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By Kyle Allyson Windisch

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ACHIEVING PHARMACOLOGICALLY RELEVANT IV ALCOHOL SELF-ADMINISTRATION IN THE RAT

For the degree of Master of Science

Is approved by the final examining committee:

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ACHIEVING PHARMACOLOGICALLY RELEVANT IV ALCOHOL SELF-ADMINISTRATION IN THE RAT

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of

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by

Kyle Allyson Windisch

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Windisch, Kyle Allyson. M.S. Purdue University, December 2011. Achieving Pharmacologically Relevant IV Alcohol Self-Administration in the Rat. Major Professor: Cristine L. Czachowski

Alcohol consumption produces a complex array of effects that can be divided into two types: the explicit pharmacological effects of ethanol (which can be quite separate temporally from time of intake) and the more temporally "relevant" effects (primarily olfactory and taste) that bridge the time from intake to the onset of the pharmacological effects. Dissociating these effects is essential to untangling the neurologic underpinnings of alcohol abuse and dependence. Intravenous self-administration of ethanol allows for controlled and precise dosing, bypasses first order absorption kinetics allowing for a faster onset of pharmacologic effects, and eliminates the confounding "non-pharmacological" effects associated with oral consumption. Intravenous self-administration of ethanol has been reliably demonstrated in both mouse and human experimental models; however, consistent intravenous self-administration of pharmacologically relevant levels of ethanol remains elusive in the rat. Previous work has demonstrated reliable elevated intravenous ethanol self administration using a compound reinforcer of oral sucrose and intravenous ethanol. The present study sought to elucidate the role of each component of this reinforcer complex using a multiple schedule study design. Male P rats had free access to both food and water during all intravenous self-

administration sessions and all testing was performed in conjunction with the onset of the dark cycle. Once animals achieved stable operant responding on both levers for an orally delivered 1% sucrose solution (1S) on a FR4 schedule, surgery was conducted to implant an indwelling jugular catheter. Animals were habituated to the attachment of infusion apparatus and received twice daily sessions for four days to condition each lever to its associated schedule. Animals were then trained to respond on a multiple FR4-FR4 schedule composed of alternating 2.5 minute components. During one component only oral 1S was presented, while in the second component a compound reinforcer of oral 1S + IV 20% ethanol was presented (25 mg/kg/injection). Both levers were extended into the chamber during the session, with the active lever/schedule alternating as the session progressed across components. Average ethanol intake was 0.47 ± 0.04 g/kg. A significant increase in sucrose only reinforcers and sucrose lever error responding was found suggesting that sucrose not ethanol is responsible for driving overall responding. The current findings suggest that the existing intravenous ethanol self-administration methodology remains aversive in the rat.

INTRODUCTION

Overview of Intravenous Ethanol Administration

Alcohol is a multifaceted drug with a complex array of effects resulting from consumption. The principle effects of alcohol consumption can be divided into the more temporally relevant non-pharmacological orosensory effects and the direct pharmacological effects of ethanol. Intravenous self-administration provides a method by which these distinct effects can be isolated to assess their component properties relevant to the reinforcing effects of alcohol. Intravenous ethanol self administration has been successfully implemented in mice (Grahame 1997, Grahame 1998, Blokhina 2004), monkeys (Gomez 2003, Karoly 1978, Williams 2004), and humans (Zimmermann 2008, Zimmermann 2009).

Benefits of Intravenous Administration of Ethanol

Intravenous self-administration of ethanol lacks the face validity of the more commonly used oral administration route. However, as venous administration of ethanol bypasses digestive tract absorption, intravenous ethanol administration allows for more precise control of the neural exposure resulting from each reinforcer dose. Because the ethanol is being directly administered into the bloodstream, the intravenous route allows for a faster ethanol exposure with nearly immediate brain exposure. Additionally, utilization of the intravenous route allows for precise standardization of ethanol exposure for each individual reinforcer dose

across animals. Overall, intravenous ethanol administration allows for a faster brain exposure of ethanol in precisely controlled doses across subjects.

Human and Primate Models Using Intravenous Ethanol Administration

Intravenous ethanol self administration has been thoroughly demonstrated in both human and primate models. Zimmermann et al. (2008) demonstrated that human subjects will willingly self administer 6% ethanol intravenous in unit doses that raise arterial blood ethanol concentration (aBEC) by 7.5 mg% per infusion across a 2.5 hour infusion session. Subjects achieved average maximal aBEC of 76.5 ± 23.6 mg% during each of three sessions. Four of the twelve subjects willingly infused ethanol to reach the 100 mg% safety limit with three participants repeatedly reaching the safely limit within a single session. Therefore, despite the novel intake method, humans have been shown to readily self administer pharmacologically relevant doses of ethanol intravenously.

Monkeys have been shown to acquire and maintain intravenous self administration of 20% ethanol (Deneau, 1969). Of the five rhesus monkeys studied, four acquired self administration for access to 200 mg/kg/injection doses without need of alternate induction methods. One animal, however, did not acquire responding for intravenous self administration of ethanol at any dose. Maximal intake of these animals was 8.6 g/kg/day with voluntary periods of abstinence observed lasting 2 to 4 days through the first 4 months of chronic access. As well, limited access studies using monkeys (Karoly, 1978) have demonstrated that rhesus monkeys will acquire and maintain operant responding for access to 100 mg/kg/injection doses of 15% ethanol during a 3-hour daily session. Animals were found to respond for access to achieve blood ethanol concentrations (BEC) of 400 mg% then reduce

responding to maintain this BEC for the remainder of the operant session. Extending the length of the session from 3 to 6 hours resulted in higher overall session intakes but did not affect the peak BEC achieved. Overall, these studies demonstrate that both humans and nonhuman primates will initiate and maintain responding for access to intravenous ethanol across repeated sessions and continually achieve pharmacologically relevant ethanol exposures.

Rodent Models Using Intravenous Ethanol Administration

Mice have been shown to self administer pharmacologically relevant levels of intravenous ethanol at relatively elevated ethanol concentrations (20-60% ethanol). Grahame et al. (1997) demonstrated that both C57BL/6J (B6) and DBA/2J (DBA) inbred mice will acquire and maintain operant responding for intravenous ethanol. Both B6 and DBA mice responded for access to ethanol doses of 60, 75, and 90 mg/kg/injection with average daily intakes during 2-hr operant sessions greater than 1.0 g/kg for both B6 and DBA mice. Although DBA mice traditionally do not orally consume ethanol to pharmacologically relevant levels, with intravenous administration, DBA mice receiving the 60 mg/kg/injection dose had higher daily intakes (1.26 g/kg) compared to the normally 'high' drinking B6 mice (1.03 g/kg). In line with the findings of Grahame et al., Kelley (1997) demonstrated that B6 mice will acquire a conditioned place preference for intravenously administered 30% ethanol at a dose of 0.82 g/kg. B6 mice were also found to generalize intravenously administrated ethanol to ethanol administered via intraperitoneal (IP) injections, demonstrating that intravenous and IP ethanol administration have similar interoceptive properties.

Blokhina et al. (2004) further confirmed that mice will self administer ethanol intravenously. During a single 30-minute session, mice operantly responded for access to

ethanol doses of 0.75 and 3.75 mg/kg/injection (using an ethanol concentration of 1-4%). Ethanol was confirmed to maintain significant self-administration behavior in both DBA and Swiss mice with session intake for ethanol of 0.089 and 0.094 g/kg respectively. Though these exposures rather low (and arguably not pharmacologically relevant exposures), the animals used in this study had no previous exposure to ethanol and were not trained to operantly respond prior to the single 30-minute test session. Overall, mice have been shown to initiate and maintain responding for intravenous self administration of ethanol and demonstrate a conditioned place preference for intravenously administered ethanol.

Several significant drawbacks have been noted with intravenous self administration in mice. Indwelling catheterization surgeries are relatively arduous with mice and have a considerable decrement in catheter longevity compared with other animals (Thomsen 2007). In contrast, rat catheterization surgeries are relatively simple and have a longer patency than mice (lasting on average 8-14 weeks when properly implanted and maintained). As well, rats tend to have more stable responding for intravenous drug administration compared to mice (Thomsen 2007). Intravenous self-administration of ethanol in rats allows for examination of the neurological role of ethanol using site specific neuro-modulation (via micro-injection of a drug into a specific brain region) and electrophysiological recording from various brain regions during acquisition of responding for ethanol and protracted pharmacological relevant exposure.

Limitations of Past Studies Using Rats

Intravenous self administration of ethanol in rats was first examined by Smith and Davis in 1974 (see Table 1. Previous Rat Studies Using Intravenous Ethanol Self-Administration).

Non-food or water deprived ethanol naïve male Holtzman Sprague-Dawley rats were shown to acquire lever responding for intravenous ethanol doses of a 0.12 mg/kg/injection during 12-hour operant sessions. Even though animals were shown to have stable self administration of ethanol via the intravenous route of administration, peak ethanol intake was low (0.048 g/kg over a 12 hour session). Although the researchers were able to achieve stable dosing, ethanol intake was extremely low and has limited utility in modeling human alcohol use and abuse. As well, similar to intravenous self administration in monkeys, over a quarter of the animals used for the study did not acquire responding for intravenous ethanol.

Following this work, Sinden and Le Magnen (1982) demonstrated that male Wistar rats would acquire operant responding for relatively higher doses of intravenous ethanol during chronic access. In this study, rats had increasing responding over five days for 0.5 and 1.0 mg/kg doses of ethanol as compared to a saline control. The 5.0 mg/kg/injection dose, however, was not found to be reinforcing and resulted in decreasing responding across sessions. Average daily intake for the 1.0 mg/kg/injection dose was 0.0625 g/kg. The majority of the responding (74%) occurred during the dark portion of the light/dark cycle in bouts of responding (2-3 responses within a 5 minute period). Bout responding never extended beyond a series of 6 responses. Although total daily intakes for this study were still low, it showed that higher doses of intravenous ethanol are reinforcing in the rat as demonstrated by increasing responding across sessions.

