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Environmental Influences on the Sign Tracking of Ethanol: A Rodent Model of Alcohol Addiction

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Environmental influences on the [illegible]tacking of ethanol:

A rodent model [illegible]hol addiction

by

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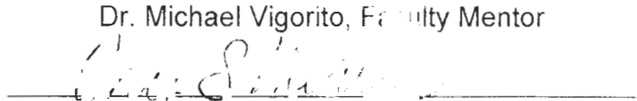

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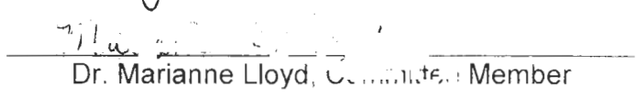

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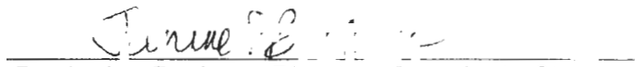

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Abstract

Rodent models of alcoholism provide a m[illegible] for [illegible]loring the factors that contribute to alcoholism. The rodent sign tra[illegible]in[illegible] [illegible]dure using a bot[illegible] (w[illegible] thanol or water) as the conditioned stimulus and a sugar [illegible]t as the unconditioned stimulus has several components that appear related to d[illegible] [illegible]buse. In this study, the environmental influences of rearing condition an[illegible] b[illegible] [illegible]ial infection were explored as possible contributory factors to the abuse of [illegible]. In Experiment 1, Sprague-[illegible]ley rats reared in an enriched environment show[illegible]st [illegible] acquisi[illegible]on of [illegible]gn [illegible]ing behavior and consumed more ethanol than [illegible] a standard environment, but neither group developed a preference for eth[illegible]ol A[illegible]tive-feature discrimination task revealed that the enriched- and standard-rea[illegible]d[illegible] not impulsive since they readily reduced sign tracking behavior on trials when th[illegible] pellet was omitted. Although, the enriched rats were more vulnerable to the effects [illegible]anol than the standard [illegible], because they were sign tracking the bottle more, inc[illegible]ed [illegible]ulsivity does not adequ[illegible]ely explain their "addiction to alcohol". In Experiment 2, [illegible]ng-Evans rats [illegible]e trained in the sign tracking procedure with or without ethanol [illegible] bottles as in the first experiment, but all rats were also given 24-hr access to eth[illegible]ol in [illegible] home cage. [illegible]tment with the bacterial endotoxin lipopolysaccharide (LPS) sig[illegible]cantly increased the rats' pref[illegible]rence for ethanol, nevertheless this greater liking for e[illegible] id not affect the sign-tracking of ethanol. Therefore the compulsive ethanol drin[illegible] in the Long Evans rats as in the Sprague Dawley rats in Experiment 1, appeared to [illegible] due to sign tracking procedure, rather than the rewarding properties of the etha[illegible] [illegible]ver, in contrast to the Sprague-Dawley rats the negative-feature discrimination [illegible] revealed substantial impulsivity of

sign tracking behavior in the Long-Evans rats. The results of both experiments suggest that environmental influences appear to have a significant impact on sign tracking performance and the responsiveness to ethanol but further research is needed to further evaluate the usefulness of the sign tracking model as an animal model of alcoholism and the underlying mechanisms that contribute to the alcoholic phenotype.

The influence of environmental experience on the signaling of ethanol:

A rodent model of alcohol addiction

Alcohol, otherwise known as ethanol (EtOH), is frequently consumed for enjoyment and the reduction of social anxiety within social situations (Enoch, 2006). Alcohol typically affects 5 main neurotransmitter systems in the brain. These five systems are the glutamate, GABA, dopamine, serotonin, and opioid systems. With glutamate, alcohol typically affects the NMDA receptor which binds this neurotransmitter. Alterations of this receptor during light drinking affect memory, but persistent heavy drinking will cause brain damage. GABA is partially responsible for the visible behavior effects of intoxication, and is involved in developing the tolerance of alcohol. Serotonin contributes to arousal and is responsible for consummatory behavior, which includes alcohol consumption. Dopamine and the opiod systems contribute to the pleasurable feeling of alcohol consumption and are believed to increase drinking consumption while decreasing during withdrawal. Since the pleasurable feelings depart when the alcohol departs, this leads some people to abuse alcohol (Chastain, 2006).

Alcohol abuse and addiction have been found to typically develop while a person is in adolescence and later continue throughout adulthood (Enoch, 2006, Walker & Ehlers, 2009). There are three stages to the addiction cycle. These stages are the anticipation, binge drinking, and withdrawal stages. The anticipation stage is characterized by the fixation or sensitization to/of a drug due to the intermittent presentation of the drug. The binge drinking stage occurs when the individual drinks to the point of intoxication due to dependence on the drug or due to other motivating

pressures, like stress. The withdrawal stage is di[illegible]hed by a negative affect due to the body's desire to re-experience the drug (Koob, 2000).

The introduction of alcohol to an individual for the sake of research would be a questionable practice, so rodent models are typically used to learn more about alcohol. Rodent models are often used because there are [illegible] common physiological elements they share with humans. The stages of addiction are replicated in rat models to learn more about the underlying processes of alcohol addiction, provided that rats can overcome the aversive taste of alcohol (Koob, 2000). One promising model of alcohol addiction is the sign tracking model, which provides a good model of the anticipatory stage of alcohol addiction. In the sign tracking model, rats are trained to consume ethanol by pairing brief presentations of a bottle with food pellets. Sign tracking will be described in further detail later in this introduction.

Through research with animals and humans many factors have been found to contribute to alcohol use and abuse. These factors include stress, genetic, behavioral (sensitization or impulsivity), and environmental factors like rearing conditions and exposure to potentially harmful substances. In human studies of adolescents, stress was found to diminish the reward system, affect the prefrontal cortex of the brain, and impair hippocampal development which in turn makes adolescents more responsive to addictive drugs. Three factors that contribute to the enhanced alcohol addiction of adolescents are the physiological changes within the prefrontal cortex during this time period which promotes risk taking behavior, neurobiological vulnerability, and the stress induced sensitization of the hypothalamic pituitary axis (HPA). (Andersen & Teicher, 2009; Enoch, 2006) Additionally, addictive drugs such as [illegible] share neural mechanisms with

natural rewards. There is strong evidence that the pharmacologic effects of PCP induce changes in the experience of rewarding stimuli, such as social and physical pleasure, to make these positive experiences feel more euphoric (Tomie, Grimes, & Pohorecky, 2008).

Genetic factors responsible for alcohol abuse include the MET158 variant of the Catechol-o-methyl transferase (COMT) gene which was found to be linked with susceptibility to alcohol. However, an individual with the alcohol vulnerability COMT gene is not doomed to abuse alcohol, because the environment that a subject is raised in (rearing conditions) interacts with the potential to develop addiction. This interaction is affected by many neurotransmitters. Specifically, the neurotransmitter serotonin has been implicated in the control of impulsivity, which is one of the many behavioral factors that contribute to alcohol abuse. Impulsivity is described in further detail later in this introduction. Additionally, early environments (rearing conditions) have an impact on behavior is described in further detail later in this introduction.

Finally, immune system activation is a potential factor in alcohol addiction. Although there is not much research on the role of the immune system in alcohol addiction, several observations suggest a potential role for neuro-immune interactions in drug abuse. Research with humans has found that there is a high prevalence of HIV positive individuals that abuse drugs (Ferrando, 2008). Research with rats has found that HIV transgenic rats show a greater methamphetamine-induced behavior sensitization than control F344 rats. Although HIV-1 transgenic rats do not have HIV-1 infection, the HIV genes that have been inserted into the rat genome produce HIV proteins (e.g., gp 120) that affects immune system functioning (e.g., altered cytokine levels) which in

turn affects neuronal functioning. Greater sensitivity of HIV-1 Tg rats to methamphetamine may be due to the greater dopamine expression in the prefrontal cortex of the HIV rats (Liu, Chang, Vigorito, Kass, Li, & Cheng, 2009). Research with alcohol preferring mice found that an intraperitoneal injection of 1mg/kg of lipopolysaccharide (LPS) promoted higher alcohol consumption, which effects lasting three months after the injection (Blednov, Benavidez, Geil, Perra, Perra, & Harris 2011). LPS is a protein found in bacterial walls that when detected by the immune system activates an innate immune defense. The Blednov et al study suggests that a single immune system activation is sufficient to cause long term changes in neuronal functioning and subsequent EtOH consumption.

The purpose of the following experiments was to explore the effects of two environmental factors on the sign tracking of Lewis rats in associative learning conditions (Experiment 1) and exposure to bacterial insult (Experiment 2). Additionally, modifications of the sign tracking procedure were introduced to further evaluate sign tracking as an animal model of compulsive alcohol use and abuse. Several studies suggest that like compulsive alcohol use, sign tracking behavior is associated with impulsivity (Tomie et al, 2008). Thus, in the following experiments modifications of the sign tracking procedures were included as potential measures of impulsivity.

Sign Tracking, Incentive Sensitization, and Drug Abuse

Sign Tracking

Sign tracking procedures are characterized by the pairing of a conditioned stimulus (CS) with the prompt delivery of an appetitive (e.g. food) unconditioned

stimulus (US). These procedures represent a variation on the Pavlovian "classical" conditioning paradigm because the CS and the US occur independent of the subjects' behavior. After animals have learned to associate the CS with the US, conditioned responses (CR) of anticipatory behavior develop that are classified as *goal tracking* or *sign tracking*. Goal tracking, which is the typical response in a Pavlovian conditioning paradigm, refers to the animals' use of the signal CS solely as a means of tracking the impending arrival of the reward US, with the anticipatory behavior being directed at the US. For example, goal tracking rats are monitored by counting the number of breaks in an infrared beam that occur when the animal inserts its head in the food tray. Sign tracking is distinguished from goal tracking by the animals' tendency to primarily track and direct its anticipatory behavior at the signal CS instead of the goal US (Robinson & Flagel, 2009). In sign tracking studies with birds, for example, investigators measure anticipatory pecks that birds direct at a keylight CS. Rats will also show anticipatory approach and investigatory behaviors toward a light CS. Sign tracking behavior was originally erroneously called autoshaping by Brown and Jenkins (1968) because they believed that the behavioral fixation on the signal for food was due to superstitious (operant) conditioning. This superstitious conditioning implies that the animal fixated on the signal because its interaction with the signal seemed to produce the US, and satisfy a perceived operant behavioral requirement. Several sign tracking studies have demonstrated that the animal's behavior will persist even when the CS-US are omitted on a substantial percentage of the trials, which suggests that the animal responding is not an operant response (Monterosso & Ainslie, 1999). The term "autoshaping" is more often referred to as sign tracking to reflect a more conceptually accurate representation of

its relationship to classical conditioning, i.e. sign tracking predicting US. Unfortunately, some investigators continue to use the conceptually inaccurate term "autoshaping" when describing this procedure.

Sign tracking behavior can be manipulated to produce more profound effects in rats by using a signal that rats may interact with using their snouts and mouth rather than a light that can only be observed by the rat. Replacing a light CS with a retractable lever CS, for example, causes many rats to direct their anticipatory behavior towards the lever. Some investigators have even added bars to the testing chamber. With this bar addition, the rats will direct their anticipatory (sign tracking) behavior of chewing, biting, or pressing to the bar. Often the rat will press the bar sufficiently enough to close a switch. With this modification, investigators will typically count the number of bar presses as an index of sign tracking behavior.

Sign tracking behaviors reveal that a neutral signal gets associated with the positive stimulus of food the neutral signal gains its own motivational qualities or incentive salience for the animal. Not long after the neutral signal has gained incentive salience, most rats develop compulsive behaviors towards the signals. These compulsive-like behaviors, also known as incentive sensitization, which develops through conditioning in a sign tracking procedure, can also occur in traditional operant or instrumental conditioning procedures (Robinson & Berridge, 2008).

The concept of incentive sensitization is explained in an addiction model in which a distinction is made between drug liking (the pleasure) and drug wanting, i.e., the craving (Robinson & Berridge, 2008). This model parallels the finding that over the course of

developing addiction and with repeated exposure, i[illegible] is a marked increase in drug wanting while there is either no change or a small [illegible] in drug liking. This may be due to different neural mechanisms being re[illegible] for the two components of drug reward, and because repeated use causes a [illegible] of the "wanting" system but no sensitization or even tolerance in the "liking" system. It is theorized that the mesolimbic dopamine system can be sensitized by repeated administration of many abused drugs and that this neural circuit may be more important in [illegible]nting than in drug liking (Robinson & Berridge, 2008).

Research indicates that individual differences in the tendency to sign-track (focused anticipatory behavior) are connected with different tendencies to attribute incentive salience to distinct reward-related cues (Flagel, Watson, Akil, & Robinson, 2008). This suggests that sign-trackers are prone to a form of plasticity (addictive phenotype) that may contribute to the development of addiction (Robinson and Berridge, 2000, 2001; Saunders & Robinson, 2010), which in turn parallels the finding that drug abusers are individuals predisposed to develop pathological levels of incentive salience attributable to reward-related cues (Tomie et al, 2000).

