THE EFFECTS OF N-ACETYLCYSTEINE ON BEHAVIORAL EXTINCTION IN MICE

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ABSTRACT

The experiments conducted herein examine: (a) the effect of NAC on the extinction and reinstatement of positively reinforced operant behavior maintained by food and (b) the potential for differential effects of NAC across different schedules of reinforcement within an animal model. Forty-seven C57BL/6J mice were trained in an operant paradigm to respond for access to food on an FR-5 or VR-5 reinforcement schedule. Extinction was then implemented concurrent with injections of NAC or vehicle. Following extinction, cued and reward reinstatement sessions were conducted. Data were collected on lever presses on active and inactive levers and head entries into the dipper throughout all phases. Results revealed an ameliorative effect on response frequency during extinction and reinstatement phases for the NAC group for the FR contingency only. No drug effect was evident for the VR schedule, and when FR and VR groups were compared to each other, no significant differential effect of drug by schedule was noted. The significance of results for the FR contingency parallel those found in the drug relapse/ reinstatement literature and may suggest consistency across different types of positive reinforcers. However, these results may be tempered by the lack of significant findings for the VR contingency, which more closely parallels naturally occurring schedules of reinforcement. The ambiguity of these findings combined with the potential for NAC to ameliorate undesirable side effects of extinction warrant continued investigation.

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CHAPTER I

INTRODUCTION

Problem Behavior in Individuals with Intellectual Disabilities

Problem behavior is one of the key clinical concerns for individuals with intellectual disabilities (ID) (Hastings & Brown, 2000). Problem behavior is defined as any behavior considered inappropriate in terms of frequency, severity, and/or typicality with respect to context resulting in detrimental effects to the individual or environment (Lowe, Felce, Perry, Baxter, & Jones, 1998; Lowry & Sovner, 1991). Among potential detrimental effects are the risk of exposure to a variety of negative outcomes for the individual (Emerson, 1995; Emerson et al., 2000; Paclawskyj, Kurtz, & Connor, 2004), impaired family functioning (Fox, Vaughn, Wyatte, & Dunlap, 2002), and monetary costs to society (Honeycutt et al., 2003).

The consensus across studies suggests the prevalence of problem behavior is between 10% and 20% among individuals with ID (Emerson et al., 1997; Emerson & Bromley, 1995; Emerson et al., 2001; Holden & Gitlesen, 2007; Jacobson, 1982; Kiernan & Kiernan, 1994; Oliver, Murphy, & Corbett, 1987). It is common for individuals to exhibit more than one type of problem behavior (Emerson et al., 1997; Emerson & Bromley, 1995; Emerson et al., 2001; Joyce, Ditchfield, & Harris, 2001; Lowe et al., 1998; Matson, Cooper, Malone, & Moskow, 2008; Nord, Wieseler, & Hanson, 1998; Smith et al., 1996), and problem behaviors also increase concurrently in prevalence, frequency, and severity with increasing disability severity (Borthwick-Duffy, 1994; Emerson, 1995; Emerson & Bromley, 1995; McClintock et al., 2003; Sigafoos et al., 1994; Smith et al., 1996). A variety of individual factors have also been linked to

the prevalence of problem behavior, including functioning level, age, gender, living situation, mobility, self-help skills, sleep problems, social skills, communication abilities, and receptive and expressive language (Borthwick-Duffy, 1994; Emerson, 1995; Emerson et al., 2001; Holden & Gitlesen, 2007; Jones et al., 2008; Kiernan & Alborz, 1996; McClintock et al., 2003; Qi & Kaiser, 2004; Sigafoos, 2000; Wiggs & Stores, 1996).

Problem behavior can take the form of multiple topographies. Four general categories of problem behavior include self-injury, aggression, property destruction, and stereotypy (Emerson et al., 2001). The most common forms of problem behavior include physical aggression, verbal aggression, self-injury, destructiveness, stereotypy, noncompliance, tantrums, and sexually inappropriate behaviors (Axelrod, 1987; Emerson, 1995; Hastings & Brown, 2000; Lowe & Felce, 1995; Neilsen & McEvoy, 2004). Other problem behaviors cited less frequently in the literature include pica (i.e., eating inedible objects), isolation/withdrawal, elopement, and overactivity (Emerson, 1995; Lowe & Felce, 1995; Neilsen & McEvoy, 2004).

Despite the variability across individuals and topographies, all problem behaviors are emitted because they serve a function for the individual. The function of problem behavior, as with all operant behaviors, is defined by the relation between the behavior and environment (Catania, 1998). Carr (1977) proposed behaviors should be considered as having three potential operant functions: positive reinforcement, negative reinforcement, or automatic reinforcement. Behaviors that serve a positive reinforcement function are emitted to obtain access to a stimulus, such as attention, tangibles, or sensory stimulation (Honig & Staddon, 1977; Iwata, Pace, Kalsher, Cowdery, & Cataldo, 1990). Behaviors maintained by negative reinforcement generally function to postpone, reduce, terminate, escape, and/or avoid an aversive stimulus (Honig & Staddon, 1977; Iwata, 1987; Iwata & Worsdell, 2005). Behavior that is considered automatically

reinforced is maintained by a non-social consequence that is generally considered an internal event (Lalli, Browder, Mace, & Brown, 1993).

Tools that address the functional aspects of problem behavior are often applied in order to design appropriate behavior interventions. Currently, the primary technique applied to this end is functional behavior assessment (FBA). FBAs are designed to assess problem behaviors as learned responses emitted to address a specific function whose form is a product of environmental factors (Sugai & Lewis-Palmer, 2004). That is, the primary goal of FBA is to identify the relevant contingencies maintaining problem behaviors (Dunlap et al., 1993; Horner & Carr, 1997; Peterson, 2002). By identifying the relevant environmental variables and contingencies maintaining behavior, FBAs facilitate the identification of strategies that will reduce or eliminate future occurrences of problem behavior (Crone & Horner, 2000; Sprague & Horner, 1995). The results of an FBA can be used to develop function-based interventions that emphasize changing environmental variables and teaching new skills (Horner, 1994; Horner & Carr, 1997; Kennedy, 2000). As such, FBAs are considered an essential step in designing effective function-based interventions (Sturmey, 1994).

Function-based interventions focus on redesigning the environment and manipulating environmental consequences for inappropriate and appropriate behavior (Gresham, Watson, & Skinner, 2001; Horner & Carr, 1997; Neilsen & McEvoy, 2004). Critical components of successful function-based interventions include explicit programming for changes in the environment and the behavior of others (Horner, Sugai, Todd, & Lewis-Palmer, 2000; Neilsen & McEvoy, 2004) and programming for the teaching of new skills and/or appropriate alternative behaviors that are functionally similar to the problem behavior (Gresham et al., 2001; Horner & Carr, 1997; Johnston & Reichle, 1993; Mace, Lalli, & Lalli, 1991; Neilsen & McEvoy, 2004).

More specifically, function-based interventions provide more appropriate treatment for problem behavior via the: (a) elimination of the reinforcement contingency for problem behavior, (b) shaping of appropriate behavior through differential reinforcement, (c) ongoing provision of differential reinforcement for appropriate alternative behaviors, (d) alteration of environmental variables relevant to the problem behavior, and (e) teaching of new skills (Arndorfer & Miltenberger, 1993; Dunlap, Kern-Dunlap, Clarke, & Robbins, 1991; Holden, 2002; Iwata, Vollmer, Zarcone, & Rodgers, 1993; Iwata & Worsdell, 2005; Mace, 1994; O'Neill et al., 1990; Sprague & Horner, 1995; Vollmer & Smith, 1996).

Extinction

Although intervention design can vary along a continuum based on functioning level of the individual, variability of the behavior, and so on, one nearly universal element of function-based consequent interventions are extinction procedures. Extinction is broadly defined as the elimination of a reinforcement contingency (Lerman & Iwata, 1995, 1996; Vollmer, 1994). More specifically, extinction involves removing and/or terminating reinforcement following a previous history of reinforcement (Ducharme & Van Houten, 1994; Keller & Schoenfeld, 1950; Skinner, 1938; Verhave, 1966). As a result of the elimination of the response-reinforcer contingency, extinction is the most direct way of producing reductions in problem behavior (Iwata, Vollmer, & Zarcone, 1990).

The means by which extinction is accomplished is determined by the relation of the targeted behavior to the maintaining consequence (Lerman & Iwata, 1995, 1996; Vollmer, 1994). During the extinction procedure, environmental manipulations occur such that there is zero probability the target behavior will produce the reinforcing stimulus. That is, the individual can

still engage in the target behavior, but it will no longer produce reinforcement. The absence of contingent reinforcement produces the most prominent feature of extinction: a decrease in the frequency of the behavior over time (Ferster & Perrott, 1968).

Characteristics. The generality of extinction effects on operant responses has been demonstrated across species, response classes, and settings, suggesting they are a fundamental aspect of operant reinforcement processes (Lerman & Iwata, 1996). During the course of extinction, several well-documented side effects can occur, including the extinction burst, extinction-induced aggression, spontaneous recovery, and the partial reinforcement extinction effect (Ducharme & Van Houten, 1994; Harris & Ersner-Hershfield, 1978; Lerman & Iwata, 1996; Lerman, Iwata, & Wallace, 1999).

The term extinction burst refers to the initial increase in responding following the exposure to extinction. Response bursts occur during the initial stages of extinction and are typically followed by a gradual decrease in behavior to zero or near zero levels (Ferster & Perrott, 1968). Bursting can occur in terms of the frequency, duration, magnitude, intensity, and/or variability of the problem behavior (Ducharme & Van Houten, 1994; Ferster & Perrott, 1968; Thompson & Bloom, 1966). This increase can be so great as to exceed the level at which it was occurring while being reinforced (Skinner, 1938).

Extinction-induced aggression (EIA) refers to the emergence and/or increase in aggressive behaviors that occur following exposure to extinction (Lerman & Iwata, 1996). Similar to the course of extinction burst responding, EIA usually occurs at the highest levels immediately after extinction exposure and then gradually decreases across the extinction period (Azrin, Hutchinson, & Hake, 1966; Rilling, 1977; Thompson & Bloom, 1966). Data suggest this

transitory increase may occur whether aggression is the targeted response or not (Goh & Iwata, 1994; Todd, Morris, & Fenza, 1989).

Spontaneous recovery is defined as the phenomenon whereby after extinction has occurred, the extinguished response reappears despite not having been reinforced (Lerman & Iwata, 1996). This can occur from anywhere to a few minutes (Sheppard, 1969) to over a month (Youtz, 1938) after extinction has been implemented, but the recurrence is transitory provided extinction remains in effect (Ducharme & Van Houten, 1994). A decrease in response strength is also characteristic of spontaneous recovery (Skinner, 1938).

The partial reinforcement extinction effect (PREE) refers to the greater resistance to extinction resulting from prior exposure to an intermittent reinforcement schedule relative to a continuous schedule (Huang, Krukar, & Miles, 1992). That is, responses that have been maintained on a less consistent schedule of reinforcement, such as a variable ratio (VR) schedule, are more resistant to extinction than are responses that have been continuously reinforced. This characteristic of extinction is particularly problematic in applied settings, where behaviors are typically maintained by an intermittent schedule of reinforcement (Ducharme & Van Houten, 1994).

Application. Operant extinction is regularly incorporated in function-based intervention treatment packages. In fact, in a review of interventions applied following FBAs, Blakeslee, Sugai, and Gruba (1994) reported that extinction was applied, either explicitly or implicitly, in over 50% of studies included in the review. Similarly, extinction has been used to treat a wide range of problem behaviors, including tantrums (Allen, Turner, & Everett, 1970; Williams, 1959), property destruction (Martin & Foxx, 1973), aggression (Allen et al., 1970; Figuero, Thyer, & Thyer, 1992; Martin & Foxx, 1973; O'Reilly, Lancioni, & Taylor, 1999; Pinkston,

Reese, LeBlanc, & Baer, 1973), elopement (Lang et al., 2009), and self-injurious behavior (Goh & Iwata, 1994; Iwata, Pace, Cowdery, & Miltenberger, 1994; Jones, Simmons, & Frankel, 1974; Lovaas & Simmons, 1969; Mazaleski, Iwata, Vollmer, Zarcone, & Smith, 1993; Pace, Iwata, Cowdery, Andree, & McIntyre, 1993; Repp, Felce, & Barton, 1988; Rose, Sloop, & Baker, 1980; Zarcone et al., 1993).

Despite the prevalence of extinction procedures, surprisingly little applied research has been conducted on the topic (Lerman & Iwata, 1996). One reason so little attention has been given to extinction may be the potential risks and side effects associated with extinction in clinical settings. For example, extinction is frequently dismissed as a viable intervention option due to the intensity of side effects, the length of treatment time necessary to achieve clinically significant reductions in problem behavior, and difficulties in implementation (Ducharme & van Houten, 1994). It may be contraindicated if the occurrence of response bursts and/or aggression may result in physical harm to the individual or others (Lerman et al., 1999). However, the use of extinction is unavoidable, as in the case of differential reinforcement. Research indicates differential reinforcement procedures work only because reinforcement is applied concurrent with extinction (Iwata, Pace et al., 1990; Iwata, Vollmer et al., 1990; Leibowitz, 1972; Mazaleski et al., 1993; Vollmer & Iwata, 1992).

Therefore, despite the associated risks, it is not surprising that extinction is considered a necessary and vital component of any problem behavior intervention (Ducharme & Van Houten, 1994). Based on the importance of extinction in intervention research, investigators have begun to explore the effects of drug on extinction. It is possible that timely use of the appropriate drug could allow interventionists to capitalize on the efficacy of extinction procedures, while minimizing the characteristic features of responding and adverse effects. Additionally, while

research has demonstrated how and often why a particular intervention can decrease or eliminate behavior, very few of these efforts have incorporated and/or examined the potentially accelerative effects of drugs on these interventions.

N-Acetylcysteine

The use of drugs to treat problem behaviors has become increasingly commonplace among individuals with ID (Emerson et al., 2000; McGillivray & McCabe, 2004; Witwer & Lacavalier, 2005). This trend has led to the increased necessity for research on the behavioral effects of drugs. These research efforts often take the form of examining the influence of drugs on basic behavioral processes in animal models. One focus has been the effect of drugs on extinction, a topic of particular interest due to the undesirable side effects of extinction and a dearth of other non-behavioral interventions. Any drug that could facilitate extinction of problem behavior while concurrently attenuating or eliminating undesirable side effects could serve as an invaluable supplement to function-based interventions.

One drug that has received attention regarding application to extinction is N-acetylcysteine (NAC). N-acetylcysteine is an N-acetylated derivative of the naturally occurring amino acid cysteine (Gass & Olive, 2008). It is a white crystalling compound sold in an over the counter tablet in health food stores, where it is typically advertised as improving mental functions (LaRowe et al., 2006). Medically, NAC has been used as a treatment for bronchopulmonary disorders (Grandjean, Berthet, Ruffman, & Leuenberger, 2000), to prevent X-ray contrast nephropathy (Birck et al., 2003), as a cognitive improvement agent in dementia patients (Adair, Knoefel, & Morgan, 2001), and most commonly, as a treatment for acetaminophen poisoning (Smilkstein, Knapp, Kulig, & Rumack, 1998).

Use of N-Acetylcysteine in animal models. In addition to traditional applications, NAC has also been recently used in drug relapse and reinstatement paradigms. Reinstatement, in the context of drug relapse models, refers to the resumption of previously extinguished operant behavior following noncontingent exposure to stimuli (Stewart & de Wit, 1987). Two frequently used drug reinstatement models use are cue-primed reinstatement and drug-primed reinstatement. Cue-primed reinstatement involves pairing sensory stimuli with response-contingent infusions of the drug (e.g., cocaine). Once a response criterion is reached, the operant response is placed on extinction (i.e., responses do not produce any programmed consequence). Once responding becomes minimal under the extinction procedures, a reinstatement phase is instituted. During reinstatement, responding results in access to the drug-associated stimuli but does not result in drug administration. Responding maintained by the drug cue therefore functions as an index for drug-seeking behavior (Kruzich, 2007; Shalev, Grimm, & Shaham, 2002; Stewart & de Wit, 1987; Tsiang & Janak, 2006). Conversely, drug-primed reinstatement procedures parallel those just presented, except that reinstatement is primed directly with administration of a small dose of drug rather than drug-associated stimuli (Fuchs, Tran-Nguyen, Weber, Khroyan, & Neisewander, 2003; Highfield, Mead, Grimm, Rocha, & Shaham, 2002; Kruzich, 2007).

With respect to drugs of abuse, NAC works by modulating glutamate within the brain. This mechanism of action is critical, as glutamate has been linked to many drugs of abuse, including cocaine, amphetamines, opiates, nicotine, cannaboids, alcohol, and inhalants (Gass & Olive, 2008). Specifically, NAC exercises influence over the glutamatergic system by virtue of its ability, once converted to cystine, to drive the cystine/glutamate exchanger (Baker et al., 2003a; Knackstedt, Melendez, & Kalivas, 2009; Pileblad & Magnusson, 1992). A general

description of NAC effects includes increased basal glutamate levels, stimulation of inhibitory glutamate autoreceptors, and reduced synaptic release of glutamate (Pittenger, Krystal, & Coric, 2005). The increased extrasynaptic glutamate that results from NAC restores homeostasis disrupted by exposure to drugs of abuse (Pittenger et al., 2005; Zhou & Kalivas, 2008) and prevents drug-induced plasticity (Baker et al., 2003b; Madayag et al., 2007). A detailed description of NAC mechanisms of action can be found elsewhere (see Baker et al., 2003a, b; Madayag et al., 2007; Moran, McFarland, Melendez, Kalivas, & Seamans, 2005; Moussawi et al., 2009).