One possible explanation for the low session intakes observed in these studies is the dilute infusate used. The use of low ethanol concentrations (i.e. <10% ethanol) resulted in high total infusion volumes during each session that may have limited ethanol dosing. To achieve a more robust and pharmacologically relevant exposure (e.g. 0.3 g/kg intake), animals receiving

the 1.0 mg/kg/injection dose would need to administer 30 ml of infusate (which is an infusion volume roughly doubling the blood volume of a 350 g rat). Administration of this large a volume would result in significant aversive physiological effect. As observed in this study, it appears that the highest session volume rats will self administer is 7 ml. This ceiling effect of total infusion volume may have contributed to the sub-pharmacologically relevant ethanol exposures observed with this and other studies. That is, the aversive effects that would have resulted from such high volumes of saline in the bloodstream would have limited that total volume of intravenous ethanol the animals were willing to self-administer.

As well, although Sinden increased the infusion dose, the infusate concentration was not markedly increased (with a 1.5% ethanol concentration used for the highest dose). Higher infusion doses were instead achieved by extending the pump duration. Though this non-manipulation of ethanol concentration had no notable difference in overall dosing observed (the animals demonstrated higher daily ethanol intakes compared to Smith et al.), the use of such a low concentration of ethanol limits the maximal dose one can administer per infusion without having extremely protracted reinforcer administration times. This protracted reinforcer administration duration limits the effectiveness of the intravenous administration model to achieve faster temporal salience compared to oral administration. In other words, if one advantage of the intravenous route of administration is to study fast rising BECs and a more discrete reinforcer delivery as compared to drinking ethanol, then use of low concentrations cancels out this advantage due to the protracted infusion time required for higher doses. Due to the apparent ceiling for total volume and protracted time of administration observed with low concentrations of ethanol, examination of the acquisition of

operant responding in rats for intravenous ethanol self administration must use higher ethanol concentrations.

To this end, Oei and Singer (1979) examined the influence of food deprivation and schedule manipulation on a rat's ability to acquire operant responding for intravenous self administration of ethanol using a higher ethanol concentration (20% ethanol). Animals in three treatment conditions [100% body weight, 80% body weight, and 80% body weight plus fixed time interval 1 minute (FI1) schedule for food reinforcement] operantly responded during daily 1-hour sessions for access to an 8 mg/kg infusion of ethanol. The non-food restricted animals did not acquire lever responding for access to intravenous ethanol. However, both food restricted groups acquired responding for intravenous ethanol, with the highest stable response rates observed in the food restricted group responding under the FI-1 schedule. Although food restriction does result in rats acquiring and maintaining responding for intravenous ethanol, it does not translate well to human alcohol use. The result observed with the non-food deprived group is similar to those of Sinden et al. for the 5 mg/kg/injection dose. From this, it appears that unit doses over 1 mg/kg/injection regardless of infusate concentrations are unable to initiate and maintain responding in non-deprived rats.

This apparent ceiling for unit dose does not allow for pharmacologically relevant intake of ethanol despite maintained stable responding. A relevant question to this end is do these seemingly sub-pharmacological exposures of ethanol have any neurological effect. Lyness and Smith (1992) examined the role of dopamine and serotonin on the acquisition and rate of operant responding. Male Sprague-Dawley rats were allowed access to 0.5, 1, 2, 4, and 8 mg/kg/injection doses of intravenous ethanol on a FR1 schedule during 8-hour sessions daily (0.5-4% ethanol). Animals acquired operant responding for intravenous ethanol doses of 1, 2,

and 4 mg/kg/injection and a substantial increase in responding (frustration) was noted when the infusion solution was switched from ethanol to saline. The peak intake observed was 0.075 g/kg/day. Animals did not acquire responding for either the 0.5 or 8 mg/kg dose. Dopamine manipulation via blockade (using haloperidol) or lesions (6-OHDA lesion of nucleus accumbens) had no effect on either acquisition or rate of operant responding for intravenous ethanol. Interestingly, serotonin blockade via the irreversible tryptophan hydroxylase inhibitor *p*-chlorophenylalanine (PCPA) resulted in a significant increase in operant responding for intravenous ethanol while serotonin stimulation via the selective serotonin reuptake inhibitor fluoxetine reduced responding. The rate of responding for intravenous ethanol in these animals returned to baseline levels within several days of exposure to serotonin treatment. Gass and Olive (2007) also found that Wistar rats previously trained to respond for a 1 mg/kg/injection dose of intravenous ethanol demonstrated reinstatement to seeking for intravenous ethanol using cue-, prime-, and stress-induced reinstatement models. From both the Lyness and Gass studies it appears that despite the low levels of intake observed, intravenous ethanol self administration in rats results discrete neurological effects that can be manipulated.

When intravenous ethanol is the only reinforcer, animals appear to maintain responding only at doses below 5 mg/kg/injection resulting in low total session intakes. Several studies have examined the role of either experimenter administered ethanol or co-administration of ethanol with other compounds in facilitating increased ethanol intakes and operant responding for higher doses of intravenous ethanol. DeNoble et al. (1985) examined the effects of co-administration of intravenous ethanol (1, 3, 10, 30, 60, 90, 180, and 360 mg/kg/injection doses) and non-reinforcing doses of pentobarbital on acquisition and rate of operant lever

responding on a FR1 during chronic access. As seen previously, rats failed to initiate lever responding when ethanol was the only reinforcer for any of the ethanol doses. As well, repeated substitutions of ethanol for pentobarbital (pentobarbital doses of 0.5, 1.0, and 2.0 mg/kg/injection) failed to maintain responding. However, when ethanol was combined with non-reinforcing doses of pentobarbital (0.125 and 0.25 mg/kg/injection), responding was maintained for ethanol doses of 1, 3, 10, 30, and 60 mg/kg/injection. Total daily intake for these animals ranged from 0.45 to 2.7 g/kg. This study demonstrates that elevated intakes are possible via intravenous administered ethanol doses above 5 mg/kg/injection when ethanol is co-administered with a positive reinforcer. However, these results may be due to a synergistic interaction between ethanol and pentobarbital. A different reinforcer complex, such as intravenous ethanol plus oral sucrose, is necessary to facilitate further analysis of the effects of ethanol using co-administration.

Numan et al. (1985) demonstrated elevated ethanol intake with intravenous ethanol self administration using cycles of forced ethanol to induce dependency. Eleven male Long-Evans rats underwent periodic cycles of forced ethanol (30%) administered in 5 hour intervals over 4-6 days with daily doses of 9-16 g/kg infused. After each cycle the rats were allowed to operantly respond for access to a 930 mg/kg/injection dose of ethanol at a 20% ethanol concentration. The criterion for stable self administration behavior (SAB) was defined as responding resulting in intake of more than 5 g/kg/day of ethanol. Failure to reach SAB resulted in rats receiving additional cycles of forced intravenous ethanol. Of the animals used in the study, three failed to acquire SAB. For the remaining animals, the average number of cycles required to establish SAB was 5.25 (range 3-8). The majority of SAB responding (62%) occurred during the dark portion of the light/dark cycle with responding predominantly

occurring in bouts. Interestingly, when intravenous ethanol self administration was performed using these parameters with naïve rats not exposed to forced ethanol cycling all of the animals failed to acquire responding. This suggests that pre-exposure to elevated levels of ethanol facilitate the acquisition of operant responding for higher doses and concentrations of intravenous ethanol. Alternatively, the intravenous ethanol could be acting as a negative reinforcer with the animals avoiding withdrawal symptoms.

Pre-exposure to seven weeks of intravenous ethanol plus fading doses of cocaine facilitated subsequent operant responding to intravenous ethanol alone. In Sprague-Dawley rats (Ikegami, 2002) the ethanol plus cocaine fade group demonstrated consistent elevated responding for intravenous ethanol alone (with intakes of 0.5-2.0 g/kg during 1-hour operant sessions) for all intravenous ethanol doses (62.5, 125, 250, and 500 mg/kg/injection using an ethanol infusate concentration of 10%). The cocaine fade only group did not acquire subsequent responding for intravenous ethanol following pre-exposure training. Rats in the ethanol plus cocaine fade group had daily intakes during the cocaine fade of 6.7-11.1 g/kg of ethanol; subsequent intake of ethanol alone was lower, but still pharmacologically relevant. Although the use of other drugs of abuse such as cocaine and barbiturates present additional confounds, these studies suggest that other positive reinforcers such as sucrose might be capable of initiating elevated responding that can be maintained by intravenous ethanol alone.

Hyytiä et al. (1996) examined the possible role of genetics in the acquisition of responding for intravenous ethanol using the AA (Alko-Alcohol) and ANA (Alko, Non-Alcohol) selectively bred lines of rats. AA rats were selectively bred for high oral ethanol intake while ANA rats were bred for low ethanol consumption. In this study, food restricted AA and ANA rats were not found to differ on either intake or break point for intravenous heroin self-

administration. However, AA rats acquired responding for 1, 2, and 4 mg/kg/injection doses of intravenous ethanol (average intake of 0.04 g/kg during 3 hour sessions) while ANA did not acquire responding for intravenous ethanol. Although the AA and ANA lines were initially selected for oral ethanol consumption, the finding that AA but not ANA rats will acquire and maintain responding for a range of intravenous ethanol doses suggests a possible genetic contribution for responding for intravenous ethanol. Further work with rat lines selected for high ethanol consumption may assist in the development of a functional methodology by which rats will intravenous self administer ethanol. Interestingly though, session responding across all three doses for AA rats remained at a fixed level of total responding regardless of dose suggesting a possible confound (possibly aversion) that limits total responding for intravenous ethanol.

