ASSESSING THE REINFORCING EFFECTS OF CAFFEINE: THE SELF-ADMINISTRATION OF CAFFEINE BY RATS

Shanna Babalonis

A Thesis Submitted to the
University of North Carolina Wilmington in Partial Fulfillment
of the Requirements for
The Degree of
Master of Arts

Department of Psychology

University of North Carolina Wilmington

2006

Approved by

Advisory Committee

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Chair

Accepted by

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Dean, Graduate School

Abstract

Caffeine is the most widely consumed psychoactive drug, with the prevalence of use approaching 80% of the world's population. In stark contrast to most stimulants, caffeine is considered an innocuous agent with advantageous behavioral effects. Nonetheless, the sustained use of caffeine can result in tolerance or sensitization to the pharmacologic and behavioral effects of the drug effects shared with other stimulants including amphetamine and cocaine. Moreover, unlike cocaine, caffeine abstinence results in unique withdrawal symptoms that are easily identified. The observation of withdrawal, dependence, and tolerance, notions usually associated with drug abuse, suggest caffeine consumption may provide an intriguing model of substance abuse. To this end, the aims of this work were to delineate environmental factors that establish caffeine self-administration in rats. The self-administration of caffeine was established and modified by a combination of behavioral and pharmacological factors including food restriction, drug dose, and infusion rate. The results suggest caffeine-maintained behavior is comparable to nicotine self-administration, but distinct from that of cocaine or heroin. These findings highlight the role of non-pharmacological factors in substance abuse and suggest that further investigations evaluating the reinforcing effects of caffeine can enhance the understanding and treatment of drug abuse.

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INTRODUCTION

Persistent behavior maintained by access to and consumption of a substance is a hallmark of substance abuse. Termed an operant by behavioral psychologists, substance abuse, similar to behavior maintained by other environmental events, is maintained by the response-dependent delivery of a drug. A malleable response, the characteristics of which are dependent on environmental fluctuations, operants may be empirically analyzed, parsing out the permutation of controlling variables. Behavior maintained by the delivery of a drug is also subject to the same functional analysis as responses maintained by non-drug reinforcers.

Assessing the determinants of reinforcement involves evaluating several classes of variables in order to specify the environmental elements controlling behavior. Requisites in understanding this process begin with evaluating the behavioral history of the subject and subsequently examining the specific combination of events occasioning the operant. Inferences involving the efficacy of a reinforcer may be drawn from specific elements of behavior, including rates and temporal patterns of response and resistance to change (Nevin, 1974). Although of great merit, significant problems emerge when evaluating reinforcing effects using these measures, a discussion which is beyond the scope of this paper. However, when the reinforcing event is a pharmacologic compound, the model allowing for the most direct assessment of variables involved in engendering and maintaining drug seeking, including relative reinforcing effects, is the animal self-administration paradigm.

Since its inception, empirical analyses of the self-administration paradigm have identified and clarified variables controlling drug maintained responding, The

information garnered from these analyses have delineated drugs which have high abuse liability, the specific neurochemistry which maintains responding, and many of the behavioral parameters which regulate drug intake. Moreover, rodent drug self-administration studies demonstrate these effects efficiently, with conservation of time and funds, situating the model as indispensable in drug development and research. Importantly, the model aligns with the current understanding of the phenomena while directing research to the imperative remaining questions (Massoud, Hademenos, Young, Goa, Pile-Spellman & Vinuela, 1998).

Animal Self-Administration as a Model of Drug Use

To better understand the phenomena associated with drug use in human beings, it is necessary to have an experimental analog which allows for the examination of specific aspects of behavior. An appropriate animal model should be consistent and reliable, with high degrees of control. Moreover, a model should have predictive utility, elucidating properties of behavior in a valid manner (Koob, 1995).

The animal self-administration model, namely the rodent model, has proven to be a very useful tool in the investigation of drug abuse. Over a series of studies, it has been established that drugs which are readily self-administered by rodents closely approximate the drugs which pose problems for human beings. Based upon this consistent correlation, the animal self-administration model has been indispensable in identifying abuse liability effects in new compounds. Used in pre-clinical trials, agents are tested to determine if the presentation of the novel drug will maintain self-administration. If the drug maintains self-administration, it may have abuse potential (Johanson & Balster, 1978; Koob, 1995).

Also within the scope of the model is the identification of the particular

neurochemical effects coinciding with drug consumption. Clarifying the translation between biological effects and behavioral effects, the observation of specific neuronal states during self-administration enhances the understanding of the biological underpinnings associated with drug consumption behavior.

Specifically, the rodent self-administration model has provided an experimental setting that elucidates some of the factors potentiating the reinstatement of drug consumption. The model contends that after self-administration of a drug is established and subsequently maintained, the operant is interrupted, whereby the previously reinforced response no longer produces drug presentation. Criterion for extinction are relatively arbitrary, but a common designation is that percentage of responding in the last extinction session must be a relatively low percentage of the behavior observed in the first extinction session. After sufficient exposure to the extinction contingency, animals will eventually cease engaging in the operant and the behavior is considered extinguished. This situation provides ideal experimental setting to examine the factors which set the occasion for the regeneration of responding.

Relapse, the initiation of drug use after a period of abstinence, is a prominent issue in drug rehabilitation. Understanding the mechanisms which potentiate this behavior will undoubtedly lead to better clinical treatment programs and aid in the development of effective prevention programs.

Through decisive publications concerning reinstatement of animal drug self-administration, it has been demonstrated that previously extinguished drug-maintained responding may be reinstated via three distinct stimulus presentations. Upon the termination of drug-maintained responding, the presentation of stimuli previously paired

with the drug precipitates responding (McFarland & Ettenburg, 1997; de Wit & Stewart, 1981). Discrete cues once paired with the drug or discriminative cues signaling drug availability have induced drug-seeking behavior. Speaking to both respondent conditioning effects as well as the stimulus control of an operant, these results indicate reinstatement behavior is sensitive to the permutation of environmental parameters present.

Moreover, response independent presentations of the previously self-administered compound engender the diminished operant as well. Cocaine, heroin and amphetamine self-administration are all reinstated through priming injections of the self-administered drug (Stretch et al., 1971; Davis & Smith, 1976; de wit & Stewart, 1981; 1983). Although few studies have been conducted using human subjects, there is evidence that this model of reinstatement has clinical applicability. When cocaine users are exposed to a series of choice trials between money and an i.v. infusion of cocaine, a priming dose of cocaine prior to the choice trials shifts preference toward cocaine choices earlier in the session (as compared to placebo) (Donny, Bigelow & Walsh, 2004). Moreover, drug liking reports increase prior to cocaine self-administration sessions when a priming dose of cocaine is administered (Walsh, Haberny & Bigelow, 2000). Thus, there is evidence that this model may also provide invaluable information in the development of pharmacotherapeutics to abate drug relapse.

In addition, high levels of stress may also re-initiate drug seeking. After cocaine or heroin self-administration responses are effectually extinguished, intermittent footshock presentations (0.5 to 1.0 mA) reliably reinstate “drug-seeking” behavior (Erb, Shaham & Stewart, 1996). This effect has been reported across various conditions,

including a 6-week drug-free phase, various training drugs (heroin cocaine, nicotine and alcohol) various drug training doses, strains of rats, footshock durations and intensities (Shaham, Erb & Stewart, 2000). However, presentation of conditioned stimuli or discriminative stimuli (paired with footshock or signaled its delivery) failed to reinstate self-administration behavior (Shaham et al., 2000).

When viewed in summation, the self-administration paradigm has provided a platform for uncovering the behavioral and neurochemical variables involved in drug initiation, maintenance and relapse. With continuing research, prevention methods may likely develop, along with effective pharmacotherapeutics, to help further understanding of drug abuse while simultaneously combating it.

Overview of Animal Self-Administration Research

At its inception, self-administration experimentation was primarily an evaluation of drug dependence, whereby behavior was initially maintained by attenuation of withdrawal symptoms. Pioneering work by Weeks (1962) reported sustained self-administration behavior in rats dependent on morphine. Specifically, female rats were given chronic *i.p.* injections of in a series of progressively larger doses of morphine. Doses were presented in an escalating manner, with the range of doses being 2 mg/kg to 40 mg/kg, totaling 122 injections. Subsequently, animals were placed in an operant chamber whereby depression of a lever resulted in an intravenous infusion of morphine, via a cannula implanted inside the right jugular vein. This model, in the absence of experimenter interference, allowed animals to allocate drug consumption during the experimental sessions. Consequently, rates of self-administration were dependent on the interaction between the ratio requirement (FR 1, FR 5 or FR 10) and the dose of

morphine available for IV infusion during the session (3.2 mg/kg or 10mg/kg). However, rates of behavior peaked when animals were in the state of withdrawal, induced by either an *i.p.* nalorphine injection or via extinction (lever presses resulting in no scheduled consequence). Specifically, this behavioral perseveration is often conjectured to be a model of drug seeking, with the establishing operation being withdrawal symptoms (surmised as drug craving) and the operant being maintained by negative reinforcement (the attenuation of physical symptoms).

Physical dependence, although increasing both the rapidity of which the self-administration operant is acquired, is by no means a prerequisite for engendering drug self-administration. Predicating an assessment of drugs that would commence and maintain self-administration, new studies began shifting focus in order to identify drugs readily self-administered by drug-naïve animals.

With few exceptions, drugs that maintain animal self-administration very closely align with those that pose problems for humans, stressing the model's utility as a predictive measure (Yokel & Pickens, 1973; Weeks & Collins, 1987; Yanagita & Takahashi, 1982). Incorporating subjects from various species, many early studies have examined numerous psychoactive compounds to determine if the agents were able to maintain self-administration, providing information about the abuse liability of the drugs (van Ree et al., 1978; Collins et al., 1984; Yokel, 1987).

