors that underlie relapse may be of a different nature than those of craving. The design of a study exposing animals to prolonged periods without exposure to drug associated cues accurately mimics human conditions in that most humans abstain by removing themselves from drug related contexts. Further this model allows for aggregation of these vectors (withdrawal related mechanisms) and, consequently, a more pronounced dependent measure. This will be discussed in greater detail in the general discussion.

Results from cocaine animal studies indicate a steady increase in "craving" up to a period of 2 months (Grimm et al., 2003). In addition, associated neurochemical changes may be indicative of withdrawal related mechanisms at work (Grimm et al., 2002). Once again, an examination of dose magnitude interactions is not presented within any of these studies. This is essential in creating accurate models that will reflect the complex nature of drug histories prior to periods of abstinence. Therefore, the design of this study employed two doses of cocaine (low and high) and 2 periods of abstinence (1 day and 30 days) in order to uncover potential differences.

Materials and Methods

Animals

Thirty three adult male Sprague-Dawley (Harlan; Houston, Texas, USA) rats weighing between 300-325 g were used for Experiment 4. Rats were individually housed, and all other animal maintenance of Experiment 4 was as described for Experiment 1.

Apparatus

The operant apparatus used for Experiment 4 was the same as in Experiment 1.

Surgeries

Surgeries were performed as described in Experiment 1.

Drugs

The Research Technology Branch of the National Institute of Drug Abuse generously supplied the cocaine HCl.

Testing

Animals were allowed to recover for 1 week after surgeries. The following day, animals were transported to the testing chambers from the home cage using individual plastic boxes. Before being placed into the testing chambers, each animal was flushed with sterile saline to ensure catheter patency. Rats were then placed in pre-determined testing chambers and connected via the exposed end of the blunted 22 ga needle. This was attached to a length of 0.02 ID Tygon tubing housed within a spring leash attached to the chamber. This was directed to another length of 0.02 ID tubing via a single channel fluid swivel. This connection then formed a closed system that would receive jugular infusions directly from the syringe on the mechanical pump.

On day 8 all animals began training using an FR-1 schedule of reinforcement. Four groups were created in order to test the effects of prolonged versus abbreviated abstinence (0.25 mg/kg-1-day of abstinence [1D-L], 0.25 mg/kg-30-day of abstinence [30D-L], 0.50 mg/kg-1-day of abstinence [1D-H], 0.50 mg/kg-30-day of abstinence [30D-H]). Each animal was trained to stable responding (< 20% variability across two consecutive days) first, then trained for an additional 15 days before being placed into their respective abstinence groups. Animals undergoing forced abstinence periods remained in their homecage for the duration of the abstinence length. Rats were not disturbed or exposed to any drug-associated stimulus. Upon completion of the abstinence period, each rat was reintroduced to the testing apparatus and began the first of 10 extinction trials.

Extinction conditions were exactly the same as in Experiments 2 and 3. Each animal was given a daily 1-hour session of free responding within the operant chamber with no over consequences. Lever presses resulted in no stimulus light or pump activation. Further, no infusions of any kind were administered during these sessions. Both active and inactive lever responding was recorded. After the completion of 10 sessions, each animal was tested in a single reinstatement preparation on the following day. Reinstatement sessions consisted of 1 hour of extinction conditions, as described above, followed by 1 hour of free responding with a stimulus light presentation as the sole consequence to an active lever response. The onset of the second hour was signaled by a single, experimenter delivered active lever prime resulting in the activation of the stimulus light cue. Each cue presentation was 12 seconds in duration. All other conditions were exactly the same as during extinction training.

Data Analysis

Terminal acquisition (last day of acquisition training) and reinstatement data were analyzed using three-way ANOVA tests (Abstinence Length x Training Dose x Levers). Extinction data were analyzed using four-way repeated measures ANOVA (Abstinence Length x Training Dose x Levers x Days). Tukey's post hocs were used when appropriate. In all cases statistical significance levels were set at $p<0.05$.

Results

Terminal Acquisition

A three-way (Abstinence Length [1-Day, 30-Days] x Training Dose [0.25 mg/kg, 0.50 mg/kg] x Levers [Active, Inactive]) repeated measures ANOVA was used in order to determine if Active Lever responding was significantly different from Inactive Lever Responding on the last day of baseline responding (terminal acquisition). The results indicated a statistically significant difference by Levers [$F(1,29)=160.47$, $p<0.001$]. The near zero levels of responding on the left lever, again, prompted the exclusion of Inactive Lever responding from any further analyses.

A two-way (Abstinence Length [15-Days, 30-Days] x Training Dose [0.25 mg/kg, 0.50 mg/kg]) ANOVA on Active Lever responding resulted in significant main effects by Dose [$F(1,29)=9.75$, $p<0.001$] but no effects by Abstinence Length [$F(1,29)=0.04$, $p>0.05$] or interaction between the two (Abstinence Length x Dose) [$F(1,29)=3.02$, $p>0.05$].

The differences by Dose were expected as per a standard biphasic dose-response curve. In other words, greater responding was observed in groups trained with the low dose of 0.25 mg/kg compared to 0.50 mg/kg cocaine ($p<0.05$).