Several rodent lines have been selectively bred for alcohol preference. The AA/ANA line was the first line of rats to be selectively bred for alcohol preference (Li, 1993). Subsequently, several additional rat lines have been developed for ethanol preference (P/NP, HAD/LAD, Sardinian sP/sNP, and University of Chile UChA/UChB lines). Use of these selectively bred lines allows for the assessment of potential genetic contributions to alcohol use and abuse. However, several key differences exist between these selectively bred lines. Of these lines, P rats show a pronounced alcohol deprivation effect (ADE) with subsequent escalation of ethanol consumption. As well, P rats exhibit a pronounced increase in ethanol consumption following foot-shock induced stress (Vengeliene, 2003). Both ADE and stress are thought to be components of escalated human alcohol consumption. Of these lines, P rats, developed here at Indiana University, serve as an excellent rodent model to assess the genetic contributions of why humans use and abuse alcohol.

Overall, the previous studies using intravenous self administration of ethanol in rats suggest that it may be possible to maintain stable responding for pharmacologically significant levels of ethanol by increasing the ethanol concentration, unit dose, combining intravenous ethanol reinforcer administration pre-exposure with a substance known to increase responding, and using a rat line selected for high ethanol consumption. These parameters should lead to the development of a model by which rats will self-administer ethanol intravenously to a level that would allow for examination of pertinent questions regarding human alcohol consumption and abuse.

Sucrose Fading

The sucrose fading technique (Samson 1986) has been shown to facilitate acquisition of oral ethanol consumption in food- and water-sated rats. Initially demonstrated in the outbred Long Evans rat, sucrose fading uses a concentrated sucrose solution to initiate operant responding (typically between 10-20% sucrose). Once stable responding is acquired the sucrose is gradually removed while gradually increasing the ethanol content. Sucrose fading is usually accomplished over a period of several weeks and results in rats that willingly consume pharmacologically relevant levels of a 10-40% ethanol solution. Though originally used for oral ethanol consumption, it is likely that the sucrose fade technique could be used to facilitate intravenous self-administration of ethanol by providing an oral sucrose solution in conjunction with intravenous ethanol administration similar to the Ikegami cocaine fade technique. Our current findings support the use of this modified sucrose fading to assist with the initiation and maintenance of operant intravenous ethanol self-administration (see preliminary intravenous ethanol self-administration findings below). Notably, fading of the oral sucrose component

from 2% sucrose (2S) to either 1% sucrose (1S) or water still elicited reliable responding for the oral reinforcer complex. It was not possible, however, to characterize the precise role sucrose plays in this reinforcer complex due to the utilization of a single component schedule design.

Use of Concurrent Schedules of Reinforcement

Concurrent schedules of reinforcement are traditionally used to demonstrate allocation of responding between multiple reinforcers. This allocation of responding allows concurrent schedules to quantitatively assess choice or preference for a reinforcer compared with the other available reinforcers. This is to say, with concurrent schedules animals are able to demonstrate which reinforcer they prefer by increasing responding for it over the other available reinforcers. Generally, manipulations of reinforcer quality (by concentration or adulteration) and the schedule requirement (ratio or time interval) can further elucidate the relative preference across reinforcers. Difficulties arise with concurrent schedules when either side bias or insensitivity for reinforcer manipulation effect responding. If the reinforcers have roughly similar efficacy, rats may not sample both reinforcers equally. Instead the animals might only respond for the reinforcer they encounter first (typically due to an innate side preference) rather than demonstrating a preference between the reinforcers. Responding is maintained by only one of the reinforcers and precludes access to the alternate reinforcer due to a side bias. Concurrent schedules also assume that the animal is sensitive to changes in schedule and reinforcer quality. Heyman and Oldfather (1992) examined ethanol choice behavior in rats with access to sucrose (10S) and a sweetened ethanol solution (10S10E) both on variable interval (VI) schedules. Responding for the sweetened ethanol solution was shown

to not change as a function of changes in the VI schedule (inelastic responding). This reinforcer insensitivity could substantially confound determination of the reinforcing properties of individual components of a compound reinforcer.

In addition to the side bias and reinforcer sensitivity, behavioral interactions across reinforcers have been noted with use of concurrent schedules. As animals demonstrate preference for session reinforcers, qualitative changes in one reinforcer will effect responding for all reinforcers. Manipulations of the schedule or concentration of one reinforcer tend to influence responding for the other reinforcers. Files et al. (1995) observed a negative behavioral contrast. Responding for a 2% sucrose plus 10% ethanol (2S10E) reinforcer declined when the alternate reinforcer concentration was increased from 2S to 5S. McSweeney et al. (1998) observed positive behavioral contrast for ethanol responding when the alternate food reinforcer was extinguished. Changes in responding for individual reinforcers due to manipulations of the alternate reinforcer generally require several sessions to allow responding to reach stable levels. Though traditional oral consumption studies can easily accommodate the additional sessions that concurrent schedules require following reinforcer manipulation, studies using intravenous administration are severely limited in study duration due to catheter patency concerns. Because of the side bias, possible reinforcer insensitivity, and behavioral contrast effects inherent to concurrent schedules, this method seems disadvantageous for use with intravenous self-administration of ethanol in rats.

Use of Multiple Schedules of Reinforcement

Multiple schedules of reinforcement with ethanol have been show to result in independent and stable responding for each individual component of the schedule (Slawecki

1997). During a multiple schedule session animals are allowed access to multiple reinforcers during discrete time intervals such that only one reinforcer or reinforcer complex is available during a given time interval. Reinforcer availability is switched in equal intervals across the session with each reinforcer paired with a specific response (typically a specific lever or nose-poke). Such a design allows for the isolation and manipulation of each schedule without significant influence on the other schedule(s). Slawecki et al. (1997) demonstrated that with multiple schedules rats will maintain responding for both sucrose plus ethanol (5S10E) and sucrose (5S). Additionally, it was found that responding during the component maintained by each reinforcer was unaffected by manipulations of the sucrose and/or ethanol concentrations of the alternate reinforcer. Therefore, responding for each reinforcer was both stable and independent. As well, Czachowski et al. (1999) successfully achieved stable and independent responding for unsweetened ethanol (10E) and sucrose (5S) reinforcers using the multiple schedule design. Interestingly, unlike concurrent schedules, increasing the sucrose concentration, and therefore its reinforcing efficacy, did not result in a decrease in responding for ethanol.

Multiple schedules have been shown to have independent and stable responding for multiple reinforcers during a single session. This stability and independence allows for easy manipulation of one reinforcer without affecting responding for another reinforcer, which significantly decreases the number of sessions required between reinforcer manipulations to reach stable responding. This decrease in total sessions required is of great benefit to studies using intravenous administration as catheter longevity limits the total study duration. Although all levers are extended into the chamber, only one lever is active during any given component. Responding for a preferential side would only result in reinforcement when that lever was

active. As animals progress across training such non-reinforced behavior is reduced as animals instead follow the active lever for access to each reinforcer. Therefore responding is less likely to be influenced by a side bias. Overall, multiple schedules allow for the study duration necessitated by catheter patency with intravenous administration studies while not allowing for side preference and sensitivity confounds.

From the literature, it seems that a methodology by which rats will intravenously self administer ethanol to pharmacologically relevant levels is plausible. For acquisition and maintenance of responding, relatively high doses of ethanol (20-100 mg/kg/injection) seem to be necessary to that insure pharmacologically active exposures of ethanol occur during initial responding. To facilitate rapid onset of these effects without risk of cardiovascular damage, relatively elevated concentrations of ethanol should be used (15-30%). A positive reinforcer (i.e. sugar) should be used to facilitate initial acquisition of elevated responding but faded quickly to establish responding for ethanol alone. Use of selected rat lines bred for high ethanol consumption (i.e. ethanol preferring P rat) may utilize possible genetic contributions that underlie escalated ethanol consumption. Preliminary work (see below) utilizing each of these aspects appears to successfully result in pharmacologically relevant intravenous self administration of ethanol in the rat. However, the precise role of sucrose in the reinforcer complex used in these studies remains elusive.

Preliminary Data

Four male P rats were operantly conditioned to respond for access to an oral sucrose reinforcer (see methods section for further detail on training). In brief, lever pressing was trained first on an FR1 schedule for an oral 5% sucrose (5S) solution. Once stable responding

was achieved the response requirement was increased from a FR1 to a FR4 schedule across several sessions with the oral sucrose concentration subsequently diminished from 5S to 2S. Animals were then implanted with an indwelling jugular catheter (as described in methods section). After a five day recovery period, the animals were habituated to the intravenous administration procedure. For this habituation, animals were first attached to the infusion tether with no infusion during two orally reinforced sessions. Then normal saline (0.9% NaCl) infusions were coupled with the presentation of the oral sucrose reinforcer for four sessions. Finally, a reinforcer complex of 10 mg/kg/injection dose of intravenous ethanol (6% ethanol by volume) with oral 2S was presented for seven sessions to insure elevated stable responding for the intravenous ethanol plus oral sucrose compound reinforcer. Various manipulations of intravenous dose (10-25 mg/kg/injection), infusion duration (3-10 seconds), and ethanol (6-30%)/sucrose (0-2%) concentrations were then performed. Blood alcohol concentrations were collected following an ethanol concentration manipulation session to verify pharmacologically relevant self administration levels.

Preliminary data indicate that selectively bred alcohol preferring (P) rats will self-administer a reinforcer complex of oral 2S plus intravenous ethanol of variable concentrations to pharmacologically significant levels. This was supported by blood alcohol concentration measurements taken directly following a 30-minute self-administration session every twenty minutes for a total of eighty minutes (Figure 1. blood alcohol concentration). Additionally, reducing the sucrose content in the oral portion of the reinforcer complex to either 1S or water resulted in no decrement in the initial trajectory of responding (Figures 2.1.-2.2. cumulative records). Interestingly, sucrose appears to predominately exert its influence on responding during the latter portion of the session resulting in a substantial increase in

responding for either of the sucrose conditions (1-2% sucrose) as compared to the oral water plus intravenous ethanol. From these data, it does not appear that sucrose is necessary for the initiation of lever responding as indicated by the similar initial slopes in responding across oral reinforcer types (2S, 1S, and 0S). Further characterization of the role of sucrose in this reinforcer complex is needed to elucidate the unique influence that the oral sucrose and intravenous ethanol have in this reinforcer complex for the selectively bred P rat.