A drug that initiates and maintains self-administration is generally considered to have reinforcing effects. However, the apparent self-administration of a drug can stem from other effects not directly related to reinforcing actions. Substantiating that drug presentation is the event maintaining behavior, an essential measure is to ensure that

responding eventually cease when the operant does not produce a drug infusion. When vehicle is substituted for the drug under examination, there should be an eventual, marked decrease in the response (Meisch, 1987; Yokel, 1987).

Further evidence that drug presentations are maintaining the operant can be provided when measuring the allocation of responses between an active manipulandum and an inactive device. The active manipulandum, a response on which produces drug infusion, should engender proportionally greater rates of response than the inactive device (behavior upon which carries no scheduled consequence) (Pickens & Thompson, 1968; Meisch, 1987; Katz, 1989). However, this measure must be employed cautiously. Misinterpretations of these data may occur if the doses of the agent under examination acts as central nervous system stimulant. General locomotor activity may increase, producing increased responding on the inactive lever, due to the direct effects of the agent, (with direct effects referring to the drug's effect on the central nervous system and the physiological effects that ensue). These direct effects may modify behavior independent of the indirect effects, with responding on either lever being artificially increased, due to the effects of the drug under analyzation. The indirect effects (also termed reinforcing effects) of the drug may be difficult to interpret due to this effect. Therefore, additional control methods are necessary to determine the underpinnings of the behavioral maintenance.

Random alternations of the active device with the inactive, within or between experimental sessions, ought to produce "tracking" of the lever or key associated with drug infusions (Pickens & Thompson, 1968). The allocation of responses should

eventually exhibits exclusivity for the active device, regardless of its proximal position (Pickens & Thompson, 1968; Corrigall & Coen, 1989).

Another property supporting the conjecture that behavior is indeed controlled by response-dependent drug presentations is a dose-dependent change in self-administration responding. Rates of responding or number of infusions obtained, (typically synonymous measures), should follow as a direct function of the dose of drug available. The function obtained is generally an inverted U-shaped function (Katz, 1989). Thus, low doses of the drug maintain relatively low rates of responding. As the dose increases, rates of responding escalate as well, with responding peaking when a "moderate" dose is available. From this point, increases in dose result in decreases in the operant. As a result, relatively lower and higher doses maintain similar rates of behavior and approximately the same number of infusions are self-administered. However, some drugs, namely nicotine and caffeine, have relatively flat dose-response curves within self-administered doses. With only a narrow range of doses that will maintain behavior, rates of responding are generally low, do not fluctuate to a great degree and will diminish outside the specific doses (Dworkin & Stairs, 2002).

Additionally, drug presentations non-contingent on behavior (pre-session or within-session injections of a drug, within- or between-subject yoked infusions) should not sustain the operant (Pickens & Thompson, 1968; Meisch, 1987). Of specific concern when psychomotor stimulants are being assessed, this measure beings to demarcate drug preference from the generalized increases in locomotor activity and direct effects of a drug (Yokel, 1987).

In concert with the aforementioned measures, the maintenance of behavior under intermittent schedules of reinforcement also bolsters conclusions about reinforcing effects (Weeks and Collins, 1987). Although some events, still considered reinforcers, do not maintain stable rates of behavior when schedule requirements exceed low ratios. However, reinforcing efficacy may be generally inferred through maintenance of steady rates of behavior when schedule parameters increase work requirements or reinforcers become less frequent.

Finally, administration of an agent that antagonizes the central action of the self-administered compound can help clarify the mechanism of behavioral maintenance. When a non-competitive, centrally acting receptor antagonist is administered, drug-maintained responding will eventually cease if drug presentation was in fact the event maintaining behavior (Yokel, 1987).

Variations in sensitivity to the behavioral effects of a drug are steeped in environmental conditions and particular permutations of stimuli (Dews, 1955). Behavioral history and previous drug exposure can affect the biological and behavioral effects of a drug as well as changing the rates of acquisition and maintenance of drug self-administration. Moreover, conditions present at the time of drug administration (stress levels, deprivation level, exteroceptive stimuli changes, schedules of reinforcement, experimental paradigm) are also influential in behavioral reactivity to drug effects. Environmental arrangements occurring after drug exposure (discriminative stimulus effects, behavioral requirements) can also impact behavior at the time of administration

Subjected to parametric variation from numerous sources, a drug noted for its behavior maintaining qualities, specifically nicotine, can dually serve as a presumably aversive stimulus as well, as evidenced by behavior maintained by postponement of or active avoidance of drug infusions of identical dose that also maintained behavior (Goldberg & Spealman, 1983, 1982; Spealman, 1983). Thus, the capacity for a drug to maintain and subsequently strengthen behavior appears not as a static effect, but rather a dynamic feature dependent on the confluence of a host of behavioral and pharmacologic elements.

Because behavioral maintenance via drug presentation is highly sensitive to pharmacological and environmental specificities, some drugs, although consumed at extremely high rates by human beings, initially do not seem to find parallel in an animal model. Despite over one billion people worldwide abusing nicotine (Vainio, Weiderpass, & Kleihues, 2001), the chemical in tobacco determined to maintain its consumption (Stolerman & Jarvis, 1995), it failed to consistently serve as a reinforcer in the animal self-administration paradigm (Griffiths, Brady, & Bradford, 1979; Dworkin, Vrana, Broadbent, & Robinson, 1993) until the mechanisms potentiating its reinforcing properties were fully elucidated. The initial published report of intravenous nicotine self-administration was conducted using squirrel monkeys with their behavior maintained under a second-order schedule (FI 1 min (FR 10:S)) (Goldberg et al, 1981). The schedule specified that each ratio completed under a FR 10 schedule result in a brief visual stimulus (flash of a cue light inside the chamber), with the first FR 10 requirement completed after 1 minute producing both a brief stimulus presentation as well as a infusion of nicotine (30 mg/kg). Behavior was maintained at high rates, the average being

roughly one response per second. The operant was nearly eliminated when either saline was substituted for nicotine or when a mecamylamine injection, a nicotinic antagonist, was administered prior to the session. Responding diminished to nearly half the aforementioned rate when the brief stimulus presentation was omitted (upon completion of each FR 10). Seminal not only in terms of demonstrating an instance of behavioral maintenance through nicotine presentation, but also because the results underscored the importance of conditioned stimuli, potentiating behavior of an otherwise operantly ineffectual drug.

Succeeding studies, exploring the conditions that more readily expose the reinforcing properties of nicotine, outlined specific parameters lending to its reinforcing efficacy. Of specific concern has been the level of food deprivation necessary to maintain self-administration. Carroll et al. (1979, 1984) stressed the influence of food deprivation as an imperative variable when examining drug maintained behavior, especially that of central nervous system stimulants (Carroll, France, & Meisch, 1979; Carroll & Meisch, 1984). Moreover, Dworkin et al. (1993) notably delineated the influence of concurrent availability of non-drug stimuli, food and water, to effectually decrease responding maintained by nicotine.

Another significant demonstration was the identification of specific experimental variables that collectively increase nicotine-maintained responding (Corrigall & Coen, 1989; Donny, Caggiula, Knopf, & Brown, 1995). To achieve relatively high, stable rates of behavior experimenters first established and maintained the operant through food presentation rather than drug presentation. The animals, male Sprague-Dawley rats, were food deprived 18 to 24 hours prior to each session, with a supplemental diet consisting of

20 grams of food per day (allowing for 133% body weight increase over 5 weeks). A narrow range of doses were available during experimental sessions (.003, .030, .060 mg/kg/inf), the latter two maintaining behavior above that of vehicle (approximately 130 responses or 20 infusions per hour, under a FR 5). Temporal variables were imparted to further furnish an environment occasioning nicotine self-administration: limited access to the drug (sessions terminated after an hour) and a 60-second timeout situated immediately after the reinforced response. Finally, the role of conditioned stimuli was further established. Experimental conditions provided a flash of a cue light coincident with each target response and reinforcer delivery as well as a compound stimulus during timeout periods (simultaneous tone presentation and ambient light alteration) (Corrigall & Coen, 1989; Donny et al., 1995). Comprehensively instantiated, the aforementioned variables both mitigate prior issues of nicotine reinforcer efficacy and provide an established model of nicotine self-administration upon which further manipulations may be subjected.

With an archetypal mainframe to demonstrate reinforcing effects, it is the focus of the current study to accordingly establish caffeine self-administration. However, as the case with initial nicotine self-administration studies, attempts at behavioral maintenance through caffeine infusions resulted in limited success.

Overview of Caffeine Self-Administration

Specifically, reports of the inability of IV presentations of caffeine to serve as reinforcing event in rodents have been documented in the literature (Atkinson & Enslen, 1976; Briscoe et al, 1998; Collins et al, 1984). Atkinson & Enslen (1976) first explored this topic with an experiment whereby animals were split into two groups: one group

receiving injections of increasing doses of caffeine for up to 98 hours prior to the session and the other group receiving no pre-treatment. When placed into an operant chamber in which lever presses resulted in an IV infusion of caffeine, animals from the pre-treated group self-administered caffeine for 3 to 4 days above the rate that was maintained by saline infusions. However, the animals that received the saline pre-treatment never acquired the operant (Atkinson & Enslen, 1976).