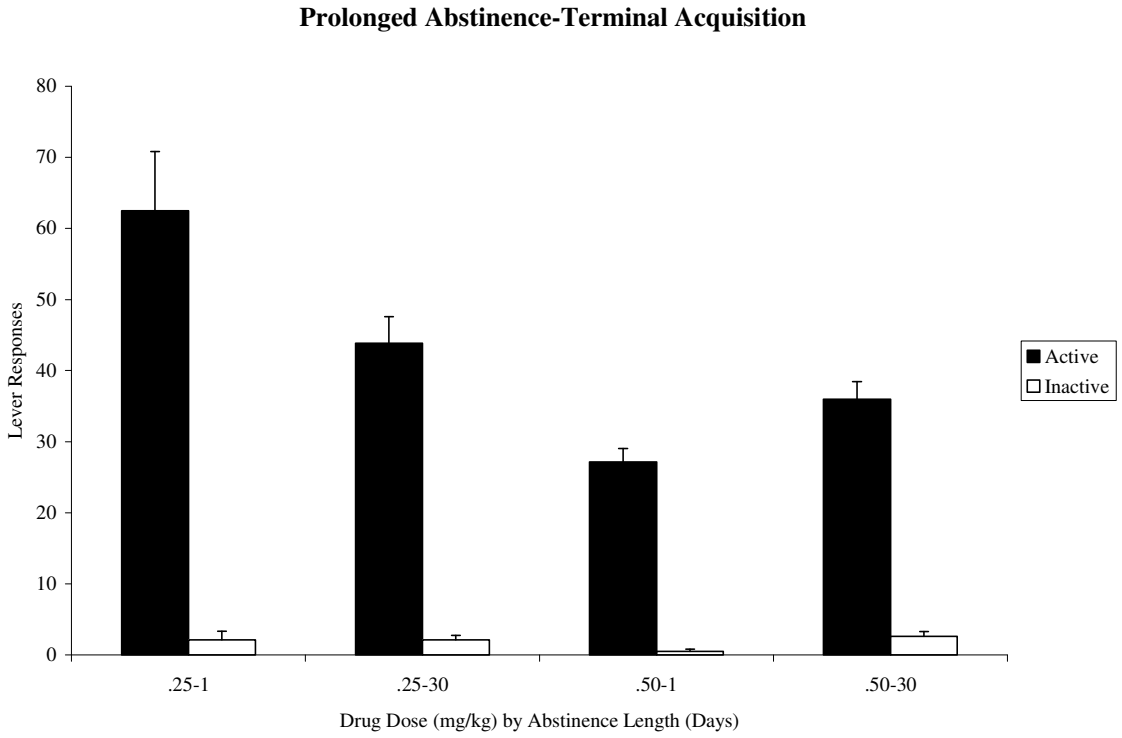

Fig. 22. Experiment 4, mean number of active and inactive lever responses during terminal acquisition. Groups trained on the low dose responded more than those trained on the high dose ($p<0.05$).

Extinction

Extinction responding was first analyzed to determine if Active and Inactive Lever responding was significantly different. Four-way repeated measures ANOVA (Abstinence Length [1-Day, 30-Days] x Training Dose [0.25 mg/kg, 0.50 mg/kg] x Levers [Active, Inactive] x Days [1-10]) revealed significant differences between Levers [$F(1,252)=268.28$, $p<0.001$]. Although statistically different from right lever responding, the elevated trends in responding evident in Figure 23 prompted the analysis of Inactive Lever responding on its own.

Inactive Lever responding was analyzed using a three-way (Abstinence Length [1-Days, 30-Days] x Training Dose [0.25 mg/kg, 0.50 mg/kg] x Days [1-10]) repeated measures ANOVA. Results revealed significant effects by Days [$F(9,261)=11.01$, $p<0.001$] and by Training Dose [$F(1,29)=4.20$, $p=0.05$] but none for Abstinence Length [$F(1,29)=5.56$, $p>0.05$]. Significant interactions (Abstinence Length x Training Dose x Days) [$F(9,261)=1.69$, $p>0.05$] were not present. Post hocs revealed that group 1D-L showed lower responding than all other groups and that responding decreased from Days 1-10 (all $ps<0.05$).

Active Lever responding was then analyzed using a three-way (Abstinence Length [1-Day, 30-Days] x Training Dose [0.25 mg/kg, 0.50 mg/kg] x Days [1-10]) ANOVA. The results indicated significant main effects by Days [$F(9,261)=63.40$, $p<0.001$] and interaction effects by Abstinence Length x Training Dose x Days [$F(9,261)=61.60$, $p<0.001$]. No main effects were observed for either Training Dose [$F(1,29)=2.68$, $p>0.05$] or Abstinence Length [$F(1,29)=1.61$, $p>0.05$].

Post hocs indicated overall increased resistance to extinction by Group 30D-L compared to all other groups ($ps<0.05$). In accordance with the incubation of craving properties possessed by cocaine and various other drugs (Grimm et al., 2003; Lu et al., 2004; Shalev et al., 2001), significant increases in responding during extinction were observed in Group 30D-L versus Group 1D-L animals on Days 2, 8 and 10 (all $ps<0.05$). Significant increases in responding during extinction were observed in Group 30D-H versus Group 1D-H animals on Day 2 but showed decreased responding on Days 3, 4, 6 and 10 (all $ps<0.05$). With the exception of Day 2, the results from these post hocs indicate differential dose sensitivity associated with craving incubation after different

abstinence periods. Significantly elevated responding was observed by Group 1D-L versus Group 1D-H animals on Day 1 and significantly depressed responding on Days 4, 6 and 10 (all ps<0.05). Significantly elevated responding was observed by Group 30D-L versus Group 30D-H animals on Days 1, 7, 8 and 10 (all ps<0.05). Higher amounts of responding by animals trained on the low dose of cocaine (0.25 mg/kg) replicate response patterns from a normal cocaine dose-effect curve. However, this was only evident during extinction after 30 days of abstinence. Accordingly, with the exception of Day 1, the directionally opposite findings from 1D abstinence groups (Days 4, 6 and 10) are of particular interest.