To date, NAC has largely been explored as a means of supporting and sustaining extinction of drug-seeking behavior within the drug relapse and reinstatement models. The behavioral result of NAC administration is the attenuation and/or prevention of drug-seeking in animal models of drug reinstatement (Baker et al., 2003b; Madayag et al., 2007; Moran et al., 2005; Moussawi et al., 2009; Zhou & Kalivas, 2008). In addition, data from several studies suggest NAC blunts or prevents escalation of drug intake in animal models (Kau et al., 2008; Mayadag et al., 2007). Pretreatment with NAC prior to daily cocaine administration has also been shown to result in reduced responding upon exposure to extinction (Kau et al., 2008; Zhou & Kalivas, 2008).

Despite the relative wealth of studies involving drugs of abuse, only one of the aforementioned studies have reported data on the effect of NAC on other positive reinforcers, specifically food (Baker at al., 2003a). Baker et al. employed a food reinforcement paradigm as a control comparison for the drug reinstatement paradigm. While NAC was shown to have significant effects on cocaine-primed reinstatement of lever pressing, no effect on food-primed reinstatement was evident.

In a recent pilot study conducted by the author and colleagues, a behavior analytic approach was taken to addressing the question of the effect of NAC on the extinction of an operant response maintained by food reinforcement. Ten C57/BL6J mice were trained to nose poke for access to a liquid reinforcer on an FR-5 schedule. During extinction, mice received daily injections of NAC (100 mg/kg) or vehicle 2.5 hrs prior to experimental sessions (N = 5 per group). Results of a mixed model, conducted on *log* transformed scores to correct for non-normality, indicated no significant effect of treatment. However, *t*-tests on Days 1 through 3 showed that response frequency was significantly higher in the NAC group on day 2 (p = .035) Day 4 (p =.030); a similar data pattern approached significance on day 1 (p = .057).. No significant differences existed across groups during cued or reward reinstatement, a trend for increased responding existed in this phase for the NAC group. Although these results were limited by the small sample size used, *t*-test findings that were significant and in the opposite direction anticipated encouraged continued study.

Use of N-acetylcysteine in humans. The ameliorative effects of NAC on drug relapse have been reported in clinical studies with humans as well. LaRowe et al. (2007) administered NAC or placebo in a double blind clinical trial to non-treatment seeking cocaine-dependent individuals before exposing the participants to cocaine-associated cues. Results indicated that while taking NAC, participants reported a decreased desire to use and less interest in cocaine slides. Investigators also reported that participants treated with NAC watched the cocaine slides for less time. In a similar study, data indicated that during NAC administration, a trend existed for a greater reduction in withdrawal symptoms and craving. Additionally, participants reported reduced cocaine use following NAC administration (LaRowe et al., 2006).

In addition to drugs of abuse, the ability of NAC to augment extinction while producing minimal side effects (LaRowe et al., 2006) has rendered this drug a potential candidate for use in treating problem behaviors. Most studies examining the effects of NAC on problem behaviors have been conducted in people with obsessive-compulsive disorder (OCD) due to the implication of glutamate dysfunction in OCD-related behaviors (Chakrabarty, Bhattacharyya, Christopher, & Khanna, 2005; Lafleur et al., 2006). Within the spectrum of behaviors related to OCD, NAC has been used to treat trichotillomania (i.e., chronic hair pulling), pathological nail biting, and pathological skin picking.

Grant, Odlaug, and Kim (2009) treated the trichotillomania of adults in a 12 week double-blind placebo controlled study. Significantly greater reductions in hair-pulling symptoms were evidenced in the group administered NAC. Additionally, the NAC group demonstrated significant improvements on assessment measures of depression, anxiety, and responder rate. It is worthy of note that these findings represent the most remarkable treatment effects to date for trichotillomania, a traditionally intractable disorder. As pointed out by the authors, NAC offers some promise in light of minimal success of other treatment options for OCD behaviors. The remaining studies on NAC and OCD are in the form of case studies and have reported promising findings (Berk et al., 2009; Odlaug & Grant, 2007).

Despite research on the efficacy of use with compulsive behaviors, the efficacy of NAC as a means of ameliorating problem behavior in individuals with ID has yet to be investigated. One case study has been reported wherein NAC was used to successfully treat self-injurious behavior (i.e., cutting) in a person with borderline personality disorder (Pittenger et al., 2005). Although the impact of these data is tempered by concerns about experimental design and validity, they suggest a potential ameliorative effect for NAC. Additionally, they are the first

data to suggest that NAC may work for behaviors similar to those commonly seen in individuals with ID.

New Applications for N-Acetylcysteine

The existing literature suggests potential applications of NAC to positively reinforced behaviors, such as drug addiction and obsessive compulsive behaviors. Further, the data may point to corresponding mechanisms within the brain. Assuming similarity exists in behavioral contingencies maintaining drug addiction, compulsivity, and problem behaviors, the application of NAC to problem behaviors in individuals with ID may be worth investigating. This is especially true in light of the research suggesting that NAC not only attenuates or prevents continued engagement in behavior once extinction has occurred but may also facilitate extinction (Gass & Olive, 2008) and ameliorate characteristics of extinction responding (Kau et al., 2008; Zhou & Kalivas, 2008).

Although the use of drugs as a supplement to behavior plan implementation is not yet supported by data, experiments using drug applications as a means of augmenting extinction processes offer promise. The current experiments seek to expand the literature by investigating the effects of NAC on the extinction of a positively reinforced operant behavior contingency maintained by food. The experimental questions addressed herein are:

a.) Does administration of NAC affect extinction responding in mice trained on a positively reinforced operant task, and

b.) Does administration of NAC differentially affect the extinction responding of mice trained on continuous versus intermittent schedule of positive reinforcement?

CHAPTER II

METHODS

Subjects

Five-week-old experimentally naïve male C57BL/6J mice purchased from Jackson Laboratory were used in this study. Upon arrival, each mouse was assigned to a home cage where it was group housed with 3 or 4 other mice in a temperature-controlled room and maintained on a 12 hr light/dark cycle. Housing conditions and experimental protocols were approved by the Vanderbilt University Institutional Mouse Care and Use Committee. An acclimation period of two weeks was observed prior to any procedures.

Following the acclimation period, each mouse was removed from the home cage individually and handled by the experimenter for 2 min daily for two experimental weeks (i.e., 10 days). Food restriction procedures were in place throughout the handling period (see Food Restriction section). Mice were allowed *ad libitum* access to water throughout the experiment, except during testing. On the final day of handling, mice were each marked uniquely to ensure individual identification throughout the study. Mice were 7 weeks old at the conclusion of pre-experimental procedures.

Materials

Apparatus. Eight Med Associates Operant Conditioning Chambers were used as the testing apparatus. The interior of the operant chamber measured approximately 15.9 cm by 14 cm by 12.7 cm. The roof and two walls of the chamber were made of .6 cm clear polycarbonate

material; the remaining two walls were constructed of metal. The floor consisted of a grid of small metal rods placed lengthwise between the two polycarbonate sides, a construction that allowed for the passage of urine and fecal material to a removable metal pan below. One of the polycarbonate sides had a raised lip and attached door that folded down to open and folded up and latched closed for inserting and removing subjects. The operant chamber itself was mounted on a white propylene base and enclosed in a sound attenuating cubicle. The latter opened via a single door, which contained a peephole for unobtrusive observation.

The operant chamber was configured with two levers and a dipper entry hole. All three elements were located on the same metal wall. For the purposes of this experiment, only the right lever was active (hereafter referred to as the "active lever") while the left lever was inactive (i.e., only lever presses on the active lever produced reinforcement based on the schedule requirement, whereas responses on the left lever did not result in any programmed consequences). The dipper entry was located between the left and active levers. The dipper entry was large enough to permit head entry by the mouse, which allowed access to the dipper cup through a small hole at the base of the dipper entry. The operant chamber was also equipped with a house light located just below the roof of the chamber on the metal wall opposite that with the levers and dipper entry. Additionally, a clicker mechanism was mounted on the exterior of the operant chamber.

All reinforcement events in the operant chamber were delivered via a dipper located external to the operant chamber. A solution of 50% water and 50% Homemade Vanilla Shake Ensure® beverage was mixed weekly for use throughout the study. The dipper arm was activated when a schedule requirement was reached, which resulted in the delivery of liquid. That is, the dipper arm rose from the retracted position, where the dipper cup was not flush with the dipper entry hole, into the activated position, where the dipper cup was flush with the dipper entry hole.

From this position, the dipper cup attached to the end of the dipper arm could be accessed via the dipper entry in the wall of the operant chamber. When the dipper was retracted, it rested in a small rectangular container holding the liquid. Therefore, when the dipper was activated, the liquid from the full dipper cup could be consumed, and when the dipper was retracted, it was automatically refilled when submerged. It is important to note activation and retraction of the dipper made an audible sound.

The computer program Med-PC® IV (Med Associates, Inc., 2010) executed all operant chamber activity related to light cues, delivery and duration of reinforcement, and timing of testing sessions. Separate programs were used for shaping and response acquisition, reinforcement schedules, extinction, and reinstatement.

Drug/vehicle injections. Mice were weighed on the day preceding the first day of extinction procedures, immediately following termination of unrestricted food access (see Food Restriction section). The weight was used to determine the amount of drug or vehicle received for injections. On testing days when injections were administered, mice received either 100 mg/kg of NAC or phosphate buffered saline (vehicle) were injected intraperitoneally 2.5 hrs prior to testing. Vehicle injections were given to control for the effect of receiving an injection. The solution received by each mouse was determined based on group assignment (e.g. vehicle vs. drug), and the experimenter was blinded to solution.

Data Collection

Data were collected on lever presses on the active and left levers. Data were also collected on head entries into the dipper, as well as on number of reinforcements received, where appropriate. All data were collected via the Med-PC IV software (Med Associates, Inc., 2010).

Food Restriction

As a result of employing a food contingency in this experiment, it was necessary to create a state of deprivation prior to experimental procedures. Therefore, food deprivation procedures were used for all mice in this study. Restricted food access was employed during pre-experimental handling, autoshaping, schedule training, baseline, extinction, and reinstatement.

On days immediately preceding any experimental procedure, animals were allowed only 4 hrs of access to food. During the period of food access, food was provided *ad libitum* and was subsequently removed from the home cage after 4 hrs had elapsed. On days not preceding a test day, animals were given continued *ad libitum* access to food until the next day that preceded a testing day. On days when no experimental procedures occurred but food deprivation needed to be implemented in preparation for a subsequent day of testing, food was removed at approximately same time as it was on the most recent day of food restriction. That is, where sessions occurred Monday thru Friday, mice were allowed free access to food from Friday following testing until Sunday evening. On Sunday evening, food was again removed in preparation for the experimental session to occur on Monday. Access to water was never restricted in the home cage.

Experimental Procedures

Group assignment. On the final day of autoshaping, each mouse was randomly assigned to one of four experimental groups. Groups were divided by schedule type, fixed ratio (FR) and variable ratio (VR) and treatment condition (NAC or vehicle). This resulted in the following groups: FR-NAC, FR-VEH, VR-NAC, and VR-VEH. Group assignments were made after the final day of autoshaping.

Experimental sessions. Mice were allowed to move freely around the operant conditioning chamber during all sessions. Throughout the experiment, one session per day, five consecutive days per week occurred, with the exception of the reinstatement phase. All mice were exposed to the same phase(s) at the same time. That is, no mouse proceeded to subsequent phases (e.g.., from autoshaping to schedule training) until the data from all subjects was deemed appropriate for a phase change. Within each phase, experimental sessions were run at approximately the same time each day (i.e., within the same 30 to 60 min period).

For all phases of the experiment, mice were removed from the housing room prior to the sessions and taken to the testing room. Once in the testing room, each mouse was removed from the home cage and placed in an assigned operant chamber. The operant and sound attenuating chamber doors were closed and the session was started individually for each mouse as it was situated. Mice were removed from the operant chamber promptly when experimental sessions ended. Mice not included in that run remained in the testing room in the home cage until the subsequent run, when the same procedures just described were used. When all mice in a given run had completed experimental sessions, the 4 hr period of food access commenced and the cage was returned to the housing room.

Autoshaping. Following completion of preparatory procedures, autoshaping began. Food restriction procedures were active during this phase. Autoshaping sessions were 1 hr in duration. The response acquisition criteria was defined as >100 lever presses and >100 reinforcements during the 1 hr autoshaping session. Following 15 days of autoshaping, all but one mouse met the response acquisition criteria. The mouse failing to meet criteria was not included in subsequent analyses.

In order to automatically shape the desired operant response, mice were reinforced on an FR-1 schedule to promote acquisition of the lever press response. That is, any time the subject emitted a lever press on the active lever, the dipper was activated and delivered reinforcement through the dipper entry. The initial experimental arrangement for autoshaping was as follows: (1) the house light came on when the session started and remained on for the duration of the session, (2) the clicker mechanism was activated, emitting one audible click when the schedule requirement was met, and (3) concurrent with the click, the dipper was activated. Based on this arrangement, the click was to serve as a discriminative stimulus signaling the completion of the schedule requirement and resulting availability of food. Once activated, the dipper remained activated indefinitely until the subject entered the dipper entry hole. Once the head of the mouse crossed the dipper entry threshold, the dipper remained activated for a further 10 s. Once 10 s had elapsed, the dipper arm retracted.

None of the mice met the response acquisition criteria while exposed to the initial autoshaping procedures, and procedural refinements were therefore deemed necessary. The first refinement involved one noncontingent presentation of the liquid; that is, the dipper was activated one time automatically concurrent with the start of the session (hereafter referred to as autoshaping with NCR). Due to the noncontingent nature of this presentation, a click was not paired with this reinforcement. This modification was intended to increase activity and response variability among the mice by granting them access to the Ensure. However, the result of this modification was not robust. The second modification involved adding a fixed-time (FT) reinforcement schedule to the autoshaping with NCR modification. On the first day, the dipper was activated every 3 min, giving the mice access to reinforcement, regardless of activity. On the second day, the time interval was increased to 9 min. On the third day, the time interval returned

to 3 min, and on the fourth day, only the autoshaping plus NCR modification was used. These modifications resulted in the expected increase in activity and response variability and led to marked increase in the number of mice meeting the response acquisition criteria. Subsequent to these procedural refinements, 73% of subjects met the response acquisition criteria.

The remaining non-responders were then individually manually shaped in one to four 30 min to 1 hr sessions. Once a mouse began to lever press independently, it resumed autoshaping sessions with parameters identical to autoshaping plus NCR procedures outlined above, and this continued until the response acquisition criteria had been reached. During manual shaping procedures, all mice having already met the response acquisition criteria did not participate in autoshaping sessions, but food deprivation procedures remained in place for all mice. Following the conclusion of manual shaping procedures, all mice were exposed to two additional experimental sessions employing autoshaping plus NCR parameters.

Schedule Training. Following completion of autoshaping, subjects were transitioned stepwise from the autoshaping schedule of FR-1 to a terminal session schedule of FR-5 or VR-5. These sessions were 30 min in duration, and schedule training sessions took place over the course of 2 experimental weeks (i.e., 10 days). Food restriction procedures were in place throughout this phase.

The initial experimental arrangement for schedule training closely mirrored that applied in autoshaping plus NCR and was as follows: (1) the house light came on when the session started and remained on for the duration of the session, (2) a single noncontingent presentation of the liquid occurred concurrent with the start of the session, (3) the clicker mechanism was activated, emitting one audible click, any time the schedule requirement was met, and (4) concurrent with click, the dipper was activated. Two notable procedural changes were made

prior to and during this phase, respectively: (1) dipper activation duration was reduced from 10s to 5 s for this and all subsequent phases, and (2) the noncontingent reinforcement component was removed for Days 3 through 10 of this phase. Initially, it was determined that continued exposure to the noncontingent presentation of the liquid at the start of each session was necessary to ensure continued contact with the liquid while subjects experienced increasing response requirements. However, data from Days 1 and 2 of schedule training suggested that this modification was no longer necessary and was immediately discontinued.

Shaping the increase in response requirement was accomplished by exposing the subjects to steady increases in response requirement. For the fixed ratio schedule, mice were transitioned from FR-1 to FR-2 to FR-3 to FR-4 to a terminal schedule of FR-5, and for the variable ratio schedule, mice were transitioned from FR-1 to FR-2 to VR-3 to VR-4 to VR-5. The schedule requirement was increased daily on Days 1 thru 5, and then mice were exposed to the terminal schedule appropriate to their group assignment on Days 6 thru 10. Responding on the terminal schedule on these days was visually analyzed and judged to be frequent and stable enough to proceed to baseline.