Moreover, Briscoe and colleagues (1998) initially maintained self-administration behavior in male Sprague Dawley rats via 0.5 mg/kg/inf IV cocaine presentations. These sessions were followed by a series of trials which caffeine, ephedrine or their combination was substituted for cocaine. After stable self-administration behavior was observed under .5 mg/kg/inf cocaine availability, each animal was randomly assigned to receive one particular dose of caffeine (.25, .5, .75 and 1.0 mg/kg/inf), ephedrine (.25, .5, .75 and 1.0 mg/kg/inf), or an infusion of their combination (.25 mg/kg/inf caffeine and .125 mg/kg/inf ephedrine; .5 mg/kg/inf caffeine and .25 mg/kg/inf ephedrine; .7 mg/kg/inf caffeine and .5 mg/kg/inf ephedrine; or 1.0 mg/kg/inf caffeine with .7 mg/kg/inf ephedrine). Each dose as well as vehicle was tested for 3 days subsequent cocaine self-administration, with eight animals being assigned to each dose. Neither caffeine nor ephedrine was able to maintain behavior above levels engendered by saline infusions. However, on the first day of substitution, 3 doses of the caffeine-ephedrine solution (.25+.125; .5+25; .7+.5) maintained behavior significantly greater than those observed under saline availability. Nonetheless, this effect was specific to the first day of the compound's availability, with behavior during successive sessions resembling behavior maintained by saline infusions (Briscoe et al., 1998). Additional substitution

testing with permutations of different doses of the caffeine and ephedrine compound revealed the same general trend. Three doses of the compound maintained high levels of self-administration behavior (.7+.5 and .7+.7 mg/kg/inf ephedrine + caffeine; .7+.5 mg/kg/inf caffeine+ ephedrine), but, again, this tendency was confined to the first session of substitution, declining almost immediately upon the following session (Briscoe et al., 1998).

Echoing the same inconsistencies, Collins and colleagues (1984) implemented a FR 1 self-administration schedule where 1.0 mg/kg/inf IV caffeine was available for infusion. The operant was established through cocaine presentations and when caffeine was substituted, highly variable behavior ensued. The caffeine dose was then eventually decreased to .1 mg/kg/inf in an attempt to re-establish and solidify responding. Although two of the rats continued to respond, they did so in an erratic manner, whilst the remaining four animals ceased responding completely (Collins et al., 1984).

Caffeine self-administration has been consistently reported as an ever-fluctuating, highly variable behavior, regardless of the species studied. Several non-human primate studies where caffeine was available under IV self-administration conditions have all presented either extreme with-in subject, with-in condition variability (Deneau, Yanagita & Seevers, 1969; Griffiths, Brady & Bradford, 1979), high degrees of between-subject variability (Schuster, Woods & Seevers, 1969) inability for the drug presentation to maintain the operant (Yanagita, 1970; Hoffmeister & Wuttke, 1973), or have necessitated experimenter-induced injections to initiate responding (Deneau et al, 1969; Schuster et al., 1969).

Moreover, results obtained from studies examining oral self-administration of caffeine have been equivocal at best. In a bottle choice condition with drug-naïve rats, preference for caffeinated water over non-caffeinated water was only evident when the concentration of caffeine was very low, resulting in negligible cumulative caffeine intake (Falk, Yosef, Schwartz & Lau, 1999). However, after an imposed 14-day "forced choice" of highly concentrated caffeinated water (the only hydration available), the animals consistently chose caffeinated water under "free-choice" conditions. Although still of great import, this situation may imply a choice occasioned by caffeine dependence or choice mitigated by negative reinforcement conditions rather than simple caffeine preference. These results also necessitate the delineation of the degree to which the discriminative stimulus effects of caffeine and the conditioned reinforcing effects of its presentation influence self-administration of the compound.

Notwithstanding the inability to consistently showcase the presentation of caffeine as a behavior-maintaining event, other paradigms have been implemented to reveal other reinforcing effects of the drug. Although not without criticism for the implications its results involve, conditioned place preference experiments have been used to superficially asses a drug's reinforcing efficacy through observation of choice behavior via spatial location preference. The trials involve a choice between an environment which has previously been paired with the discriminative stimulus effects of a particular drug and an environment which has been paired with saline or a competing drug. Inferences involving drug preference or the reinforcing properties of the elicited conditioned effects of the drug are generally concluded through the relative amount of time spent in a drug-

paired environment as compared to the alternative or via the percent of animals exhibiting the place-preference (or alternatively an aversion).

Although few studies have been conducted using caffeine as a target drug, some evidence has emerged to suggest that place preference may be sensitive to some of the reinforcing mechanisms of caffeine. At a low doses (.32, 1.0, 1.5, 3.2, 5.6 and 10 mg/kg, *i.p.*), caffeine was found to serve to occasion place preference (Patkina & Zvartau, 1998; Bedingfield, King & Holloway, 1998). However, at higher doses tested (12, 25 and 50 mg/kg, *i.p.*) it was found to potentiate place-aversion behavior (Patkina & Zvartau, 1998). To further elucidate caffeine's reinforcing effects in relation to other drugs, it has been used as a challenge drug, pinned against both ethanol and cocaine. When animals, previously exposed to both drugs, were given a choice between a cocaine-paired environment (5 mg/kg, *i.p.*) and a caffeine-paired environment (1.5 mg/kg, *i.p.*), all of the eight animals tested chose the cocaine-paired environment on every trial (Patkina & Zvartau, 1998). However, when animals were given a choice between an ethanol-paired compartment (1.2 g/kg, *i.g.*) and a caffeine-paired environment (1.5 mg/kg, *i.p.*), equal time was spent in each environment, across all trials, implying no preference between the treatments. Therefore, the conditioned place preference paradigm reveals some of the dose-related reinforcing properties of caffeine and provides a relational comparison in the context of other drugs.

Effects of Caffeine on Schedule-Controlled Behavior

Although limited research has been conducted concerning the effects of caffeine on schedule-controlled behavior, it is nonetheless a key aspect in a discussion of the behavioral effects of caffeine.

Caffeine administration has been shown to have significant effects on schedule-controlled behavior. When acutely administered, caffeine has dose-dependent effects on rates of behavior. Specifically, when behavior of squirrel monkeys is maintained on a FI 180 s schedule of food presentation, a moderately low dose (1.5 mg/kg, *im*) significantly increased rates of responding in all animals at an average of 140% of that observed under control conditions. However, after a relatively high dose (28.0 mg/kg, *im*), behavior was significantly decreased, with rates of behavior declining to 20% of the rate emitted under control conditions (Katz and Goldberg, 1987). Moreover, chronic caffeine administration did not seem to reliably affect behavior when administered after the experimental session, but when chronically administered with injections occurring prior to sessions, the same doses which increased and decreased behavior under acute administration had similar effects on behavior (Katz and Goldberg, 1987). In fixed ratio schedules of food maintained behavior, low doses of caffeine have little effect, while high doses (30 and 56 mg/kg) greatly reduced rates of responding (Glowa and Spealman, 1984). These results have been supported by similar findings, whereby acute administration of 10.0 mg/kg, *im* caffeine have increased rates and 30.0 mg/kg decreased rates of behavior under a FI 300 s shock avoidance schedule (Howell, 1993).

Decreases in food maintained behavior are also evident when caffeine is chronically administered to rats. When administered caffeinated drinking water (3 mg/ml) rates of behavior maintained under a FI 5 minute schedule of food presentation were temporarily decreased. Peak effects were characterized by a 60% decrease in response rates and 30% decrease quarter-life values and occurred 72 hours after caffeinated water availability. However, these effects soon diminished and complete

tolerance developed after peaked 5 days of caffeine exposure (Gasior, Shoaib, Yasar & Goldberg, 1998).

Caffeine also seems to alter schedule induced responding. When Sprague Dawley rats were exposed to a FI 90 s schedule of food presentation and given free access to a water spout located above the lever, caffeine differentially and dose-dependently affected the observed behaviors. When baseline rates of lever pressing were high, caffeine did not increase rates of behavior at any dose tested (10, 30, 56, 100 mg/kg), but decreased rates at higher doses (56 mg/kg, 100 mg/kg). When lever pressing baseline rates were lower, none of the doses tested altered rates of lever pressing. Regardless of an animal's baseline rates, the high dose of caffeine (100 mg/kg) suppressed licking behavior in all animals, however low doses (10, 30 and 56 mg/kg) were able to decrease licking in some animals (McMillan, 1979).

Demonstration of the Reinforcing Effects of Caffeine in Humans

Despite the presence of equivocal evidence emerging out of animal laboratories, research concerning the behavioral effects of caffeine seems to be less ambiguous. The reinforcing effects have reported in a variety of experimental settings (Griffiths & Mumford, 1995).

Aligning with basic behavioral pharmacology principles, caffeine adheres to an inverted U-shaped function in human self-administration settings. Thus, low doses of caffeine (25 mg per cup of coffee) maintain consumption at low levels, but at greater rates those engendered by control (decaffeinated coffee) (Oliveto, Hughes, Pepper, Bickel & Higgins, 1991). This behavioral maintenance was consistent among subjects, regardless of the amount of caffeine they normally ingest. Moreover, when 50 or 100

mg/per cup coffee was available, rates of consumption increased, with more caffeine being ingested by a greater percentage of the subjects (Oliveto et al., 1991). In concordance, when doses above 100mg/unit were available for self-administration, rates of behavior decreased as a function of increasing dose (100 to approximately 350 mg), with very high doses per unit (400 mg and higher) prompting avoidance behavior (Griffiths & Woodson, 1988).

Choice and consumption behavior are sensitive discriminative stimulus effects of the dose consumed as well. Evans & Griffiths (1992) conducted an experiment using a series of choice trials. Free choice trials noted preference between a caffeinated and non-caffeinated beverages, while forced-choice trials were present to ensure subjects experienced both options. Their data reflected the same aforementioned trend of a dose-dependent behavior. However, after examining the data, it was subsequently discovered that there was a strong relationship between those subjects who chose the caffeinated beverage and their reports of positive subjective effects after consuming the caffeinated beverage. Moreover, these same subjects were more likely to have reported negative subjective effects after forced-choice trials where the non-caffeinated beverage was presented. Likewise, the subjects who consistently chose the non-caffeinated beverage where those subjects who reported aversive subjective effects upon forced consumption of the caffeinated beverage (Evan & Griffiths, 1992).

Although somewhat dismissed by the animal literature, there has been equivocal evidence that the behavioral requirements following choice trials may influence self-administration of caffeine (Silverman, Mumford & Griffiths, 1994). As is the case with other drugs (cocaine, d-amphetamine and methylphenidate), caffeine self-administration

is sensitive to the conditions that follow drug availability (Stoops, Lile, Fillmore, Glaser & Rush, 2005). Therefore, the implication is that self-administration behavior of certain stimulants may increase when a task requiring attention or vigilance is necessary.