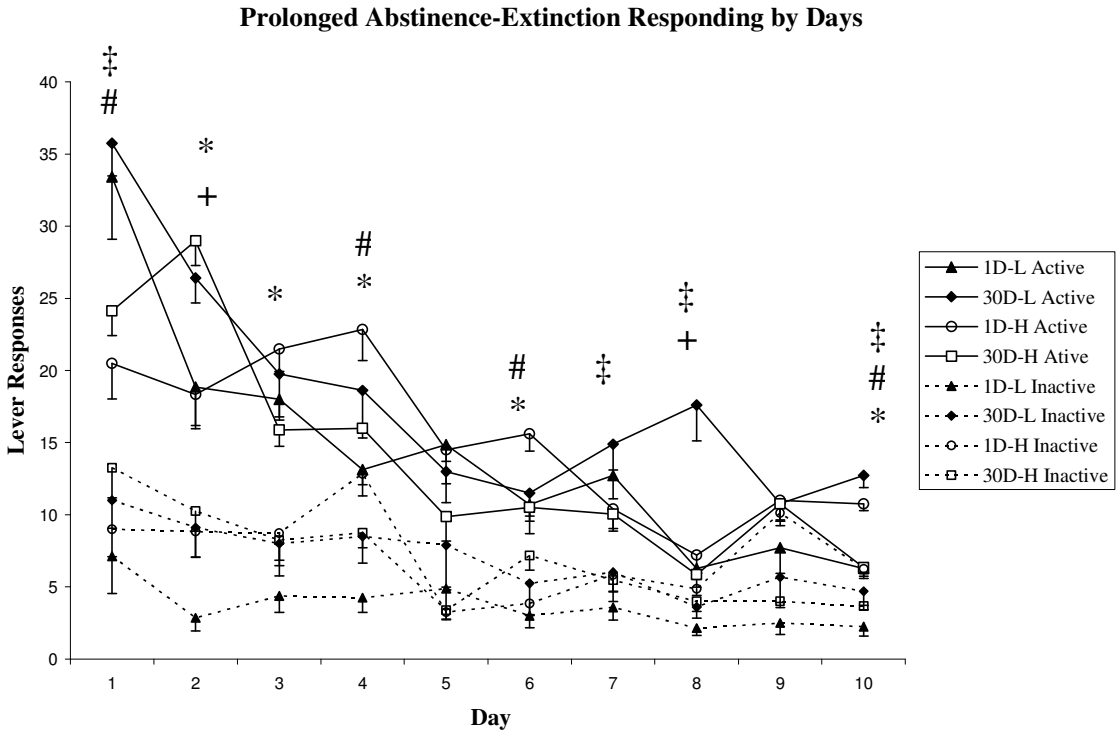

Fig. 23. Experiment 4, mean number of active and inactive lever presses during extinction training days 1-10. Significantly higher responding by group 30D-L compared to group 1D-L is denoted with a +. Significant differences between groups 30D-H and 1D-H are denoted with an *. Significant differences between groups 1D-L and 1D-H are denoted with a #. Higher responding by group 30D-L versus group 30D-H is denoted with ‡.

In addition to the standard analyses on Active Lever responding, an analysis on transformed data was performed as well. Results from the three-way (Abstinence Length [1-Day, 30-Days] x Training Dose [0.25 mg/kg, 0.50 mg/kg] x Days [1-10]) ANOVA on Active Lever responding resulted in significant main effects by Days [$F(9,261)=51.69$, $p<0.001$] but none by Abstinence Length [$F(1,29)=4.07$, $p>0.05$] or Training Dose [$F(1,29)=3.03$, $p>0.05$]. Significant interaction effects were present by Abstinence Length x Training Dose x Days [$F(9,261)=2.89$, $p<0.01$].

Subsequent comparisons of means using Tukey's post hocs showed significantly greater resistance to extinction by Group 30D-L versus Group 1D-L on days 2 [$F(1,16)=11.88$, $p<0.01$], 7 [$F(1,16)=8.12$, $p<0.05$], 8 [$F(1,16)=12.13$, $p<0.01$] and 10 [$F(1,16)=13.98$, $p<0.01$] in accordance with the notion that cocaine craving is subject to incubation periods (Lu et al., 2004). Results from post hoc tests revealed the most resistance to extinction by Group 30D-H compared to Group 1D-H on day 2 ($p<0.05$). Results from mean comparisons showed the greatest amount of resistance to extinction by Group 1D-H versus Group 1D-L on days 2, 3 and 10 ($p<0.05$). Finally, post hocs showed the greatest amount of resistance to extinction by Group 30D-H versus Group 30D-L on days 2, 4 and 9 and showed the least amount of resistance on days 8 and 10 ($p<0.05$). The latter results based on dose differences indicates increased resistance stemming from the reinforcing value of higher doses of cocaine rather than carryover effects from total amounts of lever pressing. As noted in the terminal acquisition data, lower doses typically engender greater amounts of behavioral responding, or drug-taking, under CRF schedules. This, however, does not necessarily reflect the reinforcement

value of a particular dose as detailed by economic cost/benefit examinations of self-administration (i.e. progressive ratio studies) [Rowlett, 2000].

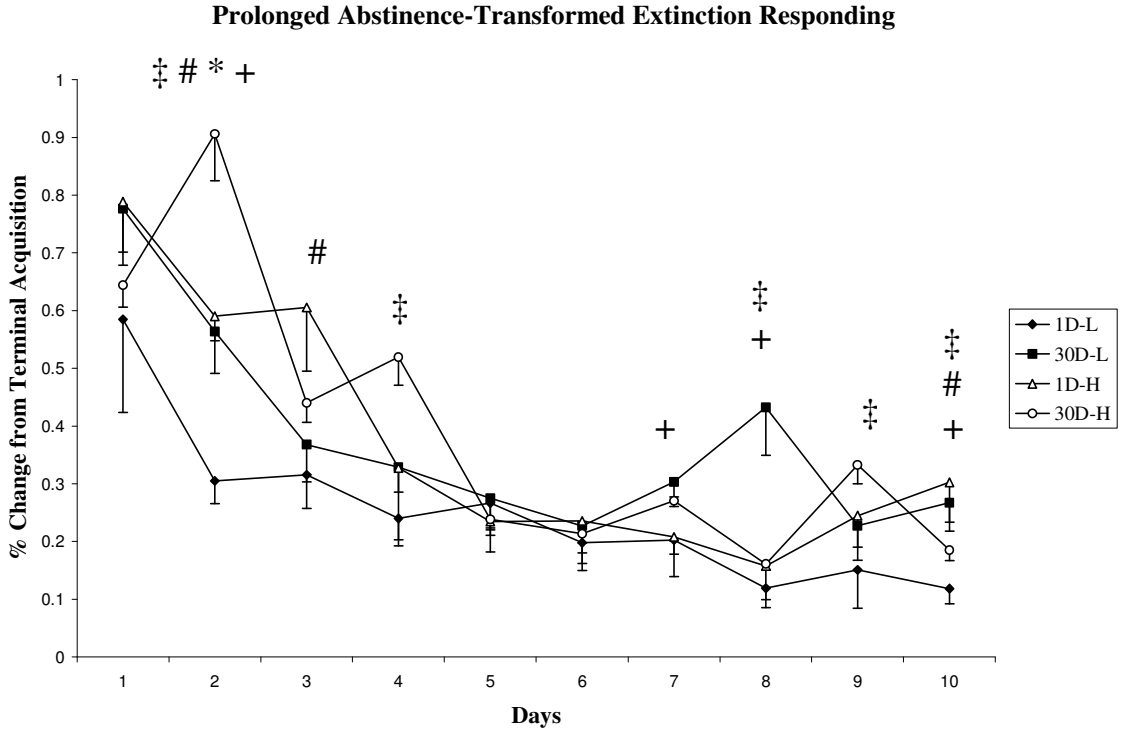

Fig. 24. Experiment 4, mean percentage changes in baseline responding from terminal acquisition to extinction training days 1-10. Greater resistance to extinction by group 30D-L versus group 1D-L is denoted with a +. Greater resistance to extinction by group 30D-H compared to group 1D-H is denoted using a *. Greater resistance to extinction by group 1D-H versus group 1D-L is denoted by a #. Significant differences between group 30D-H compared to group 30D-L are denoted with a ‡.

Reinstatement

Differences in allocation of lever responding between the Active and Inactive Lever were initially examined. A three-way repeated measures ANOVA (Abstinence Length [1-Days, 30-Days] x Training Dose [0.25 mg/kg, 0.50 mg/kg] x Levers [Active, Inactive]) showed significant differences by Levers [$F(1,29)=107.19$, $p<0.001$]. Near zero levels of

responding on the left lever prompted the exclusion of Inactive Lever responding from any further analyses.