Baseline. The terminal FR-5 or VR-5 schedules were used during all baseline sessions. Baseline sessions were 30 min in duration, and food restriction procedures remained in place. Experimental arrangements with respect to lighting and dipper activity were identical to those used during the final week of schedule training. That is, the parameters were as follows: (1) the house light came on when the session started and remained on for the duration of the session, (2) the clicker mechanism was activated, emitting one audible click, any time the schedule requirement was met, (3) concurrent with click, the dipper was activated, and (4) the dipper retracted 5s after head entry into the dipper entry. Noncontingent reinforcement modifications

were not used during baseline. The decision regarding when to transition the groups to extinction was made based on a visual analysis of stability of group means across baseline days.

Extinction. During extinction, no programmed consequences were available for the lever press. Extinction sessions were 30 min in duration, and food restriction procedures continued to be observed. Criterion for extinction was an average of less than 2 responses per minute across three consecutive days for each mouse. The experimental arrangements were as follows: (1) the house light came on when the session started and remained on for the duration of the session, (2) clicks were not emitted by the clicker mechanism, regardless of activity, and (3) the dipper remained in the retracted position. The chambers were cleaned prior to starting the extinction phase, and the liquid container was also removed for extinction sessions so as to remove olfactory stimuli associated with the food reinforcement.

Injections of NAC or vehicle occurred during the extinction phase. These injections were given i.p., as mentioned previously, 2.5 hr prior to the experimental session. This time frame was based on similar studies in the literature (see Zhou & Kalivas, 2008), where administration times have varied between 1 hr and 4 hr prior to testing. Mice were removed from their home cage individually and administered the solution by the experimenter. Following the injection, mice were returned to the home cage and remained in the housing room until removal for the experimental session.

Pre-reinstatement break. Prior to testing reinstatement effects, a pre-reinstatement phase break of 1 week occurred. A period of 1 week was chosen to ensure that a break was granted while taking care to not miss any potential lasting effects NAC may have beyond termination of administration. During this time, mice were not exposed to food deprivation, injection, or experimental procedures.

Cued reinstatement. Cued reinstatement sessions were 30 min in duration and food restriction was in place for 2 days prior to the first session and throughout the phase. It was necessary to restrict food access for 2 days prior to the cued reinstatement phase to ensure food deprivation levels were equivalent those in baseline and extinction. Two consecutive days of reinstatement sessions were conducted.

Experimental arrangements with respect to lighting, clicker, and dipper activity were as follows: (1) the house light came on when the session started and remained on for the duration of the session, (2) the clicker mechanism was activated, emitting one audible click, any time the schedule requirement was met (FR-5 or VR-5), and (3) the dipper remained retracted throughout the session. That is, the discriminative stimulus (i.e., the clicker) was issued when the schedule requirement was met, but no reinforcement was given. This arrangement was intended to measure the degree of reinstatement of responding occasioned by the discriminative stimulus (i.e., the click). Additionally, the liquid container was restored to its position and filled with liquid. This was done to in order that the cued reinstatement phase parameters mimicked, as closely as possible, those applied in baseline.

Reward reinstatement. Following the completion of cued reinstatement, a 4-day break ensued wherein mice were not exposed to any experimental procedures. On the fifth day after cued reinstatement, reward reinstatement testing took place. The procedures used for reward reinstatement paralleled those used in the drug relapse/reinstatement literature. The reward reinstatement session was 30 min in duration and food restriction was in place for 2 days prior to the first session and throughout the phase. As with the cued reinstatement phase, it was necessary to restrict food access for 2 days prior to the reward reinstatement phase to ensure food

deprivation levels were equivalent those in baseline, extinction, and cued reinstatement. One reward reinstatement session was conducted.

Experimental arrangements for reward reinstatement were as follows: (1) the house light came on when the session started and remained on for the duration of the session, (2) the clicker mechanism was activated, emitting one audible click, any time the schedule requirement was met (FR-5 or VR-5), (3) a single noncontingent presentation of the liquid occurred concurrent with the start of the session, (4) the dipper retracted 5 sec after head entry into the dipper entry, and (5) following that initial noncontingent activation, the dipper remained retracted for the duration of the session. The liquid container was present and filled with liquid in this phase as well, in parallel with parameters applied in baseline. The experimental arrangement in this phase was intended to measure the degree of reinstatement of responding occasioned by access to the liquid and continued exposure to the discriminative stimulus (i.e., the click).

Table 1 displays a complete timeline and description of experimental procedures.

Table 1

Procedural Timeline

	Phase	Duration of Procedure	Food Restriction	Drug Administration	Schedule of Reinforcement
Wk 1	Pre-handling	2 min	Yes	No	
Wk 2	Pre-handling	2 min	Yes	No	
Wk 3	Autoshaping	1 hr	Yes	No	FR1
Wk 4	Autoshaping/ Manual Shaping	1 hr	Yes	No	FR1
Wk 5	Autoshaping/ Manual Shaping	1 hr	Yes	No	FR1
Wk 6	Schedule Training	30 min	Yes	No	FR1 → FR-5/VR-5
Wk 7	Schedule Training	30 min	Yes	No	FR1 → FR-5/VR-5
Wk 8	Baseline	30 min	Yes	No	FR-5 or VR-5
Wk 9	Baseline	30 min	Yes	No	FR-5 or VR-5
Wk 10	Extinction	30 min	Yes	Yes	
Wk 11	Extinction	30 min	Yes	Yes	
Wk 12	Pre-reinstatement Break			No	
Wk 13	Reinstatement	30 min	Yes	No	

Statistical Analyses

The IBM SPSS Statistics 18 (SPSS, Inc., 2010) package was used to conduct all statistical analyses. Histograms of raw data indicated a non-normal distribution; however, *log* transformation of the data resulted in normal distribution. All data analyses were therefore carried out with *log* transformed values. The data were analyzed using mixed model analysis

methods, also known as a multilevel linear model or a hierarchical linear model. Within the mixed model, methods of applied longitudinal data analysis were followed to accommodate for the presence of repeated measures in the data. Additional analyses included *t*-tests and ANOVAs for comparing individual data points from the mixed models. Statistical significance was set at $p \leq 0.05$.

Due to irregularities in responding presumably caused by unrestricted food access on the weekends, data from the first session of each week were not included in the final analysis. To ensure vital extinction data were not discarded, the first session of the first week of extinction was run using baseline session parameters. Similarly, each reinstatement session was preceded by one day of the staggered food deprivation schedule, so as to mimic the food access available on days when experimental sessions were run. That is, the procedures on each of the days preceding the first cued and reward reinstatement sessions mirrored those on applied on the first session of each week, with the exception that subjects were not actually exposed to experimental sessions.

CHAPTER III

RESULTS

Data from this study were analyzed via mixed-model analysis, *t*-tests, and ANOVAs. Results of baseline *t*-tests, which were conducted to ensure that no unexpected pre-experimental differences existed, are discussed first. Next, analysis of extinction drug effects is discussed. Results of mixed-model and *t*-test analyses for the FR groups are presented, followed by results of the same analyses for the VR groups. Analysis of extinction schedule effects are then addressed in the presentation of mixed-model and ANOVA results of the FR and VR groups combined analysis. Lastly, results of *t*-test analyses for the reinstatement data are presented.

Baseline Analyses

Baseline *t*-tests were conducted on Day 1 to determine whether differences existed for the frequency of active lever responses between the FR-NAC and FR-VEH groups, as well as between the VR-NAC and VR-VEH groups. Equal variances were assumed for all *t*-tests. Both *t*-tests were nonsignificant, indicating that the two FR groups were not significantly different from one another and the two VR groups were not significantly different from each another at Day 1 of baseline. Corresponding *t*-tests for number of reinforcers earned and head entries also produced nonsignificant results. Significant differences were found between the FR-NAC and FR-VEH ($p = .016$) and between the VR-NAC and VR-VEH ($p = .016$) groups for left-lever presses. However, as this was a low frequency behavior and not the primary variable of interest,

the significant *t*-test results were not considered problematic. The results of *t*-tests from day 1 of baseline are shown in Table 2.

Table 2

Baseline t-tests: Baseline Day 1

		t	df	*p* (2-tailed)
Active	FR Groups	.557	21	.584
	VR Groups	.080	22	.937
Left	FR Groups	2.685	17	.016
	VR Groups	2.642	20	.016
Head Entries	FR Groups	-1.787	21	.088
	VR Groups	-1.205	22	.241
Reinforcers	FR Groups	.509	21	.616
	VR Groups	.192	22	.849

A second baseline *t*-test was conducted on Day 8 of baseline. To compare responding on the FR schedule to that of the VR schedule, mice from the NAC and VEH groups were collapsed within each schedule type. That is, all FR mice were collapsed into one group (N=23) and all VR mice were collapsed into a second group (N=24). The median of the final three days of baseline was taken for each mouse, and this number was used to calculate the *t*-test. Results indicated that active lever presses were significantly higher in the VR group, relative to the FR group (p = .038). A significant difference in active lever presses across schedule types was desirable for two

reasons: (a) it aligned with known differences between patterns of FR and VR operant responding, and (b) when taken in concert with the lack of a significant difference across groups for the number of reinforcers earned, it suggested groups were sensitive to the schedule requirement. No significant differences were found between the collapsed FR and VR groups for left lever, head entries, or number of earned reinforcers. Further, no significant differences were found for number of earned reinforcers between FR-NAC and FR-VEH groups and between VR-NAC and VR-VEH groups. It is important to note that baseline was the only phase in which reinforcement data were analyzed, because the reinforcement contingency was terminated with the onset of extinction. The results of these *t*-tests are shown in Table 3.

Table 3

Baseline t-tests: Median of Baseline Days 6 to 8, Collapsed Groups

	t	df	*p* (2-tailed)
Active	-2.134	45	.038
Left	-1.070	41	.291
Head Entries	.925	45	.360
Reinforcers	-1.676	45	.101

Extinction Drug Effects

Mixed-model analyses were conducted on the complete extinction dataset (e.g. all 8 extinction sessions). Additional mixed-model analyses were conducted on the restricted extinction dataset (e.g. the first three days of extinction), which allowed for isolation of the days when the most robust effect of the drug was expected. Similar analyses have been conducted

within the literature based on the repeated finding that the effect of NAC administration is most marked within the first few days of exposure to extinction (Baker et al., 2003a; Kau et al., 2008; Zhou & Kalivas, 2008). For all mixed-models, data from each of the 3 dependent variables, active lever, left lever, and head entries, was compared across the two FR groups and the two VR groups. This analysis allowed for a direct comparison of the drug and vehicle groups for each schedule. Corresponding *t*-tests provided more detailed comparisons of individual sessions.

Figure 1 shows data for all dependent variables from the FR-NAC and FR-VEH groups for all phases. Within the FR groups, a mixed-model conducted on the complete extinction dataset showed administration of NAC produced a significant decrease in the frequency of active lever responses relative to VEH ($p = .018$). This result is displayed in Figure 2. However, no significant drug effect was observed on the active lever in the restricted mixed-model analyses. Some clarification of this disparity was provided via preplanned independent *t*-tests analyses conducted for Days 1, 2, and 3 of extinction. Active lever presses were significantly decreased in the FR-NAC group only at Day 2 ($p = .041$). Differences at Days 1 and 3 were nonsignificant.

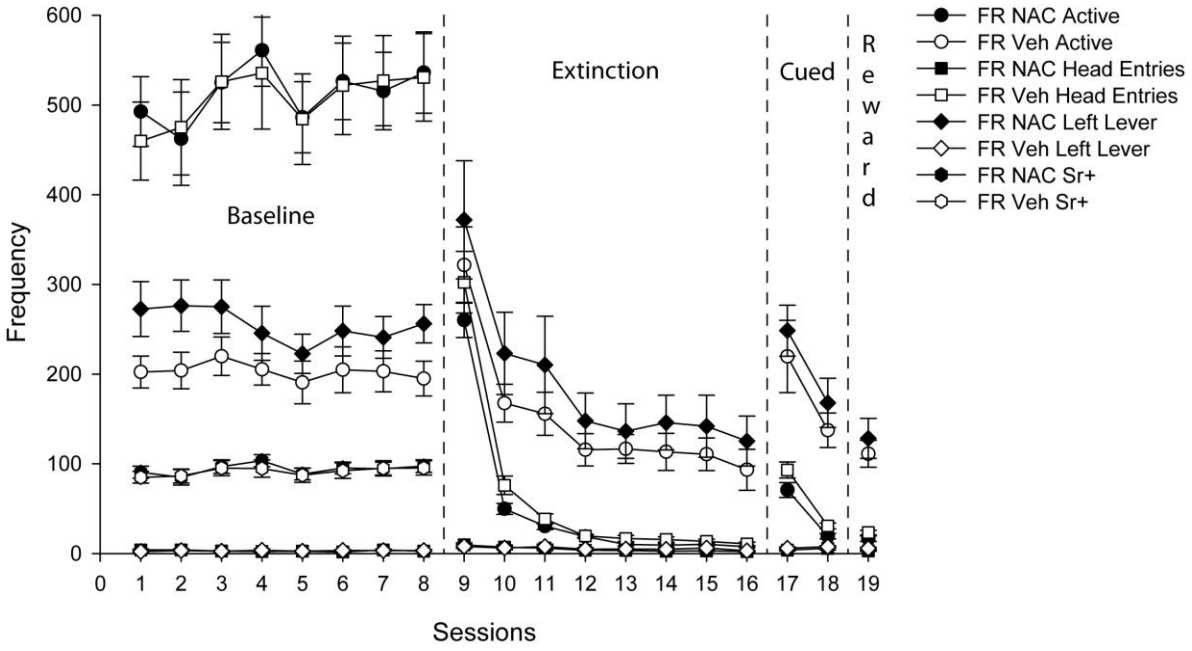

Figure 1. Fixed Ratio Groups: All Dependent Variables

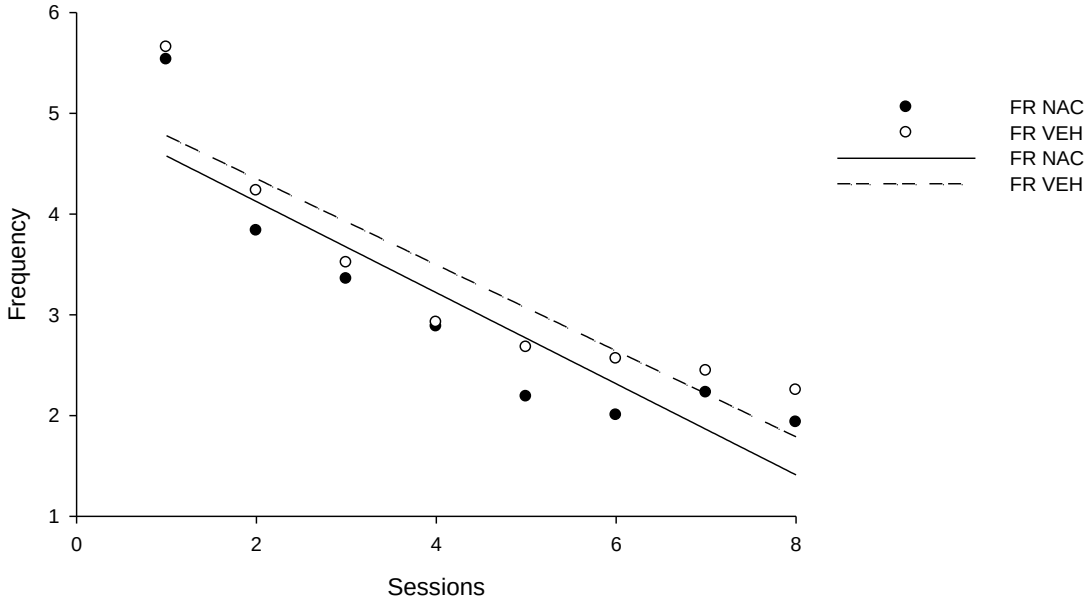

Figure 2. Fixed Ratio Extinction Active Lever: *Log* Linear

Based on the pattern of mixed-model and *t*-test results for active lever presses among the FR groups, additional *t*-test analyses were conducted for the remaining extinction sessions (e.g., Days 4 through 8). Response frequency was significantly decreased in the FR-NAC group on Day 5 ($p = .038$), and a similar pattern of responding approached significance for Day 6 ($p = .071$). The complete and restricted mixed-model analyses produced a nonsignificant effect of NAC on left-lever presses and head entries, and *t*-test results for Days 1 through 3 were correspondingly nonsignificant for these variables. The treatment x time interaction was not significant for any dependent variable in either FR mixed-model analysis. Results of the complete and restricted mixed-model analysis are represented in Tables 4 and 5, respectively, and *t*-test results are displayed in Table 6. Figures 3 through 5 represent data for each of the three dependent variables for the FR groups for the extinction and reinstatement phases only.