Another factor influencing caffeine self-administration is dependence. Although the mechanism is unclear, withdrawal symptoms may have discriminative stimuli effects which potentiate caffeine consumption. Moreover, subsequent to ingestion, subjects report caffeine administration as assuaging the aversive subjective effects of withdrawal while concomitantly increasing the subjective effects of the dose consumed (Griffiths, Bigelow & Liebson, 1989; Hughes, Hunt, Higgins, Bickel, Fenwick & Pepper, 1992; Hughes, Oliveto, Bickel, Higgins & Badger, 1993).

Emerging evidence suggests that the presence of withdrawal symptoms do not exclusively set the occasion for caffeine consumption. When partial deprivation is experimentally induced, the effects are incongruous with the aforementioned findings. While complete deprivation was associated with the subjective effects of withdrawal, partial deprivation did not occasion significant withdrawal symptoms. Regardless, neither partial nor complete deprivation prompted a substantial increase in self-administration, lending to the conclusion that deprivation nor aversive subjective effects solely potentiate caffeine consumption (Mitchell, de Wit & Zancy, 1994). Therefore, as evidenced by the literature from the human laboratory, caffeine availability serves as a reinforcing event under specific circumstances.

Pharmacology of Caffeine

At behaviorally active doses, caffeine, a neuromodulator, has multiple effects on the central nervous system. The primary action of caffeine is one of adenosine receptor

blockade, competitively inhibiting the binding of adenosine receptor ligands (Snyder, Katims, Annau, Bruns & Daly, 1981). Hence, the behavioral effects of adenosine analogs are effectively antagonized by caffeine in a manner that implies competitive interaction at the receptor level, inferred through a rightward shift in the adenosine dose-response curve (Barraco, Coffin, Altman, & Phillis, 1983; Coffin & Spealman, 1987).

Of the four adenosine receptors identified in human brains, $A_{1,}$ $A_{2A,}$ $A_{2B,}$ and A_3, caffeine exhibits the greatest amount of affinity for A_{2A}, A_1 and A_{2B} receptors, with the antagonism potency being most complete at the A_{2A} receptor (Fredholm, Battig, Holmen, Nehlig, & Zvartau, 1999; Daly & Fredholm, 1998). The primary behavioral correlate of adenosine antagonism is a dose-dependent heightening of locomotor activity, an enhancement of which is four-fold of that under vehicle-treatment conditions, albeit exhibited with less consistency than that of amphetamines (Snyder et al., 1981). Moreover, as previously mentioned, administration of methylxanthines produces an increase of schedule-controlled behavior in both rodents and monkeys (McKim, 1980; Glowa & Spealman, 1984; Spealman & Coffin, 1988). Further behavioral support of the aforementioned action is evident in A_{2A} knock-out mice, (animals which do not have the receptor), upon which a caffeine injection produces only behavior suppressing effects, similar to that of an agonist (Ledent, Vaugeois, Schiffmann, Pedrazzini, El Yacoubi, Vanderhaeghen, 1997).

Additional effects, non-centrally generated, are hypothesized as resultant effects of antagonism of endogenous adenosine and include lipolysis (the breakdown of fat stored in adipose tissue cells), increased heart rate, increased release of catecholamines, and increased renal blood flow (Fredholm, 1985).

Previously suggested to be a function of benzodiazepine receptor blockade, the effects of methylxanthines, particularly caffeine, are significantly more potent at adenosine receptors than benzodiazepine receptors (reported as much as 100 times so) and a correlation between the activation of the two mediated by methylxanthines has been disregarded (Snyder et al, 1981).

Of great behavioral and biological import is caffeine's secondary action of dompaminergic activity enhancement. Mediated through adenosine antagonism, this interaction appears to be a function of the colocalization of the two receptors (Ferre et al, 1992; Garrett & Griffiths, 1996), with dense populations of A_{2A} receptors found in the caudate/putamen, nucleus accumbens, and tuberculum olfactorium (Daly & Fredholm, 1998). Thus, A_{2A} receptors are strictly positioned in dopamine-innervated areas and follow the same postsynaptic arrangement as postsynaptic D_2 and D_1 type receptors (Ferre et al., 1991, 1992). This colocalization has been purported to induce two types of interactions between the two receptor sub-types: a decrease of D_2 agonist affinity via activation of A_{2A} receptors and a decrease of cyclic AMP (induced through A_{2A} receptor stimulation) through the activation of D_2 receptors (Ferre et al., 1992). Thus, an agent with properties of A_{2A} receptor antagonism, such as caffeine, may both intensify the potency of endogenous dopamine, an effect specific to D_2 receptors, and diminish the agonizing effects of cyclic AMP on GABAergic neurons (Daly & Fredholm, 1998).

Translating this biochemical interaction into behavioral terms has resulted in research which garners support for a dompaminergic mediation of the ensuing behavior upon caffeine administration. Akin to other drugs which principally act as dopamine agonists, caffeine increases locomotor activity, with these effects attenuated by selective

D_1 and D_2 receptor antagonists (Garrett & Holtzman, 1994a). Accordingly, animals which display locomotor activity tolerance to caffeine exhibit cross-tolerance behavior to both D_1 and D_2 receptor agonists (Garrett & Holtzman, 1994b). Moreover, when dopamine synthesis is inhibited, caffeine-induced locomotor activity enhancement is likewise diminished (Finn, Iuvone & Holtzman, 1990; Garrett & Griffiths, 1996).

In consideration of the aforementioned research implications, it is the focus of the current study to effectively establish a caffeine dose-response curve, utilizing a rodent self-administration model to attempt to further qualify IV caffeine presentations as a behavior maintaining event, stressing the importance of both behavioral and pharmacological variables.

METHOD

Subjects

Serving as subjects were 32 experimentally naïve, drug naïve, male Sprague-Dawley rats (Harlan Intl., Indiana, IN). The animals were approximately 3 months old at the beginning of the experiment and were kept at 75% of their free-feeding weight (approx. 250-350 g), with supplemental feedings consisting of 15-20 grams. Individually housed, the animals were kept in conditions which provided constant free access to water and saline catheter flushes every hour. Animals were housed in a temperature and humidity controlled environment with lights operating on a 12:12 reverse light/dark cycle (lights on 1900 to 0700 hours). The experimental facility was accordance with the guidelines of the Institutional Animal Care and Use Committee and the Guide for Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources.

Surgery

Under sodium pentobarbital (50 mg/kg, *i.p.*) and atropine methyl nitrate (10 mg/kg, *i.p.*) anesthesia (SIGMA Chemical Co., St. Louis, MO), a chronic in-dwelling intravenous catheter was implanted into the rats' right jugular vein. Passed subcutaneously and secured to the vein with silk sutures, the catheter exited through the animals back via a protective back-plate covering (composed of Nalgene® plastic, Teflon, plastic screws and stainless steel covering). The external portion of the catheter was protected by a stainless steel leash, attached to both the back-plate and to a liquid swivel. Post-operatively, tetracycline and heparinized saline (.05ml) were administered (*i.v.*). Catheter patency was ensured by an *i.v.* infusion of sodium methohexital (Brevital,

0.05 ml/injection), conducted at least once every 3 days, 2 hours prior to the experimental session.

Drug

Caffeine anhydrous ($C_9H_{10}N_4O_2$; 1,3,7-trimethylxanthine), obtained from SIGMA Chemical Co. (St. Louis, MO) was dissolved in physiological saline and made available in doses of .400 mg/kg/inf; .625 mg/kg/inf; and .750 mg/kg/inf.

Apparatus

Experiments were conducted operant chambers encased in sound attenuating receptacles (MED Associates, St. Albans, VT). The exterior of each chamber, specifically modified to accommodate drug-self administration, was equipped with a high-speed microliter drug syringe pump (MED Associates, model PHM-103), a counterbalanced arm (designed to provide the appropriate pressure on the catheter's encasing material), a food pellet dispenser (Gerbrands, model G5100), a tone generator, a 28 V houselight, and a ventilation system. Each chamber, constructed of stainless steel side walls and Plexiglas anterior and posterior walls and ceiling lid, displayed exterior dimensions of 23 cm x 21.5 cm x 21.25 cm (MED Associates). The interior of the chamber (21.75 cm x 20.25 cm x 20 cm) consisted of a gridded floor area (comprised of 15 cylindrical metal bars, spaced 1.25 cm apart) and was equipped with two levers, each located 3 cm above gridded floor. One lever (designated the active lever, initiating either the drug pump or the feeder) was located on the right wall, with a green cue light located 5 cm directly above the lever and the food aperture located 5 cm to the left of the lever. The inactive lever (responses upon which were recorded but had no scheduled consequence) was located on the left wall, 4

cm from the chamber's posterior wall, and had a red cue light located 5 cm above the lever.

Procedure

Prior to surgery, lever pressing behavior was shaped and maintained by food pellet presentation (45mg), (active lever responses only). The initial schedule, a FR 1, gradually increased until the terminal schedule of reinforcement, FR 10, was reached and subsequently maintained. Ambient chamber conditions were: an extinguished houselight, an illuminated green cue light above the active, an illuminated red light above inactive lever, with white noise present. Each lever press response was accompanied by both a brief (.5 seconds) cue light extinguishment and a feedback click. After each food pellet delivery, a 60 second time-out ensued, characterized by a tone presentation and illumination of the chamber via the 28v houselight, with both stimuli presented for the full 60 seconds of the time-out period. Responses during timeout periods had no scheduled consequence, but were recorded. The same held true for inactive lever responses during the session and in time-out periods. Sessions were 60 minutes in duration with no limit on the number of reinforcers obtained.