Within a different exploration of compulsive behavior, Tomie (1996) introduced the concept of "Cue and Manipulandum" (CAM). Cue refers to the [illegible] or the passive object, and manipulandum refers to an interactive object. Essentially, CAM represents an alternative method of describing incentive salience. In the typical operant conditioning experiment the subject is required to act on a manipulandum to obtain a reward. The reward (and cues associated with it) is usually located at a distance from the manipulandum. CAM occurs when the experimenter pairs a reward cue very near or on an

object that must be manipulated during an instrumental response. The close spatial relationship between the manipulandum and reward facilitates a compulsive response toward the manipulated object. The compulsive and excessive behaviors persist even though they serve only to delay or prevent the acquisition of reward. Tomie found that although the operant procedure required that the animal only make a response then retrieve the reward, the close proximity of reward to the manipulandum induced sign tracking of the manipulandum which interacted with the simple operant requirement. This finding indicates that the sign-tracking behavior is not under strict voluntary control. Furthermore, Tomie's findings suggest that the animals' maladaptive behavior in the CAM situation is due to conditioning and poor self-regulation (Tomie, 1996). Tomie (1995) suggests that the exaggerated responses to objects can also be found in humans that consume drugs (a reward) using only one method of administration (like an alcoholic to a beer glass) or when the object that delivers the drug is directly related to the drug's reinforcing effects (like the consumption of drug in pill form). These compulsive behaviors are also acknowledged by addiction researchers as being reminiscent of the fixated behavior that drug addicts exhibit toward their desired paraphernalia of administration. Additionally, addiction researchers suggest that these behaviors are typically activated by subjective internal motivational states that contribute to the impulse use of the drug, which increases the likelihood of drug consumption (Tomie et al, 2008).

Tomie suggested that the sign tracking procedure be modified to more closely model the acquisition of compulsive behaviors directed toward drug-delivering paraphernalia in humans by replacing the response lever in the sign tracking procedure

with a bottle. Thus, a rat sign tracking a bottle will lick at the spout and therefore self administer any drug contained within the bottle.

Tomie's (2005) study found that repeated intermittent presentations (sign tracking procedures) of an ethanol sipper tube induced more ethanol intake than did continuous access to the EtOH sipper tube. Also more general activity was found in an intermittency condition than in a continuous access condition, which is perhaps indicative of higher levels of arousal. Therefore, one factor causing excessive responding in sign tracking is the experience with repeated insertions and retractions of the sipper tube which induces a state of arousal or sensitization, increasing the likelihood that an active rat would contact and drink EtOH from a sipper tube. Tomie also found that although random presentations of the bottle and food US did not generate sign tracking behavior, paired bottle-US presentations produce sign tracking. This indicates that behavior directed toward the bottle increases only when the bottle becomes a signal for the US. Thus, EtOH intake in the sign tracking procedure appears to be due to intermittency-induced arousal plus Pavlovian CS and US responding (Tomie, Gittleman, Dranoff, & Pohorecky, 2005; Krank, 2003).

Thus, the sign tracking procedure using a bottle as the CS has three components that appear related to drug use and abuse. First, individuals prone to drug abuse (addictive phenotype) are more likely to respond to the intermittent presentations of the bottle, resulting in compulsive responding toward the bottle appropriate addictive behavior. Second, the presence of a Pavlovian relationship between the bottle CS and food US attaches incentive salience to the bottle, making the reward US further increasing bottle-directed behavior. Finally, when the bottle contains a drug, the

compulsive behavioral interaction with the bottle [illegible]ld may further contribute to the maintenance of the compulsive behavior since the i[illegible]ction with the bottle [illegible]u[illegible] in administration of a drug (e.g. alcohol).

Behavioral Sensitization and Drug Abuse

Another way that sign tracking behavior is [illegible]lated to drug abuse is through behavioral sensitization. Behavioral sensitization is [illegible]ated as an increase in the locomotor-stimulating effects of a drug, such as [illegible]nine, after [illegible]osure to a consistent drug dose. The increased sensitivity [illegible] drug with repeated experience is believed to be a determinant factor of addictive behavior in rats and humans, and may be a result of direct changes in the circuitry of the [illegible] Neuroimaging studies describe prefrontal activity alterations and striatal activity [illegible] resulting from [illegible]avioral sensitization. It is believed that altered prefrontal [illegible] evidenced by problems with emotional stress regulation and inhibitory c[illegible] with heightened striatal responses to addicted drug and drug-related salient stimuli perpetuate habitual drug seeking (Li & Sinha, 2008; Feil et al, 2010). Sign tracking responses and the psychomotor activation syndrome appear to be similar behavior because both behavior types are skeletal-motor responses. Skeletal motor responses include forward locomotion actions as well as directed approaches that include contact and manipulation responses, which culminate in consummatory-like responses, such as gnawing, licking, sniffing, chewing, and swallowing (Tomie et al, 2008). The [illegible] increase of sign tracking behavior as a result of repeated exposure to paired CS and US may be related to the increase in drug induced behavior (sensitization) as a result of repeated drug exposure.

Evidence of a relationship between sign-tracking and psychomotor sensitization has also been reported. In rats, sensitization has been shown with many stimulant drugs (e.g. cocaine) as well as with morphine. Although it is more difficult to demonstrate behavior sensitization with EtOH, cross-sensitization has been shown between EtOH and morphine (Nestby et al., 1997; Lessov et al., 1997). Cross sensitization is the experience in which an individual is initially sensitized to one substance (morphine) that consequently sensitizes the individual to a different substance (EtOH). This is usually due to a relationship between substances, such as similar neurobiological effects. The cross-sensitization between EtOH and morphine may be mediated by a common interaction on the opioid system. Evidence that there is a "cross-sensitization" between sign tracking and stimulant induced sensitization comes from a study reporting that rats that develop predominant sign-tracking behavior show an enhanced tendency to exhibit psychomotor sensitization to cocaine, when compared to rats that develop predominant goal-tracking behavior (Flagel et al., 2008).

Previous studies of the nucleus accumbens core (NAC) of the brain demonstrated that the crucial structure for sign tracking is the same structure that is implicated in drug relapses within addiction. Flagel et al suggested that sign-trackers are susceptible to a form of plasticity that may contribute to the development of addiction. In support of this, Flagel et al also reported that predominant sign-trackers exhibited higher levels of D1 mRNA in the NAC relative to predominant goal-trackers after the first day of training with sign-tracking procedures (Flagel, Watson, Kelly, & Akil, 2007), but after 5 days of training, sign-trackers showed dulled dopaminergic expression patterns relative to goal trackers, including lower levels of tyrosine hydroxylase, dopamine transporter, and

dopamine D2 mRNA relative to goal-trackers (Flagel et al., 2007). These data are consistent with the hypothesis that behavioral [illegible] induced by sign-tracking procedures are related to changes in the dopaminergic system in a manner well-known by addiction researchers. Furthermore, levels of the D1 receptor was found to be integral for sign track learning (Dalley et al, 2005) and levels of the D2 receptor have been associated with increased reports of "drug-liking" in humans (Volkow et al., 2002).

Impulsivity and Drug Abuse

Impulsivity is closely related to drug use and abuse, both as a contributor to use and as a result of use. Impulsivity has been used to refer to a wide range of seemingly unrelated maladaptive or inappropriate behaviors including the inability to wait, difficulty in withholding responses, excessive presence of [illegible] responses, and insensitivity to negative or delayed consequences (Dalley et al., 2011). In addition, impulsivity is a risk factor for drug experimentation, problematic drug use, and contributes to the inability to refrain from drug use. Brief fluctuations in decision-making or inhibition may have especially negative consequences for drug users who are trying to abstain from drug use, because momentary lapses in control or inhibition could increase the risk of drug use. Extended exposure to a drug may also result in impaired inhibitory capacity, which may be due to long-term neurological damage from chronic drug use (de Wit, 2009).

Drug addiction has specifically been related to impulsivity by studies reporting that rats that are intolerant of reward delays subsequently self-administer more EtOH than do delay tolerant rats (Poulos, Le, & Parker, 1995; Poulos, Parker, & Le, 1998). Poulos et al have shown that rats, exhibiting intolerance to a 1 delay by choosing small

immediate rewards over larger delayed rewards, subsequently consumed more EtOH than rats that were less delay-intolerant. Their work suggests that impulsivity and EtOH drinking are linked phenomena (Poulos, Parker, & Le..., 2007), and provides support for the hypothesis that rats that perform more sign-tracking tend to be more impulsive and drink more EtOH (Tomie et al, 2008).

Impulsivity's link to sign tracking was tested by Tomie through the use of a delay-discounting (impulsive choice) test. Impulsivity was tested by using a two-choice lever-press operant procedure. In this procedure, the rat had a choice between two levers that could be pressed. The left lever would be readily available and if pressed would generate an immediate small reward of one pellet, while the right lever would be available less frequently but if pushed would generate a three to five pellet reward. Rats that demonstrated prior predominant sign tracking behavior were more impulsive-like and would respond to both levers, while goal trackers primarily responded on only one lever. Additionally, during sign tracking sessions the adaptive (impulsive) group acquired sign tracking faster, and with more CR than the goal tracking group. Impulsivity was also reported after injections of dopamine agonist-like compounds such as cocaine, amphetamine, and methamphetamine (Tomie, Agarwal, Fohorecky, & Benjamin, 1998).

There have not been too many studies on strain differences in impulsivity, but one study did explore how Lewis (LEW) and Fischer (F344) rat strains differ on a number of physiological characteristics, such as hypothalamic–pituitary–adrenal (HPA) axis activity, as well as on behavioral tasks, including the sign tracking task (Stearns, Gomez-Serrano, Weiss, & Riley, 2006). Since sign tracking has been linked to HPA axis functioning, impulsivity and drug taking, Kearns et al compared LEW and F344 rats on

their rate of attainment and presentation of the sign tracking response. Rats were trained on a *negative automaintenance* procedure. In the negative automaintenance procedure, the rat was first trained on the sign tracking procedure. Later, the sign tracking procedure was changed so that the sign tracking responses directed at the manipulandum (interactive object) were then punished by the cancellation of the food pellet delivery. While sign tracking behaviors were diminished in the negative automaintenance procedure, they were usually not eradicated entirely (Monterosso & Ainslie, 1999). The animals that were affected the least by the "punishment" were considered more impulsive. While there were not significant differences between strains within the negative automaintenance procedure, LEW rats did acquire the sign tracking response faster and performed the sign tracking response at a superior rate to the F344 rats. This is consistent with existing research that indicates that LEW rats behave more impulsively, are more sensitive to the rewarding effects of drugs, and more readily self-administer drugs of abuse than F344 rats. These findings also indicate that the HPA axis may have a modulatory impact on sign tracking behavior.

Measures of Impulsivity

Impulsivity is a multi-dimensional construct, with various impulsivity measures reflecting separate underlying processes. One process is impulsive choice which is measured by the delay discounting procedure that measures impulsive choice and behavior disinhibition as described above (de Wit, 2009). Another process includes impulsive response-inhibition, such as responding on a schedule which measures the inability to withhold a response (e.g. Differential Reinforcement of Low Rates procedure) (de Wit, 2009; Monterosso & Ainslie, 1999). A third behavioral impulsive process is

impulsive action, which is measured in the negative automaintenance procedure also described previously (Killeen, 2003). A different procedure is non-discriminated appetitive conditioning which is measured by conditioned locomotor activity that demonstrates behavior disinhibition (de Wit, 2008; Winstanley et al, 2004). In non-discriminated appetitive conditioning, rats are fed at the same time each day and their locomotor activity is assessed. Typically, an increase in activity is found to be present prior to the expected delivery of food which represents a lack of behavioral inhibition. This increase in activity is due to the association between the specific time of day and the food delivery (Winstanley et al, 2004). In each of these paradigms impulsivity is implicitly or explicitly associated with the effect of delay on the value of reward. (Monterosso & Ainslie, 1999).

In the present experiments we evaluated the negative-feature discrimination procedure as a potential measure of impulsivity. In this procedure a target conditioned stimulus (bottle) is paired with food US as usual, but in the presence of a negative-feature stimulus (a light, smell, or sound) the bottle CS is not followed by the food US. The ability to use the negative-feature to predict that food will not occur is known as *negative-feature discrimination.* This task is used specifically as an impulsivity metric to investigate whether there are differences in the acquisition of negative-feature discrimination between sign tracking rats with different environmental experience. If sign tracking rats in one condition are more impulsive than sign tracking rats in another condition, they may show poorer acquisition of the negative-feature discrimination.

Environmental Influence [illegible] aring Condi[illegible]

Flagel et al (2010) have noted that rats [illegible]ly bred for high respo[illegible]vity to environmental novelty are almost exclusively sig[illegible]rs in appetitive conditioning procedures and rats selectively bred for low resp[illegible]ity to environmental n[illegible]elty are almost [illegible]clusively goal-trackers. When these rats [illegible] used in sign-tracking procedures with a cocaine US, the same results were found. [illegible]-responders t[illegible]ward novelty all acquired predominant sign-tracking CR pert[illegible]hile none of the low r[illegible]sponders did so. Thus, the high responsivity phenotype [illegible]r[illegible]minant sign trac[illegible]g in procedures employing either food US or coc[illegible] while the l[illegible]nsivity phenotype does not exhibit sign-tracking to s[illegible] either food [illegible] or cocaine [illegible].