Table 4

Fixed Ratio Complete Mixed-Model Analyses

	Source	Numerator df	Denominator df	F	*p* (2-tailed)
Active	Intercept	1	21	3087.097	<.001
	Treatment	1	21	6.525	.018
	Time	7	147	166.941	<.001
	Treatment*Time	7	147	.994	.438
Left	Intercept	1	19.273	100.275	<.001
	Treatment	1	19.273	.641	.433
	Time	7	126.195	4.984	<.001
	Treatment*Time	7	126.195	1.008	.429
Head Entries	Intercept	1	21	1742.197	<.001
	Treatment	1	21	.767	.391
	Time	7	147	22.270	<.001
	Treatment*Time	7	147	.599	.756

Table 5

Fixed Ratio Restricted Mixed-Model Analyses

	Source	Numerator df	Denominator df	F	*p* (2-tailed)
Active	Intercept	1	21	3973.251	<.001
	Treatment	1	21	2.709	.115
	Time	2	42	309.623	<.001
	Treatment*Time	2	42	1.381	.262
Left	Intercept	1	17.655	117.662	<.001
	Treatment	1	17.655	.026	.874
	Time	2	34.927	2.889	.069
	Treatment*Time	2	34.927	.054	.947
Head Entries	Intercept	1	21	2180.439	<.001
	Treatment	1	21	.374	.547
	Time	2	42	33.989	<.001
	Treatment*Time	2	42	.283	.755

Table 6

Fixed Ratio t-tests for Extinction

			t	df	*p* (2-tailed)
Active	FR Groups	Day 1	-.972	21	.342
		Day 2	-2.175	21	.041
		Day 3	-.809	21	.428
		Day 4	-.264	21	.794
		Day 5	-2.209	21	.038
		Day 6	-1.899	21	.071
		Day 7	-.957	21	.349
		Day 8	-1.599	21	.125
Left	FR Groups	Day 1	-.304	17	.765
		Day 2	.232	19	.819
		Day 3	-.129	19	.899
Head Entries	FR Groups	Day 1	-.252	21	.803
		Day 2	-.673	21	.508
		Day 3	-717	21	.481

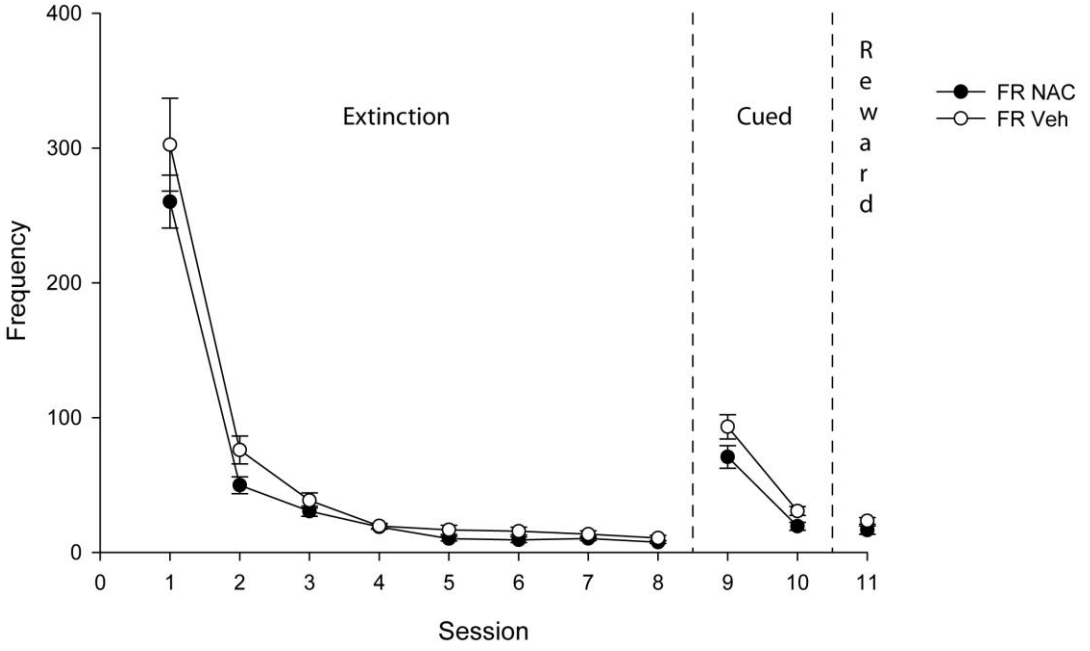

Figure 3. Fixed Ratio Extinction and Reinstatement: Active Lever

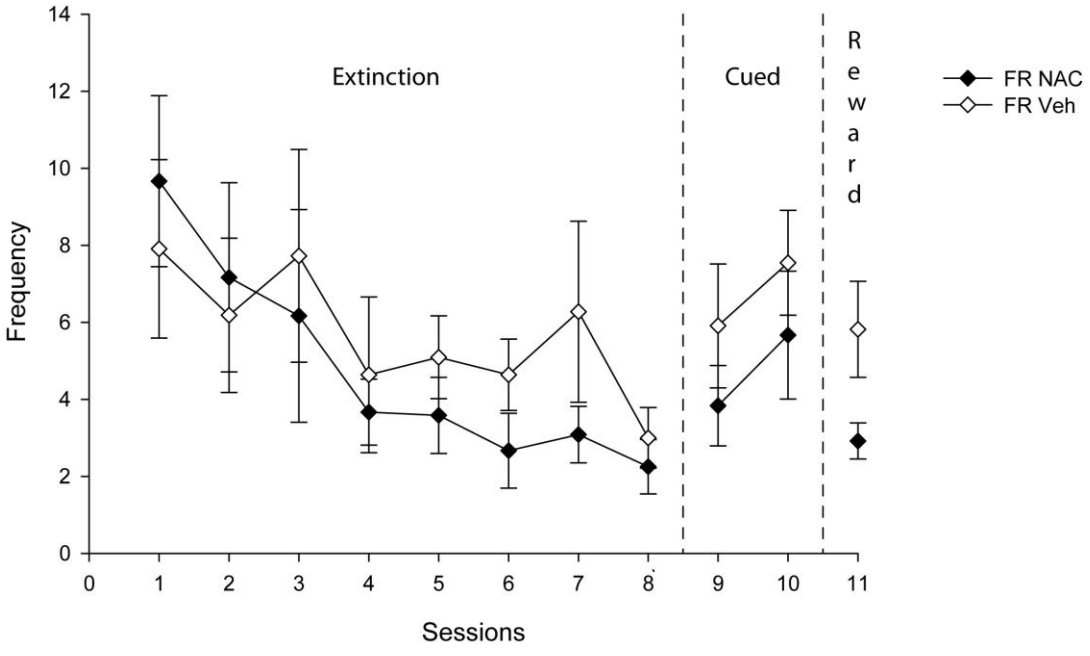

Figure 4. Fixed Ratio Extinction and Reinstatement: Left Lever

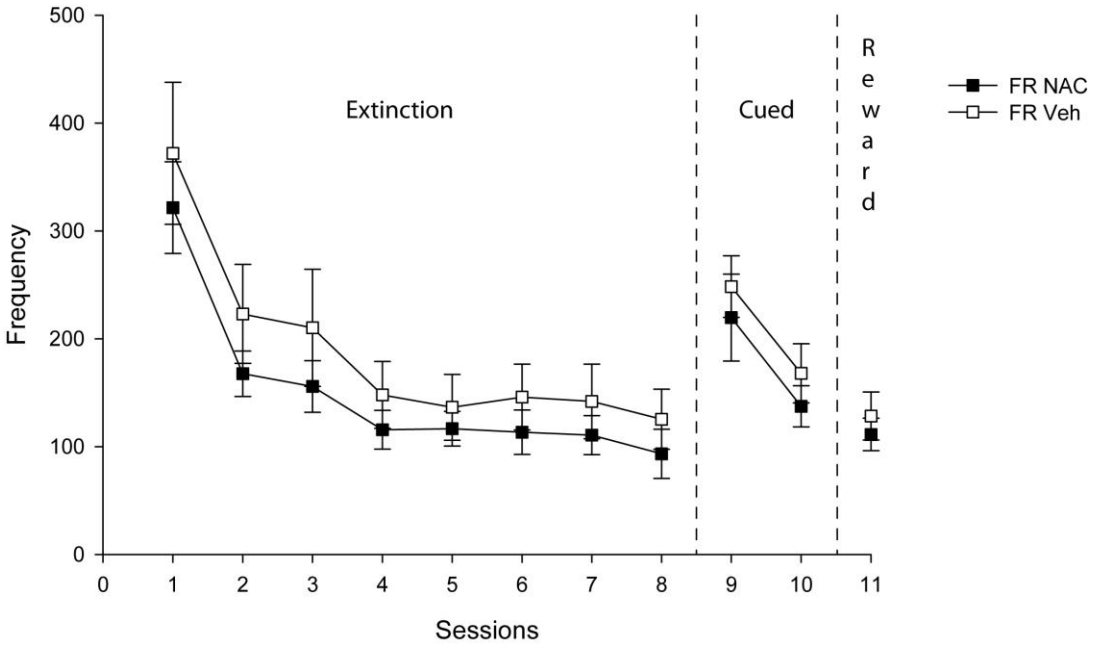

Figure 5. Fixed Ratio Extinction and Reinstatement: Head Entries

Figure 6 displays data on all dependent variables from the VR-NAC and VR-VEH groups for all phases. Among the VR groups, NAC did not produce a significant effect on active lever presses, left-lever presses, or head entries. Similarly, no significant drug effect was observed for active lever presses or head entries in the restricted mixed-model, but response frequency on the left lever was significantly different across groups (p = .049). Corresponding *t*-tests for extinction Days 1 through 3 were also nonsignificant. The interaction of treatment x time was nonsignificant for all dependent variables. Results of the complete mixed model analysis are shown in Table 7, restricted mixed-model analyses in Table 8, and *t*-test results in Table 9. Figures 7 through 9 show data for each of the three dependent variables for the FR groups for the extinction and reinstatement phases only.

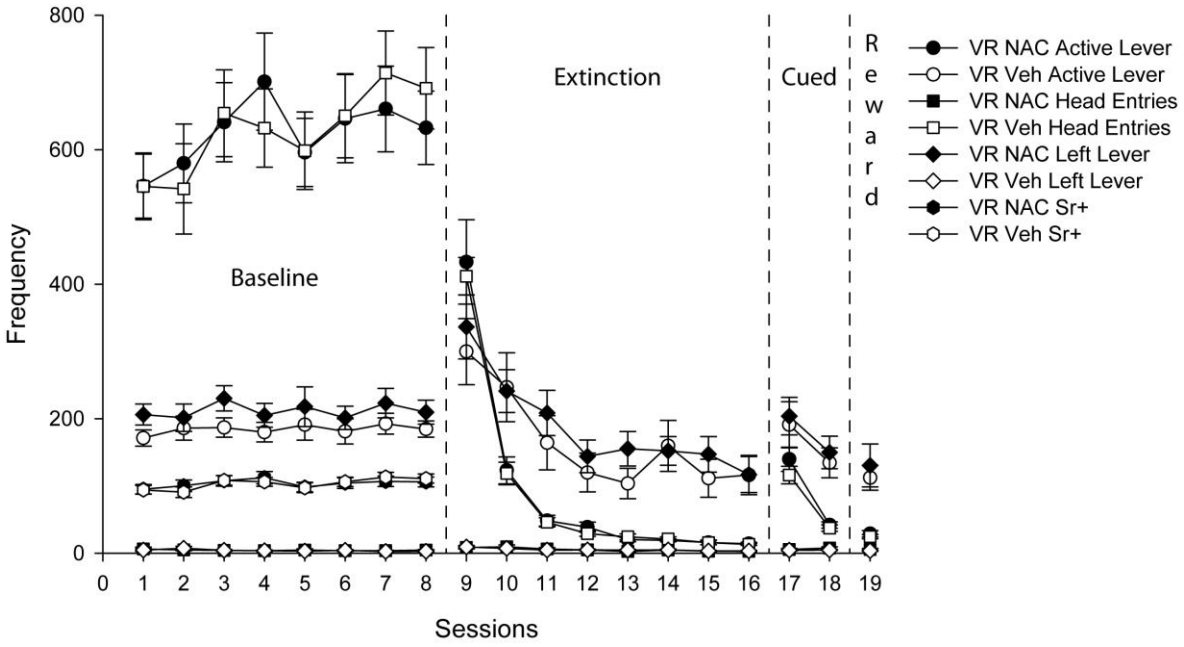

Figure 6. Variable Ratio Groups: All Dependent Variables

Table 7

Variable Ratio Complete Mixed-Model Analyses

	Source	Numerator df	Denominator df	F	*p* (2-tailed)
Active	Intercept	1	22	2706.486	<.001
	Treatment	1	22	.004	.949
	Time	7	154	202.927	<.001
	Treatment*Time	7	154	.458	.863
Left	Intercept	1	20.863	88.572	<.001
	Treatment	1	20.863	.001	.979
	Time	7	131.963	7.271	<.001
	Treatment*Time	7	131.963	.816	.576
Head Entries	Intercept	1	22	2047.809	<.001
	Treatment	1	22	1.010	.326
	Time	7	154	24.337	<.001
	Treatment*Time	7	154	.894	.513

Table 8

Variable Ratio Restricted Mixed-Model Analyses

	Source	Numerator df	Denominator df	F	*p* (2-tailed)
Active	Intercept	1	22	4310.886	<.001
	Treatment	1	22	.008	.930
	Time	2	44	302.222	<.001
	Treatment*Time	2	44	.033	.968
Left	Intercept	1	19.052	101.796	<.001
	Treatment	1	19.052	.570	.460
	Time	2	36.828	3.273	.049
	Treatment*Time	2	36.828	.668	.519
Head Entries	Intercept	1	22	2146.777	<.001
	Treatment	1	22	.689	.415
	Time	2	44	20.396	<.001
	Treatment*Time	2	44	.966	.388

Table 9

Variable Ratio t-tests for Extinction

		t	df	*p* (2-tailed)
Active	Day 1	-.345	22	.733
	Day 2	-.159	22	.875
	Day 3	1.126	22	.272
Left	Day 1	.454	21	.654
	Day 2	.922	20	.367
	Day 3	1.282	18	.216
Head Entries	Day 1	-.112	22	.912
	Day 2	.466	22	.646
	Day 3	-.626	22	.537