Subsequent to surgery, caffeine was available under a FR 1 schedule of *i.v*. drug presentation (50 ml of caffeine, 100 ml/sec). Responding produced the same permutation of stimuli as the food maintained behavior, with the only difference being the initiation of the drug pump instead of the firing of the feeder. Doses of caffeine were: .400 mg/kg/inf; .625 mg/kg/inf; and .750 mg/kg/inf (with heparinized saline used as vehicle for probe trials).

The self-administration behavior of two groups of animals was examined. One group of animals (*n*=4) were first given access to .400 mg/kg/inf initially, then when the behavior of each animal stabilized, the dose was switched to .750 mg/kg/inf availability. The other group of animals (*n*=4) began with .750 mg/kg/inf availability and were subsequently given access to .625 mg/kg/inf. Probe trials, in which saline was substituted for caffeine for the entire duration of the session, were implemented when an animal displayed consistent self-administration of the dose currently available for administration. Novel dose availability or saline probe sessions were instantiated when a particular animals' behavior had stabilized and the current dose was reliably self-administered. Criteria for stability were based upon review of each animals' data for: a least five days of relatively consistent self-administration behavior, behavior which did not approach levels resembling behavior under saline availability, or responses on the active lever proportionally greater than those on the inactive lever once reliable self-administration was observed. Moreover, it was also considered essential to observe an appreciable decrease in behavior upon saline substitution before reintroducing a dose of caffeine, with probe trials being presented to each animal at least once during the series of sessions in which a particular dose of caffeine was available.

After each drug self-administration session, while animals were still experimental chamber, catheters were flushed with .1 ml of heparinized saline to clear the drug completely from the line. Animals were then immediately placed into home cages and their catheters connected to saline pumps. One hour afterward (post-session), all animals were fed 15 to 20 grams of Lab Diet rat food, depending on their weight.

RESULTS

Effect of Dose & Schedule on Number of Infusions

Each group's (*n*=4) data was analyzed using a separate Two-Way ANOVA, analyzing the main effects of dose and schedule on the number of infusions obtained. The first group was exposed to the following conditions: 0.4 mg/kg/inf under a FR 1, 0.75 mg/kg under a FR 1 and FR 2, and saline under FR 1. There was a significant effect of dose on the number of infusions self-administered under a FR 1 schedule (F (3, 62)= 14.712, $p < .05$). There was also an effect of schedule under the 0.75 mg/kg/inf condition (F (1, 32) = 7.956, $p <.05$). Holm-Sidak Pairwise Multiple Comparison revealed that each dose under a FR 1 schedule maintained responding at a level significantly different from that of vehicle under a FR 1 schedule (.04mg/kg/inf dose: (t=2.225, p=.027); 0.75 mg/kg/inf: (t=4.219, p=.02)).

The second group's (*n*=4) data was also analyzed using a Two-Way ANOVA, examining the effects of dose and schedule on number of infusions. This group was exposed to the following conditions: 0.625 mg/kg/inf under both FR 1 and FR 2, 0.75 mg/kg/inf under FR 1 and FR 2, and saline under FR 2. There was a main effect of dose (F (2, 59) = 13.21, $p < .05$), a main effect of schedule (F (1, 48), $p < .05$), and an interaction between schedule and dose (F (2, 76) = 10.92, $p < .05$). A Holm-Sidak Pairwise Multiple Comparison delineated the effects. The .625 mg/kg/inf dose maintained behavior to a significantly greater degree than the .75 mg/kg/inf dose (t=5.119, $p < .01$) under a FR 1 schedule. There were no differences detected between the two doses under an FR 2 schedule and neither dose was significantly different from vehicle FR 2 conditions (0.625 mg/kg/inf: t = 1.231, $p > .05$; 0.75 mg/kg/inf: 1.002, $p <$

.05). There was a significant interaction between dose and schedule (F (1, 45) = 8.071, p < .05) so that behavior observed under the .625 mg/kg/inf FR 1 was significantly different from all other schedule permutations (t = 4.44, p < .05).

Effect of Dose & Schedule on Pause Duration

Again, each group's (n=4) data was analyzed using a separate Two-Way ANOVA, examining the effect of schedule and dose on pause duration. The first group was exposed to the following conditions: 0.4 mg/kg/inf under a FR 1, 0.75 mg/kg under a FR 1 and FR 2, and saline under FR 1. There was a significant effect of dose on the pause duration under a FR 1 schedule (F (3, 62)= 12.933, p < .05). There was also an effect of schedule on pause duration under the 0.75 mg/kg/inf condition (F (1, 32) = 7.956, p <.05), with the FR 2 condition occasioning longer pauses than those observed under the FR 1 condition. Holm-Sidak Pairwise Multiple Comparison indicated that each dose under a FR 1 schedule induced pausing that was significantly shorter in duration than pausing observed under vehicle FR 1 conditions (.04mg/kg/inf dose: (t=2.225, p=.027); 0.75 mg/kg/inf: (t=4.219, p=.02)).

Pausing behavior was analyzed via a Two-Way ANOVA for the data of second group (n=4) as well. There was a main effect of dose (F (2, 59) = 12.82, p < .05), a main effect of schedule (F (1, 48) = 10.952, p < .05), and an interaction between schedule and dose (F (2, 76) = 11.014, p < .05) on pause duration, A Holm-Sidak Pairwise Multiple Comparison was used to identify the specific results. The .625 mg/kg/inf dose occasioned a significantly shorter pause than the .75 mg/kg/inf dose (t=4.53, p<.05) under a FR 1 schedule. Moreover, both doses engendered pauses that were significantly different than those observed under vehicle conditions (.625 mg/kg/inf: t = 6.781, p < .01;

.75 mg/kg/inf: 4.441, $p < .05$). There were no differences in pause duration detected between the two doses under an FR 2 schedule, but behavior under both doses was significantly different from behavior under vehicle FR 2 conditions (0.625 mg/kg/inf: $t = 3.946$, $p < .05$; 0.75 mg/kg/inf: $t = 2.75$, $p < .05$). There was a significant interaction between dose and schedule so that mean pause duration observed under the FR 1 schedule implementing the .625 mg/kg/inf was significantly different from all other schedule permutations (F (1, 45) = 7.923, $p < .05$).

Effect of Dose & Schedule on Cumulative Consumption

Figure 5 presents cumulative caffeine intake as a function of both the dose available for self-administration and the ratio requirements instantiated. Expressing the average amount of caffeine consumed per session in milligrams, this graph simply converts the infusion data in Figure 1 to convey average intake levels. Each group's (n=4) data was analyzed using a separate Two-Way ANOVA. When analyzing the data from the first group, there was no significant effect of dose detected on the amount of caffeine self-administered under a FR 1 schedule (F (1, 46)= 4.322, $p > .05$). However, there was an effect of schedule under the 0.75 mg/kg/inf condition (F (1, 32) = 7.956, p <.05), with the FR 1 schedule occasioning more caffeine consumption than the FR 2 schedule.

When analyzing the second group's data, there was a main effect of dose (F (1, 35) = 11.452, $p < .05$), a main effect of schedule (F (1, 48) = 10.74, $p < .05$), and an interaction between schedule and dose (F (2, 76) = 10.677, $p < .05$) on amount of caffeine consumed. A Holm-Sidak Pairwise Multiple Comparison delineated the effects. The .625 mg/kg/inf dose engendered total consumption to a significantly greater degree

than the .75 mg/kg/inf dose (t=5.119, $p < .01$) under a FR 1 schedule. There were no differences detected between the two doses under an FR 2 schedule (0.625 mg/kg/inf: $t = 1.031$, $p > .05$; 0.75 mg/kg/inf: 2.313, $p > .05$). There was a significant interaction between dose and schedule (F (1, 45) = 8.071, $p < .05$) so that total consumption observed under the .625 mg/kg/inf FR 1 was significantly different from all other schedule permutations (t = 5.23, $p < .05$).

DISCUSSION

As evidenced in Figure 1, behavior seems to be sensitive to both the caffeine dose available for self-administration as well as the schedule requirements for its infusion. Duly, the dose which occasioned the highest levels of responding (.625 mg/kg/inf) was only the most effective in maintaining the behavior when a FR 1 schedule was in effect. When the schedule parameters were raised to a FR 1 schedule, regardless of dose available, behavior substantially decreased, lending to the conclusion that increases in ratio values have a considerable effect on behavior maintained by caffeine presentations.

Although not following the strictest of interpretations, the data (Figure 1) may loosely adhere to the typical inverted U-shape function, observed with most drugs self-administered by animals. While still a relatively flat function, inferences may still be viable, specifically that there seems to be a narrow range of doses able to maintain behavior. Additionally, observed levels of self-administration, although deemed distinct from those engendered by saline availability, where neither elevated nor excessive, relative to other drugs. Albeit, support for these conclusions may be garnered from nicotine self-administration studies which purport the same trends (Corrigall & Coen, 1989; Donny, Caggiula, Knopf, & Brown, 1995). However, when individual subject, with-in trial data were examined, at the highest levels observed, although quite intermittent and infrequent, self-administration behavior approximated the maximum allowable by session parameters.

Moreover, analyzing behavior occurring during probe trials, it appears that substituting saline for caffeine was very effective in decreasing behavior to minimal levels. However, more research is warranted to hypothesize more accurately about the

mechanism of behavioral maintenance. It remains unclear if the indirect effects of caffeine presentation were maintaining behavior. The degree to which the direct effects of the drug (generalized increases in locomotor activity) controlled self-administration need to be further analyzed. Nonetheless, in the confines of the present experiment, the allocation of behavior between the active and inactive lever was examined and revealed that after repeated exposure to the experimental arrangement the clear majority of behavior was distributed toward the active lever. Not without limitations and interpreted as preliminary, this effect has been an established observation in self-administration studies concerning drugs of abuse (Meisch, 1987).