Since high responsivity toward enviro[illegible]elty is typical behavior of rats raised in enriched housing conditions, the invest[illegible]ion of rearing conditions on sign tracking behavior in the presence of EtOH p[illegible]iting avenue of explora[illegible]. The two main rearing conditions are the star[illegible]g condition and the enriched rearing condition. The idealized standard re[illegible] of rats often consists of the inclusion of two rats in a cage with no other [illegible] at th[illegible] disposal, while the idealized enriched environment rearing cond[illegible] consist o[illegible]e housing of several rats (typically 4 or 5) in larger than average [illegible] that contain various stimulating items such as running wheels, tunnels and s[illegible] may be alte[illegible]d on a regular basis. Studies of enriched environments have [illegible] that the enriched c[illegible]dition brings on various neurobiological and behavior[illegible]ications wh[illegible] may have an impact on drug sensitivity and addiction (L[illegible]an, Macrì, Solinas, & Jab[illegible] 2008).

Findings from studies on environmental enrichment suggest that this condition might act on precise brain regions that handle responses to novelty or conflict (such as the hippocampus, amygdala, and the cingulate). Additionally, environmental stimulation, especially applied throughout adolescent development, adjusts the neurobehavioral systems as is evident in learning, memory and defensive responses (Laviola et al, 2008). The behavioral modifications include, among other things, the decrease of anxiety-like behavior. This adjustment change highlights the continued plasticity of the systems mediating emotion beyond the age of weaning and illustrates the importance of an animal's physical environment (Holmes, le Moal, Vogel, Mirochnik, Leman, & Belzung, 2005). This type of adjustment plasticity might be the reason why environmental interventions protect against the effects of genetic and/or acquired vulnerabilities (Laviola et al, 2008).

Previous drug research with rats has shown that rats reared in an enriched condition are more sensitive to the acute effects of amphetamine (dopamine agonist) than rats reared in an isolated condition (Green et al 2001). Yet, enriched condition rats self-administer less amphetamine than isolated condition rats (Lorenes & Bonaguera, 2008), which contrasts the results of an experiment with voluntary EtOH intake that indicated that enriched animals consumed greater amounts of EtOH than isolated animals within a two bottle (EtOH vs. water) preference task (Cooper, Gibson, & Blanchard, 1982). In an effort to corroborate the different accounts, this study used cocaine to further explore the environmental enrichment behavioral phenotype. In this study, enriched condition and isolated condition rats were studied with a cocaine conditioned place preference (CPP) behavior test while cocaine self-administration was measured. Enriched condition

rats exhibited less cocaine self-administration, despite showing enhanced cocaine CPP. It appears that this is because the enriched condition may exhibit a protective phenotypic plasticity against addiction (Green et al, 2010). However, this effect is paradoxical because enriched rats are more sensitive to the locomotor-activating, dopamine-releasing, and rewarding effects of drugs. Therefore, environmental enrichment seems to diminish addiction liability without decreasing drug sensitivity (Green et al, 2010). Essentially, rats would be expected to show the sensitization of cue signs during use (as measured by sign tracking), without the addictive preference for drug (as measured by self-administration procedures such as the previously mentioned preference task).

Experiment 1

Rearing [illegible]

Sign tracking behavior is believed to [illegible] t in rats with a high propensity of assigning incentive salience to stimuli associated with rewards, as is typically found in the addictive phenotype (Tomie et al, 2008). [illegible] periment sought to assess the effect of rearing condition (enriched vs. stand[illegible]) [illegible] tracking of a bottle filled with EtOH or with water. The observation that a[illegible] [illegible] environment can [illegible] sign tracking performance suggests that rearing conditions [illegible] the addictive phenotype (Lavicia et al, 2008). Since groups that differ in susceptibility to sign tracking also differ in measures in impulsivity (Tomie et al, 2008), [illegible] animals in a negative-feature discrimination task as a potential measure of [illegible] impulsive rats [illegible] expected to show poorer discrimination than [illegible] ve rats because discrimination tasks require that rats learn to inhibit conditioned [illegible] g (i.e., licking the water bottle) on days when the bottle is not followed by [illegible] US.

Moreover, by comparing sign tracking of [illegible] with sign tracking of w[illegible] is possible to determine if the additional cons[illegible] of the addicti[illegible] drug [illegible] while sign tracking further enhances sign tracking beh[illegible] For example, it is possible that rats drinking EtOH will show greater sign track[illegible] than rats drinking water because the EtOH has become rewarding and has motivated [illegible] to consume more Et[illegible] In addition to looking for greater sign tracking in [illegible]-exposed rats, we also took advantage of the negative-feature discrimination [illegible] ure to evaluate the [illegible]warding properties of ethanol. If the rats drinking ethanol find the ethanol to be rewarding then

they should not show discrimination because the s...ru...n in the bottle is motivating their drinking, not just the bottle as a signal for the fo... let ...s. Thus rats sign tracking water should sho w discrimination, but not rats c... ...thanol if the ...tle... is itself rewarding. To evaluate the effectiveness of thi... ...gy, some rats ... ... giv... a highly preferred Polycose solution in the bottle they w... ...cking. It is well known that rats ...nd Polycose highly rewarding. Therefore rats ... n t... ...r a Polycose solution should not show discrimination since drinking from the bott... ...ir... ited by the Polycose and ... just the food pellet US.

...t...

Subjects

The subjects were 17 male Sprague- ... ... from Harlan (I... anapolis) that were born on November 3, 2009 and were p...vi... y u...d in other e...per...ments. All rats within this experiment were previously used in ... ...ditioning and mo...line conditioned place preference experiments. A... ra... ... experienced mor...ine treatment ...the previous experiment, thus it was not ne... ... to counterbalance ... ...n assigned to the present experiment. Eight rats had ... ...sed in enr...hed environments in groups of four since rats were 6 weeks of age. T... ...nriched environments consisted of weekly toy rotation and 15 minute rodent ha... ...g. The other ... ... stan... ... rats were housed in pairs within shoebox cages. These ho... ...; ...ditions ... maintained throughout the experiment except the last 2 ... ..., ... enriched environment rats were transferred in pairs to shoe box cages to fr... ... enrichment cages for other experiments. All rats were maintained on a 12 h... ...ght-dark cycle, with the light

turning on at 8 am. All rats were given water and food *ad libitum*, with one exception. Standard rats experienced a 7 day food deprivation via daily 1 hour food access during the first 7 days of EtOH's introduction into the sign tracking paradigm. One standard rat was dropped from experiment prior to EtOH introduction to reduce running time of experiment. This experiment was approved by Bucknell's Institutional Animal Care and Use Committee. All guidelines for the care and use of animals set by the United States Public Health Service were firmly followed.

Apparatus

Sign Tracking Chambers

Rats were trained in four standard (23 x 21 x 23.5 cm) operant conditioning chambers that were modified to accommodate a 250 ml bottle. The four training chambers were constructed similarly, but there were some differences. All chambers had cue lights and a lever that were located on the same metal wall as the food tray, but they were not used for these experiments. Additionally, there were speakers located between the two pairs of sign tracking cages that provided background white noise for these experiments. All equipment was controlled by software written in MedPC (Med Associates Inc., St. Albans, Vermont).

Chambers 1 and 2 have cue lights for are located on the top left of one of the metal walls 10 cms above the grid floor. The lever is located in the middle of the same metal wall as the cue lights 9.5 cms above the grid floor. The food trays are approximately 4.3 cms x 4.3 cms, and are located in the middle of the same metal wall 2.5 cms above the grid floor. Chambers 3 and 4 have cue lights are located on the top left

of one of the metal walls 8.5 cms above the grid floor, and 2.5 cms above the food tray. The lever is located in the middle of the sand tray as the cue light 7 cms above the grid floor. The food trays are approximately 5 cms x 5 cms, and are located in the left (2.5 cms away from plastic wall) of the same metal wall 1 cm above the grid floor. All four chambers were installed with a retractable bottle mechanism from Med Associates on the plastic wall closest to the food tray. A hole in the plastic wall that received the bottle sipper tube was approximately 2.5 cms from the grid floor. The bottle was retracted between trials. During CS presentation, the bottle was advanced so that the sipper tube was flush with the plastic wall so that the rat could lick the sipper tube but not touch it with its paw. This permitted the monitoring of licking. The rats' approach to the US in the food tray (i.e., head pokes) were recorded with infrared sensors from Med Associates that are attached to the clear sides of the food tray. Lickometers from Med Associates that were connected to the bottle sipper tubes and also to the grid floor were used to monitor licks.

Holding Cages

Each day, prior to testing in the sign tracking procedures the rats were placed in suspended stainless steel mesh cages (20.3 cms x 20.3 cms x 22.9 cms) in the sign tracking room for a waiting period of about 5 min. These cages were also used for acceptance and preference tests by mounting one (acceptance tests) or two (preference tests) bottles to the front of the mesh cages.

Procedure

Rats were weighed daily and tested 5 days per week, Monday to Friday, during the early afternoon. The rats were tested in square Plexiglas boxes. Rats were transported to the testing room and placed in the holding cages for approximately 5 minutes before being transferred to the sign tracking chambers. The body weights in the sign tracking procedures were weighed before and after a session to calculate the rats' intake in grams. The start of a session was signaled by the onset of a white noise. At the end of the session the white noise was turned off and the rats were returned to their home cages.

Phase 1 – Adaptation and magazine training

In order to adapt the rats to the chambers the rats were placed in the chambers for 15 minutes with five 45 mg sucrose pellets (P.J. Noyes Company, Lancaster, PA.) in their food trays. If all of the pellets were not consumed, the rats would be exposed to a day of magazine training. In the magazine training, the rats would be placed in their chambers for 15 minutes with pellets being dispersed after each minute. This magazine training would train the rat to associate the magazine's click with the presentation of food. If the rats were having trouble making the association, the rats would be exposed to another day of magazine training. The rats received 2 days of adaptation training before being introduced to sign track training.

Phase 2 - Induction of sign tracking and goal tracking

All rats were initially exposed to 10 days of sign track training with water in the bottle. During training, the bottle (CS) was presented for 10 seconds followed immediately by the disbursement of a 45 mg sucrose pellet (US). After an intertrial

interval (ITI) of 60 seconds, the CS-US was presented again for a total of 30 trials. Since the standard-housed rats took longer to develop sign tracking behavior, they experienced 10 additional days of sign track training with water (total of 20 days) before being switched to EtOH.

Phase 3 – Introduction of Ethanol

In the next phase water was replaced with 1% EtOH for 4 rats in the enriched condition and 4 rats in the standard condition. The other half of the enriched and standard-housed rats would continue with water to serve as controls. Because it was unclear how sign tracking performance would proceed, and we were interested in getting the EtOH rats to consume as much EtOH as possible, the four most sufficient sign trackers were given EtOH and the remaining rats given water. EtOH started at 1% concentration, and gradually increased to 9% concentration in one to three day increments dependent on rat performance. The enriched rats reached 9% concentration, while the standard rats stopped at 6% concentration. The enriched rats were then reduced to 6% concentration for direct comparisons of 7 days of sign tracking performance. During this phase some additional minor manipulations were introduced as pilot tests of dishabituation (4 days) and spontaneous recovery (4 days). Dishabituation trials consisted of a single presentation of a stimulus change (house light lights off) prior to the 23rd trial of a session. Spontaneous recovery involved testing rats twice in the same day with varying delay intervals between tests. These manipulations did not affect sign tracking performance and will not be reported in this thesis.

Acceptance and Preference Tests

After the completion of Phase 3 all r[illegible]d in one bottle, 20 minute acceptance tests within the holding cages. To [illegible]ts to [illegible]nking in these cages they were given several days to drink a highly pr[illegible] sucrose solution [illegible] 100 ml plastic graduated cylinders (results will not [illegible]) followed by 1 d[illegible] with 3% EtOH solution, 1 day with 6 % EtOH solution, and 1 day with 9% [illegible] solution. The acceptance tests were followed by 4 days of 20 minut[illegible] two-bottle p[illegible]ence tests. The preference test assesses the rats' choice and consum[illegible]n of either a wat[illegible] solution or an EtOH solution. Greater preference for EtOH [illegible] ethanol has gained [illegible] warding val[illegible]. There were 2 days with 3 % EtOH sol[illegible] followed by 2 days w[illegible]h 6 % [illegible] solution. The position of the bottle with EtOH [illegible] was counterbalanced acr[illegible] days.