Analysis of Active Lever responding during reinstatement using a two-way ANOVA (Abstinence Length [1-Days, 30-Days] x Training Dose [0.25 mg/kg, 0.50 mg/kg]) showed main effects by Abstinence Length [$F(1,29)=4.55$, $p<0.05$] but no effects by Training Dose [$F(1,29)=0.11$, $p>0.05$] or interaction effects (Abstinence Length x Training Dose) [$F(1,29)=1.99$, $p>0.05$].

Visual examination of Figure 25 clearly shows that differences incurred by Abstinence Length were due to the increases in responding during cue-maintained reinstatement by Group 30D-L ($p<0.05$). Comparably elevated responding was not present in the high dose of cocaine therefore it is assumed that there is a dose dependent function in relapse responding after prolonged periods of forced abstinence. This further supports the robust Abstinence Length effects observed during extinction training, particularly at the lower dose of cocaine.

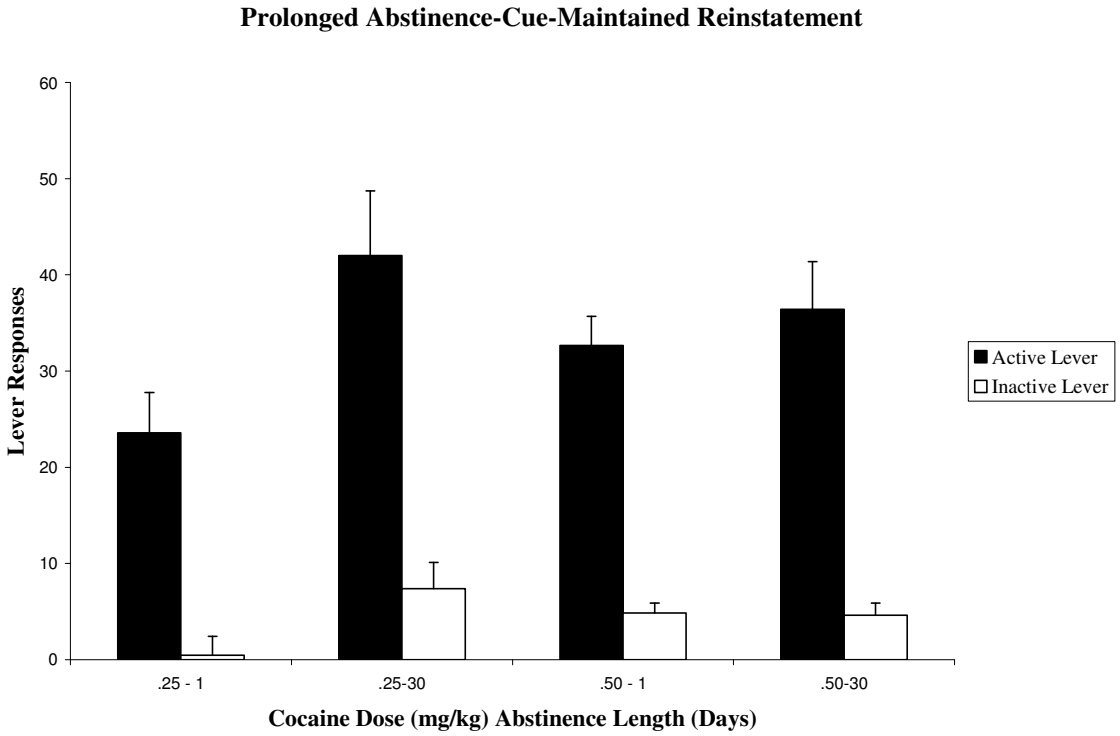

Fig. 25. Experiment 4, mean number of active and inactive lever responses during cue-maintained reinstatement. Main effects were observed by abstinence length.

Discussion

The results from Experiment 4 partially support the hypothesis of increased incubation of cocaine craving during prolonged abstinence. Dose sensitivity appears to be a critical factor in producing incubation results in the case of the examined cocaine concentrations. It was evident that the high dose of 0.50 mg/kg produced mixed results compared to the strong incubation effects seen at the low dose of 0.25 mg/kg cocaine.

Terminal Acquisition

Data from terminal acquisition showed no significant differences between training doses. Although some variability is visually apparent, the only significant difference occurred between the two training doses where the low dose showed greater responding as predicted.

Extinction

Extended abstinence periods showed "incubation" of cocaine craving (or increased drug seeking) when trained on the low (0.25 mg/kg) dose of cocaine. Results from rats trained on 0.50 mg/kg cocaine showed directionally opposite effects, indicating dose-sensitivity regarding craving effects. In addition, response patterns similar to a standard biphasic dose-effect curve were not present in the groups abstaining for 1 day. Within these groups, the greater amount of active lever depressions was observable at the high dose of cocaine (0.50 mg/kg) compared to the low (0.25 mg/kg). The opposite pattern is typically produced during standard self-administration. Dose-sensitive mechanisms are again in play in terms of the effects of cocaine craving on extinction responding.

Therefore the tenets of craving incubation are likely applicable to particular settings. In other words, incubation of cocaine craving may not occur in all situations, i.e. after different training doses or extinction training conditions. Reasons for reward magnitude sensitivity may relate to factors that modulate intake patterns during training and/or classical conditioning factors that affect assigned saliency to extra-drug cues.

Clearly, more scrutiny is required before a full range of drug history effects can be made in this area.

Pertaining to methodological issues, it must be noted that the procedures employed in Grimm et al. (2002) and Lu et al. (2004) are not precisely the same as those employed here. Animals were trained, solely, using a cocaine dose of either 0.50 or 1.00 mg/kg for periods of 6 H/day. Most certainly, overall drug intake was elevated compared to the 2-hour sessions employed for Experiment 4. Associated changes in glutamate protein expression, adenylate cyclase and cAMP-dependent protein kinase enzymes were evident after training on a high dose of cocaine (1.0 mg/kg) [Lu et al., 2003]. Increases in brain-derived neurotrophic (BDNF) factor were also evident after withdrawal from 1.0 mg/kg cocaine (Grimm et al., 2003). Without any comparable data for animals trained on lower doses of cocaine, it cannot be inferred that these changes are enacted in a similar and diminished fashion, or at all.

In addition, extinction conditions consisted of stimulus light presentations after an active lever response in Lu et al. (2004), whereas the conditions in experiment 4 did not employ the stimulus light. As is readily shown in the previous 3 experiments, the appearance of the stimulus light, after formerly being paired with a drug reinforcer, is a powerful cue that surely will aid in the onset of approach behaviors. Finally, extinction testing consisted of 6-7 consecutive 60 minute sessions per day. For those reasons, the results from this experiment should be cautiously compared to those of any other study.