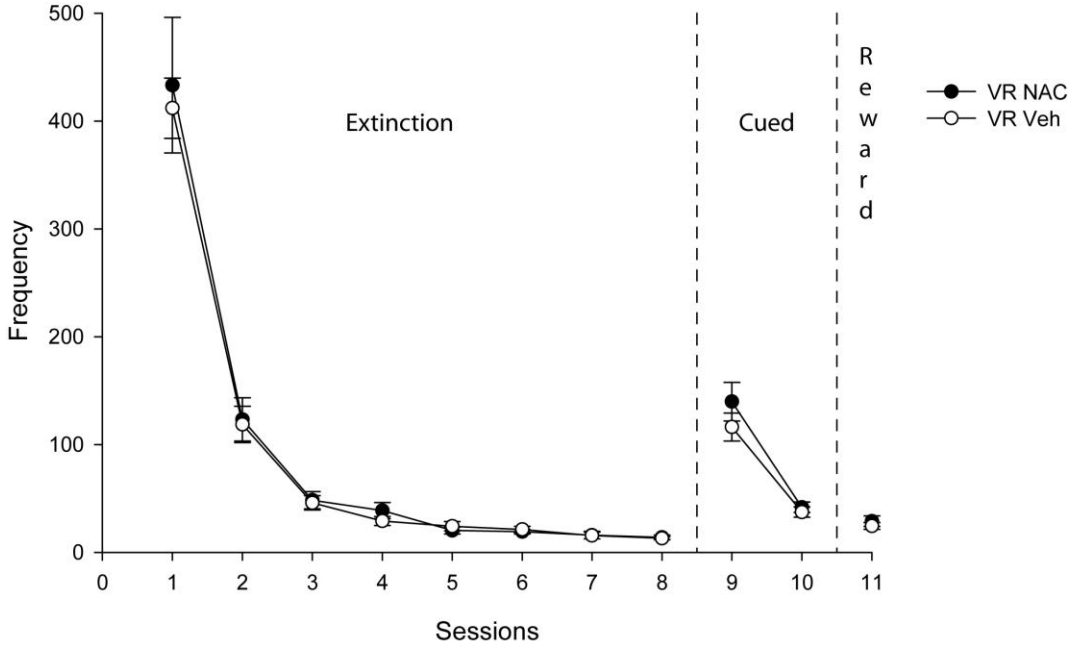

Figure 7. Variable Ratio Extinction and Reinstatement: Active Lever

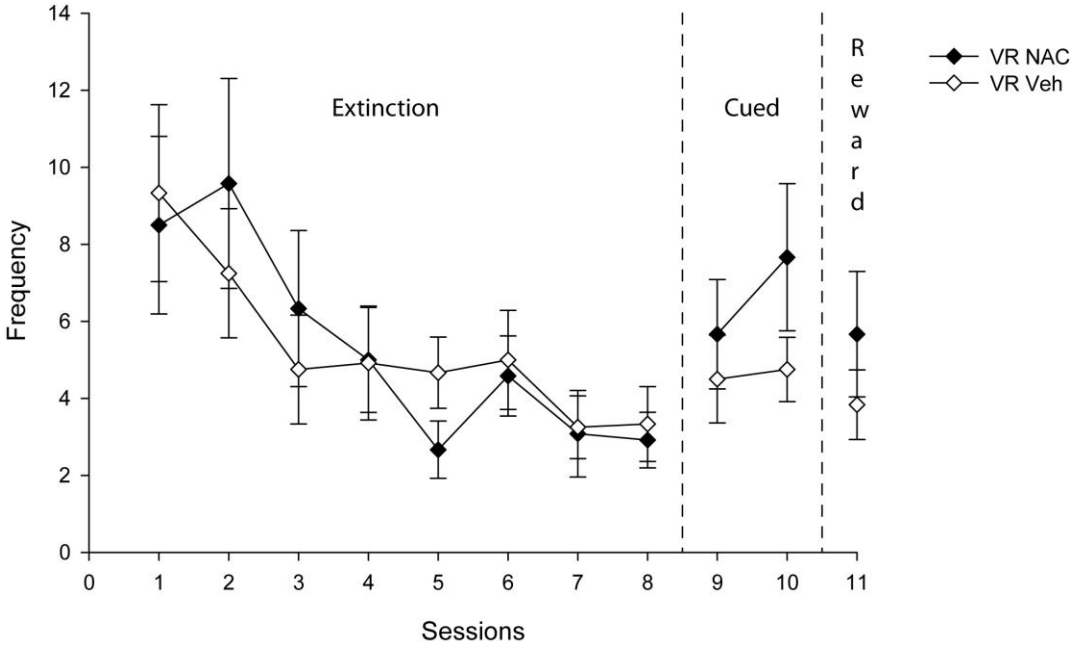

Figure 8. Variable Ratio Extinction and Reinstatement: Left Lever

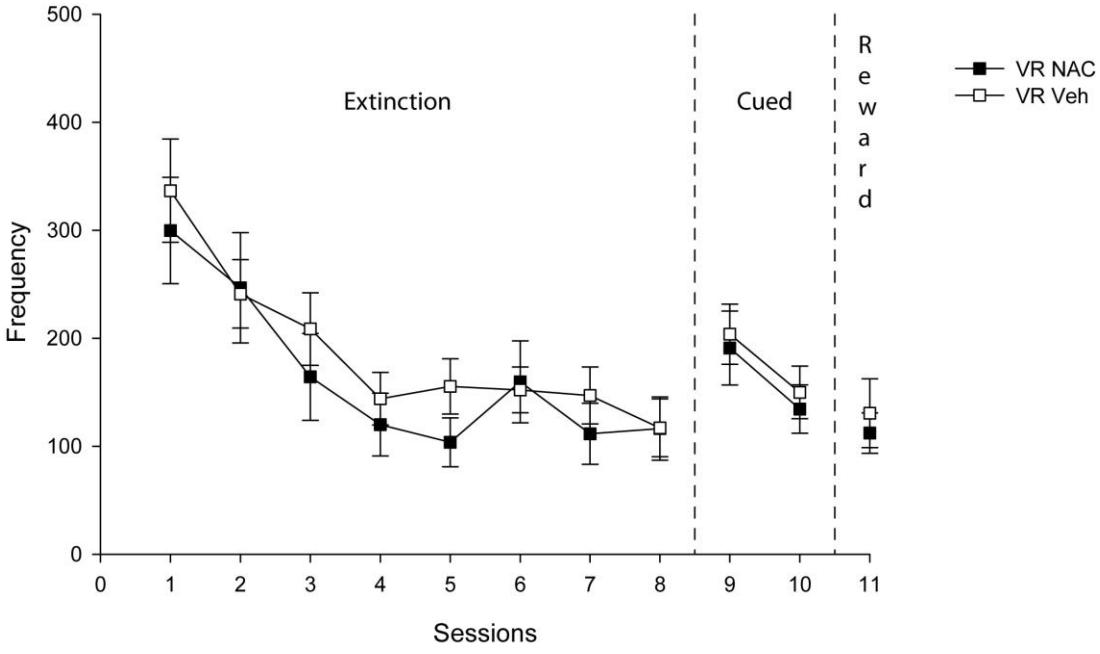

Figure 9. Variable Ratio Extinction and Reinstatement: Head Entries

Analysis of covariance parameters, which addressed the random effects of behavioral differences between individual mice, was calculated for all of the mixed-models discussed above. The resulting intracorrelation coefficients (ICCs) reveal the degree to which response frequency across sessions was correlated in individual mice. ICCs were large for all dependent variables in all models, a finding which suggests large individual differences existed across mice.

Extinction Schedule Effects

Full-factorial mixed-model analyses were run on the complete extinction data set of three dependent variables (e.g., active lever press, left lever press, and head entries) and four experimental groups (e.g., FR-NAC, FR-VEH, VR-NAC, VR-VEH). This analysis allowed for the testing of interactions in the data, such that differential effects of drug administration across schedules would be detected. Significant effects of schedule and time were observed for the active lever ($p < .001$). That is, response frequency for the active lever was significantly greater in the VR groups relative to the FR groups and responding for all groups decreased significantly across sessions. This finding was anticipated, as differences in frequency and patterns of extinction responding for fixed versus variable ratio schedules are well-documented (Bacon, 1965; Hearst, 1961; Longstreth, 1964; Perkins & Cacioppo, 1950; Skinner, 1938; Stimbert, 1970). Response frequency for the left lever and head entries decreased significantly over time ($p < .001$), but differences in response frequency across VR and FR were nonsignificant for both dependent variables. All interaction terms were nonsignificant for all dependent variables. Results of the full-factorial mixed-model are represented in Table 10. Figures 10, 11, and 12 show the data for all phases and groups for each of the three dependent variables, respectively.

Table 10

Full Factorial Complete Mixed-Model Analyses

	Source	Numerator df	Denominator df	F	*p* (2-tailed)
Active	Intercept	1	223.021	1790.924	<.001
	Schedule	1	43.060	25.447	<.001
	Treatment	2	76.003	1.435	.245
	Time	7	300.125	364.274	<.001
	Treatment*Time	7	300.125	1.191	.308
	Schedule*Time	7	300.125	1.243	.279
	Schedule*Time*Treatment	8	169.245	.582	.792
Left	Intercept	1	140.472	103.458	<.001
	Schedule	1	40.075	.048	.828
	Treatment	2	71.001	1.079	.345
	Time	7	257.141	11.280	<.001
	Treatment*Time	7	257.141	1.327	.238
	Schedule*Time	7	257.141	1.095	.367
	Schedule*Time*Treatment	8	151.000	.567	.804
Head Entries	Intercept	1	96.762	2292.819	<.001
	Schedule	1	43.012	.162	.689
	Treatment	2	79.338	1.197	.308
	Time	7	300.030	44.941	<.001
	Treatment*Time	7	300.030	.300	.953
	Schedule*Time	7	300.030	1.122	.349
	Schedule*Time*Treatment	8	169.116	1.079	.380

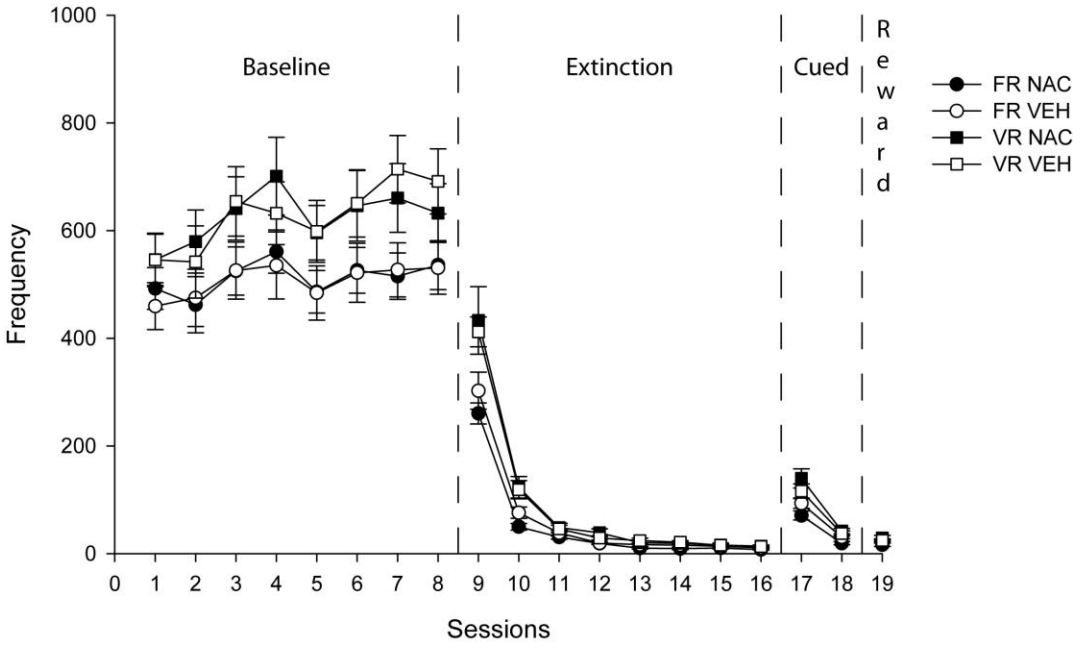

Figure 10. Fixed and Variable Ratio: Active Lever

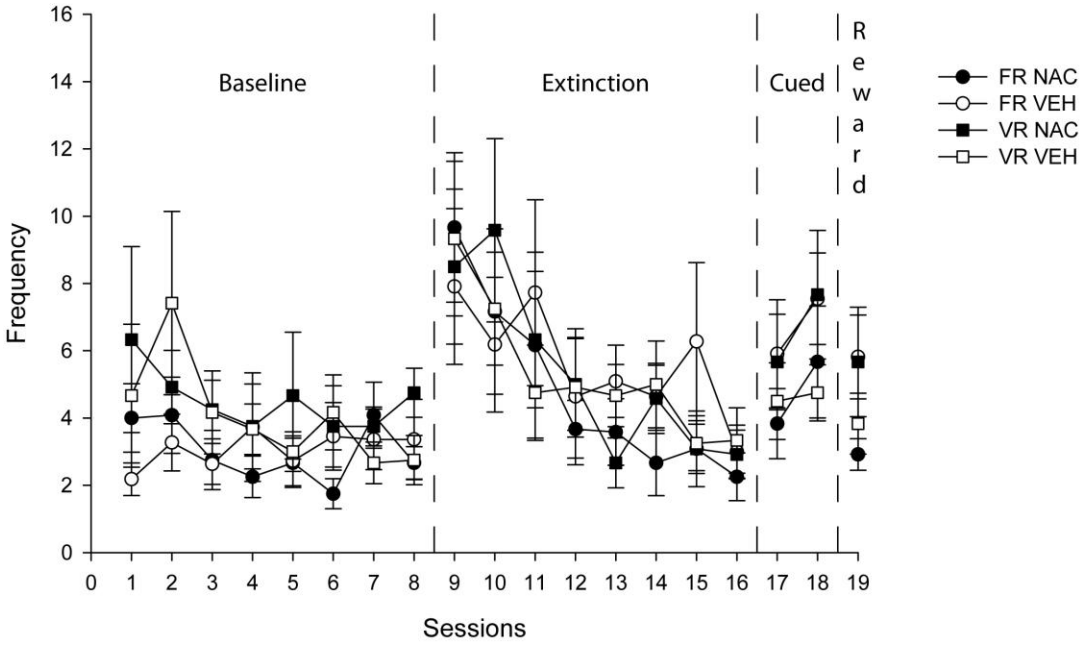

Figure 11. Fixed and Variable Ratio: Left Lever

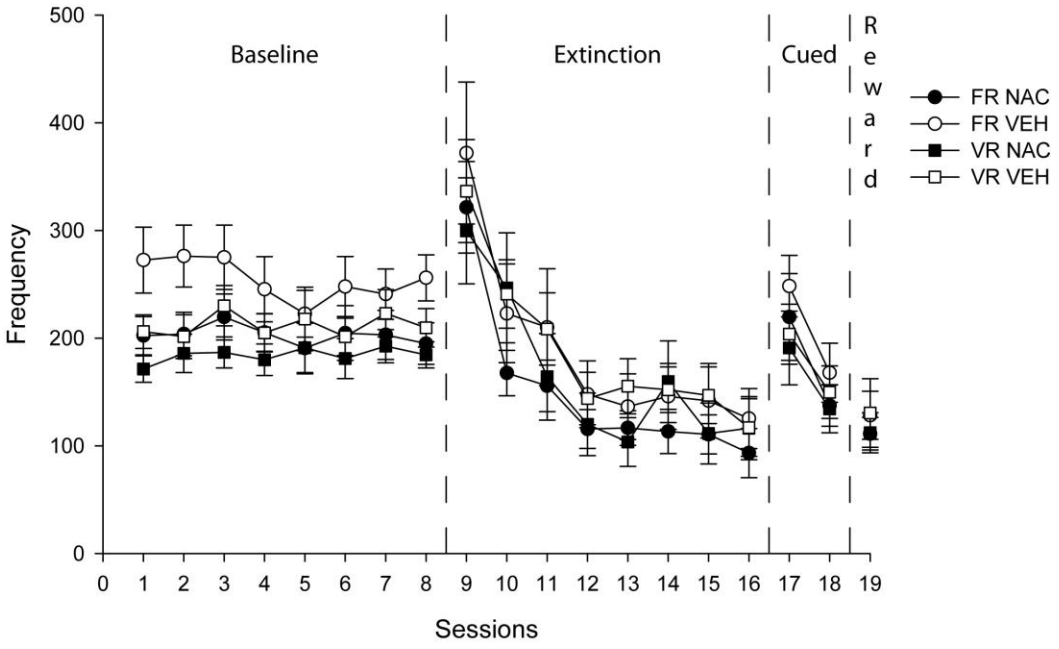

Figure 12. Fixed and Variable Ratio: Head Entries

An additional 3 full-factorial restricted mixed-model analyses were run on extinction data for days 1, 2, and 3 only for active lever press, left lever press, and head entries for the four experimental groups. This analysis allowed for the testing of interactions in the data on the days when the largest differences across groups were expected. As with the analyses run on the complete extinction dataset, response frequency on the active lever was significantly greater in the VR groups relative to the FR groups ($p < .001$), and active lever responses for all groups decreased significantly over sessions ($p < .001$). Once again, this finding was anticipated, as the effects of time and fixed versus variable ratio schedule on extinction are well-documented (Hearst, 1961; Parker, 1967; Romanczyk , 1977; Skinner, 1938). A significant decrease in response frequency was observed for left lever presses ($p = .013$) and head entries ($p <.001$); however, no significant effect of schedule was found for either of these dependent variables. The

interaction of schedule x time was significant for the active lever (p = .027), a finding that indicated different slope in active lever response frequency over time across schedules. All remaining interaction terms were nonsignificant for all dependent variables. Results of the restricted full-factorial mixed-models are represented in Table 11.

Analysis of covariance parameters, which addressed the random effects of behavioral differences between individual mice, was calculated for the full-factorial mixed-models discussed above. ICCs were large for all dependent variables in all models, a finding that suggests large individual differences across mice.

The final schedule effect analyses conducted consisted of a one-way analysis of variance (ANOVA). An ANOVA was conducted for change scores from: (1) Baseline Day 8 to Extinction Day 1, (2) Extinction Day 8 to Cued Reinstatement Day 1, and (3) Cued Reinstatement Day 2 to Reward Reinstatement for all experimental groups. These analyses were conducted in order to determine whether any significant differences existed in the degree of change of behavior across phase changes. Change scores were calculated by taking the absolute value of the difference between *log* transformed scores for the two days of each pairing. An ANOVA was conducted so that differences between and across schedule types could be determined.

For the first pairing, Baseline Day 8 and Extinction Day 1, analysis of change scores indicated that no significant differences existed for any dependent variable. An identical finding was evident for all three of the remaining pairings mentioned above. No *post-hoc* tests were conducted due to the failure of any change score analysis to reach significance. Results of these ANOVAs are displayed in Table 12.