Phase 4 – Negative-Feature Discrimination

A negative-feature discrimination ta[illegible] was introduced as a potential measure of the differences in impulsivity between the [illegible]t rat conditions and as a second measure of the rewarding properties that may have [illegible]ued to the EtOH. The sign tracking procedure was continued during this ph[illegible], [illegible] two changes made to the procedure. First, pellets were omitted on half of [illegible] days and a cue (the "negative-feature") would be added to signal the abse[illegible] [illegible] pellet US. [illegible], the trials w[illegible] reduced from 30 trials to 15 trials in order to lim[illegible] t[illegible] bility of b[illegible] inc[illegible]. On the days of food omission, an odor stimu[illegible] [illegible] to signal the omission of food. This odor stimulus was a vanilla dryer shee[illegible] [illegible] ed in the t[illegible] below the grid floor. Days with food are designated A+, w[illegible] ithout food are [illegible]signated A-.

The days of food omission were chosen randomly, with no more than two consecutive days with the same condition for a total of 10 days. In addition, the sequence of days was: A+, AB-, A+, AB-, A+, AB-, A+, AB-, A+, AB-. Additionally the bottle liquids were changed to be 1 of 4 possible combinations. The four possible solutions were 5% Polycose solution, 7% EtOH and 5% Polycose mixture, 7% EtOH, or water.

Data Analysis

The primary independent variables in each phase were the housing condition (enriched vs. standard housing condition) and the days of training, with housing condition as a between-subjects factor and days as a within-subjects factor. When EtOH was in the bottles the type of Solution in the bottle (EtOH vs. water) was a within-subjects factor, and the EtOH Concentration (days at ethanol concentration vs. sampling combination of days with water) was a within-subjects factor. During negative-feature discrimination training (Phase 4) an additional independent variable was the pairing in the presence (A+) and absence (AB-) of the negative-feature (within-subjects). The dependent variables were licks and milliliters of solution consumed for measures of sign tracking, while head pokes was the dependent variable for measures of goal tracking. The dependent variables for each phase were analyzed by separate ANOVAs followed by post hoc comparisons using SPSS. The Phase 2 and Phase 3 data were analyzed as Rearing Condition (2), Days (5) mixed factorial ANOVA. In Phase 3 and 4 (when EtOH is introduced) the data were analyzed with a Rearing Condition (2) x Ethanol Concentration (6 or 9) x Solution (2) mixed ANOVA. The negative-feature discrimination were analyzed with a Rearing Condition (2) x Days x Negative-feature (2) ANOVA. Additional ANOVAs were calculated as needed.

Starting with the first 10 days of sign tracking acquisition with water, the enriched rearing condition had begun to show an impact in sign tracking acquisition. As seen in Figure 1, the sign tracking performance as determined by licks (or approaches to CS) show performance differences which began at beginning training. A Rearing Condition (2) x Days (10) mixed factorial ANOVA revealed a significant interaction of Days x Rearing Condition, $F(9,135) = 8.023$, $p < .001$. Thus, these findings suggest that enriched rats acquired and demonstrated more pronounced sign tracking behavior than standard rats.

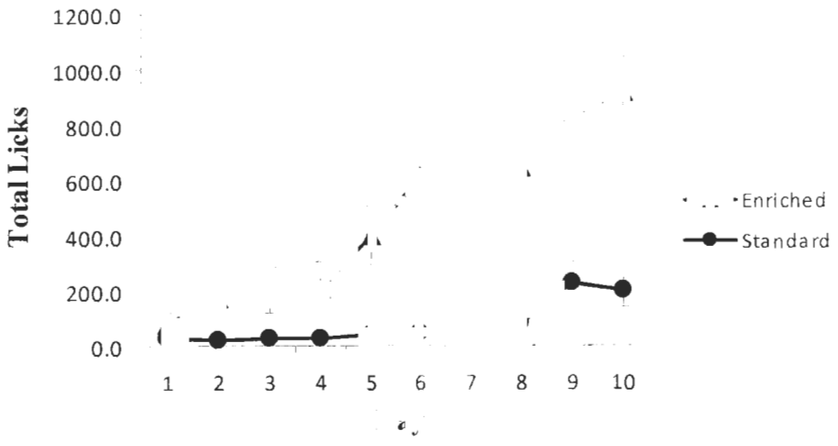
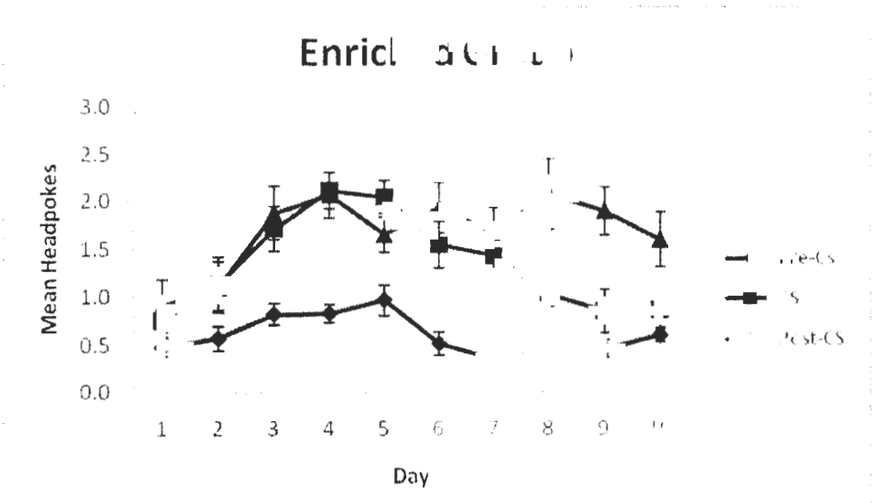


Figure 1. Phase 2- Acquisition of Sign Tracking with Water in the Bottle

Concurrently, with sign tracking acquisition the rats also showed evidence of classical conditioning as demonstrated by head entries into the food tray during the presentation of the bottle CS as shown in Figure 2. This conditioning is seen by comparing head poking 10 seconds prior to the CS (Pre-CS), during the CS, and the 10 second period following the CS (Post-CS). This is that the bottle CS was associated

with the food pellet US is indicated by greater responding during the CS compared to the Pre-CS period. Typically conditioned responding continues into the Post-CS period before declining later in the ITI. A Rearing (2) x Time (3) x Day (10) mixed factorial ANOVA revealed a significant interaction, $F(18,270) = 3.572$, $p < .001$. This interaction was evaluated with t-tests for theoretical interpretation.

T-tests revealed no significant differences between the Pre-CS and CS head pokes on Day 1 with the enriched, $t(7)=-2.317$, $p > .05$ or standard, $t(7)=-1.61$, $p > .05$ rat groups. But, by Day 2 the CS head pokes were significantly greater than Pre-CS head pokes with the enriched, $t(7)=-2.768$, $p < .05$ and standard, $t(7)=-3.022$, $p < .05$ rat groups indicating conditioned head poking. With repeated testing, head poking in the CS remained in the enriched rats, but not the standard rats. By Day 10 the head pokes no longer differed between the Pre-CS and the CS period in enriched rats, $t(7) =-1.231$, $p > .05$ but continued to differ in the standard rats, $t(7)=-4.277$, $p < .05$. The decline in the enriched rats was due to the much greater increase in tracking in the enriched rats compared to the standard rats.



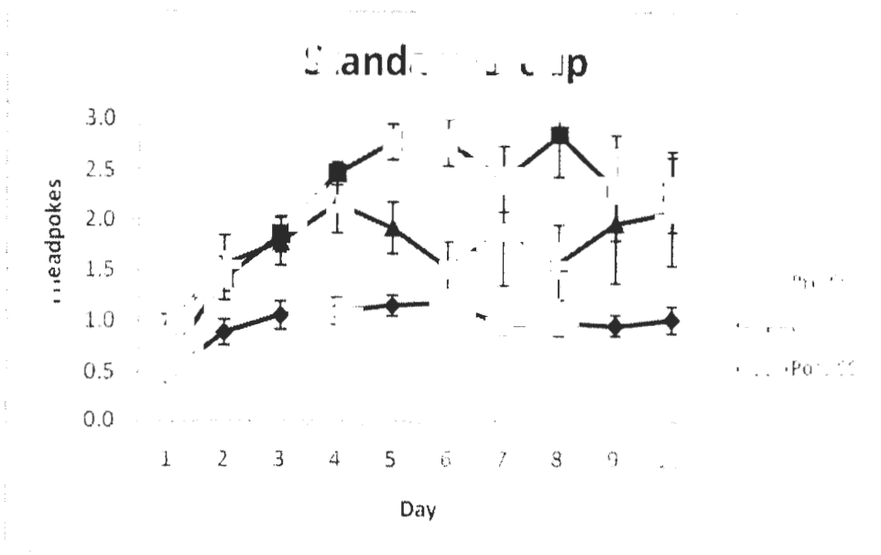


Figure 2. Phase 2 - Acquisition of Sign Tracking

Figure 3 shows the mean consumption of EtOH or water in milliliters for the rats raised in both rearing conditions in Phase 3 when the EtOH rats were receiving gradually increasing concentrations of EtOH. This figure shows EtOH concentrations as blocks, which are composed of the mean intakes on the days of the same EtOH concentration. This concentration is then compared to the same portion of days as the controls that consumed water. The EtOH concentration is confounded with days of training in the sign tracking procedures since with each increasing concentration the rats had more experience in the sign tracking procedure. However, to facilitate the presentation of the data the days were averaged by EtOH concentration because the standard rats received fewer days of training in this phase and the rats received only up to 6% EtOH, only the first 6 concentrations were analyzed in a Rearing Condition (2) x Solution (2) x Concentration (6) mixed factorial ANOVA. The analysis found an interaction of Concentration x Rearing Condition, $F(5, 60) = 2.51$, $p < .05$, and an interaction of

Concentration x Solution, $F(5, 60) = 6.354$, $p < .001$. The difference between the standard rats tracking EtOH and the standard rats tracking EtOH was pronounced early in Phase 3 when EtOH was 1%, $t(6)=2.835$, $p<.05$, but was no longer significant at the end of the phase when the EtOH rats were drinking 8% EtOH, $t(6)=-.012$, $p>.05$. This level of difference was due to the rats sign tracking more (with the rats initially poor sign trackers) increasing their sign-tracking behavior with EtOH drinking. The difference when EtOH was 1%, $t(6) =2.470$, $p<.05$, also tended to differ between the enriched rats tracking EtOH and the enriched rats tracking water, and the two groups no longer differed, $t(6)= -.530$, $p>.05$. This analysis suggests that the observed differences between the EtOH drinking and water drinking groups was due to the strength of sign tracking performance and not influenced by the availability of EtOH. Essentially, consumption rates were higher in the enriched rats than the standard rats and EtOH consumption was higher than water consumption in both conditions. However, this does not indicate that the EtOH sign tracking rats experienced EtOH as rewarding. Therefore, we decided to introduce another manipulation within the discriminated task to look for a hint of reward.

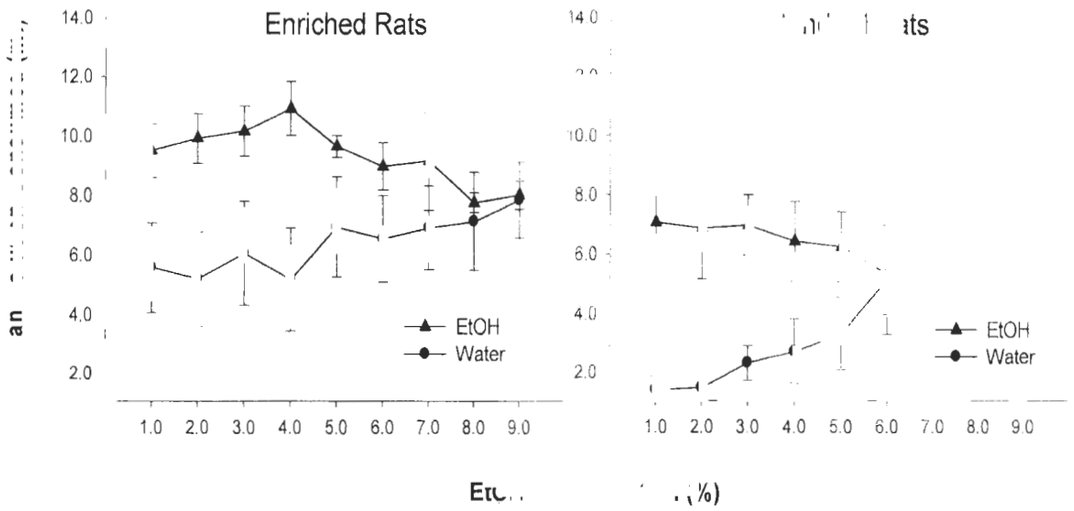


Figure 3. Phase 3 - The Introduction of Ethanol. [illegible] solution [illegible]d by groups sign tracking water or gradually increasing con[illegible]nt [illegible]. Intak[illegible]s were averaged across days with the same EtOH concentra[illegible]ilable for th[illegible]l groups.