Results from the transformed data sets lend support to the original hypothesis. While data from the groups trained on the high dose of cocaine were not compelling, animals trained using the low dose showed patterns in line with our predictions. Rats that

were forced to abstain for a period of 30 days showed the greater amount of resistance to extinction compared to those abstaining for 1 day. Further, the high dose of cocaine showed greater responding amounts than the low dose in both abstinence conditions. As mentioned, this is a critical finding in that it suggests drug seeking modulation in terms of dose reward/reinforcement value rather than a replication of typical dose-response patterns. Therefore, carryover effects from terminal acquisition were overcome, or not a factor, in revealing the relative saliency of each dose during extinction conditions after 2 periods of abstinence.

Reinstatement

Reinstatement results showed comparable results to that of extinction. Trends were evident that suggest that animals undergoing prolonged abstinence were more likely to return to active drug seeking. In particular, this was evident in animals trained on the low dose of cocaine. Stimulus light presentations, therefore, posses associative saliency that reliably activates cocaine seeking that parallels extinction patterns.

Collectively, these data support previous studies that have shown robust evidence for cocaine craving incubation. These data also extend the effects to include strong cue-maintained reinstatement after 10 days of extinction. However, the critical finding is the dose-sensitive mechanisms that could very readily result in different results depending on drug self-administration histories. Specifically, the discrepancies in results from other laboratories and Experiment 4 indicate that incubation may preferentially be expressed at

relatively moderate doses (0.25 mg/kg) and very high (> 0.50 mg/kg) doses of intravenous cocaine.

The area of extinction remains a relatively overlooked area of drug addiction research. As this series of experiments has revealed, there are numerous factors that may potentially affect behavioral outcomes. Furthermore, it is evident that extinction responding is as sensitive to modulation and as dynamic as responding during acquisition, maintenance and reinstatement. As such, it is important that examinations of extinction receive equally as detailed and careful attention. Increased attention to this area is of particular importance due the potential relevance of extinction training as an animal model of cessation therapies.

GENERAL DISCUSSION

Summary

The formulation of novel and effective therapies to combat chronic drug use/abuse in humans has proven to be a monumental undertaking. The engrained nature of behavioral and biochemical alterations that ensue after drug use becomes compulsive necessitates the creation of equally as persistent and permanent therapies. Since the investigation of drug addiction in humans is essentially limited to behavioral interventions and data collection in addicted populations, it falls to animal models to provide a vehicle for outlining the consequences of drug use and examining possible interventions. As discussed earlier, a realistic representation of human addiction is necessary in order to effectively conduct drug research and has yet to be created.

Models of self-administration still remain the most likely candidates for targeting and studying addicted populations that possess key traits to that of human addicts. As such, the area of drug research is dense with variations of the classic self-administration model first employed over 40 years ago (Weeks, 1962). However, it is only recently that the various areas of addiction that have classically been studied in isolation, have been combined to create protocols that may distinguish "addicted" animals from within a self-administering cohort (Deroche-Gamonet et al., 2004; Vanderschuren and Everitt, 2004). This is a critical milestone in drug research for obvious reasons. While many humans have self-administered some kind of drug at some point, most do not become compulsive users. Further, even within populations that have willingly taken a drug with a high potential of abuse, relatively few become clinically addicted (Anthony et al., 1994). The

lack of attention to this detail has placed addiction researchers at a disadvantage for 4 decades. Clearly, there are traits that predispose some individuals to become compulsive users while others are spared. Without a method for separating these groups within animals, analyses of compulsive drug taking/seeking have produced few applicable results in the way of human therapies.

The above mentioned studies (Deroche-Gamonet et al., 2004; Vanderschuren and Everitt, 2004) have created a paradigm that includes various characteristics of human drug intake. Prolonged access to drug, negative consequences as a result of continued use and phases of testing that mimic maintenance, extinction and relapse and humans are some of the key aspects of this model that give it increased validity. Along these lines, it has been the theme of this series of investigations to address the extinction/forced abstinence portion of addiction with equal attention to its dynamic nature. It was the aim of these studies to contribute to the creation of models that accurately represent the complexities of human addiction by addressing self-administration histories in rats. Therefore, in order to ascertain the rate to which extinction responding can be modulated by drug history, careful attention was given to include a range of variables including multiple doses, schedules, training lengths, abstinence lengths and extra-drug cues. Moreover, it is likely that extinction training holds an untapped potential for uncovering behavioral and neurochemical patterns that may aid in developing effective therapies due to its direct relevance as a model for active cessation. However, little attention has been given to this area of addiction and was therefore an additional reason for the topics addressed within this report.

Drug History

Clearly, the overarching conclusion from the experiments reported here is the importance of prior drug history on subsequent behavioral measures of addiction. While some effort has been made to link drug history with behavioral outcomes in self-administration (Carroll, 1993; Morgan et al., 2002; Morgan and Roberts, 2004), there is still relatively little information within the available literature. As such, studies approach the investigation of drug addiction within animal models by studying phases (acquisition, maintenance, extinction, reinstatement) of compulsive drug use in isolation (Baker et al., 2003; Carroll and Lac, 1997; Cornish et al., 1999; Crespo et al., 2002; Fuchs et al., 2004b; Grimm et al., 2001; Johanson, 1976). The results from this series of experiments demonstrate how critical it is to conduct exhaustive studies that incorporate histories that are as dynamic as those of human drug users.

It cannot be discounted that an exact representation of human addiction may be a lofty goal, given the numerous factors that are likely involved. However, it is clear that the models that are currently prevalent throughout addiction research lack complexity. What is within reason are studies, such as those reported here, that explore known factors that influence drug abuse and combine them into models that are more representative of human addiction. For instance, of current interest are the differences produced when animals are allowed prolonged access to drugs of abuse (Morgan, Smith, et al., 2005; See et al., 2004). These lengthened sessions produced markedly different response (such as dysregulation of intake, greater intake and self-imposed abstinence periods) patterns that closer approximate human intake. Other vectors possibly predisposing organisms to

addiction that have been investigated include impulsivity (Perry et al., 2005), preference for natural rewards (Morgan, Dess, et al., 2005) and submissiveness (Miczek et al., 2004). However, polydrug abuse, dose interactions, dose orders, training lengths and abstinence periods are some of the more obvious traits of human addiction that are not being modeled in animals. As was detailed during the introduction of this manuscript, the lack of attention to extinction or abstinence also falls under this category.