Table 11

Full Factorial Restricted Mixed-Model Analyses

	Source	Numerator df	Denominator df	F	*p*
Active	Intercept	1	105.159	3862.921	<.001
	Schedule	1	43.000	19.963	<.001
	Treatment	1	43.000	1.130	.294
	Time	3	90.513	409.219	<.001
	Treatment*Time	2	86	.570	.568
	Schedule*Time	2	86	3.773	.027
	Schedule*Time*Treatment	3	64.241	1.014	.392
Left	Intercept	1	94.706	86.503	<.001
	Schedule	1	36.522	.066	.799
	Treatment	1	36.522	.507	.481
	Time	3	75.486	3.820	.013
	Treatment*Time	2	71.432	.185	.832
	Schedule*Time	2	71.432	1.197	.308
	Schedule*Time*Treatment	3	53.959	.220	.882
Head Entries	Intercept	1	78.156	2838.934	<.001
	Schedule	1	43	.071	.791
	Treatment	1	43.000	1.041	.313
	Time	3	89.814	34.815	<.001
	Treatment*Time	2	86.000	.861	.426
	Schedule*Time	2	86.000	2.448	.093
	Schedule*Time*Treatment	3	64.241	.303	.823

Table 12

ANOVA Analysis of Change Scores

	Phase Change	F	df	*p*
Active	Baseline Day 8 → Extinction Day 1	1.347	46	.272
	Extinction Day 8 → Cued Reinstatement Day 1	.190	46	.902
	Cued Reinstatement Day 2 → Reward Reinstatement	.576	46	.634
Left	Baseline Day 8 → Extinction Day 1	.456	45	.715
	Extinction Day 8 → Cued Reinstatement Day 1	.495	45	.687
	Cued Reinstatement Day 2 → Reward Reinstatement	.548	45	.063
Head Entries	Baseline Day 8 → Extinction Day 1	.070	46	.976
	Extinction Day 8 → Cued Reinstatement Day 1	1.529	46	.221
	Cued Reinstatement Day 2 → Reward Reinstatement	.337	44	.770

Reinstatement Drug Effects

Independent samples *t*-tests were conducted for the FR groups and VR groups, respectively, for Cued Reinstatement Days 1 and 2 as well as Reward Reinstatement. For *t*-tests comparing FR-NAC to FR-VEH, significant differences were found for active lever presses on Cued Reinstatement Day 2 ($p = .015$) and Reward Reinstatement ($p = .039$); results for Cued Reinstatement Day 1 were on the cusp of significance ($p = .050$). Significantly fewer left lever responses occurred in the FR-NAC in Reward Reinstatement ($p = .001$) but no significant difference was present on either day of Cued Reinstatement. Head entries were not significantly different for any reinstatement day. Results of *t*-tests comparing VR-NAC to VR-VEH across all reinstatement days were nonsignificant for all dependent variables. However, a trend existed for increased responding in the NAC group, which is opposite to the pattern of findings for the FR groups. The *t*-tests for left lever responses and head entries were nonsignificant across all

reinstatement days. These results are displayed in Table 13. Figures 13, 14, and 15 show active lever press reinstatement data for each of the three reinstatement sessions, respectively, for the FR groups, and Figures 16, 17, and 18 display the same data for the VR groups.

Table 13

t-tests for Reinstatement

			t	df	*p* (2-tailed)
Active	FR Groups	Cued, Day 1	-2.076	21	.050
		Cued, Day 2	-2.665	21	.015
		Reward	-2.200	21	.039
	VR Groups	Cued, Day 1	.652	22	.521
		Cued, Day 2	.937	22	.359
		Reward	.631	22	.535
Left	FR Groups	Cued, Day 1	-.724	20	.478
		Cued, Day 2	-1.064	18	.302
		Reward	-3.808	19	.001
	VR Groups	Cued, Day 1	.934	19	.358
		Cued, Day 2	1.613	21	.122
		Reward	1.338	19	.197
Head Entries	FR Groups	Cued, Day 1	-1.036	21	.312
		Cued, Day 2	-.637	21	.531
		Reward	-.334	21	.742
	VR Groups	Cued, Day 1	.168	22	.868
		Cued, Day 2	.363	22	.720
		Reward	.239	22	.813

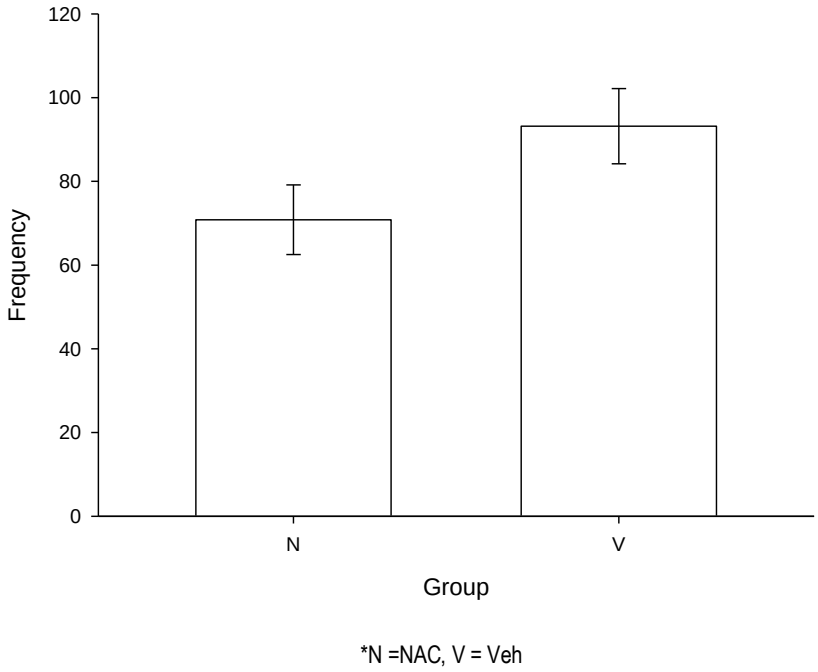

Figure 13. Fixed Ratio Cued Reinstatement Day 1: Active Lever

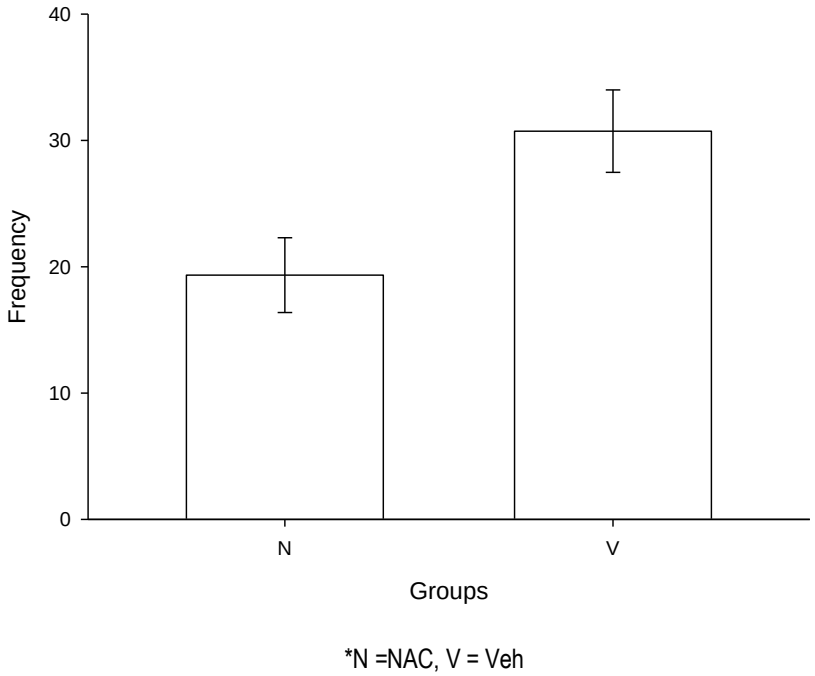

Figure 14. Fixed Ratio Cued Reinstatement Day 2: Active Lever

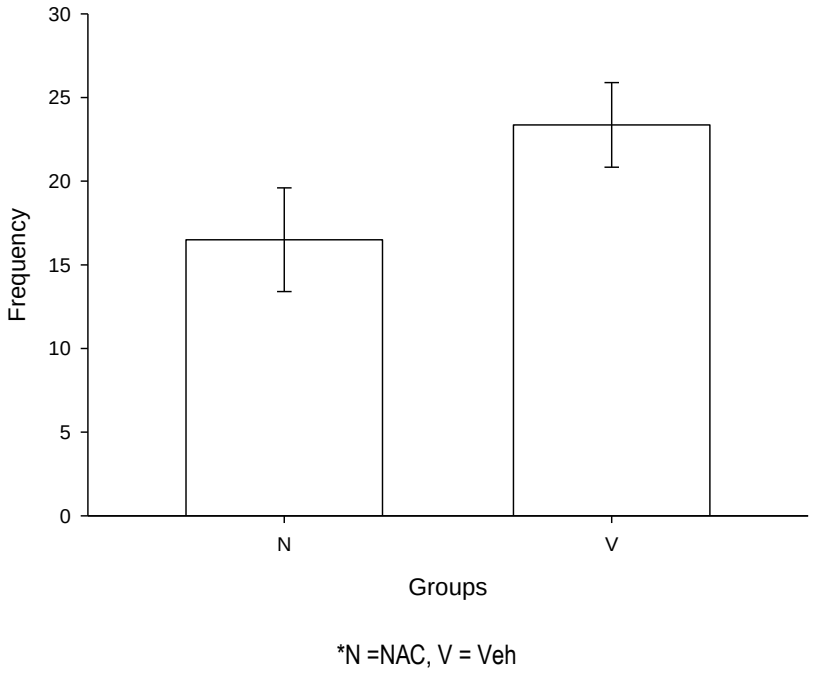

Figure 15. Fixed Ratio Reward Reinstatement: Active Lever

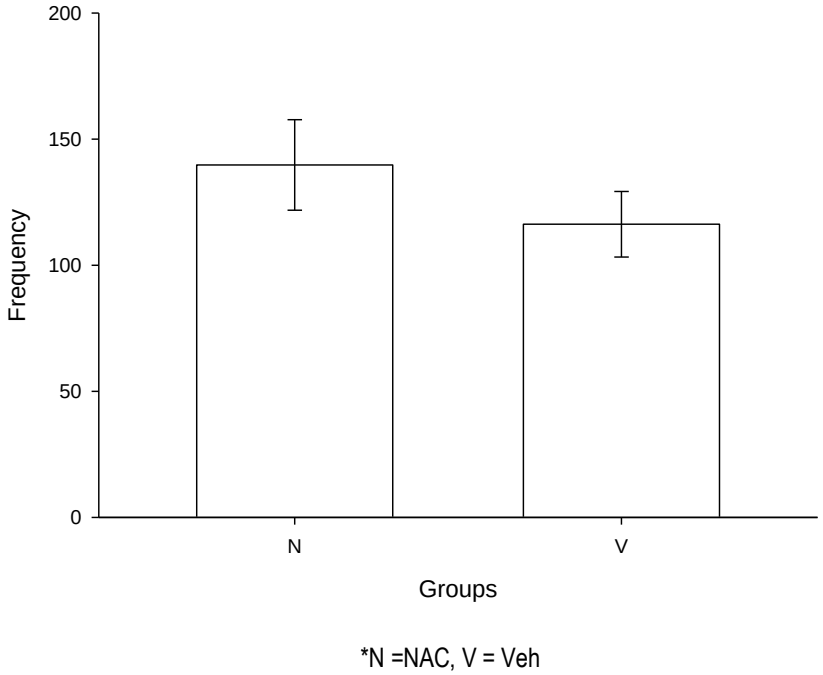

Figure 16. Variable Ratio Cued Reinstatement Day 1: Active Lever

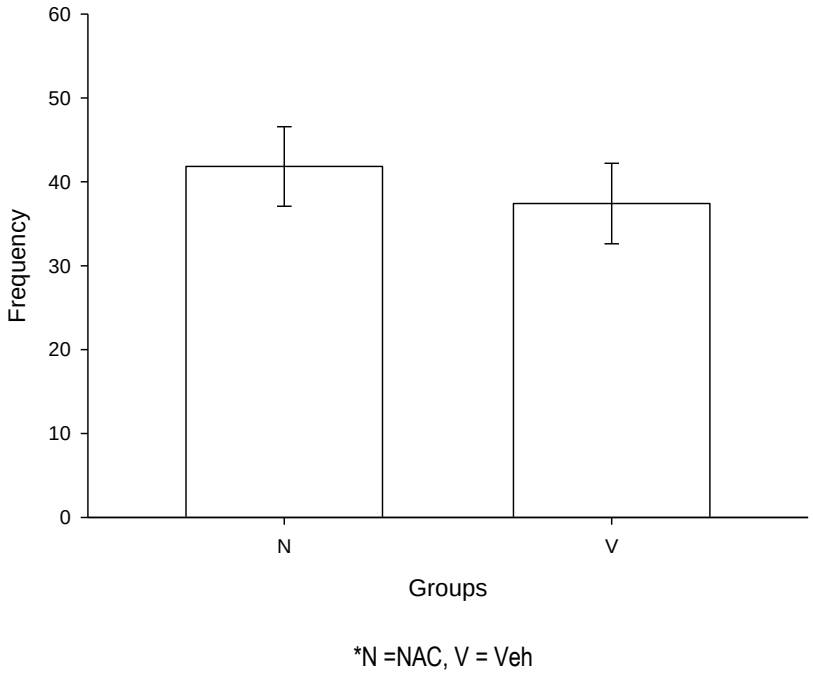

Figure 17. Variable Ratio Cued Reinstatement Day 2: Active Lever

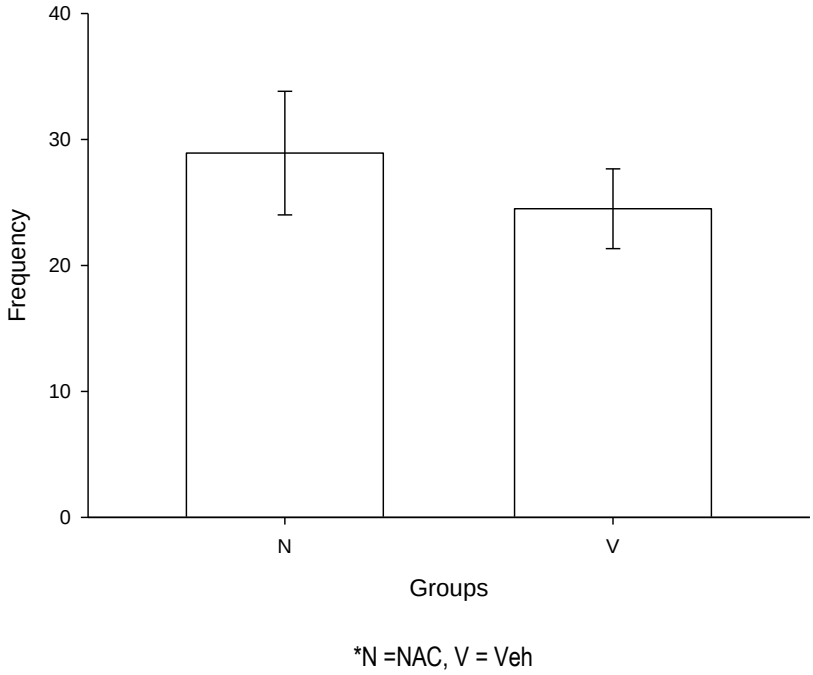

Figure 18. Variable Ratio Reward Reinstatement: Active Lever

Visual Analysis

Visual analysis of bin data was used to determine whether any differences existed between schedule groups. Figures 19, 20, and 21 display extinction data for days 1 through 3, respectively, in 1 min bins for the active lever variable for the FR groups, while Figures 22, 23, and 24 illustrate the same information for the VR groups.