In a more detailed analysis, the data that appear in Figure 4 display pause duration as a function of both caffeine dose and schedule parameters. Pause length is a measure, typically collected when employing fixed schedules, which quantifies the time between the onset of the last infusion and the initiation of responding (and, in the present experiment, the consistent inclusion of the 60 second time-out period). Informative in its implications, pause length has been observed to be positively correlated with increases in reinforcer quantity (Lowe, Davey and Harzem, 1974; Harzem and Harzem, 1981), with the same relationship evident when ratio requirements are increased (Ferster & Skinner, 1957). As displayed in Figure 4, the latter statement seems to be in effect for each dose tested. However, the incongruence with the former notion is a question framed in response-reinforcer relations. If the notion of increased pause duration as occasioned by increased reinforcer magnitude is thought of in the same manner of increases in behavioral maintenance then there seems to be less of a discrepancy, further established by the greatest pausing occurring during saline sessions. Moreover, the U-shaped trend of

pause duration, usually referred to as an inter-injection interval, as a function of dose availability has been well documented in the behavioral pharmacology literature (Katz, 1989).

The last figure introduced, Figure 5, displays mean caffeine consumption per session, across animals. Presented for the perspective which it demands, the data presented is only striking when relationally framed. When behavior was most potent (emitted under a FR 1 schedule where .625 mg/kg/inf was available), the average amount of caffeine consumed approximated 5 mg. With the average animal's weight being 300 grams, this resulted in 16.67 mg/kg in one session. Relationally, if the same dose were to be administered to an average 70 kg human being, it would equate to 1166.67 mg, or 1.17 grams of caffeine. With the average mug of coffee containing approximately 60 mg of caffeine, this tabulates metaphorically as consuming almost 20 mugs of coffee in an hour's time. The least amount of cumulative consumption observed, about 1 mg, would equate to almost 4 cups of coffee, if the analogy may be extended, considering the disparate routes of administration.

Although a modest experiment, the data presented may carry implications which are of interest and import. Not generally typified as a drug of abuse, caffeine has a profile of a more whimsical dependence: a necessary collegiate companion, a cigarette's complement, a morning essential. However, there is a contingent expressing a heightened level of concern about its ubiquitous consumption. Compelling in its contentions, the hypothesis builds the evidence for the assertion that caffeine consumption may be a model of drug abuse (Holtzman, 1990; Griffiths & Mumford, 1995). Removed from the political classification of drugs and the laws which follow, the distinction between a drug

which carries abuse liability and those which are deemed harmless is subject to the interpretation of the available data.

Evidence supporting a model of drug abuse stem from the observation of withdrawal symptoms, tolerance, discriminative stimulus effects and reinforcing effects associated with caffeine consumption in animal laboratories. Although there is not a great deal of research concerning withdrawal symptoms in animals, the behavioral effects occurring after chronic caffeine consumption cessation include disruption of both schedule controlled behavior (Carroll, Hagen, Asencio & Brauer, 1989) and locomotor activity (Finn & Holtzman, 1986). A dose-dependent effect, these interruptions appear when doses exceeding 70 mg/kg/day (in water) are consumed. Moreover, these effects may be observed 24 hours after the last dose of caffeine was ingested and may last for a few days (Finn & Holtzman, 1986).

Tolerance to the behavioral effects of caffeine have also been consistently demonstrated in animals. Rats became completely tolerant to increases in locomotor activity after repeated consumption of 40 mg/kg/day via water. Tolerance has been observed to be so marked that doses 10 to 30 times greater than those producing a significant effect in drug-naïve animals did not overcome the locomotor tolerance exhibited by the rats that chronically consumed caffeine (Finn & Holtzman, 1986). Moreover, rats receiving daily 32 mg/kg, *i.p.* injections before operant experimental sessions displayed complete tolerance after a week of caffeine exposure. The dose response curves revealed a six-fold shift to the right. There was a disruption in behavior when caffeine was initially administered, however the effects were temporary, lasting only a few days (the initial few days of the experiment) (Carney, 1982). Thus caffeine

tolerance is exhibited rather rapidly and with continuous use seems to be insurmountable (Holtzman, 1983; Finn & Holtzman, 1986; Nehlig, 1999). Cross-tolerance to locomotor activity effects appears exclusive, only exhibited upon administration of other methylxanthines and not displayed toward the discriminative stimulus effects of amphetamine, methylphenidate or cocaine (Finn & Holtzman, 1987, 1988; Holtzman, 1990).

The discriminative stimulus effects induced by caffeine injections are readily distinguished from those of saline, evidenced by rats responding to drug-appropriate stimuli in drug-discrimination trials. However, when lower doses of caffeine were administered (10 mg/kg, *i.p.*) the effects were more likely to be associated with the discriminative stimulus effects of amphetamine than were higher doses of caffeine (30 mg/kg, *i.p.*) (Holtzman, 1986).

Although previously not very well established, the reinforcing effects of caffeine have been observed in animals. Conditioned place preference studies have demonstrated that lower doses of caffeine (.32, 1.0, 1.5, 3.2, 5.6 and 10 mg/kg, *i.p.*), can prompt a preference for environments associated with caffeine presentation over an environment paired with saline (Patkina & Zvartau, 1998; Bedingfield, King & Holloway, 1998). When response-dependent *iv* infusions are available, regardless of species tested, inconsistencies have emerged, involving within-subject, within-condition variability (Deneau, Yanagita & Seevers, 1969; Griffiths, Brady & Bradford, 1979) and between-subject fluctuations (Schuster, Woods & Seevers, 1969). However, the incorporation of the results of the present study provide evidence that *iv* caffeine infusions can maintain behavior under specific circumstances and reinforce behavior necessary in accessing the

drug. When the evidence is examined in its entirety, caffeine consumption in animals very well may align with the assertion that the behavior surrounding the ingestion of caffeine is comparable to that, if not serving as a model of, drug abuse.

Examining the DSM-IV for the criteria outlining substance dependence, there are four listings which align with the current human caffeine research (Griffiths & Mumford, 1995). In an attempt to delineate how caffeine consumers fit these criteria, Hughes and colleagues (1998) conducted an interview-style study whereby caffeine consumers were asked to identify which symptoms they felt they exhibited. Although requiring a tentative interpretation due to the self-report nature of the data collection, the authors reported that the majority (56%) of the 162 participants confirmed that in spite of repeated attempts and a strong conviction to cease, control or minimize use, their caffeine consumption had continued, an indication of substance abuse as listed in the DSM-IV. Furthermore, half of the respondents reported "spending a lot of time with" caffeine, approximately a third of participants reported that they regularly consume more intended, and 20% met the requirements for clinical withdrawal symptoms (Hughes et al, 1998). Thus, with confirmatory symptoms aggregated, the estimate of participants interviewed who exhibit the symptoms which meet the criteria for caffeine dependence approached 30% (Hughes et al., 1998). Therefore, while usually relegated as a harmless ingredient or food additive, there remains evidence which counters this notion, propelling concern of dependence potential.

Beyond hypotheses of dependence, there is further reason to explore caffeine's effects, as there are data which suggest that the drug may potentiate the discriminative stimulus effects of other drugs, agents which carry high abuse liability. Specifically, a

well documented effect has been when cocaine self-administration (0.5 mg/kg/inf) is established in rats and is subsequently extinguished, caffeine injections prior to the session or within the experimental setting (5.0, 10.0 and 20.0 mg/kg, *i.p.*) have occasioned re-instatement of cocaine seeking (a resurgence of the previously extinguished behavior) (Worley, Valadez & Schenk, 1994; Schenk, Worley, McNamara & Valadez, 1996; Schenk & Partridge, 1999; Green & Schenk, 2002). To further delineate the biochemical correlates of this effect, the adenosine A_2 antagonist, DMPX, has been administered upon extinction and has not reinstated self-administration behavior (Green & Schenk, 2002). Moreover, when a non-selective A_1/A_2 agonist (NECA) is administered prior to caffeine, it does block caffeine's reinstatement effects, however it also induced hypothermic and extreme sedative effects, so conclusions may be considered provisional (Green & Schenk, 2002). However, the A_1/A_{2A} antagonist CGS15943 both maintains self-administration and reinstates cocaine seeking in baboons, while leaving food-seeking behavior unaffected (Weerts & Griffiths, 2003).

To reveal the dopaminergic underpinnings of caffeine-induced cocaine seeking, the D_1-like antagonist, SCH 23390, and the D_2-like antagonist, eticlopride, administered separately prior to caffeine in extinction trials, markedly attenuated caffeine-induced reinstatement (Green & Schenk, 2002). Additional support for a dopaminergic mechanism has been the report of cocaine reinstatement via pretreatment of a D_2-like agonist (7-OH-DPAT), and a D_2/D_3 agonist (quinpirole), while a D_1 agonist did not mimic the aforementioned effect (Garret & Holtzman, 1994a). Thus, the current hypothesis is one which disregards the influence of an A_2 antagonism mechanism,

attributing caffeine's ability to reinstate cocaine seeking to a dopaminergic-mediated mechanism.

Similarly, caffeine alters the behavioral effects of nicotine as well. Rats chronically exposed to high doses of caffeine (3 mg/ml) in their drinking water acquire nicotine self-administration (.03 mg/kg/inf) at a rate well above that of control animals not exposed to caffeine (Shoaib, Swanner, Yasar & Goldberg, 1999). Likewise, when maintained at low doses of caffeine (.25 mg/ml), rats acquire nicotine discrimination (.4 mg/kg, *i.p.*) at an accelerated rate, compared to animals maintained at higher doses of caffeine (1.0 mg/ml) as well as control animals (Jaszyna, Peters & Goldberg, 2000). In addition, this effect translates to humans maintained on higher doses of caffeine (200 mg/70 kg, *p.o., t.i.d.*). Increasing the stimulant-like discriminative stimulus effects of both low (1.0 mg/kg, *i.v.*) and high (2.0 mg/kg, *i.v.*) doses of nicotine, the oral caffeine maintenance also depleted the negative effects reported upon administration of the low dose of nicotine under placebo maintenance (Jones & Griffiths, 2003). Moreover, nicotine administration has been shown to have little to no effect on anxiety levels when consumed alone (via nicotinized smoke); however caffeine-induced anxiety (generated by coffee drinking) may diminish upon nicotine consumption (Rose & Behm, 1991).