Figure 4 shows the mean EtOH consump[illegible] as grams of [illegible]ned per kilogram of body weight. This figure shows only t[illegible] rats that rece[illegible]d [illegible]l during sign track training. For analysis, by controlling for b[illegible]ight and remo[illegible]ng water sign trackers it is possible to see that enriched rats co[illegible]l more EtOH re[illegible]tive to body weight than standard housed rats. A Rearing C[illegible] (2) x Cor[illegible]tration (6) mixed factorial ANOVA supports this finding with a [illegible]ion of Concentration x Rea[illegible]ng Condition, $F(5, 30) = 6.072$, $p < .05$. Additionally, [illegible] no di[illegible]n consumption over the 7% to 9% EtOH concent[illegible]n the enriched rats revealed by a one way repeated measures ANOVA, $F(2, 6) = .253$, $p > .05$. This means that EtOH concentrations of 7- 9% do not appear to fu[illegible] mean consumption of E[illegible]H with enriched rats. These findings suggest th[illegible] enriched environments generate

more pronounced sign tracking behavior which in turn indicates higher EtOH consumption thereby increasing vulnerability to [illegible].

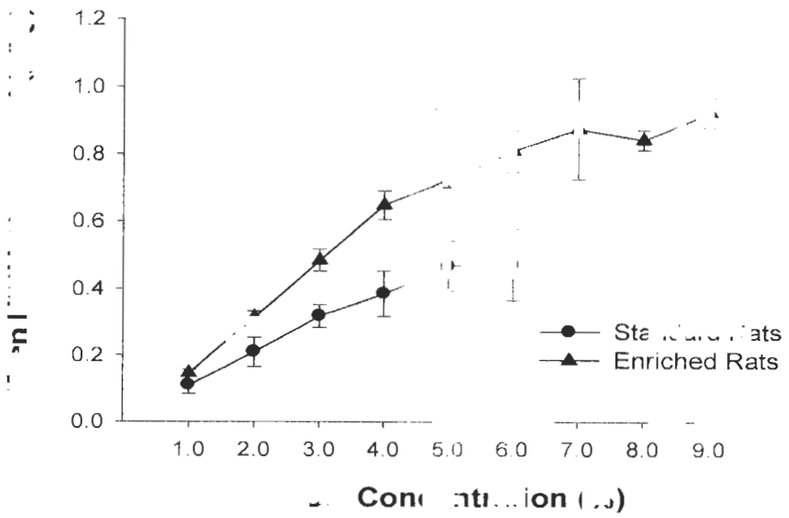


Figure 4. Phase 3 – The Introduction of Ethanol [illegible] intake [illegible] as grams of EtOH consumed per kg of body weight

Enriched rats were found to drink more [illegible] at higher concentrations than standard rats within the sign tracking procedure. However, this finding does not translate to mean that enriched rats are addicted. The data suggests that the sign tracking procedure was generating the drinking behavior and the addition of EtOH did not affect drinking behavior. In order to determine whether or not EtOH had become at all reinforcing to these rats, EtOH was provided [illegible] of the sign tracking procedure.

A one bottle acceptance test was used as a preliminary procedure to determine if the rats voluntarily accept the solution. With this test, the greater intake means the greater acceptance of solution. This experimental acceptance test with 2%, 6%, and 9% EtOH solutions. This procedure was then followed by 3% and 6% EtOH solutions.

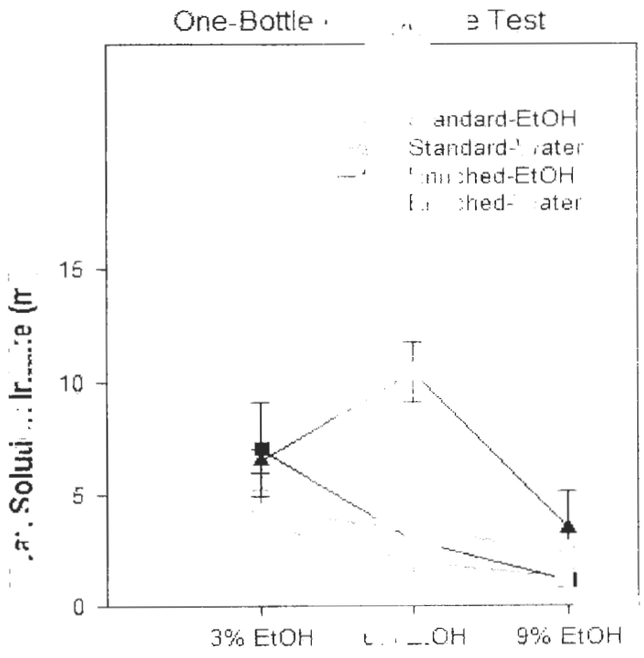
preference tests. The two bottle preference test was used to assess drug seeking behavior which is associated with addiction. Rats that find EtOH to be rewarding will seek the EtOH and drink more of it over water. Figure 5 shows the results of the acceptance (top) and preference tests (bottom) that occurred at the end of Phase 3. A mixed factorial ANOVA of Rearing Condition (2) x Drug Solution (2) x Concentration (3) revealed an interaction of Rearing Condition x Solution within the acceptance tests, $F(2, 24) = 4.597$, $p < .05$. This interaction was due to the enriched rats that sign tracked EtOH showing a greater preference for 6% than the other groups, but no group differences at other concentrations. Additionally, there was an effect of concentration, $F(2, 24) = 10.818$, $p < .001$. This supports the overall decreasing trend seen in Figure 5 of decreasing consumption within the higher concentrations.

The preference tests were calculated as percent EtOH consumed using the formula:

$$\frac{\text{mls of EtOH}}{(\text{mls of EtOH} + \text{mls of water})} \times 100$$

A score of 50% indicates no preference for either, a score above 50% indicates a preference for EtOH, and a score less than 50% indicates a preference for water. The graphs (see Figure 5) show that the groups generally demonstrated no preference for EtOH. Although the figure suggests a preference for 3% EtOH in the standard rats, a mixed factorial ANOVA of Rearing Condition (2) x Ethanol Concentration (2) calculated

on the preference data revealed a non-signifi... ...ction of Co... ...tration x Rearing ...ndition, $F(1,12) = 1.76$, $p > .05$. All other int... ... a main effe... were also not significant. The results from the acceptance t... ... that d... cite the considerable consumption of EtOH during the sign tracking ... ... the EtOH did not become sufficiently rewarding to establish a preference f... ... The fact ...at the ...riched rats drinking EtO... while sign tracking drank mo... ...% st... ...n the other grou... during the acceptance test may reflect some hab... ...n to the a... ... taste quality of EtOH, si... these animals consumed the mo... ...ing P... 3 of sign tracking (see Figure 3).



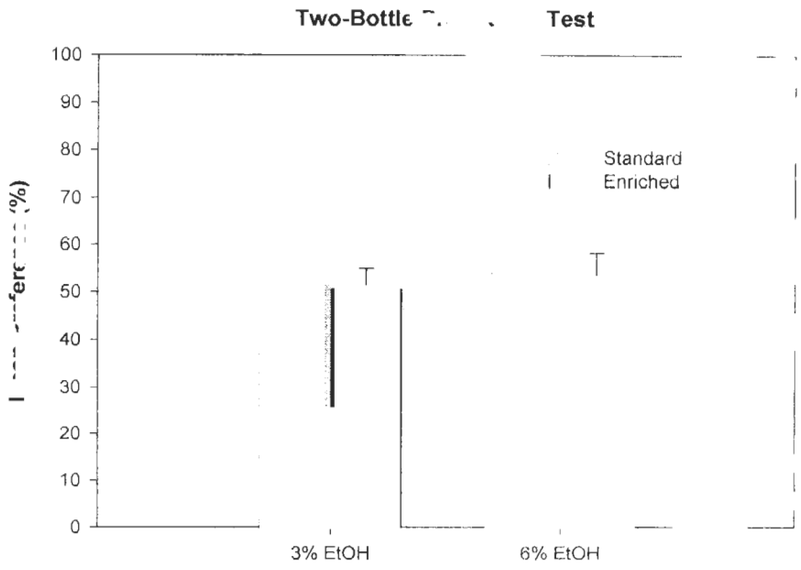


Figure 5. One-bottle acceptance tests (top graph) and two-bottle preference tests (bottom graph) following the Phase 3.

Thus, the accumulated evidence suggests that because the enriched rats are better sign trackers, they consume more EtOH. However, because the enriched rats showed no preference for EtOH compared to the standard rats, there is no evidence that enriched rats are addicted to EtOH. While this data does not provide a complete model of addiction, the data does suggest a lack of drug seeking behavior within the two bottle preference task.

Because the enriched rats consume more EtOH and water than standard rats within the sign tracking procedures, it is possible that the enriched rats are engaging in more impulsive responding toward the bottle. For this reason, a negative-feature discrimination procedure was introduced as a potential impulsivity measure. Within the negative-feature discrimination, impulsive behavior might be demonstrated in two potential ways. First, as in the previous test, responding might be greater in the enriched than

controls on A+ days in which the negative-feature (the vanilla odor) is not present and they receive a US, would suggest an impulsive [illegible]. Second, impulsivity might also be demonstrated by slower acquisition of [illegible] learning. That is, impulsive rats should have greater difficulty learning to [illegible] responding during non-reinforcement (A-).

It is possible to use the negative-feature discrimination task to further evaluate the rewarding quality of EtOH. It may be that the two-bottle test was not sufficiently sensitive to detect the rewarding properties of EtOH. If the EtOH became rewarding to the rats sign tracking EtOH they should also show poor discrimination learning because the solution in the bottle is motivating their behavior just the bottle as a signal for the food pellet US. Thus rats sign tracking water should show discrimination since water is not reinforcing to non-thirsty rats, but sign tracking EtOH should not show discrimination if the EtOH is itself rewarding. To evaluate [illegible] of this strategy, Polycose was added to the bottles of half the rats sign tracking water (Polycose) and half of the rats sign tracking 7% EtOH (EtOH-Polycose), the remaining half of the original group continued to receive water or EtOH. Therefore, rats sign tracking a Polycose solution should not show discrimination since drinking from the bottle is motivated by the Polycose and not just the food pellet US.

Based on an initial analysis, rats exposed to EtOH and water solutions responded similarly within negative-feature discrimination tests. There was no apparent EtOH effect or interaction of Polycose and EtOH. This analysis is limited to the low numbers of rats per condition (N=4). Thus, rats were divided into two groups for further analysis. The rats sign tracking EtOH or water were combined to form the Group

Non-Polycose and the rats exposed to the Polycose solutions were combined to form the Group Polycose. The negative-feature discrimination was analyzed as a mixed factor ANOVA of Group (Polycose / Non-Polycose) x Licking Condition (quinine-adulterated vs. standard) x Discriminative Stimulus (SD) (A+, A+/X-) x Days (5). Figure 6 depicts the responding (sign tracking licks) within the negative-feature discrimination tests. The ANOVA showed an interaction of Days x SD x Licking Condition, $F(4, 48) = 3.417$, $p < .05$. The enriched and standard groups sign tracking licks did not show an effect of discrimination, $F(1, 6) = 66.887$, $p>.05$, which supports the argument that when a rewarding solution is in the bottles the rats will not show discrimination. It does not matter that the Polycose group is not getting a sugar pellet on A/X- trials, they drink because they like what is in the bottle. Additionally, the Non-Polycose rats did show an effect of discrimination, $F(1, 6) = 37.434$, $p<.05$, and they learned it very quickly. This means 2 things. First, the Non-Polycose group does not find quinine or water rewarding, which confirms the preference tests with regards to quinine. Second, enriched rats are not impulsive. Even though they are sign tracking at very high levels the discrimination task suggests that the enriched rats are not impulsive. Additionally, the enriched rats' discrimination was better with the Non-Polycose solution than the standard rats, suggesting that they may be less impulsive than the standard rats.

Rats in the Polycose groups showed the highest overall responding, with greatest responding demonstrated on the days in which the quinine preceded the sugar pellet. Additionally, standard Polycose drinking rats showed the highest responses with enriched EtOH responders generating higher responses than standard EtOH responders. The negative-feature discrimination findings support two interpretations. First,

discrimination was learned by the standard and [illegible] ts in the Non-Polycose group. Second, the findings support the rewarding properties of Polycose. Third, there was higher overall responding and better discrimination in the enriched rats in the negative-feature discrimination tests.

To sum up these findings from Experiment 1, enriched rats showed greater acquisition of sign tracking and thus consumed more ethanol than standard rats. Nevertheless, the consumption of EtOH during conditioning did not establish a preference for EtOH in either housing group. The negative-feature discrimination tests revealed that the enriched rats were not impulsive as they readily reduced responding when the sugar pellet reward was not presented on EtOH- trials. Food discrimination performance also confirmed that the EtOH additive was not reinforcing during sign tracking, since the Polycose conditions demonstrated that when a rewarding solution is in the bottle, rats do not display discrimination deficits, although the enriched rats were more vulnerable to the effects of EtOH than standard rats because they were sign tracking more, increased impulsivity as measured by the discrimination task and an "addiction to alcohol" does not adequately explain drinking behavior.