As noted, numerous important discoveries with regards to self-administration and extinction responding appear to be dose related (Lynch and Carroll, 2001; Nation et al., 2004). Some vary directly in magnitude with dose (e.g. sensitization to conditioned stimulus [CS] light presentation during maintenance) [Schenk and Partridge, 2001] while others may not exist at different concentrations of the drug (e.g. incubation of cocaine craving differences at doses < 1.0 mg/kg [Grimm et al., 2001, compared to the results of Experiment 4]). Drug histories must be addressed with regards to all aspects of self-administration and in particular, the extinction phase must be given increased importance due to its direct relevance to the cessation of habitual behaviors.

While it remains important to examine singular effects of one dose or one schedule, for instance, there must be an increased focus on combining these findings in order to create models that are realistic. This will surely facilitate breakthroughs in addiction research and hopefully lead to successful pharmacological and/or behavioral countermeasures to addiction. Along these lines, the following sections outline some promising leads in addressing the mechanics that underlie extinction within drug abuse research and may shed some light as to the mechanisms underlying the results of Experiments 1-4.

Conditioned Cues

Studies of extinction are indirectly, also, studies of cue-induced drug seeking. Behavioral extinction is a well documented phenomenon within classical reward literature (Bouton, 2004). Coincident with findings from this report, one of the more interesting and less understood aspects of extinction is spontaneous recovery. This seemingly random recurrence of a previously extinguished response is thought to be mediated by subtle cues that are somehow prompting craving or seeking due to an associated salience with the reward. These cues could be contextual, discriminative etc., but it makes a case for how difficult it is to completely remove extra-reward signals. It could be argued that data from Experiment 1 (by 15 minute bins) shows evidence of within session spontaneous recovery (Figures 6 and 8). The recovery of responding observed seems random at first glance but may actually be interpretable within the framework of spontaneous recovery. Furthermore, given the presentation of a CS to an animal that has "extinguished" a previously rewarded response, reinstatement of this response is robust as shown in the reinstatement portion of Experiments 2-4 (Figures 16, 21, and 25). This is not unlikely given the discriminative properties that might have ensued as a result of the stimulus light being omitted during extinction training. Subsequent presentation during reinstatement likely signaled the availability of cocaine however, to varying degrees depending on the drug history of each group.

Contextual cues are powerful signals of drug availability and incur neurochemical changes as well. The presence of salient cues will prolong extinction responding at its onset (Neisewander et al., 2000) and prevent a depression of catecholamine metabolism

(Antkiewicz-Michaluk et al., 2004). Given their usual dose heroin addicts are more likely to overdose in a novel environment than a familiar, drug-paired context. Just the act of being in a drug-paired context, is enough to incur neurochemical changes that are neuroprotective and prevent a fatal overdose (tolerance) [Shavit et al., 1986]. In addition, contextual cues may act as discriminative cues as well. In an attempt to remove the efficacy of a contextual cue, various studies allow the test animals to live in the test chamber. This removes the ability of transport to the testing apparatus to signal the availability of a drug-reward and further reduces the ability of the context to elicit distinct associations, and perhaps any biochemical consequences, with drug reward/reinforcement.

Finally, the presence of cocaine-associated stimuli is known to facilitate self-administration (Arroyo et al., 1998; Goldberg et al., 1979; Ranaldi and Roberts, 1996). In other words, salient cues may functionally increase the efficacy of the drug reward. As such, the removal of associated cues during drug self-administration will change rates of responding during maintenance, most often by decreasing the overall amount of responding; however the opposite might be true during extinction. In particular, this may occur when the same context is used for training and extinction. Abused drugs have the ability to elicit very strong CS associations, in some cases perhaps more than food or water reward. This is, of course, one of the key aspects of drug profiles that make continued abstinence in addicts so difficult. Therefore CS-unconditioned stimulus (US) learning becomes almost permanent. Any CS employed may develop a great deal of saliency and be responsible for prolonged responding during extinction in the presence of non-reward. As shown by the removal of saline in Experiment 1, the CS (saline) might

have generalized to act as a reinforcer of a much lesser magnitude than cocaine. This also interacted with the method of training and dose of cocaine employed in that responding varied even after saline was removed. Of interest in explaining the role of cues in extinction responding are the biochemical changes that ensue as a result of withdrawal and subsequent stimulus presentation as follows.

Presentations of cues predictive of cocaine availability will result in neurochemical alterations that may aid in uncovering the mechanisms of extinction resistance. Data from Fuchs et al. (2004a) show that manipulation of the nucleus accumbens (NAcc) core might be more relevant in mediating cue reactivity than the shell. Specifically, inactivation via muscimol and baclofen resulted in abolishment of stimulus light-tone complex induced reinstatement. Elsewhere, D_1 receptor antagonist administration into the NAcc attenuated responding to a discriminative stimulus that predicted cocaine availability (Yun et al., 2004). Extracellular dopamine (DA) levels in the amygdala also were elevated following a 1-month period of withdrawal and showed a potentiated reaction to CS presentation (Tran-Nguyen et al., 1998). In addition, dopamine transporter (DAT) protein levels were elevated in the prefrontal cortex (PFC), an area associated with choice and impulsivity, following either a 1 or 15 day withdrawal period and subsequent cue-induced reinstatement of cocaine seeking (Grimm et al., 2002). However, no DA associated activity in mesolimbic and sensorimotor striatum was evident in the presence of environmental cues that predicted cocaine (10 minute presentation) [Bradberry and Rubino, 2004]. Interestingly, within the same study, serotonin (5-HT) changes were evident in response to cue presentation. Other regions that show activity dependent on cue presentations include the lateral orbital frontal cortex

(lOFC) which resulted in abolishment of reinstatement after excitotoxic lesions, post-extinction training (Fuchs et al., 2004b). This may indicate lOFC involvement in assessing the recent motivational significance of cocaine conditioned stimuli. Also, recent evidence from Carelli et al. (2002, 2003) shows interesting classification of neurons within the basolateral amygdala (BLA) and the NAcc. In response to a stimulus light-tone complex, certain neurons appear to be activated seconds before in anticipation of the cue, seconds after in response to the cue, and some are inhibited before and/or after the cue. This presents neuronal distinctions between seeking, taking, and immediate inhibition of craving or withdrawal, a topic that has been of recent interest within behavioral drug research. Finally, extracellular signal regulated kinase (ERK) signaling pathway in the central amygdala (CeA) was elevated in response to cocaine-associated cues after 30 days of abstinence (Lu et al., 2005). Additionally, inactivation of the CeA resulted in decreased cocaine seeking. While these studies allude to possible neurochemical and anatomical distinctions that may be preferentially involved in cue-induced relapse to drug seeking, they do not detail the mechanisms that control extinction learning. Other theories and mechanisms may be useful in addressing the abstinence period that precedes relapse or reinstatement to a CS.