Research Question/Hypotheses

In order to further characterize the precise role that sucrose and ethanol have in the reinforcing properties of the previously described oral sucrose and intravenous ethanol compound reinforcer, a two-lever choice multiple schedule study design was performed using the selectively bred alcohol preferring P rat. The initial hypothesis was that responding for the intravenous ethanol plus oral sucrose schedule would be more reinforcing than sucrose alone as demonstrated by a significant increase in responding during the intravenous ethanol plus oral sucrose schedule compared to responding for the oral sucrose only schedule.

METHOD

Animals

Eight alcohol preferring P rats (weighing 235 ± 9 g) were obtained from the Indiana University School of Medicine, Indianapolis, IN. Animals had ad libitum access to food and water throughout the study except as noted during the initial training phase. Animals were housed individually in a controlled environment with a 12-hour light/dark cycle (lights on at 8:00 am). All procedures were performed in accordance with NIH guidelines for animal care and usage.

Apparatus

Rat operant conditioning chambers (Coulbourn Instruments, Lehigh Valley, PA, USA) contained within sound-attenuating chambers were used for daily sessions. The front and rear walls of the chamber were composed of Plexiglas with the side panels composed of aluminum. The right side panel contained the response panel. A sipper tube was located in the center of the right panel with a sensor to record lick data. Discrete dosing of fluid into the sipper tube was achieved by computer activation of a valve located on the exterior of the sound-attenuating chamber. Retractable response levers were located on either side of the sipper with a multi-color (red, yellow, green) LED display located directly above each lever. A house light was located at the top right of the back wall. Intravenous reinforcers were delivered via a Coulbourn computer controllable infusion pump located outside of the chamber with the

infusion line connected via a rotating swivel tether allowing for relatively unrestricted movement about the chamber. All session relevant input and output data were controlled and recorded on a Windows PC using Coulbourn Graphic State software.

Procedure

Initial Training

Upon arrival, animals were allowed two days to acclimate to the animal facility. Following this, the animals were weighed and handled at least twice during the five days prior to the start of operant conditioning (see Figure 3. time table for multiple schedule training). Home cage water was removed 18 hours prior to the initiation of operant training with restricted water access during the subsequent five days to facilitate the initiation of lever press responding. Following initial training, animals had ad libitum access to both food and water for the remainder of the study. Operant lever press responding was established during four 1-hour long training sessions using an oral 5% sucrose (5S) solution on a fixed ratio one (FR1) schedule with shaping for increasingly appropriate responses during the first session. Animals unable to acquire lever responding within the first two sessions were given a twelve-hour overnight training session. For this portion of the training, only the sucrose lever was extended into the chamber and correct responding was reinforced with access to 0.1mL of a (5S) solution administered into the sipper tube. Sucrose lever side was alternated across animals to minimize the potential confound of a lever bias. Once lever responding was acquired, the animals then progressed incrementally over several sessions from a FR1 to a FR4 schedule. When animals were reliably responding under the FR4 schedule, a 7 second time-out (TO) period was added following reinforcer administration so that a consistent reinforcer duration

was established across all reinforcer types (i.e., when intravenous infusions are introduced which last 5 seconds followed by a 2 second time out, reinforcer access time can be equated). The sucrose component was then faded across several sessions to a final concentration of 1% sucrose (1S).

To introduce the second lever, the animals were briefly trained to respond on the "other" lever for an oral 1S reinforcer on a FR1 schedule. For these sessions the previously paired sucrose lever was retracted. The schedule was then incrementally increased from a FR1 to FR4 across several sessions. This completed training for sucrose reinforcement on both levers, and from this point on, one lever was associated with only oral reinforcers ("sucrose lever") and the other with intravenous reinforcer plus oral reinforcer ("IV ethanol lever").

Surgical Procedure

Once stable responding on a FR4 schedule was achieved for both levers, an indwelling jugular catheter was placed following standard catheterization surgery procedures (Manzardo 2002) while animals were under pentobarbital anesthesia. Briefly, jugular catheters were constructed from Silastic® tubing (0.020 in I.D. x 0.037 in O.D., Dow Corning) cleansed with toluene then flushed with TDMAC to suppress clot formation at the catheter tip. Tubing was then attached to a 22-gauge guide cannula (Plastic One, Roanoke, VA) using 3-0 surgical suture silk and molded using dental cement with a one-inch square of Bard monofilament polypropylene mesh (Davol, Cranston, RI) attached to the dental cement. A one-cm square of Bard mesh was then attached 3 cm from the tip of the catheter to serve as an insertion indicator. In addition to the use of Bard mesh to expedite healing, both penicillin (0.2 mL IM Combi-Pen) and atropine (1.0 mg/kg subcutaneous) were administered prior to surgery. The

prepared catheter was inserted into the right jugular vein such that the tip was located just above the right atrium. The tubing was then passed subcutaneously over the shoulder to a 3 to 4 cm midscapular incision. Threading of the catheter over the shoulder to the dorsal access point was facilitated by a 3-cm incision located 4-cm above the right hind leg so to diminish site damage and scar tissue formation around the catheter port. Incision sites were then sutured with non-dissolvable 4-0 surgical silk and treated with a topical antibiotic ointment. After surgery, catheter patency was facilitated by daily administration of a Heparin-Gentamicin maintenance flush. Patency was assured with weekly infusions of the fast acting barbiturate Brevital (1% methohexital sodium). Animals with either delayed or no response were removed from the study. Animals were allowed at least five days to recover from surgery prior to attachment of infusion tether.

Acclimation to the Tether

Following surgery, animals completed daily sessions responding for an oral 1S solution on the sucrose lever for seven sessions (see Figure 3. time table for multiple schedule training). During the final two sessions the animals were attached to the infusion tether without infusate administration to habituate the animals to the infusion related apparatus. Animals then had twice daily training sessions with only one of the levers extended into the chamber. During these sessions the stimulus light was introduced. The light above the extended active lever was illuminated serving as a discriminative stimulus. The green light was illuminated indicating active lever, green + yellow lights illuminated indicating reinforcer delivery, and green +yellow + red lights illuminated indicating post-reinforcer time-out period. The morning session occurred within one hour of the onset of the light portion of the light/dark cycle (0700-0800)

and only the oral sucrose reinforcer lever was accessible. The evening session occurred within one hour of the initiation of the dark cycle (1900-2000) and only the IV lever was extended into the chamber. During the first two days of this period, the IV lever was paired with a reinforcer complex of intravenous normal saline (0.9% NaCl) and oral 1S solution. Following this, the IV lever was paired with a reinforcer complex of 25 mg/kg/injection dose of intravenous ethanol [20% v/v in half normal saline (0.45% NaCl)] with an oral 1S solution.

Multiple Schedules Training

Once responding stabilized on each lever, animals then began training on the multiple schedules. Daily 32-minute sessions were conducted with both levers available. A two-minute wait period occurred at the initiation of each session during which time both levers were retracted. Following the wait period, both levers were extended into the chamber and lever activation for reinforcer access alternated between the two reinforcer types on a fixed time interval. Progression to the subsequent reinforcer component was independent of responding during the individual components. The discriminative stimulus remained in effect with the light on above only the active lever. The initial active lever was alternated between sessions. The multiple schedule training started with 2 components, each 15 minutes in length, which progressively shortened over days to components of 7.5 minutes, 5 minutes, and finally 2.5-minutes in length. Animals received two sessions at each component length with intravenous ethanol as the first schedule following each change. There was no effect on ethanol component responding noted as the component interval time shortened; therefore the 2.5-minute component length was used for all subsequent testing. Criterion for stable responding

under the multiple schedules was set at less than 10 "incorrect" responses (responses on the inactive lever) during each component.

Data Analysis

Total responses for each schedule component were recorded. Total sucrose and intravenous infusate volumes were measured post session. Total daily ethanol intakes (g/kg) were calculated by dividing volume of infusate delivered by the measured body weight.

Mean reinforcers and error responses for each schedule component were averaged across 2.5-minute multiple schedule sessions for ethanol first and sucrose first sessions. Average component reinforcers and error responding were analyzed using a two-way within-subject repeated measures analysis of variance (ANOVA) with session and component as factors for within session data. Post hoc comparisons were performed using Student-Newman-Keuls *t* tests ($p < 0.05$).

RESULTS

One animal did not acclimate to the tether apparatus and was removed from the study. An additional animal lost patency prior to completion of acquiring the 2.5 minute multiple schedules and was removed from analysis. For the 6 remaining animals, the average weight at the end of the experiment was 416 ± 10 g. Average ethanol intake across sessions was 0.47 ± 0.04 g/kg (range of 0.04-1.05 g/kg). Individual session data for reinforcers and error responding were plotted (see Figures 4.1.-4.2. Cumulative Record for reinforcers and error responding for Sucrose only first and Sucrose + Ethanol first sessions). Two-way repeated measures (RM) ANOVAs (day X reinforcer type) found no significant main effect for total session reinforcers over days [Ethanol first $F(6, 12)=1.046$, $p=0.444$), sucrose first $F(5, 5)=0.25$, $p=0.18$] or total error responding over days [Ethanol first $F(6, 12)=0.782$, $p=0.60$), sucrose first $F(5, 5)=1.157$, $p=0.44$]. Therefore, for subsequent analysis, data were averaged across days for each component and session type (sucrose only first or sucrose plus intravenous ethanol first).