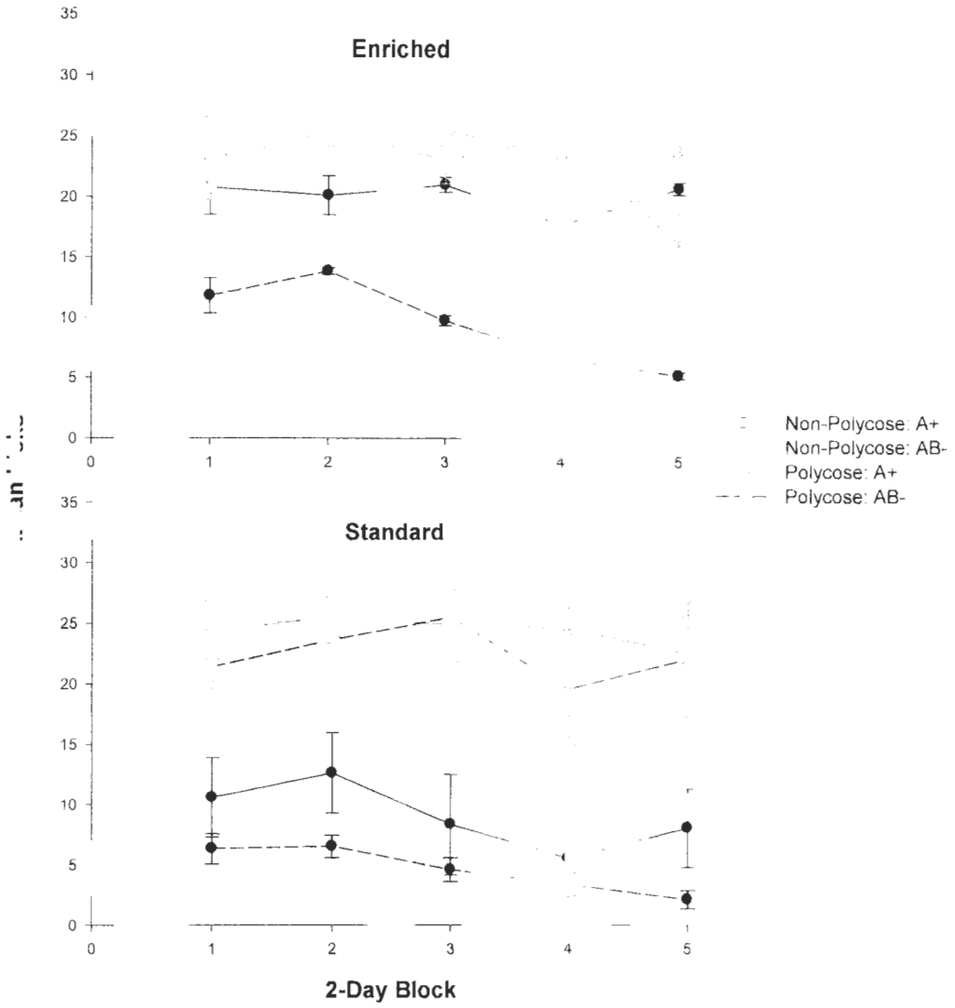


Figure 6. Phase 4 – Negative-feature [illegible] task. A+ denotes the trials in which the bottle is followed by the sugar pellet. AB- denotes the trials in which the bottle is not followed by the sugar pellet.

Exp[illegible]

Environmental Activati[illegible] [illegible]ne System

In the past two decades researchers [illegible] fields of study have discovered that the nervous system and the immune system [illegible] intimately in response to foreign substances entering the body including viruses, bacteria, and drugs of abuse. This discovery has led to a new interdisciplinary field called *Neuroimmune Pharmacology* (Ikuzu & Gandelman, 2008) Thus the neuro-immune response to drugs of abuse may share characteristics similar to the neuro-immune response to bacterial infection, suggesting that immune system activation by one foreign invader (e.g., bacteria) may affect the subsequent neuro-immune response to another foreign invader (e.g., alcohol). Since the nervous system is involved, some of these immune alterations may be behavioral. One way to investigate activation of the immune system is to expose subjects to lipopolysaccharides (LPS) rather than to actual bacteria.

LPS are large molecules consisting of a lipid called endotoxin and a polysaccharide that are found in the outer membrane of gram-negative bacteria (Raetz & Whitfield, 2002). LPS serves as a physical barrier that provides bacteria protection from its surroundings and is recognized by the immune system as a mechanism for the detection of bacterial pathogen invasion. LPS is responsible for the development of inflammatory responses and in extreme cases, endotoxic shock (Rosadini & Kushner, 2006). In addition, LPS act as endotoxins that elicit strong immune reactions in [illegible].

LPS stimulates production of inflammatory cytokines in the brain and blood serum. Cytokines are small proteins, peptides, or glycoproteins that are secreted by cells

of the immune system that are used extensively in cellular communication including tumor necrosis alpha (tnf-α), interleukin-1beta (IL-1ß), and interleukin (IL-6) (Staples, Malellari & Chang, 2008).

LPS have been found to cause acute sickness in rats with such features as hyperthermia, reduced food intake, or inactivity. Exposure to LPS may have a long-term impact on the nervous system which may generate nervous system pathology and behavioral changes and in turn produce enhanced susceptibility to drugs of abuse. Rodent models could accommodate a better understanding of the immune-nervous system interactions. In Blednov et al's (2011) LPS study mice they found that exposure to LPS caused alcohol-preferring mice to drink more alcohol as long as 3 months after a single injection. Experiment 2 examines if the effect of LPS on subsequent alcohol intake is also observed in rats that were not selectively bred to prefer alcohol. However, there are several substantial differences between the Blednov et al study and the present experiment. Whereas Blednov measured the preference for 10% EtOH in 24-hour two-bottle tests, in the sign tracking procedure the rats are restricted to small volumes of EtOH in brief daily sessions. Tomie et al (2004) and the results of Experiment 1 show that although rats will consume EtOH while sign tracking they do not develop a preference for alcohol as measured by separate two-bottle tests, although the sign tracking procedure induces alcohol consumption, the short daily exposure to EtOH is not sufficient to induce a preference for alcohol over water. Therefore, in Experiment 2 EtOH was introduced in the home cage to provide 24 hour access. This addition to the experimental procedure permitted an evaluation of the effects of LPS on compulsive ethanol consumption in the sign tracking procedure and 24-hour two bottle preference

tests in the home cage, and in short-term two-[illegible]ence tests in a test cage. The rat strain was changed to Long Evans rats because they are suggested to be better sign trackers and are the exclusive strain used in Flagel's studies (Flagel, 2008).

Method

Subjects

The subjects were 24 male, 40 day old, Long Evans rats from Harlan (Indianapolis) raised in pairs within shoebox cages. The rats were given food and water ad libitum. These rats were maintained on a 12 hour light-dark cycle, with the light turning on at 8 am. This experiment was approved by Boston Hall's Institutional Animal Care and Use Committee. All guidelines for the ethical use of rats set by the United States Public Health Service were firmly followed.

Apparatus

This experiment used the same apparatus as Experiment 1, with the following modification. For the negative-feature discrimination task, a buzzer sound was used as the signal for non-reward instead of the vanilla odor that was used in Experiment 1. A Piezo-buzzer (RadioShack 273-0066) was mounted on the top of the ceiling of all four chambers.

Procedure

LiCl Treatment

At the age of 55 days, 12 rats were injected intraperitoneally with 1 ml/mg/kg of LiCl (from Salmonella enterica, Cat#L6511, Sigma, St. Louis, MO) dissolved in saline,

while the other 12 rats were injected with the equ[illegible] amount of saline. Injections were aligned with rat pairing (each cage-mate [illegible] the same injection treatment) to minimize confounds, and for ease of measurem[illegible] rats were given 1 week of recovery time prior to the progression of ac[illegible]. Additionally, rat body weights were recorded from 1 day prior to injec[illegible] and the following 15 days.

Phase 1 – Adaptation and magazine training

This procedure was the same as Exper[illegible] within this [illegible] experienced 2 days of adaptation before continu[illegible] Phase 2.

Phase 2 - Induction of sign tracking and goal trac[illegible]

This procedure was the same as Experiment 1 [illegible] experienced 9 days of sign track training prior to Phase 3.

Phase 3 – Introduction of Ethanol

Water was replaced with EtOH for 7 rats in the [illegible] injected condition and 7 rats in the saline injected condition. The other 10 sign [illegible] rats continued with water to serve as controls. With the exception of two pairs (one saline-treated pair and one saline-treated pair), the rats were housed with a partner that drank the same solution within the sign tracking chamber. EtOH started at 1% [illegible] and worked up to 10% concentration in one to three days increments [illegible] on rat performance. This procedure continued for 58 days. Concurrently, beginning on day 28 and continuing for the duration of the sign tracking of EtOH procedure, a second bottle which contained EtOH was introduced into the rats' home cage which followed a similar concentration

progression as the sign track training. The EtOH and water bottle positions were alternated daily. This was followed by 5 days of 20 minute preference tests with the holding cages. There were 3 days with 6% EtOH solution followed by 2 days with 9% ethanol solution with the left/right position of the bottles counterbalanced across days. The same bottles as Experiment 1 were used. Thus, the procedural sequence for this phase was: 30 days of sign tracking with ethanol, 28 days with ethanol in the testing chamber and home cage, 3 days at 6% EtOH solution preference tests, and 2 days at 9% ethanol preference tests.

Phase 4 – Negative-Feature Discrimination and Extinction Training

There were several changes made to the negative-feature discrimination procedure that was used in Experiment 1, for the purpose of exploring alternative methods of administration. In the previous experiment, the reinforced (A+) and the non-reinforced (AB-) trials were given on alternating days with the same trial type within a day. In the present experiment, the two types of discrimination trials were given in the same day. The A+ and AB- trials would occur in 5-trial blocks, with the AB- block starting a session on a random half of the negative-feature training days. This negative-feature discrimination task was run for 9 days with 10% ethanol solution in the bottles of the EtOH groups and water in the other groups. The negative-feature training was followed by 4 days of extinction training in which the CS would appear each trial within its typical schedule, but without the pairing with sucrose pellet. Home cage EtOH bottle remained available for only the first 5 days of negative-feature training. Thus, the negative-feature discrimination procedure sequence was 5 days of training with

concurrent home cage EtOH followed by 4 days of training without home cage EtOH and then 4 days of extinction training.

Data Analysis

The primary independent variables in this phase were the LPS treatment (LPS or saline) and days of training. The dependent variables were licks and milliliters of solution consumed for measures of sign tracking, while the dependent variables were head pokes for measures of goal tracking. The dependent variables for each phase were analyzed by separate ANOVAs followed by post hoc comparisons. The Phase 2 and 3 had 2 dependent variables were analyzed with an immune system Condition (2) x Days or Concentration (10) x Solution (2) mixed factor ANOVA. Immune system condition and Solution are between subject variables and Days (or concentrations (i.e., days at Ethanol concentration vs. same combination of days at water)) are within subject variables. Additional ANOVAs as described in Experiment 1 will be conducted, except that LPS Treatment replaces rearing condition as the third independent variable.

Results and Discussion

Body weights of LPS treated and saline treated rats were recorded from one day prior to injection, to 2 weeks after injection to assess the effects of LPS on subsequent body weight change. A mixed factorial ANOVA of Injection Condition (2) x Days (2) revealed only an effect of days on the body weight from the day prior and the day of injections, $F(1, 22) = 6.822$, $p < .05$. However a mixed factorial ANOVA of Injection Condition (2) x Days after injection (11) revealed an interaction between Days x Injection Condition, $F(10, 220) = 3.137$, $p < .001$. As evident in Figure 7, the LPS

injection resulted in lower mean body weight change that continued to show with the weight changes being approximately the opposite of the two weeks. Thus LPS was effective in inducing weight change, and a physical acute illness as a result of immune system activation.

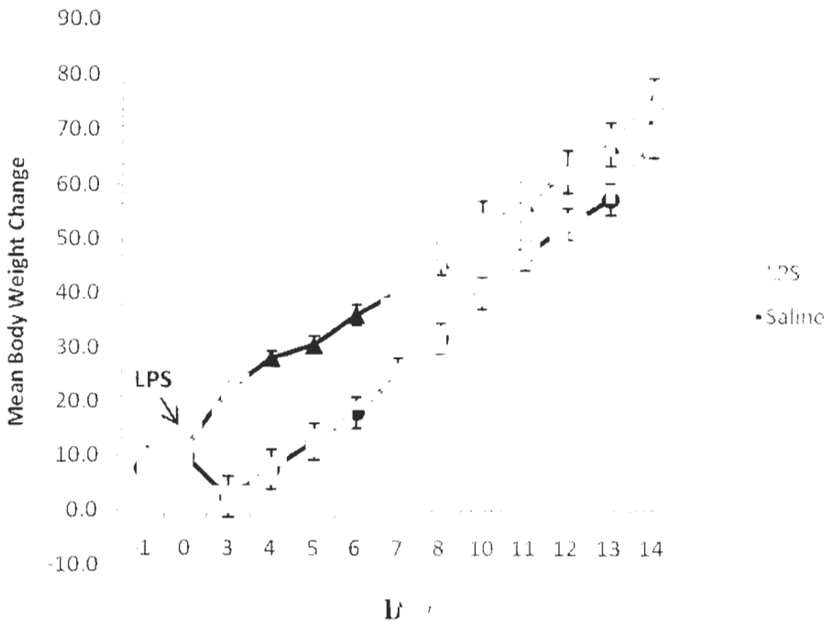


Figure 7. Mean body weight change following treatment with LPS or Saline (Day 0). Body weight was not recorded on Days 1, 2, and 9.