Moreover, the researchers found that chronic caffeine also produced sensitization the effects of both amphetamine and cocaine, with both drugs (amphetamine: .56, 1.0 and 1.7 mg/kg, *i.p.*; cocaine: 5.6, 10.0 and 17 mg/kg, *i.p.*) potentiating increases in response rates. Interestingly, nicotine did have rate increasing and quarter-life effects, but the doses tested which had a behavioral effect (.17, .30, .56 and 1.0 mg/kg, *s.c.*) affected

control animals and caffeine-treated animals similarly, with no sensitization apparent (Jaszyna et al, 1998).

Considering the range of behavioral effects engendered by caffeine administration, of great applied concern may be the incidence of caffeine ingestion by children. Caffeine is nearly ubiquitously self-administered by adults, so it follows reason that it is also the psychoactive drug most readily self-administered by children, with approximations of 77% regularly ingesting the drug (Tanda & Goldberg, 2000). However, caffeine is also the most consumed psychotropic by pregnant and nursing women. Although it is doubtful that teratogenic effects occur, caffeine readily passes through the placenta and enters the fetal bloodstream, also passing into breast milk (Julien, 2001). However from prenatal stages to at least 7 months of age, absent are the enzymes necessary to demethylate the drug, causing a drug half-life anywhere from 32 to 149 hours (Parsons & Nemis, 1981), with 4 hours being average for an adult (James, 1991).

However, little experimental research that has been conducted with reference to caffeine and children has yet to give way to any concern. When adolescents were given choice trials between caffeinated and non-caffeinated soda pop (experiencing each blindly prior to the trials), only 22% of the children (four of the eighteen) exhibited a consistent choice with only one child meeting the researcher's criteria for reliable caffeine self-administration (Hale, Hughes, Oliveto & Higgins, 1995).

Thus, chronic caffeine appears to generate multiple behavioral effects, both clinical and in the laboratory, when combined with other stimulant drugs, consequently

potentiating their reinforcing and discriminative stimulus effects, implicating the need for a more detailed analysis of caffeine's role in concomitant drug use and drug relapse.

Although displaying a large proportion of characteristics which typify compulsive drug use, caffeine remains unregulated, presumably because of the absence of deleterious consequences associated with its consumption. Even with inconsistent reports regarding both the specific manner and extent of behavioral enhancement the drug affords, the literature is nonetheless rich in reports of its ameliorative effects. When combining its high therapeutic index, ease of availability and low cost with its ergogenic, attention bolstering effects, it is subsequently dismissed as a psychotropic drug with abuse potential and reassigned as a harmless if not helpful food additive. Not only is caffeine a recreational agent which aids in augmented alertness, attenuating fatigue , mood assuage, decreased reaction time and enhanced attentional focus, it also has properties of medical utility. Caffeine is often administered to alleviate bronchial constriction (Henderson, O'Connell & Fuller, 1993), ease headaches (Julien, 2001), prevent apnea in newborns (McNamara, Nixon & Anderson, 2004) boost athletic performance without steroids and alleviate symptoms of narcolepsy (Julien, 2001). Moreover, frequent caffeine consumption is associated with increased metabolic efficacy, moderate weight loss and decreased risk of type II diabetes (Greenberg, Axen, Schnoll & Boozer, 2005).

Therefore, the present experiment, employing self-administration techniques for the behavioral analysis of caffeine's effects, has provided some evidence for the agent's behavior maintaining effects in animals. However, it is the position of this paper that a more detailed analysis is warranted to further parse out the relative role of the direct

effects of the drug, the specific neurochemical correlates which allow for behavioral maintenance and the associated conditioned stimuli paired with the drug's presentation.

FIGURES

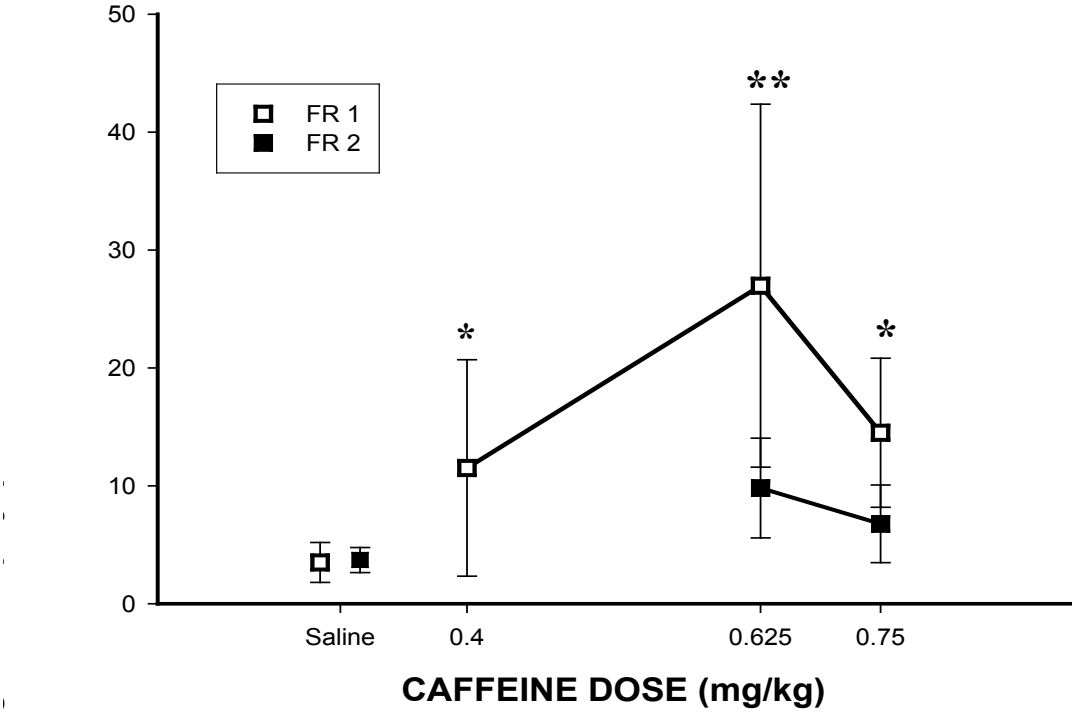

Figure 1. Mean number of infusions self-administered per session as a function of dose and response requirement. Asterisks signify a statistically significant difference from that of vehicle (*p<.05; **p<.01). Error bars represent one standard error of the mean.

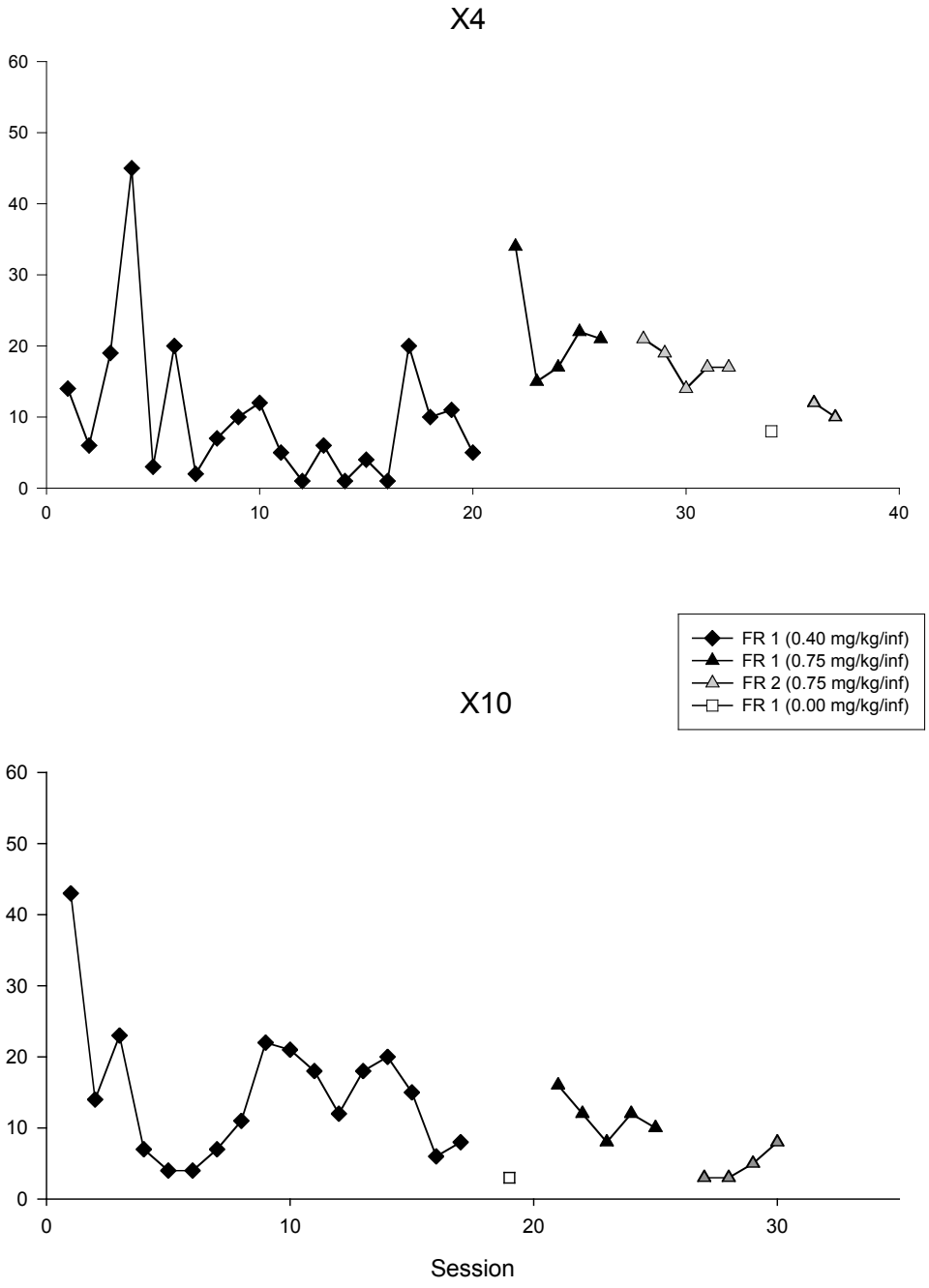

Figure 2. The number of caffeine infusions self-administered across sessions, with each panel depicting one animals' behavior. Changes in schedule or dose conditions are represented by a change in symbol and a break in the data. Two of the four animals experiencing these conditions are displayed.