The Role of Anxiety

Frustration as an emotional reaction to non-reward has been mentioned several times throughout this report. Certainly, frustration has negative emotional connotations that may correlate with aversive states such as anxiety. As an additional means of

interpreting and explaining the behavioral outcomes of extinction, the role of anxiety or arousal is examined within this section.

Several studies indicate that the behavioral responses to non-reward do include anxiety. The removal of primary reward is shown to incur aggression in animals trained to acquire a reinforcer (Azrin et al., 1966; de Almeida and Miczek, 2002). Further, results from Daly (1974) showed that animals learned to escape contexts formerly associated with a reward after removal of the primary reinforcer. Of interest, this also occurred after training on a partial reinforcement (PRF) schedule suggesting that other mechanisms are interacting with Amsel's theory of frustration-mediated approach (Amsel, 1967; 1992). Leslie et al. (2004) has taken this idea further by incorporating extinction-induced anxiety, or fear as the authors equate it, within Gray and McNaugton's behavioral inhibition system (BIS). The theories proposed in Leslie and colleagues review suggest that frustration is equal to fear (and subsequently avoidance) and may thereby be manipulated using known anxiolytics. For example, benzodiazepines ($GABA_B$ agonists) given during extinction learning after a continuous reinforcement (CRF) schedule results in perseveration in a straight alley runway test. In accordance with the consequences of PRF, as per frustration theory, animals given anxiolytics during training on a PRF schedule showed less resistance to extinction due to a disruption of frustration-mediated approach learning (Leslie et al., 2004).

Examinations of hypothalamic-pituitary-adrenal (HPA) axis and corticotropin releasing hormone (CRH) related mechanisms also argue for anxiety as a critical factor of cocaine withdrawal. It is known that cocaine withdrawal leads to elevated levels of anxiety (Gawin and Klever, 1986) and that CRH levels play a role in responding to

various stressors (Koob and Heinrichs, 1996). Measures of mRNA expression in the paraventricular nucleus of the hypothalamus (PVN) show decreased CRH levels immediately after cocaine administration (alleviation of stress) but no changes in expression after 5 and 10 days (Crespo et al., 2003). This suggests that anxiety during cocaine withdrawal is mediated in areas outside of the hypothalamus.

Along these lines, evidence exists to implicate the amygdala as a mediator of many of the observed behaviors during extinction training. Animals tested in an elevated plus maze showed increased anxiety profiles 48 hours after cocaine administration (Sarnyai et al., 1995). Further, CRH levels within the amygdala showed correlational increases. Inactivation of CRH in the amygdala abolished this heightened anxiety. A study by Richter and Weiss (1999) showed a 400% increase in CRH levels within the CeA after a 12-hour cocaine self-administration session and subsequent 12-hour withdrawal period. Accordingly, increased vocalizations, indicators of anxiety or stress, were evident after a 12- or 48-hour cocaine self-administration session (binge) in response to an air-puff stressor (Mutschler and Miczek, 1998). Measures of CRH responses in this prolonged access model would be of particular interest given its increasing relevance as a more realistic model of human addiction (Koob et al., 2004; Vanderschuren and Everitt, 2004).

Surely, anxiety plays a role in the motivational factors that force organisms to succumb to withdrawal. Overtly, it is obvious that use and cessation of psychostimulants results in elevated anxiety/paranoia/fear. Also, it is likely that extra-hypothalamic CRH and the amygdala are crucial in the cascade that results from cocaine abstinence. Most important, manipulation of this system shows evidence that may aid in facilitating the

"un-learning" that occurs during extinction training using stress alleviations. This arena of drug addiction research may hold promise as a means of diminishing one of the internal states that may catalyze drug seeking. Regarding these experiments, the use of frustration theory to explain many of the observed behavioral findings may certainly involve anxiogenic states. Successful manipulation of the frustrative states experienced by these animals could result in faster rates of cessation which could ultimately aid in creating effective therapies for human addicts.

Emerging Biochemical Theories

A variety of neurotransmitter systems contribute to the effects of cocaine and any involved learning mechanisms. Of the other neurotransmitters that may be mediators in processes that underlie extinction learning, glutamate seems to be critically and directly involved. The NAcc and DA are known to be involved in drug reward but it appears that glutamate may play a preferential role during extinction and reinstatement as will be detailed. Of critical importance, are the glutamatergic projections between the PFC and the NAcc that may modulate accumbens activity and are involved in choice and impulsivity. Chemical stimulation and inactivation of these areas results in behavioral changes during extinction and reinstatement.

For example, alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), but not DA, antagonists infused directly into the NAcc prevented reinstatement in animals trained to self-administer cocaine (Cornish and Kalivas, 2000), indicating a preferential role for glutamate in the activation of drug seeking. The presentation of a

cocaine prime after repeated intraperitoneal (IP) cocaine and subsequent extinction resulted in increased mPFC glutamate (Williams and Steketee, 2004) furthering this hypothesis. Tissue assays from the NAcc in animals trained to self administer cocaine showed elevated GluR1 and *N*-methyl-*D*-aspartate receptor (NMDAR) 1 subunit expression on extinction days 1 and 90, and elevated GluR2 on days 1 and 30 (Lu et al., 2003) indicating withdrawal-induced physiological change. In addition, assays from the ventral tegmental area (VTA) resulted in elevated NMDAR1 levels through day 90 and elevated GluR2 on day 1. However, Baker et al. (2003) reported decreases in extracellular glutamate-cystine exchange during withdrawal. Further, stimulation of glutamate exchange with *N*-acetylcystine abolished cocaine-primed reinstatement. As will be further detailed, glutamate activity in cortico-limbic areas is directly correlated with the negative effects of withdrawal and craving and may be ameliorated by extinction training.

Most relevant to this report, alterations in the structure and function of the glutamatergic system are occasioned by extinction training that suggest an alleviation of the changes that are associated with increased tendencies to relapse. While data regarding this subject are still not entirely clear, some intriguing trends do exist. Specifically, extinction training appears to alleviate deficits in NMDAR1 and AMPA GluR1 and GluR2/3 subunits (Self et al., 2004) originally engendered by withdrawal. Extinction training also reduced depreciations of NMDAR1 subunits as shown by gene expression (Crespo et al., 2002). Finally, extinction training up-regulated AMPA receptors and consequently reduced cocaine seeking behavior in a reinstatement test (Sutton et al., 2003). Self et al. (2004) proposes that the changes from extinction training

are indicative of long term potentiation and may be involved in inhibitory control over cocaine seeking. In other words, glutamate increases are associated with the learning of the new response that allows the animal to dissociate the lever with the availability of cocaine.