Classical conditioning was demonstrated by head pokes to the food tray during the presentation of sucrose pellets, as seen in Figure 8. As a reminder, this conditioning is seen by comparing the time point of 10 seconds head poking prior to CS (Pre-CS), to the head poking during CS, and the 10 seconds of head poking following the CS (Post-CS). An Injection Condition (2) x time Period (3) x Days (9) mixed factorial ANOVA revealed a significant interaction between Injection Condition, $F(16, 352) = 2.$, $p < .05$. Additionally, there was an effect of Period [$F(2, 44) = 13.771$, $p < .001$], a non-

significant three-way interaction [F (16,352) = 1 . . p .05], and non significant main effect of injection condition [F (2, 44) =.18 . p> . . . results indicate that classical conditioning does not appear to be affected by . . ons.

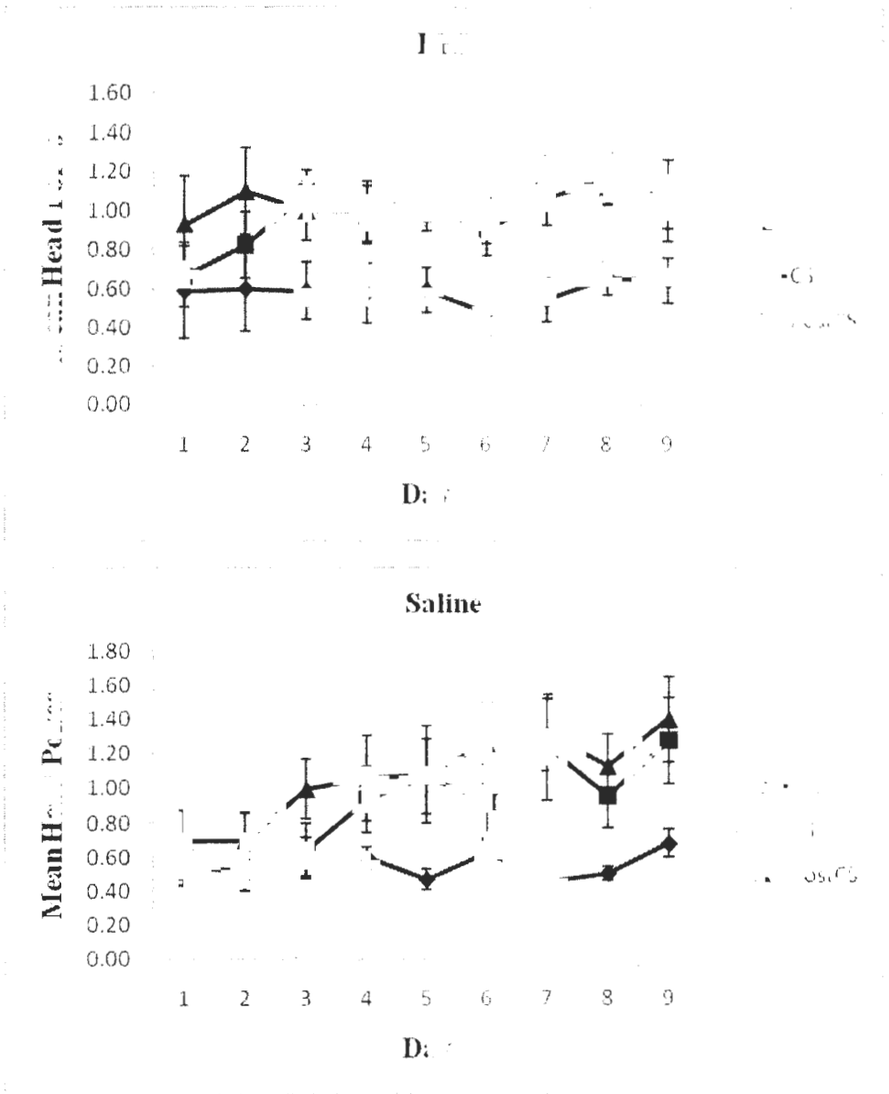


Figure 8. Phase 2 - Acqu . . ad po . ng

Sign tracking acquisition is shown in . . here were no significant differ . ces between sign tracking acquisitio . pe . . with w . . tween the

Injection Conditions, $F(8, 160) = 1.079, p > .05$. [illegible] only an [illegible] of sign tracking performance over Days, $F(8,160) = 3.[illegible] < .05$, confirming the acquisition of sign tracking in both groups.

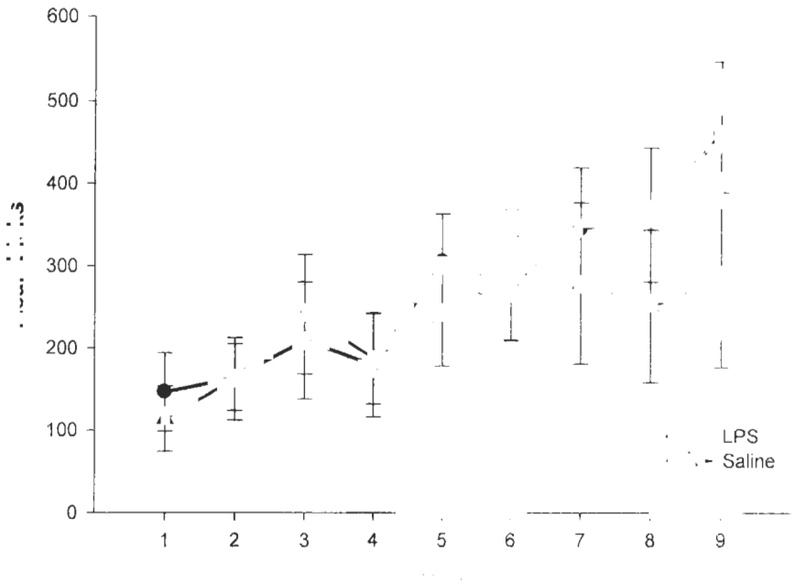
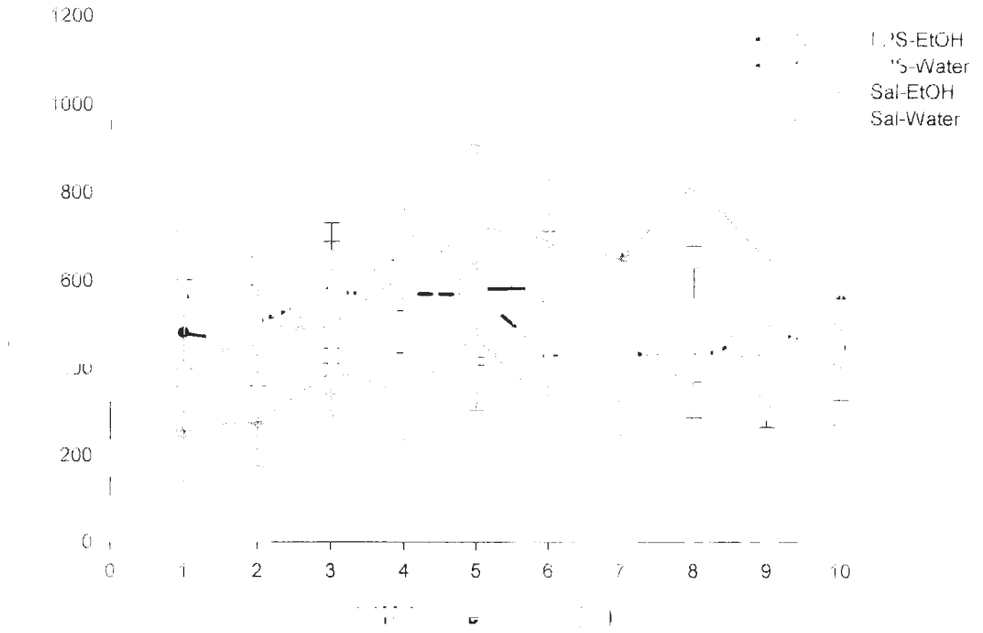


Figure 9. Phase 2 - Acquisition of [illegible] with Within the Bottle

An Injection Condition (2) x Solution (2) [illegible] centration blocks (10) mixed factorial ANOVA on the sign tracking data during the induction of [illegible] (Phase 3) revealed significant interactions of EtOH concentration by Injection Condition, $F(9, 180) = 2.758, p < .05$, and of Solution x EtOH Concentration, $F(9,180) = 2.064, p < .05$. Yet, the expected three way interaction failed to be significant, $F(9,180) = 1.267, p > .05$. The interpretation of these data is complicated with [illegible] "improvement" in sign tracking performance. Figure 10 shows the injection condition by EtOH concentration interaction, with the 4 groups on separate plots. EtOH concentrations are [illegible] the days

that the rats received a given EtOH concentration (1%-10%). These concentrations were compared to the same combination of days as the rats that consumed water. Inspection of the graph suggests that EtOH may have increased tracking in the saline-treated rats, but not the LiCl-treated rats. Note that although the LiCl-treated rats showed similar lick rates at all concentrations, the mean lick rate of the saline-treated rats was increasing in the beginning of Phase 3 when the EtOH concentrations were low which most likely reflects increased sign tracking with practice.

Although Tomie (2008) found that the addition of EtOH to the bottle can increase sign tracking in Long Evans rats, it seems unlikely to explain the apparent difference between the EtOH and water drinking saline-treated rats because the difference was observed at the very beginning of this phase, when the EtOH concentrations were very low and unlikely to produce significant pharmacological effects.



[illegible] The interaction of [illegible] sign tracking [illegible] increasing concentration [illegible] was [illegible] concentration available [illegible] groups.

[illegible] further evaluate the data from [illegible] (2) x Concentration blocks (10) [illegible] ANOVAs were conducted [illegible] and Sal- [illegible] groups. For the LPS-treated groups there [illegible] no [illegible] concentration, $F(9, 90) = .70$, $p > .05$, or solution consumed, $F(1, 10) = .016$, $p > .05$, and [illegible] no [illegible] factors, $F(9, 90) = .74$, $p > .05$. The [illegible] that the [illegible] performance of the LPS-[illegible] and gradual introduction [illegible] EtOH [illegible] saline-treated groups the [illegible] more complex. A [illegible] main effect of Concentration blocks, $F(9, 90) = 4.71$, $p < .001$, and [illegible] significant [illegible] solution interaction, $F(9, 90) = 1.645$, $p > .05$, revealed that [illegible] tracking performance of saline-treated rats [illegible] significantly in the phase [illegible]

ou[illegible] consumed. Moreover, the m[illegible]on fa[illegible] to be sig[illegible]
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adc[illegible] bottle significantly in[illegible] of [illegible] may be that th[illegible] is a sub[illegible] but it [illegible] not [illegible]
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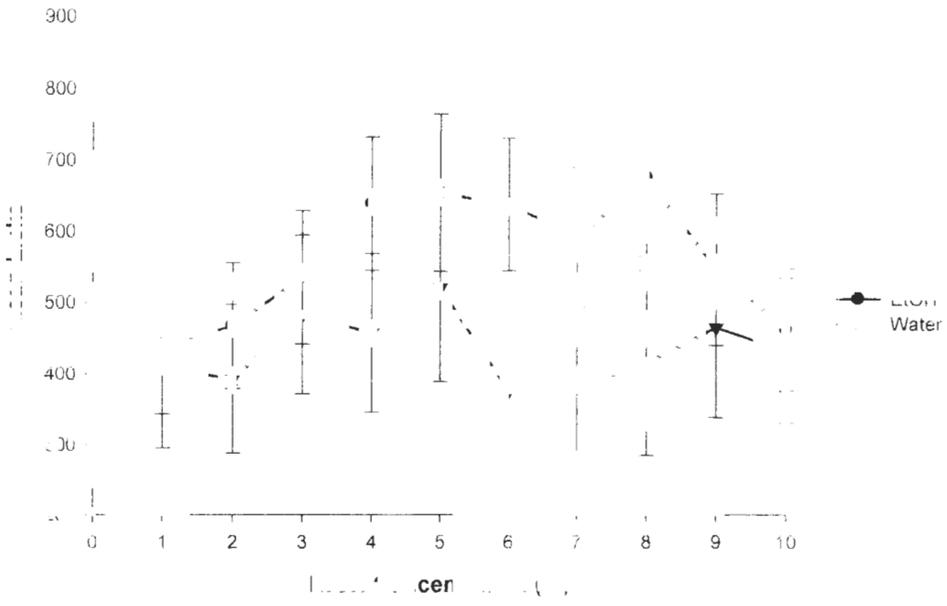


Figure 11. Ethanol concentration [illegible] injection in Phase 3

To further explore the differences in [illegible] Saline and ethanol, both injection conditions were [illegible] milliliters consumed in grams (mean ± SE) per kilogram of body weight (g/kg) [illegible] Figure 12. [illegible] and ethanol drinkers were compared [illegible] significant differences in consumption [F (9, 108) = .677, p >.05] as to [illegible] factor [illegible] of injection condition (2) by concentration blocks (10), [illegible] consumption [illegible] ethanol drinkers [illegible], F (9,108) = [illegible], p < .001.