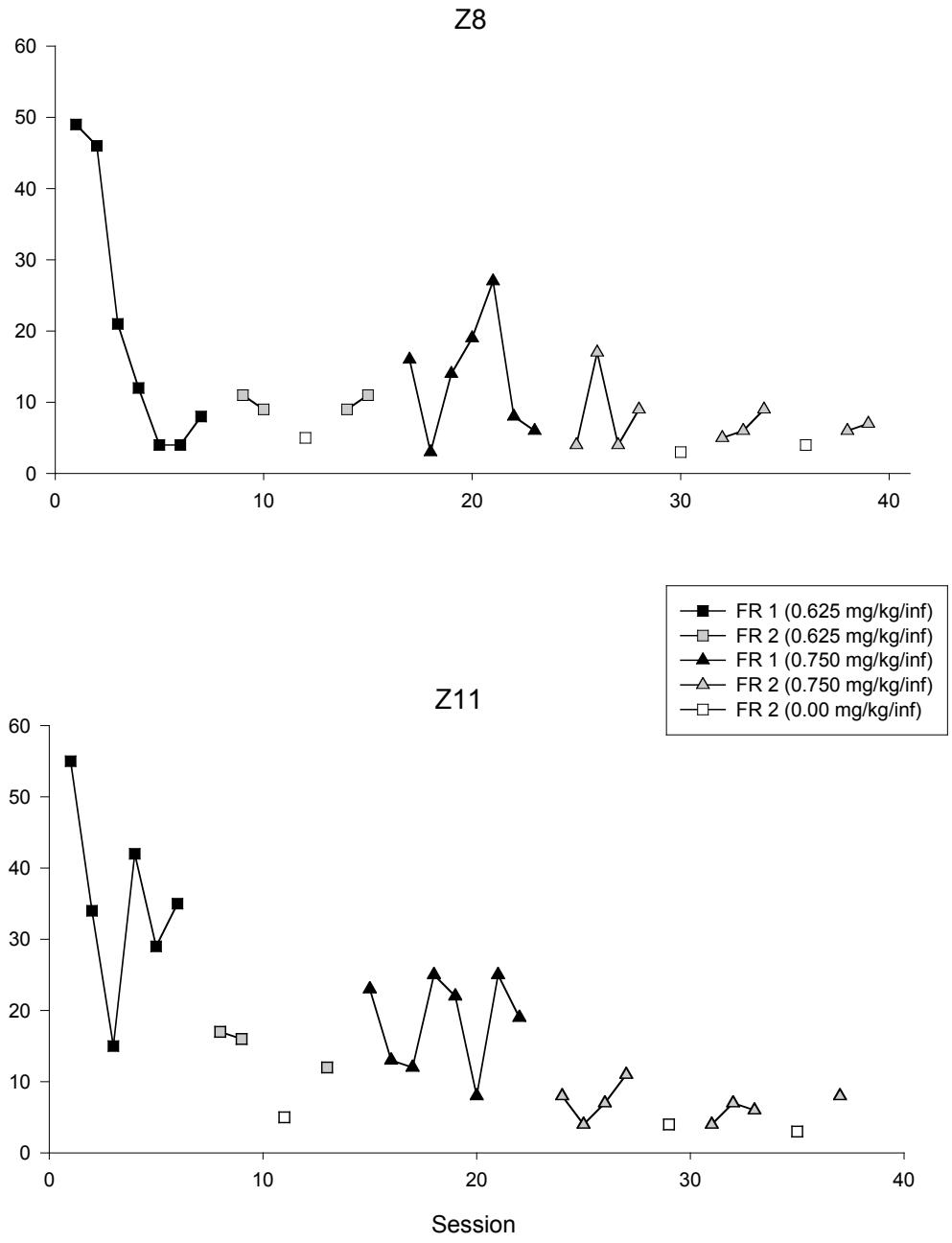

Figure 3. The number of caffeine infusions self-administered across sessions, with each panel depicting one animals' behavior. Changes in schedule or dose conditions are represented by a change in symbol and a break in the data. Two of the four animals experiencing these conditions are displayed.

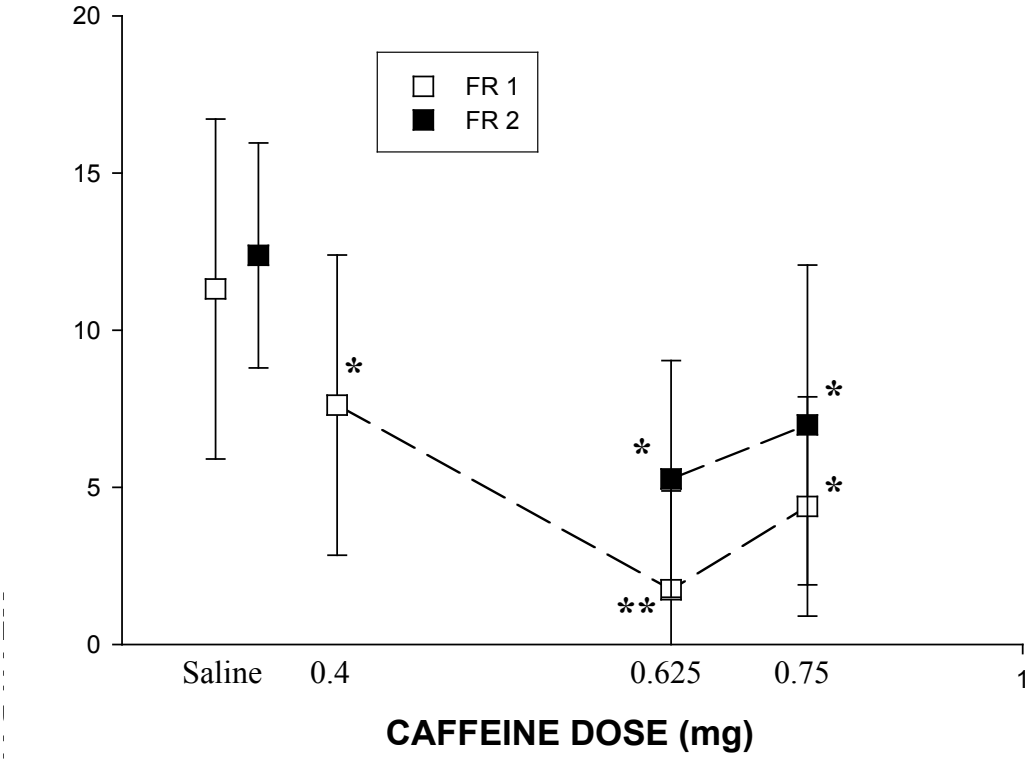

Figure 4. Mean pause duration per session as a function of dose availability and response requirement. Asterisks signify a statistically significant difference from that of vehicle (*p<.05; **p<.01). Error bars represent one standard error of the mean.

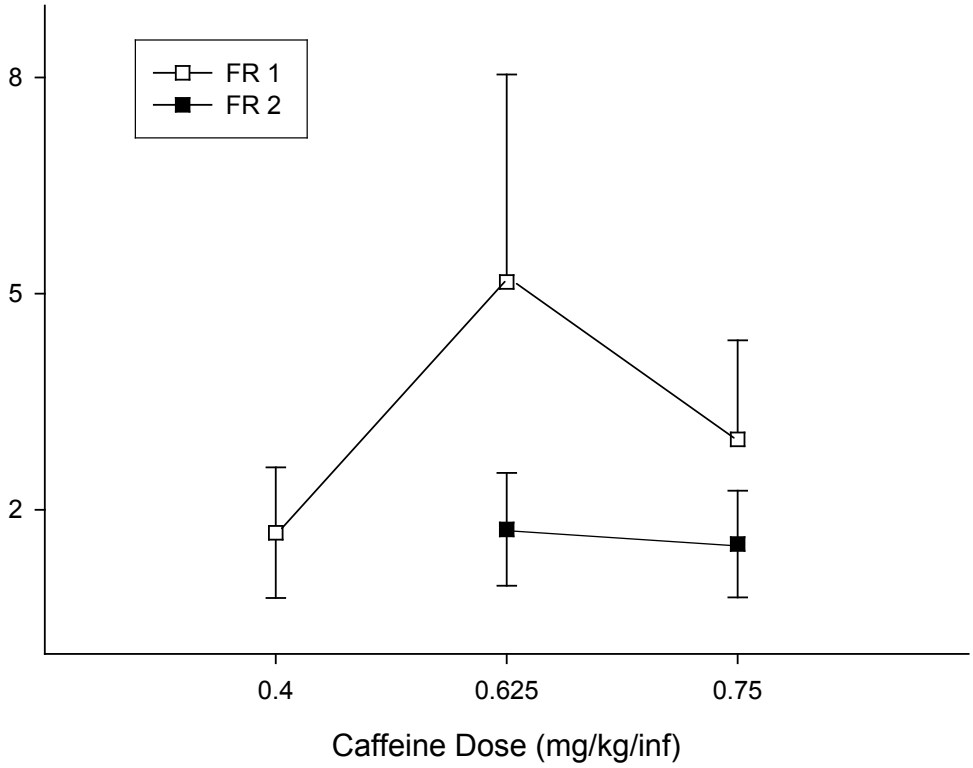

Figure 5. Mean cumulative (mg) intake per session as a function of the dose available for self-administration. Error bars represent one standard error of the mean.

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