Average total session correct responding on the sucrose only lever was 64.4 ± 31.3 (mean ± SEM) when sucrose alone was the first component and 54.7 ± 25.0 when sucrose + IV ethanol was the first component. Average total session correct responding on the sucrose + IV ethanol lever was 30.3 ± 14.1 when sucrose alone was the first component and 40.3 ± 17.4 when sucrose + IV ethanol was the first component (see Figure 5.1. Average Total Session Correct Responding). The average total session error responding (i.e. responding on the

inactive lever) for the sucrose only lever was 40.2 ± 14.0 when sucrose alone was the first component and 54.0 ± 17.3 when sucrose + IV ethanol was the first component. Average total session error responding on the sucrose + IV ethanol lever was 20.7 ± 7.8 when sucrose alone was the first component and 21.0 ± 8.6 when sucrose + IV ethanol was the first component (see Figure 5.2. Average Total Session Error Responding).

Analysis of Correct Responding

No significant difference in correct responding was shown for session type (first component being either sucrose only or sucrose + IV ethanol) [$F(1, 55)=3.339$, $p=0.127$]. A significant main effect of order was observed across components for correct responses [$F(11, 55)=12.109$, $p<0.001$)] (see Figure 6. average reinforcers across components). As well, a significant interaction of session type and component was observed [$F(11, 55)=13.07$, $p<0.001$]. Post-hoc Student-Newman-Keuls pair wise comparisons for reinforcers revealed a significant increase in correct responding on the sucrose only lever when sucrose alone is the first component (1^{st} component versus 3^{rd} component $p<0.001$). A significant increase in correct responding on the sucrose lever as compared with correct responding on the ethanol lever was observed for the first seven components [with the exception of the fourth component] when compared between session types.

Analysis of Error Responding

No significant difference in error responding was shown between session type (first component being sucrose only or sucrose + IV ethanol) [$F(1, 55)=0.573$, $p=0.483$]. A significant main effect of order was observed across components for error responses [F(11,

55)=4.881, p<0.001)] (see Figure 7. average error responding across components). As well, a significant interaction of session type and component was observed [F(11, 55)=5.547, p<0.001]. Post-hoc Student-Newman-Keuls pair wise comparisons for error responding revealed a significantly greater in error responding on the sucrose lever compared with the ethanol lever for the first six components when compared between session types.

DISCUSSION

Lever responding was stable across sessions for both sucrose and sucrose plus intravenous ethanol. A statistically significant increase was observed for both correct sucrose lever responding (as demonstrated by reinforcers) during sucrose components and error responding on the sucrose lever during sucrose + IV ethanol components when compared between session types. Although the use of multiple schedules for the study precludes a direct analysis of animal "preference" for the individual reinforcers, the increased error responding on the sucrose lever suggests that despite oral sucrose being available during the sucrose + IV ethanol components the animals persist in responding for access to sucrose only. This suggests either a partial acquisition of the multiple schedules response requirements or a "preference" for the sucrose only reinforcer. Although it is possible that the animals did not completely acquire the response requirements for the multiple schedule (due in part to use of a highly visually specific light cues to indicate active lever and lever retraction post surgery), the prolonged stability in responding across sessions suggests that animals acquired proper responding for both components of the multiple schedules. As well, error responding on the ethanol lever was below the ceiling criteria for stable responding (i.e. less than 10 error responses per component). Though incomplete acquisition of responding for the multiple schedules cannot be completely ruled out, the error responding appears more likely an indication of a degree of "preference" for sucrose alone over the sucrose plus intravenous ethanol.

Since the same sucrose concentration is available with both schedules, variations in responding should reflect the role of intravenous ethanol as a reinforcer. If responding during the IV components remained equal to the response rate of the sucrose only components then intravenous ethanol is neither reinforcing nor punishing. If responding during the IV components increased, intravenous ethanol would be shown to have positive reinforcing qualities. However, if responding during the IV components decreased, intravenous ethanol would be shown to have aversive or punishing qualities. Interestingly, when intravenous ethanol was the first component, responding on the IV lever was only marginally decreased. However, when sucrose was the first component intravenous ethanol responding was substantially lowered (see Figures 8.1.-8.2. Average session reinforcers across components for ethanol and sucrose first sessions). One possible explanation for this difference is that the infusate cooled during the initial sucrose session. This reduction in infusate temperature could have resulted in pain during infusion beyond that which normally occurred with ethanol infusion resulting in lower responding for the intravenous ethanol schedule across sucrose first sessions. Taken as a whole, these findings (increased sucrose lever responding, sucrose lever error responding, and increased first component responding for sucrose) suggest that the intravenous administration of ethanol is aversive particularly when sucrose was the first component. The responding observed during this study resembles responding typically observed during conflict or punishment testing.

Testing Conflict/Punishment

Conflict or punishment testing examines anxiety involved when crossed or opposing motivations are presented concurrently (Commissaris 1992). This is to say, conflict testing

examines responding for a reinforcer with both positive qualities (e.g. food or drug) and simultaneously aversive qualities (e.g. foot shock or noxious odor). Conflict arises as the positive reinforcer increasing the likelihood of responding while paradoxically the aversive stimulus decreases the likelihood of responding. Conflict testing is readily demonstrated by allowing a food restricted animal to respond for access to a compound reinforcer of food plus foot shock. The animal is motivated to access and consume the food while also motivated to avoid the foot shock, thus resulting in conflict. When responding is maintained by access to a positive reinforcer, adding punishment produces a decrease in overall responding for the schedule (Domjan 2003). One of the first theories on punishment, Conditioned Emotional Response (CER), was proposed by Estes in 1944. CER is based upon the observation that when a stimulus previously conditioned (CS) with operant lever responding to acquire food reinforcer is paired with a foot shock (punishment) a decrease in responding of food-reinforced behavior will occur in the presence of the CS. Which is to say, the acquisition of fear to the CS results in the disruption of the food reinforced lever responding. As animals in the current study had stable intravenous ethanol responding that was relatively close to the responding for sucrose, a substantial CER does not appear to be occurring.

Another way to examine punishment is the Geller-Seifter conflict paradigm. Originally proposed by Geller and Seifter (1960), the Geller-Seifter paradigm uses food deprived animals responding during multiple schedules for non-punished and punished (via foot shock) access of a food reinforcer. A substantial drop in responding during the punished component is observed while elevated responding during the unpunished component is

maintained. Use of the multiple schedules design allows for the examination of response rates during the separate components typically with drug manipulation.

Koob et al. (1987) used a modified Geller-Seifter paradigm to examine the effects of intraperitoneal administration of ethanol (0.75 g/kg IP) across several sessions. The IP dose used by Koob is slightly higher than the average intravenous ethanol intake observed with the animals in the present study (0.47 g/kg). Ethanol was found to produce a substantial "anxiolytic" effect (by increasing the rate of punished responding from baseline) but this was found to undergo rapid tolerance resulting in a return of punished responding to baseline levels within several sessions of ethanol administration. Ethanol was shown to also have a sedative action in the rats shown by a decrease in the rate of unpunished responding. Rats were slower to develop tolerance for this sedative effect of ethanol. Similarly, Baldwin et al. (1991) used a modified Geller-Seifter conflict test to examine the anxiolytic and sedative effects of various doses (0.25, 0.5, 0.75, and 1.0 g/kg) of IP ethanol on Wistar, P, and NP rats. P rats were found to be less sensitive to the anxiolytic effects of ethanol (increasing punished responding following only the higher 0.75 and 1.0 g/kg doses) than NP (sensitive to the anxiolytic effect at all ethanol doses) or Wistar rats (initial response at 0.5 g/kg dose). Sedative effects were significantly noticed with decreases in unpunished responding at all ethanol doses for NP rats while both P and Wistar did not decrease unpunished responding until the 0.75 g/kg dose. P rats appear to be less sensitive to both the anxiolytic and sedative effects of ethanol compared with both NP and Wistar. Together these findings suggest that P rats tend to be less sensitive to the anxiolytic effects of ethanol and these effects quickly undergo rapid tolerance. Therefore, although administration of higher quantities of ethanol could potentially counteract the aversive qualities of intravenous ethanol administration, with

chronic administration a tolerance to these anxiolytic properties quickly develops and several administrations must be endured prior to the onset of anxiolytic relief from ethanol.

Grupp and Stewart (1983b) sought to specifically examine the aversive properties of intravenously administered ethanol. Active avoidance of intravenous ethanol exposure was investigated using a shuttle jump response. Wistar rats were training to jump over a hurdle set to variable heights to avoid receiving an intravenous infusion of ethanol. Grupp and Stewart demonstrated that rats will actively avoid exposure to intravenously administered ethanol doses of 0.2, 0.4, and 0.8 g/kg while the saline control group did not acquire jump response. As well, they examined if rats would actively self administer intravenous ethanol using a runway experiment. Animals were trained to traversed the runway and enter the goal box. Upon goal box entry, animals received a 30-second infusion of either saline or ethanol. It was found that for all ethanol doses rats would passively avoid intravenous ethanol exposure by not entering the goal box after several trials. This active and passive avoidance to intravenous ethanol administration suggests that intravenous ethanol acts as a punisher in rats.

McKearney (1968) demonstrated that animals would willingly self-administer electrical shock. In this study squirrel monkeys were initially trained using a shock-postponement schedule. For this schedule, shocks were programmed to occur every 10 seconds, however, each response postponed shock presentation for 30 seconds. Once robust responding under this schedule was achieved, an additional schedule was added concurrent to the shock-postponement schedule in which responding after 10 minutes resulted in shock presentation (FI 10-minute schedule). When the shock-postponement schedule was removed, the animals maintained responding for the FI-10 minute shock. Grupp and Perlanski (1983a) found that

the same experimental design that engenders responding for shock presentation is not successful in establishing intravenous self administration of ethanol for 200 and 400 mg/kg/injection doses. These findings suggest that intravenous ethanol is aversive.

Though the animals in the current study were not food deprived, the perseveration on the sucrose lever during a sucrose + intravenous ethanol suggests a conflict of wanting the oral sucrose reinforcement without the administration of intravenous ethanol. This is also demonstrated by the increase in first component sucrose responding when sucrose only is the first reinforcer. If intravenous ethanol administration is aversive, it is probable that the pharmacologically significant intake of ethanol observed in this study was due to the sucrose portion of the IV ethanol plus sucrose compound reinforcer rather than the ethanol. However, if sucrose alone were driving responding one would expect food and water sated animals to extinguish responding during the intravenous ethanol component and shift responding to the non-aversive sucrose only component. Such extinction of intravenous ethanol responding was not observed. All animals maintained stable, though decremented, responding during the intravenous ethanol components.