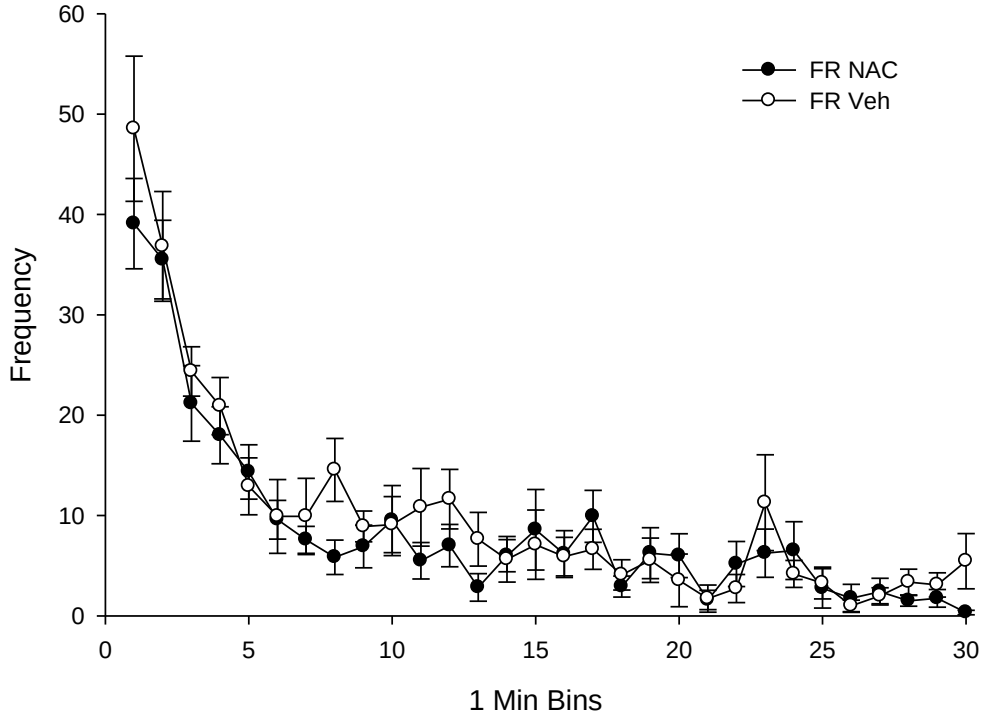

Figure 19. Fixed Ratio Extinction Day 1: Active Lever Bin Data

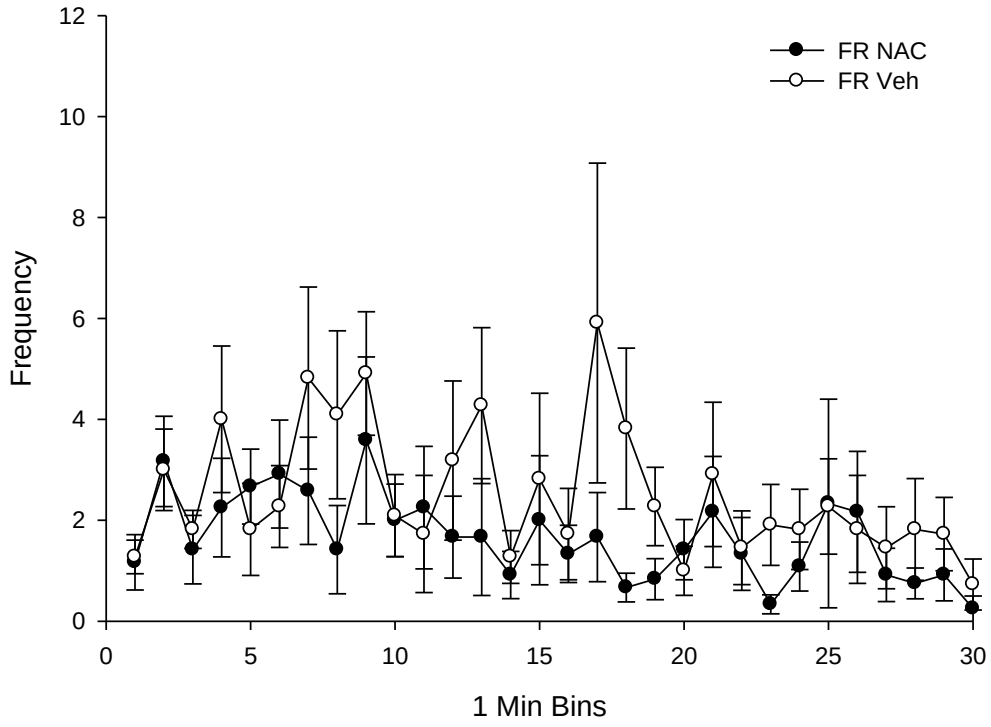

Figure 20. Fixed Ratio Extinction Day 2: Active Lever Bin Data

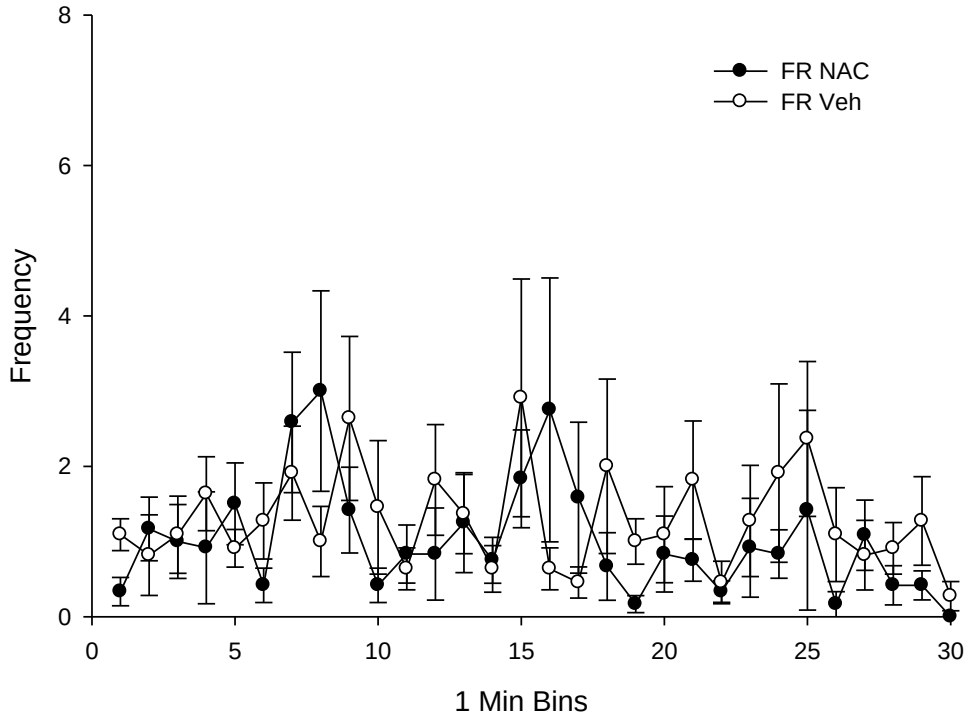

Figure 21. Fixed Ratio Extinction Day 3: Active Lever Bin Data

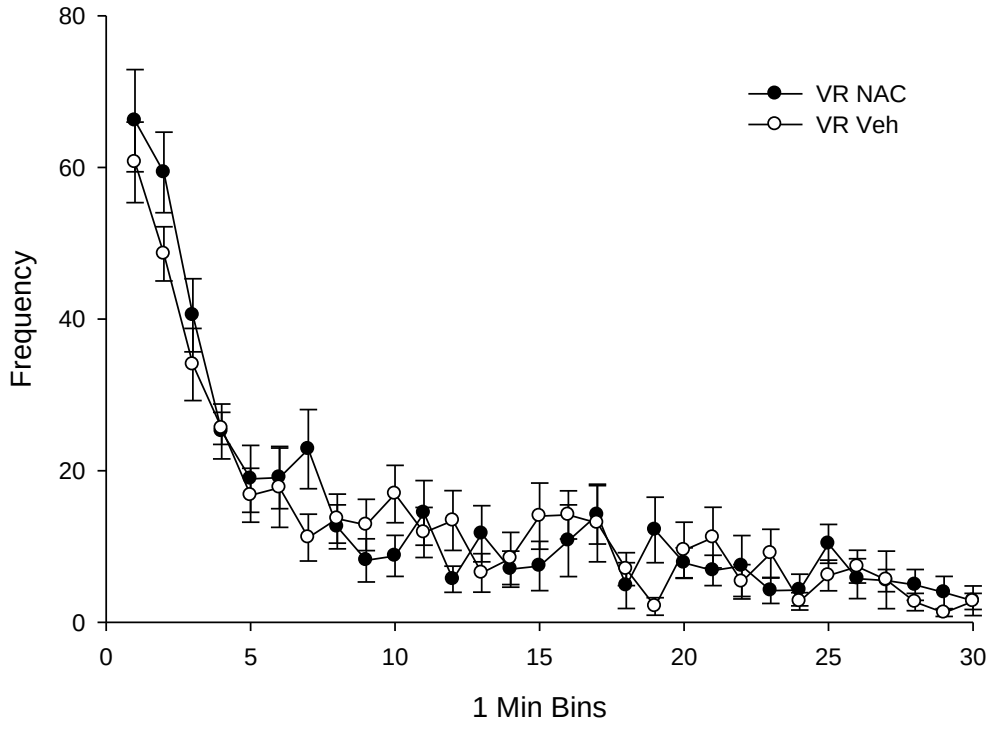

Figure 22. Variable Ratio Extinction Day 1: Active Lever Bin Data

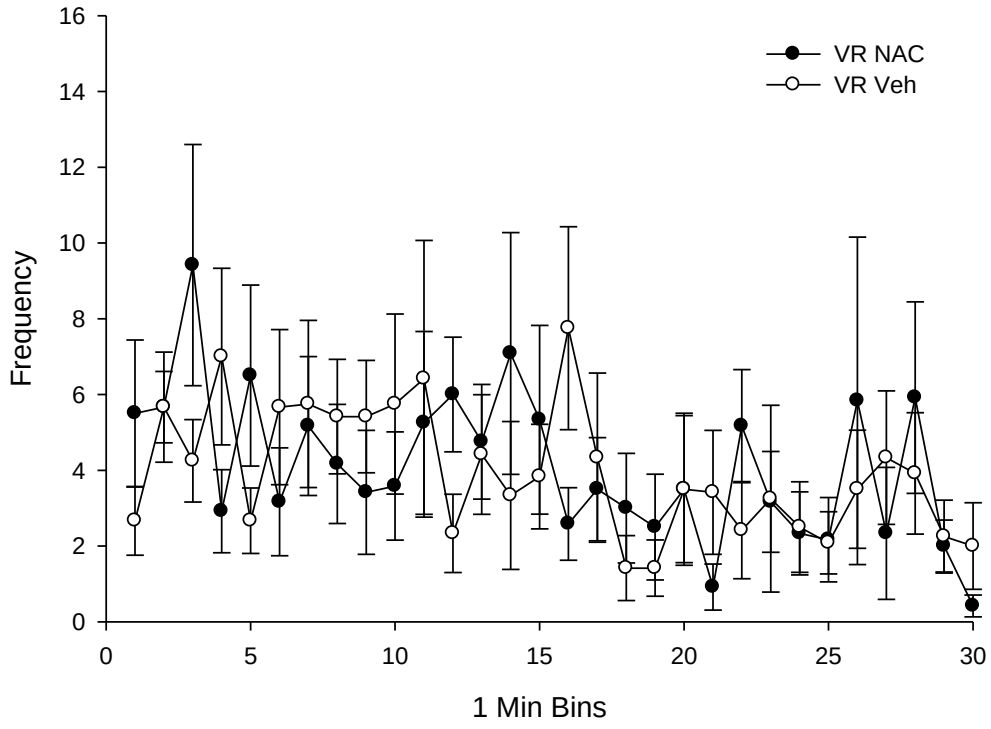

Figure 23. Variable Ratio Extinction Day 2: Active Lever Bin Data

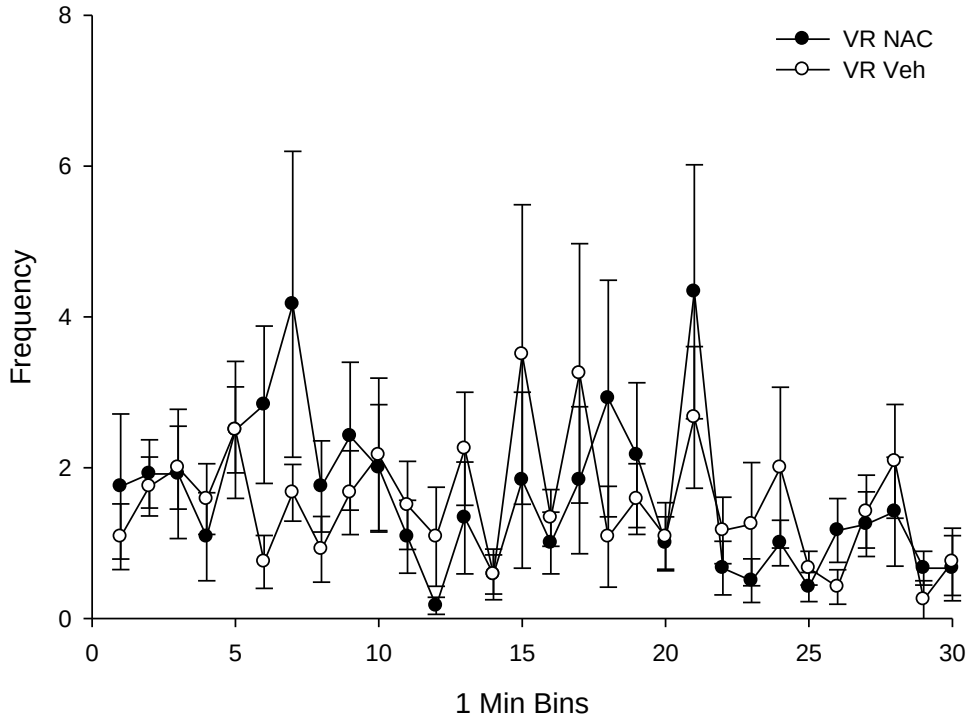

Figure 24. Variable Ratio Extinction Day 3: Active Lever Bin Data

Summary

Results of *t*-test analyses suggested no baseline differences at Day 1 and expected significant differences at day 8 across the FR and VR groups. Results of the mixed-model analyses indicated response frequency decreased significantly in extinction across nearly all analyses and dependent variables. A significant treatment effect was observed only in the mixed-model analyses of FR groups. Specifically, active lever response frequency was significantly augmented in the FR-NAC relative to the FR-VEH group on Day 2 and Day 5 of extinction. Significant drug effects were found across the FR groups in the reinstatement phase but were not observed across the VR groups. No other significant effects of treatment or interaction effects were observed. Additionally, no significant differences were found in change scores calculated at

phase changes. Covariance parameters in all of the mixed-model analyses suggested large individual differences across mice.

CHAPTER IV

DISCUSSION

This experiment investigated the effects of NAC on the extinction of an operant responding maintained by positive reinforcement. The use of FR and VR schedules of reinforcement allowed for the examination of differential effects of NAC on extinction based on reinforcement history. Data were collected on three dependent variables: active lever presses, left lever presses, and head entries. Complete and restricted datasets for each dependent variable were analyzed via mixed-models, and individual data points and change scores were analyzed via *t*-tests and ANOVAs.

Findings will be discussed for the FR groups first, followed by findings for the VR groups analyses, and, finally, results of the combined analyses for the FR and VR groups together. The next section will present a critical examination of the potential explanations for the results. Finally, the paper will conclude with a discussion of limitations and future directions for research.

Interpretation of Findings

The first round of mixed-model analyses were conducted on the full extinction dataset (i.e., all eight days), with separate analyses conducted for the FR and VR groups, respectively. Conducting separate analyses for each schedule of reinforcement in this way facilitated identification of drug effects for that schedule, as combining FR and VR groups immediately and evaluating an overall drug effect, irrespective of schedule, may have obscured or exaggerated

effects for either schedule. Therefore, separate analyses of FR and VR groups ensured accurate characterization of drug effects for each schedule. Isolating the groups based on schedule of reinforcement in this initial analysis directly addressed the first experimental question: Does administration of NAC effect extinction responding on a positively reinforced operant task?

The restricted mixed-model analyses were conducted only on data from Days 1 through 3 of extinction. The rationale for an analysis of this type can be found in studies of NAC in drug relapse/reinstatement paradigms, wherein data strongly suggests NAC exerts the most prominent effects in the first three days of extinction (Baker et al., 2003a; Kau et al., 2008; Zhou & Kalivas, 2008). Following the initial decay, levels of responding within this paradigm may become negligible, leaving little opportunity for evidence of a drug effect. Additionally, the first few days of exposure to extinction is when the extinction burst and subsequent decreases in response frequency may be expected to be the most prominent (Skinner, 1938). Isolating the days when the largest drug effects might be anticipated enhances the likelihood of finding an effect because it eliminates the days when the two treatment (e.g. NAC and VEH) groups might be expected to be the most similar. Therefore, an analysis of this sort aids in the avoidance of Type II error. These models were also executed on the FR and VR groups separately for the same reasons outlined above.

The final round of mixed-model analyses were complete and restricted full-factorial models that incorporated data sets from all four groups (e.g., FR-NAC, FR-VEH, VR-NAC, and VR-VEH). These analyses allowed direct comparisons of the two schedules of reinforcement, which provided an answer to the second experimental question: Does administration of NAC differentially affect the extinction responding on continuous versus intermittent schedules of

positive reinforcement? This was accomplished because the full-factorial mixed-model produced a measure of the three-way interaction term: schedule x treatment x time.

Evaluation of all other data points of interest that could not be addressed within the mixed-models, such as comparison of individual days or change scores, was accomplished via *t*-tests or ANOVAs. The former were used where one group was compared to one other group, such as FR-NAC to FR-VEH. Specifically, responding on extinction Days 1 through 3, cued reinstatement Days 1 and 2, and reward reinstatement were compared within schedules of reinforcement using *t*-tests. Where a comparison of all four groups was desired because a potential difference within and between schedules of reinforcement was anticipated, ANOVAs were executed. Change scores for all phase changes were analyzed via this method.

FR schedule. The complete mixed-model analysis resulted in a significant drug effect for the active lever. This result indicated that response frequency on the active lever was significantly different between the NAC and VEH groups, and a visual analysis of the graphed data reveals that FR-NAC response frequency was less than that of the FR-VEH group for each extinction session. This finding represents the first significant effect reported for NAC on an operant response maintained by food reinforcement. Additionally, this finding parallels significant findings in the drug relapse/reinstatement literature, suggesting consistency of effects across different types of positive reinforcers. The failure of the treatment x time interaction term to reach significance for the active lever suggests the difference may be specific to response frequency, rather than the shape of the extinction curve. Taken together, these results indicate that although NAC may not influence the overall shape of the extinction curve for operant responding maintained by food reinforcement, it does result in a consistent pattern of decreased response frequency.

No significant main effects or interaction terms were found for the left lever or head entries variables between the FR groups. The left lever was taken to be a measure of response variability and, as such, different response frequencies may have been expected across NAC and VEH groups upon exposure to extinction. However, a significant difference was not observed. This result suggests that either responses on the left lever were not a measure of response variability, as was anticipated, or that NAC exercised no significant effect on response variability as measured by left lever presses. Head entries were an imprecise measure of motivation, as reinforcement could only be retrieved via a head entry into the dipper entry. As with the left lever, nonsignificant findings may suggest either an incorrect assumption regarding the index measured or a lack of drug effect.