Within these studies from Self, Crespo and Sutton, withdrawal periods showed associated decrements in select glutamate receptor expression. This is not entirely in line with the previously mentioned glutamatergic findings but was consistent throughout the 3 experiments summarized. Differences in findings amongst glutamate-targeting studies could certainly be due to procedural differences, which varied greatly among experiments.

Lastly, the findings that have not yet developed into a clear role within extinction learning, but are nonetheless intuitive, will be presented within this section. Singular studies that provide insight into the directions that extinction research might take are beginning to appear more often within animal literature. For instance, increases in 5-HT_{2A} signaling within the PVN and amygdala are observed following cocaine withdrawal and may be resultant of increase G-protein signaling, an important indication of cell acitivty, rather than neurotransmitter signaling or receptor density (Carrasco et al., 2004). Results from this study indicated time dependent increases in $G\alpha_{11}$ and $G\alpha_{9}$ proteins by day 1 in the PVN and day 5 in the amygdala. Increases in 5-HT_{2A} activity in the PVN results in increased secretion of adrenocorticotropin-releasing-hormone (ACTH), coritocosterone, oxytocin and prolactin (Van der Kar et al., 2001) and may therefore be involved in the previously mentioned anxiogenic response to withdrawal and perhaps non-reward.

Zangen and Shalev (2003) examined β-endorphin levels in the NAcc during extinction training. The NAcc has a high density of opioid receptors and is innervated by arcuate nucleus of the hypothalamus β-endorphin receptors. Extinction training resulted in increased levels of the opioid peptide. Of particular importance in terms of this finding, are the similar increases observed after intermittent footshock. This indicates a possible role for β-endorphin involvement in responding to aversive or anxiety provoking stimuli (frustration).

Finally, data from Grimm et al. (2003) suggest a role for brain derived neurotrophic factor (BDNF) in cocaine craving. BDNF is involved in neural plasticity and is thought to play a role in substance abuse due to morphological changes in mesocorticolimbic systems after exposure to cocaine (Robinson and Berridge, 2003). Grimm and colleagues found increased levels within the VTA, NAcc and amygdala both during extinction training and cue-induced reinstatement after periods of withdrawal of 1, 30, and 90 days. Furthermore, BDNF levels steadily increased through day 90. A reorganization of synapses within critical areas involved with decision making and reward/reinforcement could be indicated by BDNF changes. Of great value would be any evidence that experimenter manipulations of BDNF levels resulted in behavioral changes during extinction or reinstatement.

The extinction of behaviors that formerly resulted in the presentation of drugs of abuse still needs to be fully detailed. As such, studies are exploring a variety of mechanisms, both behavioral and biochemical, which may be relevant to models of abstinence. Without question, future studies will only add to the complexity of extinction related mechanisms. Within the scope of animal research, it is critical that researchers

keep exploring this stage of drug abuse, and that models are consistently measured against human patterns of drug addiction in order to be accurate and effective.

Conclusions and Final Comments

It is of critical importance to note that the majority of the studies mentioned within this report employed doses of 1.00 mg/kg to train animals and subsequently test for various measures. It is likely that the logic was to engender maximal effects resulting from cocaine intake in order to easily measure changes. However, in light of the findings presented within this article, it must be considered that the occasioned effects at this high a dose may not be present at lower doses. Furthermore, if drastic dose-related differences do exist then the validity of these animal models is further questioned. Specifically, human drug use patterns would have to equate those of current animal models to be maximally applicable. However, the likelihood of an addict using one of the highest doses tolerable for an extended period of time, without any other drug or dose interactions throughout his/her lifetime seems very unlikely. Therefore, while current drug research data are pertinent as initial studies, they do not address the dynamics that take place in human patterns of abuse and must be interpreted carefully.

Along these lines, the definition of "extinction" throughout the cited articles is another valid concern. Criteria varied, sometimes widely, between studies and laboratories. Often, an active lever response threshold was set per hour or session. Once animals fell below this set point, they were considered to be "extinguished." Usually, a period of 7-10 days is considered sufficient. However, very few rats within these studies

ever reach zero levels of responding. Regarding our procedures, the animals employed for this report maintained very steady levels of responding for up to 10 days typically, without ever consistently reaching zero levels. Interestingly, observations from a previous THC study using intermittent saline sessions between test doses produced extinction responding that could not be terminated over the course of several weeks highlighting the difficulty of truly extinguishing a response (unpublished observations). As mentioned this may be manifested to some degree within natural reward (food, water) literature as spontaneous recovery. However, the potency and ability to enact seemingly permanent changes in the brain make self-administration extinction much more complex persistent. A suitable approach to this problem might be to quantify extinction of self-administration using a hierarchy, or "levels," rather than absolutes. In any case, there needs to be a consensus in order to better examine the mechanics of extinction and compare results effectively.

Increasingly, distinctions within these models are being made that more accurately describe the nuances of extinction. Therefore, as more data are collected on this subject, the definitions of extinction will likely be redefined to include differences between extinction training (forced abstinence) and voluntary abstinence. Further, coupled with increasingly dynamic models of acquisition, abstinence and reinstatement, extinction may eventually reveal novel and effective therapies that may aid human addicts in cessation of harmful, habitual drug use.

The value of a successful therapy for drug addiction is incalculable. To the addict it would mean a return to a normal life. Compulsive drug use destroys the health and priorities of the addict and often leads to death or incarceration. To society, a viable

therapy would alleviate the financial burden that stems from drug sale and use. Furthermore, any impact on drug related crimes would undoubtedly have a profound effect in curbing violence and illegal activities. Therefore it falls to drug addiction researchers to provide a vehicle by which to carry out discoveries. Novel and insightful investigations are essential if significant progress is to continue. Researchers must incorporate the mass of findings over the past 40 years into simple, elegant models that can function as effective and accurate testing grounds for potential therapeutics. The nature of addiction makes this a daunting task but one that is already showing progress in the right direction.

In summary, the results from Experiments 1-4 show that the extinction, or forced abstinence, phase of animal models of drug addiction is a dynamic area that can be modulated by several factors. Among the variables that changed response patterns were cocaine training dose, training length, schedules of reinforcement, and abstinence length. In addition, the applicability of Amsel's general theory of persistence provided a means by which to explain many of these effects. Furthermore, these data are important in that extinction training in animals could potentially be used as an animal model of cessation therapies for humans. A database of increasingly complex and realistic models of human addiction may provide a vehicle for discoveries in treating human addiction. The benefits of which would be incalculable to both addicts and society in general.

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Psychology

SELECTED ARTICLES

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