It is possible that the increased responding for sucrose during the first component is because of a qualitative difference in the reinforcers due to a novel taste component relevant to intravenous ethanol administration. A tiny portion of the ethanol administered is expired during normal respiration. Though small, this quantity of ethanol is theoretically detectable by the animal as a distinct taste and/or smell. It is therefore plausible that the orally consumed sucrose is qualitatively different following the first ethanol component. This qualitative difference in oral sucrose explains the observed increase in responding for sucrose when sucrose during the first component. This qualitative difference in sucrose

following ethanol administration does not, however, explain the elevated error responding on the sucrose lever observed across the session.

Implications

This study demonstrates that the current method of intravenous self administration of ethanol using a compound reinforcer of 1% oral sucrose plus 20% intravenous ethanol remains aversive in the rat. The data suggest that the animals were primarily responding for access to the oral sucrose component rather than access to either the intravenous ethanol or reinforcer complex. The data show a decrease in responding for intravenous ethanol suggesting that the intravenous ethanol is punishing or a qualitative difference exists between the oral reinforcers for the two schedules. A method by which rats will self administer pharmacologically relevant levels of ethanol with responding driven the reinforcing effects of ethanol remains elusive. This is due to in part or whole to the punishing qualities of intravenous ethanol administration.

Future Directions

Responding for the compound reinforcer was shown to be driven primarily by the sucrose component of the reinforcer complex with the potential of lingering aversive effect of intravenous ethanol administration. Further work attempting to establish a viable method by which rats with self administer pharmacologically relevant levels of ethanol must address these aversive effects as well as demonstrate a preference for intravenous ethanol. Use of a sucrose fade after surgery, similar to the cocaine fade used by Ikegami, may help bridge the gap between the aversive and reinforcing effects of intravenous ethanol. As well, warming the

infusate across the entire length of the tether apparatus might decrease a possible pain response to intravenous ethanol administration. Findings from the preliminary studies demonstrated a substantial increase in responding for intravenously administered ethanol when the infusate solution was warmed prior to the start of the session. At the present time, no methodology of intravenous ethanol self administration in rats achieves pharmacologically relevant levels of ethanol exposure that is primarily driven by responding specific for the intravenous administered ethanol.

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TABLES

Table 1

Previous Rat Studies Using Intravenous Ethanol Self-Administration

Citation	strain of rat	deprivation	concentration EtOH infusate	dose (mg/kg/infusion)	intake (g/kg)	length of session	volume infused (mL)	length of infusion	estimated BAC
DeNoble, 1985	Long-Evans	Food	20%	1, 3, 10, 30, 60, 90, 180, 360	1.65	24 hour	0.2	4 sec	not stated
Gass, 2007	Wistar	Food	1%	1	0.02	1 hour	0.03	1 sec	not stated
Grupp, 1983a	Wistar	Food	10-25%	200, 400	2.4	75 minute	not stated	not stated	not stated
Grupp, 1983b	Wistar	Active no; Passive yes	10-40%	200, 400, 800	0.2 - 0.8	single exposure	0.2mL/100g body weight	90 sec	not stated
Hyytiä, 1996	AA, ANA	Food	0.5-2%	1.0, 2.0, 4.0	AA= 0.04, ANA=0.015	3 hour	0.1	4 sec	not stated
Ikegami, 2002	Sprague-Dawley	no	10%	125 (training), 62.5, 125, 250, 500	6.7-11.1 training (EtOH + cocaine); 0.5-2.0 (EtOH alone)	1 hour	variable	not stated	44-221 mg/dL
Lyness, 1992	Sprague-Dawley	no	0.5-4.0%	0.5, 1, 2, 4, 8	0.15	8 hour	~0.06 (calculated)	not stated	not stated
Numan, 1981	Long-Evans	no	20%	avg 0.093 (range 0.08-0.11)	10.43	24 hour	0.2	1 sec	not stated
Oei, 1979	Wistar	Food	19.90%	8	0.2 (food dep), 0.5 (food dep + FT1)	1 hour	0.07	5 sec	not stated
Sinden, 1982	Wistar	no	1.50%	0.5, 1.0, 5.0	0.024 (0.5 mg/kg/dose), 0.0625 (1.0 mg/kg dose), 0.0115 (5.0 mg/kg/dose)	24 hour	0.1	3 sec	not stated
Smith, 1974	Holtzman	no	0.25%	0.12	.048 (highest)	12 hour	0.018	0.2 sec	not stated

FIGURES

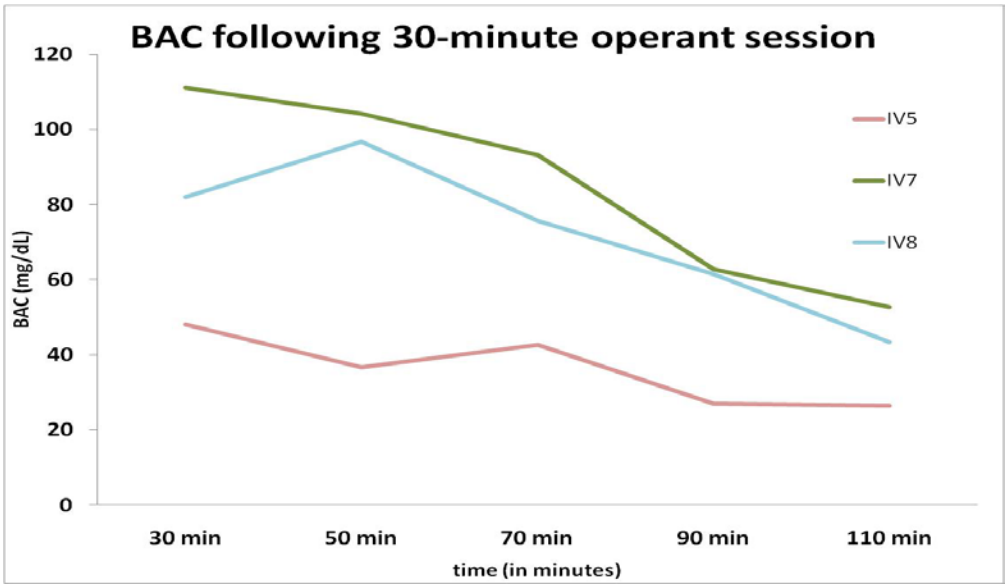

Figure 1. Blood alcohol concentrations. Tail blood samples collected every 20 minutes following a 30-minute self-administration session with animals responding for a reinforcer complex of oral 2S and varied IV ethanol concentrations.

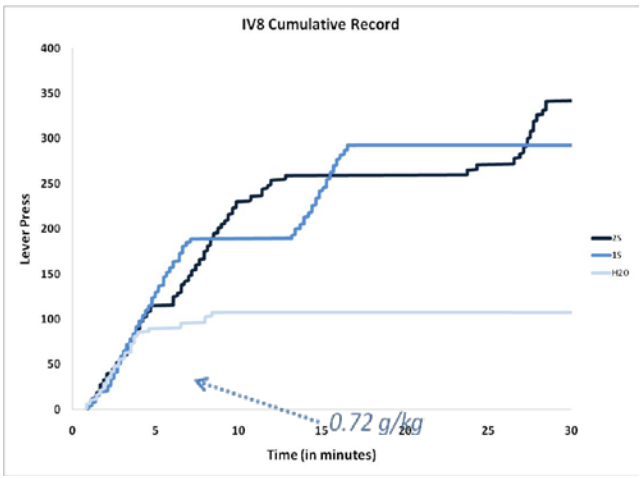

Figure 2.1. Cumulative Record Data for IV8. Lever responding during 30-minute sessions for access to a 20% intraveous ethanol (25 mg/kg/dose) plus varying oral sucrose concentration (2S, 1S, and water).

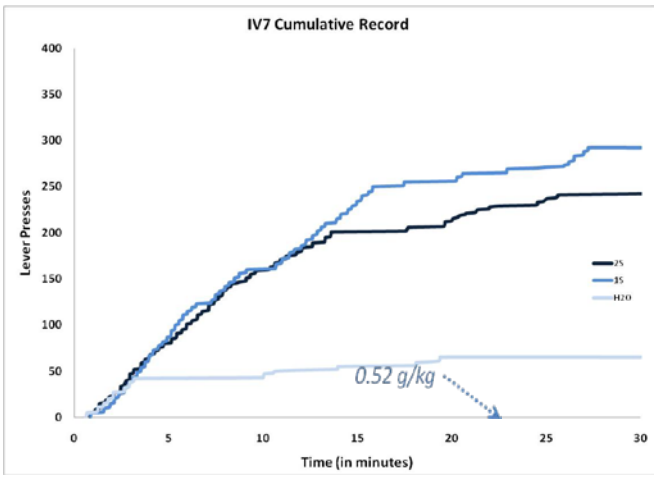

Figure 2.2. Cumulative Record Data for IV7. Lever responding during 30-minute sessions for access to a 20% intraveous ethanol (25 mg/kg/dose) plus varying oral sucrose concentration (2S, 1S, and water).