The restricted mixed-model analysis did not produce any significant findings. This result is particularly surprising for the active lever variable, as the juxtaposition of a significant drug effect in the complete mixed-model and use of a restricted dataset that targeted days with the largest anticipated between groups differences would have been expected to yield another significant drug effect. Instead, this finding revealed analysis of the restricted dataset was not sufficient to produce a significant difference across groups. This in turn suggests the differences across groups were not confined to the first few days of extinction, but rather persisted throughout extinction.

With respect to *t*-test results for the first three days of extinction, only Day 2 produced a significant result for the active lever. It may be expected that active lever responses would be significantly different on all three days due to the significance of the treatment term in the complete mixed-model analysis, as the largest between groups differences were expected on these days. However, the nonsignificant *t*-test findings for the active lever on Days 1 and 3

provide some degree of explanation for the lack of significant findings in the restricted mixed-model analysis. The additional *t*-test analyses of the remaining days suggest a potentially sustained pattern of drug effects, as differences were significant on Day 5 and approached significance on Day 6. The sustained pattern of significant differences across several days of extinction, rather than confined to the first few sessions, likely explain the presence of a significant drug effect in the complete mixed-model.

When the *t*-test results are considered in concert with the nonsignificance of the treatment x time interaction term in both the complete and restricted mixed-models, this pattern of results suggests that while the slope of the decrease in active lever responding was similar for both groups, the frequency of responding across extinction sessions was significantly decreased after administering NAC. That is, the necessity of including all eight days of extinction data for a significant drug effect to emerge reflects a difference in the magnitude, rather than the shape, of the entire extinction curve. These results suggest, in the case of food reinforcement, that NAC may exert an ameliorative effect on operant responding throughout exposure to extinction (i.e., until responding ceases or stabilizes).

In addition to the presence of a drug effect during extinction for the FR schedule groups, NAC also resulted in significantly decreased frequency of active lever responses during reinstatement. While *t*-test results for Cued Reinstatement, Day 1 fell right at the cusp of significance, responding was significantly less in the FR-NAC group for Cued Reinstatement, Day 2 and Reward Reinstatement. This finding is particularly interesting because it parallels findings in the drug relapse/reinstatement literature, such that following extinction of an operant response, NAC decreases the degree of reinstatement of the previously reinforced response upon exposure to a cue or reward. Additionally, this difference was preserved across the different

reinstatement phases as an effect was seen whether a cue or a reward was used to reinstate responding. The practical implications of these results with respect to problem behavior are promising. For example, an appropriately timed administration of NAC following extinction of a targeted problem behavior may prevent re-exposure to discriminative stimuli or relevant reinforcers from occasioning renewed engagement in the behavior.

The failure of *t*-test results to reach significance for left lever presses and head entries across extinction and reinstatement days tested, with one exception, invites the same conclusions as discussed in the mixed-models. That is, these variables are either inaccurate measures of the indexes or are accurate, but without evidence of a drug effect. The one exception to this statement was the *t*-test for the left lever during Reward Reinstatement, a term that proved to be highly significant. Given the number of *t*-tests conducted, it is possible that this result could represent a Type I error as a chance significant finding. The alternative explanation is that NAC may significantly decrease response variability, assuming the index is accurate, when responding is reinstated with exposure to the reinforcer. Based on the tentative nature of the measure and finding alike, a replication of this finding is warranted.

VR Schedule. Results of the complete and restricted mixed-model analyses for the VR groups did not produce any significant drug effect for any dependent variable. In fact, statistical terms did not approach significance for active lever in either mixed-model. Visual analysis of the active lever data shows similar extinction curves for the VR-NAC and VR-VEH groups. The absence of a drug effect for the VR groups stands in contrast to the significant effects found for the FR schedule. Although a difference in the magnitude of drug effect may have been expected, opposite findings such that NAC produces no effect on the operant response for one schedule type while producing a significant effect for the other were unanticipated. The disparity of these

findings suggests a fundamental difference in the way extinction responding on FR versus VR schedules of reinforcement interacts with NAC.

From a practical perspective, the nonsignificant findings for the VR mixed-models may actually mitigate the significant findings for the FR mixed-models. Although the findings are promising, very few behaviors are on a naturally occurring FR schedule of reinforcement. Instead, naturally occurring schedules of reinforcement are typically intermittent, or variable, in nature, and therefore, the VR schedule in this experiment more closely approximates natural schedules of reinforcement. As a result, the VR groups comparison may be taken to predict more accurately the effect of NAC on operant behavior outside of an experimental context. The results of the FR groups analysis, despite achieving significance, may therefore have restricted application to the natural environment.

FR and VR schedules. The complete full-factorial mixed-model did not produce significant main effects for treatment, or any significant interaction terms for any dependent variable. This result suggests the combined total effect of NAC, when the FR and VR schedules were considered together, was nonsignificant. When taken in concert with results from the complete and restricted mixed-models for both schedule groups, this result is not surprising as the lack of drug effect evidenced in the VR groups likely neutralized the significant drug effect observed in the FR groups. The interaction term of interest, schedule x treatment x time, addressed the effect of treatment on different schedule groups over time. This interaction term reveals whether NAC exerts differential effects on responding based on schedule assignment. The nonsignificance of this interaction term within the complete full-factorial model suggests that reinforcement history does not moderate the drug effect. However, this finding is difficult to reconcile with the significant versus nonsignificant findings for the treatment term in the FR and

VR groups analyses, respectively. This seemingly disparate set of findings may reflect insufficient power.

The restricted full-factorial mixed-model produced similarly nonsignificant results. The only significant term of interest in this analysis was the interaction of schedule x time for the active lever press. The significance of this interaction suggests changes in response frequency were significantly different for distinct schedules across time (e.g., the first three days of extinction for this analysis). However, the origin of this result is difficult to specify. Differences in response frequency could be due to difference in reinforcement histories across FR and VR groups, varied drug effects across schedule groups for the first three days of extinction, or a combination of both of these factors. The most notable disparity for either of these factors would be anticipated in the first few days of extinction, making it further difficult to interpret the interaction term. Some clarification is provided by examining the schedule x treatment x time term. The nonsignificance of this term could suggest the significance of the schedule x time term is a product of reinforcement history. If a drug effect were driving the difference across schedule types, it would be expected that adding the treatment term would not markedly affect the significance value in a negative way. The restricted mixed-model results mirror those of the complete mixed-model in revealing a lack of differential drug effect relative to schedule across time.

Differential drug effects were also explored via evaluation of change scores, which were analyzed for all groups and dependent variables via ANOVAs. This analysis was conducted because it was necessary to explore the possibility that a difference existed between and across reinforcement histories in change scores at phase changes. That is, it was feasible that a drug effect might be observed when examining the change in response frequency from day 8 of

baseline to day 1 of extinction, day 8 of extinction to Cued Reinstatement Day 1, and so forth. However, an ANOVA conducted for all four groups simultaneously (e.g., FR-NAC, FR-VEH, VR-NAC, and VR-VEH) did not reveal any significant differences for any phase change for any dependent variable. The lack of differential drug effects at phase changes suggests that NAC does not exert any affect on the degree to which responding is regained or lost at a phase change, contingent on the parameters of each phase.

Possible Explanations for Findings

Many potential explanations exist for the findings just discussed. When the experimental procedures, visual analysis of data, and statistical analyses are considered together, two specific explanations emerge as the most feasible. They are: (a) the findings are accurate but require further elucidation of the variable effects relative to reinforcement history, and (b) the findings are tenuous, given previous failure of NAC to produce an effect on extinction of operant behavior maintained by food reinforcement, and require further consideration of potentially divergent brain mechanisms.

The first potential explanation for the findings indicates the effects of NAC are confined, within the parameters of this experiment, to extinction and reinstatement of operant behavior maintained on an FR schedule. This finding aligns with those seen in the drug relapse/reinstatement literature, which in turn suggests consistent drug effects for NAC across different types of positive reinforcers. However, the results for the FR groups are at once promising and difficult to reconcile with the complete absence of drug effect observed in the VR groups. The parallel between VR schedules and naturally occurring schedules of reinforcement

portend greater practical relevance and could suggest that, despite significant results, findings from the FR groups may be of limited applicability to the natural environment.

Alternatively, the argument could be made that the experimental arrangement was insufficient to solicit a drug effect from the VR groups. For example, investigation of differential effects across schedules may have been facilitated by longer training and baseline periods and/or more disparate schedule requirements. The distinction between FR-5 and VR-5 schedules of reinforcement may not have been sufficient to reveal differential effects of NAC. Although the apparent lack of any recognizable drug effect for the VR schedule, even upon visual analysis, may make this a questionable argument, it remains a valid concern. That is, one or more aspects of the VR-5 schedule may have been insufficient to: (a) produce the type of extinction curve necessary to reveal a drug effect for a variable schedule of reinforcement, (b) represent an approximation of naturally occurring schedules of reinforcement, or (c) render it adequately different from the FR-5 schedule to suitably address the question of variable effects for constant versus intermittent reinforcement schedules.

The latter argument may be the least plausible, given the significant differences seen in active lever press frequency across FR and VR groups in the final day of baseline and the first two days of extinction. However, some merit is restored to the same assertion when the lack of a significant schedule x time interaction term in the complete full-factorial mixed-model is considered. This term suggests a high degree of similarity in the slope of the complete extinction curves (i.e., all eight extinction sessions) of the FR and VR groups. This similarity between two curves that should be dissimilar lends credence to the potential inadequacy of the VR extinction curve. That is, the characteristics of the extinction curve sufficient to produce drug effects for the FR schedule may not have been sufficient to reveal the same for the VR schedule. If it is

assumed that a drug effect does exist for the VR schedule, it may be required that an increased schedule requirement, longer training period, or multiple exposures to extinction are necessary to reveal it.

In addition to the potential experimental limitations that exist for the VR groups data, it is difficult to predict the social significance of the FR groups findings. Despite achieving significance, the differences between the FR-NAC and FR-VEH groups are subtle. This assertion is supported by results of the statistical analysis as well as visual analysis of the bin data. Many would argue that such subtlety is an insufficient foundation upon which to build an argument for pharmacological augmentation of behavior intervention plans. The present failure of the VR comparison to produce significant results only fortifies such an assertion. Even if the drug effects proved to be consistent when applied to the rare problem behavior that finds itself on an FR schedule of reinforcement, it could be difficult to justify NAC administration when only a minor effect may be anticipated. Then again, the earliest papers on extinction procedures reported the severe and sometimes permanent injuries sustained as a result of engagement in problem behavior. In cases like these, a decrease in the frequency of problem behavior, however subtle, may have made a substantial difference for the individuals involved.

The second potential explanation for the ambiguous nature of these findings is that they may reflect the limited effect of NAC on positive reinforcers other than drugs of abuse. The results may then be considered tenuous in light of previous research on NAC and extinction of operant responding maintained by food. For example, findings from the present study do not align with findings from the pilot study presented, wherein drug effects in the opposite direction were shown. That is, response frequency was shown to be elevated, instead of reduced, during extinction in the NAC group relative to the VEH group. While the pilot study may have been

insufficiently powered, absence of a significant drug effect in the mixed-model paired with significant *t*-test results in the opposite direction for Days 1 through 3 of extinction also stand in contrast to the findings for the FR groups from the present study. Another disparity is found in the lack of significant differences across groups for during any reinstatement session in the pilot study. It is difficult to reconcile that two nearly identical studies were conducted, even when concerns regarding power are considered, and produced opposite results. The presence of significant results for the FR groups also stands at odds with findings in the Baker et al. (2003a) experiments, where NAC was shown to have no effect on food-primed reinstatement.

In addition to ambiguous behavioral data, the literature suggests the possibility that discrete pathways within the brain mediate operant behavior maintained by different types of positive reinforcers, such as drugs of abuse and food. For example, Carelli, Ijames, & Crumling (2000) recorded cell firing in nucleus accumbens in rats trained to lever press on multiple schedule for food, water, or cocaine. Results showed that 93% of neurons recorded exhibited firing patterns that did not overlap across food/water and drug conditions, a finding that suggests separate and distinct circuits mediate natural reinforcers and drugs of abuse. Within the NAC literature, Baker et al. (2003a) suggested that NAC may be selectively ameliorating a pathology produced by drugs of abuse. This assertion was supported elsewhere when it was shown that reinstatement of lever pressing for food did not elevate extracellular glutamate; instead, it was reported that increased glutamate selectively reinstated responding for drug reinforcement. In fact, it was shown that food-induced reinstatement does not depend on activity within the nucleus accumbens (McFarland, Lapish, & Kalivas, 2003). These results suggest food-induced reinstatement neither produces elevated levels of glutamate nor is it mediated in the nucleus accumbens, a violation of two necessary requisites of NAC drug effects. Alternatively, the

ventral pallidum has been shown to be pivotal in food-induced reinstatement (McFarland & Kalivas, 2001), a site which is not at present known to be influenced by NAC.

The absence of significant drug effects prior to this study taken together with the literature on brain mechanisms mediating different types of rewards raise the possibility that application of NAC to extinction of operant behavior maintained by food reinforcement is misdirected. Despite the significant effects found for the FR groups, the aforementioned subtlety of effects paired with the absence of drug effect for the VR groups may in fact reinforce the alternate pathways argument. These are critical considerations in thinking about future studies, as no degree of experimental manipulation will result in a significant drug effect if NAC does not exert an effect on the relevant pathways.

Limitations

The major limitations in this study fell into one of two categories: (a) experimental procedures and (b) statistical analyses. Upon examination in retrospect, several features of the autoshaping procedure contributed to difficulties in acquisition of the response. The resulting failure of the mice to autoshape initially produced an experience that could have been akin to extinction, although it cannot be termed that as the issue was not termination of but rather failure to contact the reinforcement contingency. However, this history may have affected responding throughout baseline and extinction. The same can be said for the mice exposed to manual shaping, a procedure that by nature is variable in several identifiable and unidentifiable ways. The variability across mice in the type, duration, and order of shaping procedures almost certainly impacted the data in ways that cannot be defined or measured.

Concerns regarding the effect of food restriction procedures on responding represent a second major limitation. Deprivation was employed as a motivating operation in order to increase desire for food and therefore responding within the operant response paradigm. While consistency in the application of these procedures may be considered a strength of the experiment, it is also a weakness as it essentially constituted a predictable noncontingent or fixed time reinforcement schedule. That is, access to food was granted at the same time following each experimental session. The expectation of food delivery may have impacted levels of operant responding, especially in the extinction phase. Additionally, weight loss in mice was negligible throughout the course of the study, which suggested mice were ingesting the same amount of food in the 4 hr period of access as they had been when food access was unrestricted prior to the experiment. It could be surmised from this that mice were not experiencing the intended deprivation state prior to experimental sessions.

The third and final experimental limitation that warrants mention concerns response accuracy across schedules of reinforcement. Cumulative records were not available for any session for any mouse throughout this experiment. Lack of access to this information resulted in the inability to: (a) confirm that the intended discriminative stimulus had taken on the appropriate properties, (b) monitor the degree to which mice were tracking the response requirement, and (c) examine changes in response patterns within and across sessions. Ultimately, this shortcoming eliminated access to subtleties within the data that may have revealed details regarding the how, when, and why of drug effects or lack thereof.

Only one limitation exists with respect to statistical analyses, and it is concerned with the number of analyses conducted. It is worthy of note there that all analyses were preplanned, based on anticipated effects and/or precedents within the literature, with one exception. The exception

was the *t*-tests conducted on Days 4 through 8 of extinction for the FR groups, and it was determined *post hoc* that these analyses were necessary in light of the pattern of results for that schedule. However, a total of 18 mixed-models, over 50 *t*-tests, and 9 ANOVAs were conducted. A large number of analyses increase the likelihood of a chance significant finding. Future studies should seek to increase power and reduce the number of analyses necessary to answer the experimental questions.

Conclusion

This study examined the effect of NAC on the extinction of operant responding maintained by food reinforcement across two different schedules of reinforcement. Results revealed an ameliorative effect on response frequency for the NAC group for the FR contingency only. No drug effect was evident for the VR schedule, and when FR and VR groups were compared to each other, no significant differential effect of drug by schedule was noted. A variety of practical and experimental considerations were discussed in an effort to interpret and explain these results.

Although the significant findings from this study were not as robust as expected, they warrant continued exploration. Additionally, the lack of consistent findings across the FR and VR groups raises a variety of questions worth investigating. Researchers should replicate this study to determine whether the effects of NAC are specific to certain schedules of reinforcement. Manipulation of different aspects of the VR schedule may also help to reveal subtleties in the data with respect to NAC that were not apparent here. Additionally, it is critical that neuroscience researchers continue to explore the potential divergence of pathways mediating different types of positive reinforcement. Similarly, a more refined understanding of the site and

mechanism of action for NAC will help elucidate the true potential of this drug and its ability to ameliorate characteristics of extinction responding. The literature on the effect of NAC and extinction of operant behavior maintained by positive reinforcement are at once ambiguous and potentially promising enough to warrant continued investigation, and the potential for this drug to positively affect the lives of individuals with IDD and problem behaviors only encourages this enterprise.

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