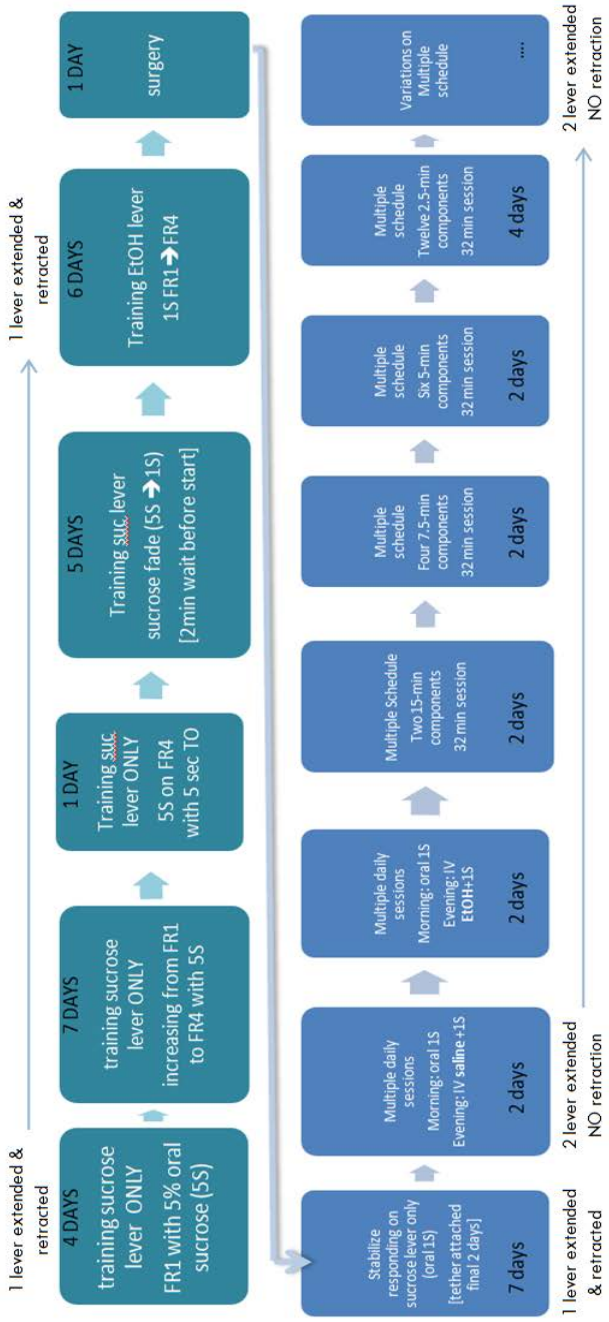

Figure 3. Time Table for Multiple Schedule Training.

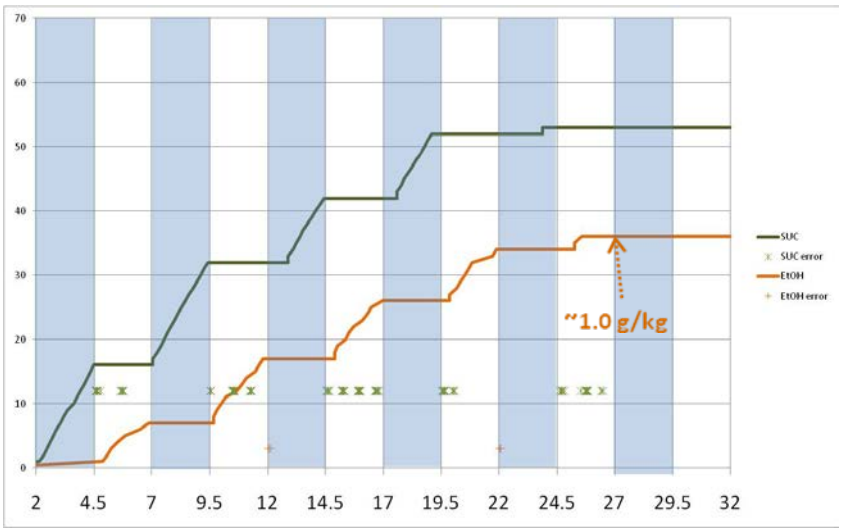

Figure 4.1. Cumulative Record Sucrose Only First Component for IV15. Green equals sucrose reinforcers administered while orange is intravenous ethanol reinforcers. The green "X" symbols designate error responding on the sucrose only lever while the orange "+" symbols designate error responding on the intravenous ethanol lever. Shading across x-axis indicates progression to next component. For this animal, total ethanol intake during this session was approximately 1.0 g/kg. The animal emitted a high rate of error responses on the sucrose only lever across the session, while error responding on the intravenous ethanol lever was restricted to the time around each component transition.

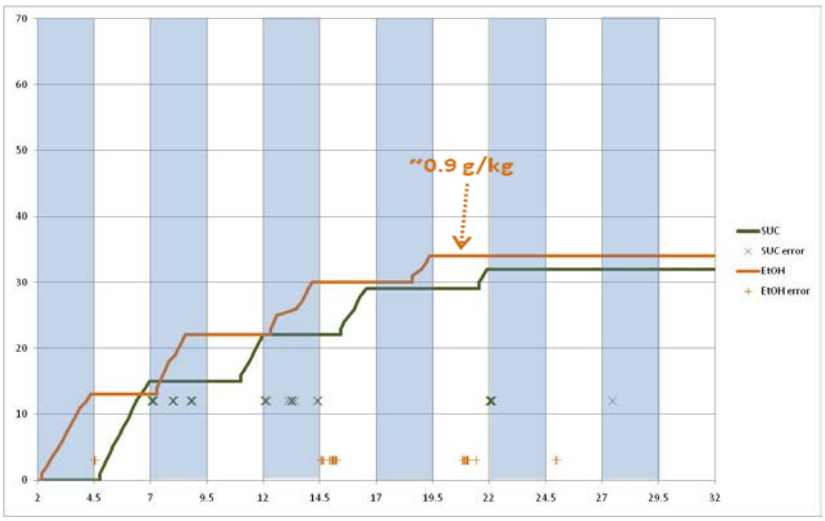

Figure 4.2. Cumulative Record IV Ethanol Plus Sucrose First Component for IV15. Green equals sucrose reinforcers administered while orange is intravenous ethanol reinforcers. The green "X" symbols designate error responding on the sucrose only lever while the orange "+" symbols designate error responding on the intravenous ethanol lever. Shading across x-axis indicates progression to next component. For this animal, total ethanol intake during this session was approximately 0.9 g/kg. The animal emitted a less error responses on the sucrose only lever across the session [compared with sucrose first session (Figure 4.1.)]. Error responding on the intravenous ethanol lever was relatively restricted to the time around each component transition with an increase in intravenous ethanol error responding compared with the sucrose first session (Figure 4.1.).

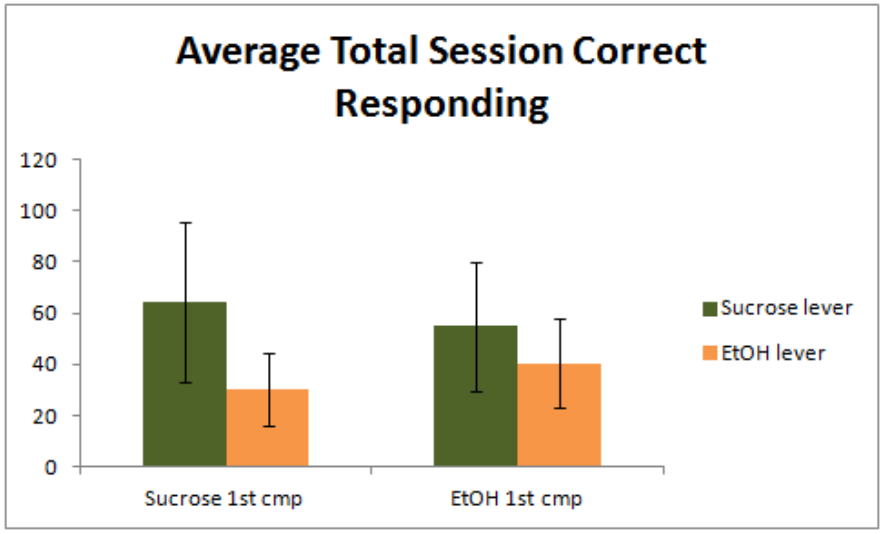

Figure 5.1. Average Total Session Correct Responding. Green is correct responding on the sucrose only lever, while orange is correct responding on the intravenous ethanol lever. On the left are the data for the sucrose first sessions while the data for ethanol first sessions is on the right. No statistically significant difference was noted with correct responding for either session type or component type.

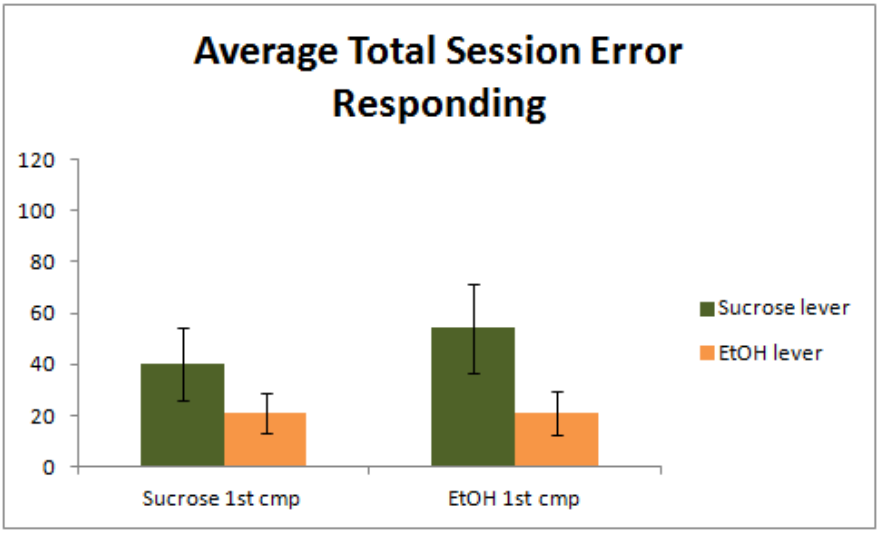

Figure 5.2. Average Total Session Error Responding. Green is error responding on the sucrose only lever, while orange is error responding on the intravenous ethanol lever. On the left are the data for the sucrose first sessions while the data for ethanol first sessions is on the right. No statistically significant difference was noted for error responding for either session type or component type.

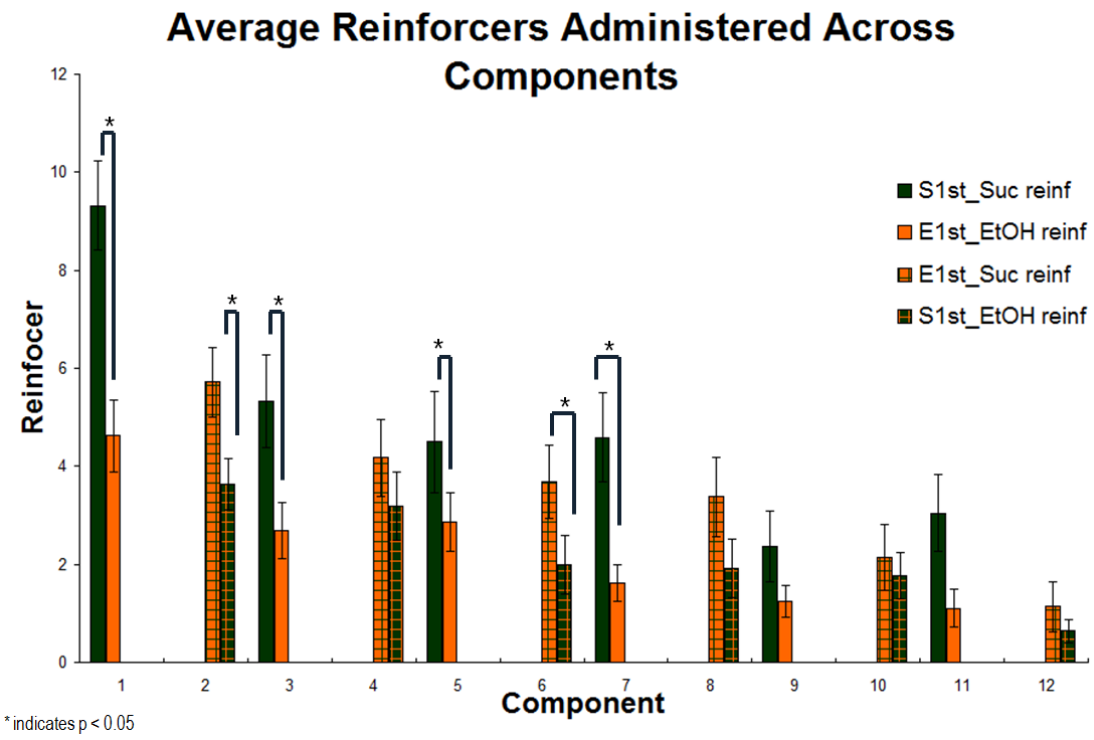

Figure 6. Reinforcers Administration for Sucrose and IV Ethanol Sessions. The primary color indicates session type (green for sucrose, orange for ethanol). The left column of each component is sucrose reinforcers, while the right column is ethanol reinforcers (with minor shading indicating reinforcer type where necessary). As significant increase ($p < 0.05$) in sucrose reinforcers is noted for the first seven components (except for component 4).

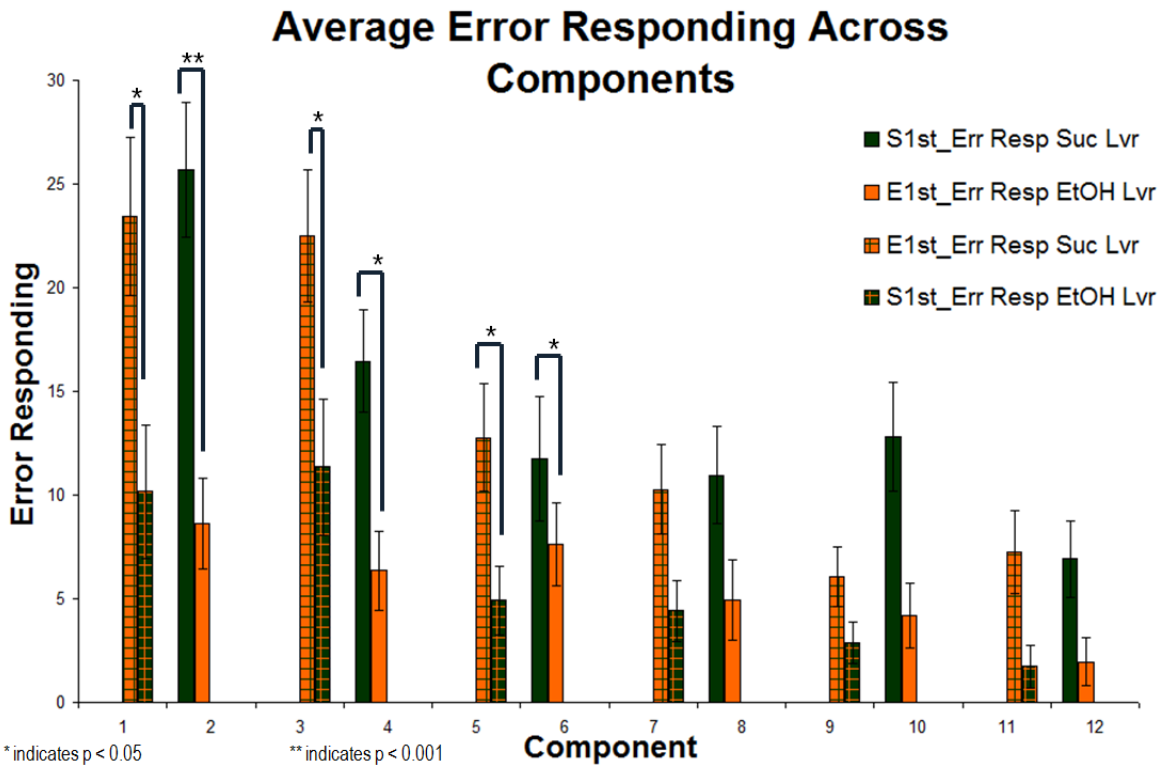

Figure 7. Error Responding for Sucrose and IV Ethanol Sessions. The primary color indicates session type (green for sucrose, orange for ethanol). The left column of each component is sucrose error responding, while the right column is ethanol error responding (with minor shading indicating reinforcer type where necessary). As significant increase ($p < 0.05$) in sucrose error responding is noted for the first six components.

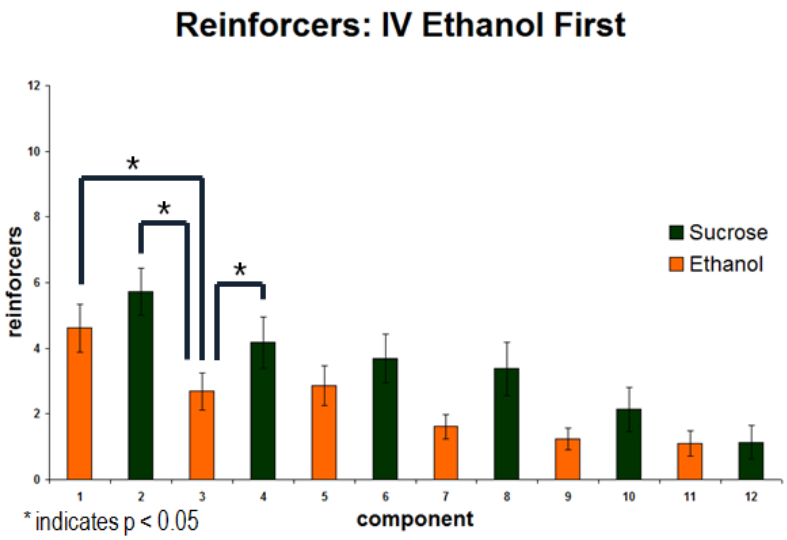

Figure 8.1. Reinforcers Intravenous Ethanol First Sessions. Green is sucrose reinforcers; while orange is intravenous ethanol reinforcers. A significant difference is noted between the first ethanol component and the subsequent ethanol component indicating a substantial drop in responding between the first and second ethanol schedule components.

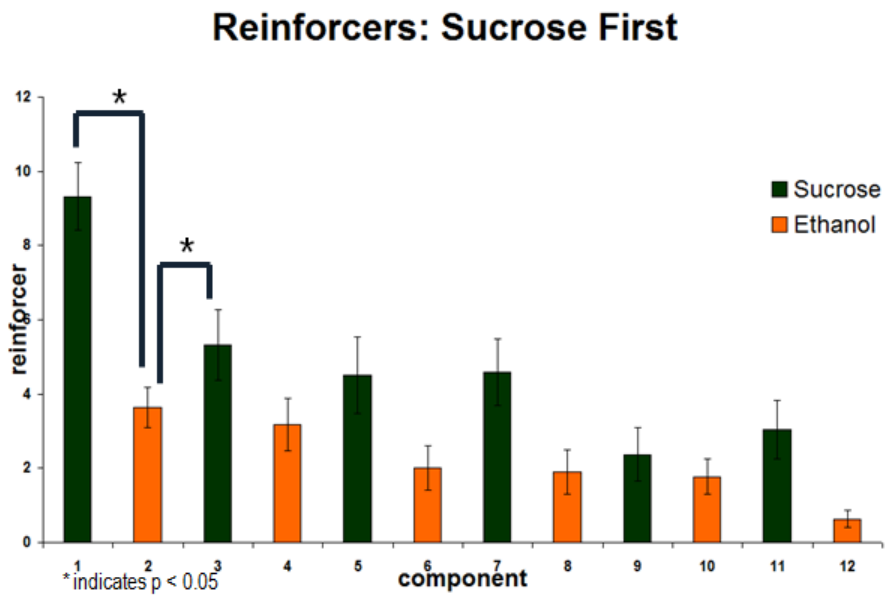

Figure 8.2. Reinforcers Sucrose First Sessions. Green is sucrose reinforcers; while orange is intravenous ethanol reinforcers. A significant difference is noted between the first ethanol component and the first and second sucrose components.