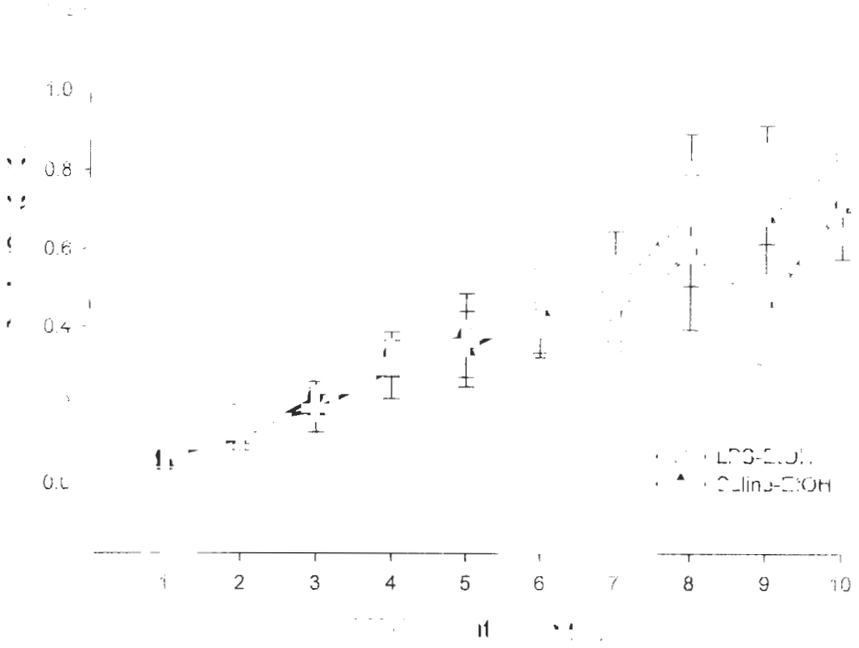
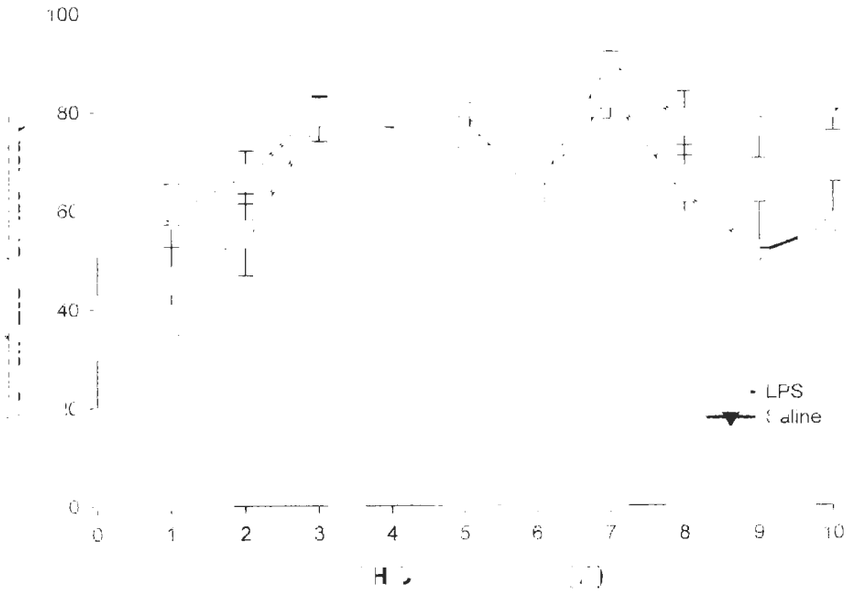


Figure 12. [illegible] 3 – The Introduction of [illegible] of Ethanol consumed p[illegible]nt.

[illegible] to further explore the relationship [illegible] (Li injection) [illegible] Et[illegible] consumption [illegible] their home cages. The [illegible] it was [illegible] how much each individual rat consumed. [illegible] housed with rats that had the same [illegible] the solution while sign tracking. The 2 pairs that drank differ[illegible] were not included in this analysis. Additionally, 2 rats had to be [illegible] plotted based on the averages of the 3-trial [illegible] one or two [illegible] not ideal, but it is still [illegible] and we also did not [illegible] the individual rats to confirm [illegible] Et[illegible] scale [illegible] the mean preference for each concentration than days [illegible]

...ed as percent EtOH co... ...formula [(... fraction / ...ters) x 100]. A mi... ...of ... (2) x ...on (10) was perform... on the pr... ...Th... ...significance, $F(9, 90) = ...$, ...using ... for ... with ... ...ation. Inspect... ...13 ...ows that ... ...urly dev... ...greater ... (at ... ...of ... ...) ... ...-treated an...als. W... ...t-t... ...one of the ... ...(16%) at the end of th... ...



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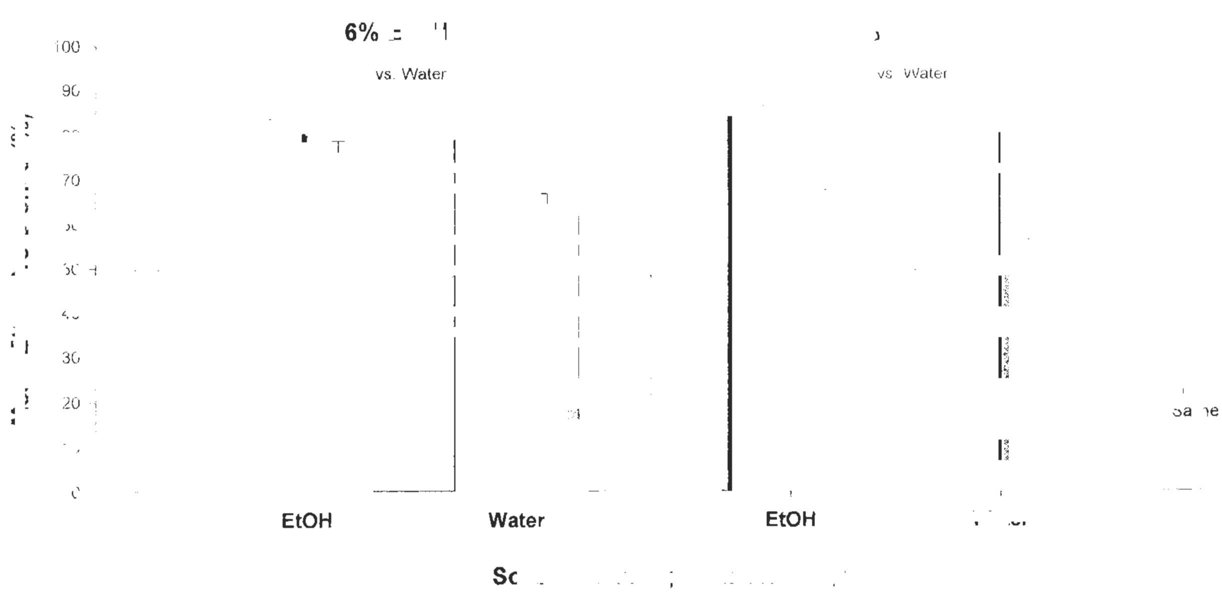

...ure 14. Tw... bottle preference ..., the ... hase 3

[illegible] evidence suggests that [illegible] not [illegible] [illegible] of [illegible] during sign tr[illegible] [illegible]d a [illegible] prefe[illegible] [illegible]ced by daily home-cage [illegible]

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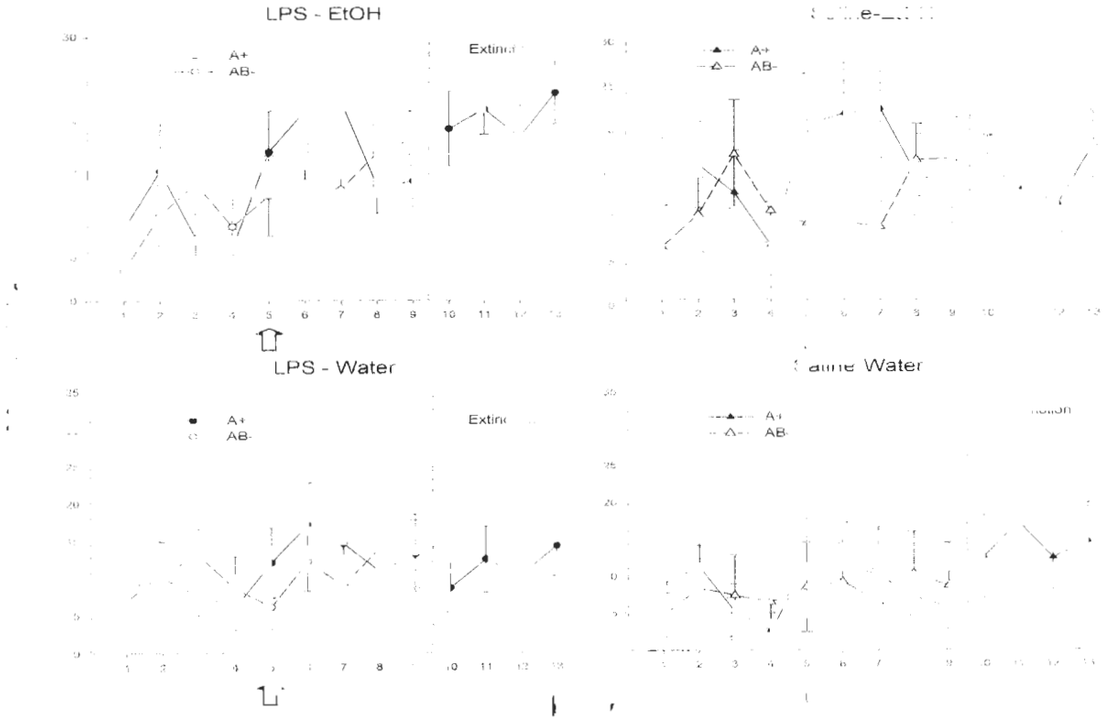
Th[illegible] [illegible]rt the effect of EtOH on [illegible] [illegible]able within the rats' home ca[illegible] [illegible]ve-[illegible] tra[illegible] [illegible]ed for the followi[illegible] [illegible] task [illegible] with a 10% [illegible]OH [illegible] the neg[illegible] [illegible] followed by 4 days of [illegible] in [illegible] its ty[illegible] schedule, [illegible]

[illegible] discr[illegible]minat[illegible] [illegible] on [illegible] ssive inhi[illegible]ion [illegible] [illegible]ot [illegible]cur in the presence [illegible]-f[illegible] ([illegible]), but [illegible]uld

[illegible] A+ trials. Rats that [illegible] ng A+ trials and th[illegible] were analyzed with a mixed fac[illegible] (2) x (2) by Discrimina[illegible] Stimulus [illegible] (2) x [illegible] (9). There was an in[illegible] $F(1,160) = 2.$[illegible], $p < .05$, and [illegible] $F(1, 20) = 4.$[illegible], $p <$[illegible] However [illegible] were not significant. [illegible] be [illegible] Figure 15.

Examination of Figure 15 reveals th[illegible] ng [illegible] not [illegible] asymptotically overtaken in any of the 4 g[illegible] This finding [illegible] discrimination learning in [illegible] that [illegible] stimulus [illegible] with the bu[illegible] and/or [illegible] function that prevented the rats [illegible] task. We [illegible] this [illegible] with the habituating data. [illegible] a significant SD x Day [illegible] difference between [illegible] and [illegible] on Day 5 and the [illegible] was withdrawn and the differe[illegible] re[illegible] to [illegible] to [illegible]. Also, this diff[illegible] in the animals' [illegible] indicated by the SD [illegible] difference betw[illegible] persist, and [illegible] not [illegible] 2 days [illegible] long-Evans rats sho[illegible] Sprague-Dawley rats in [illegible] with the long-Evans [illegible] have than the enriched [illegible] rats.

To analyze the extinction data, the responses of [illegible] on the 4 days of extinction [illegible] being [illegible]d by the [illegible]. Analysis of this [illegible] Treatment (Session (2) x [illegible] (2) x Day (5) [illegible] yielded a [illegible] Day interaction, $F(4, 80) = 3.316$, $p < .05$, [illegible] Further tests revealed that on Days 3 and 4 of extinction, [illegible] was significantly greater than [illegible] $p < .05$). Since the main effect of [illegible] $F(1, 20) = 0.262$, $p > .05$), the difference was due to group [illegible] responding. Thus, there was no evidence of [illegible]



[illegible] 15. [illegible]ise 4- [illegible]egative-fea[illegible] [illegible]scri[illegible] A+ [illegible]s the tri[illegible]s in [illegible]ich [illegible] [illegible]s fol[illegible]/ed by the sug[illegible]p [illegible]et. [illegible] - [illegible] tri[illegible]s in wh[illegible] the bot[illegible] [illegible] [illegible]s [illegible]d by the sug[illegible] pellet. On Day 5, [illegible] [illegible]d fr[illegible]m [illegible]s hor[illegible]

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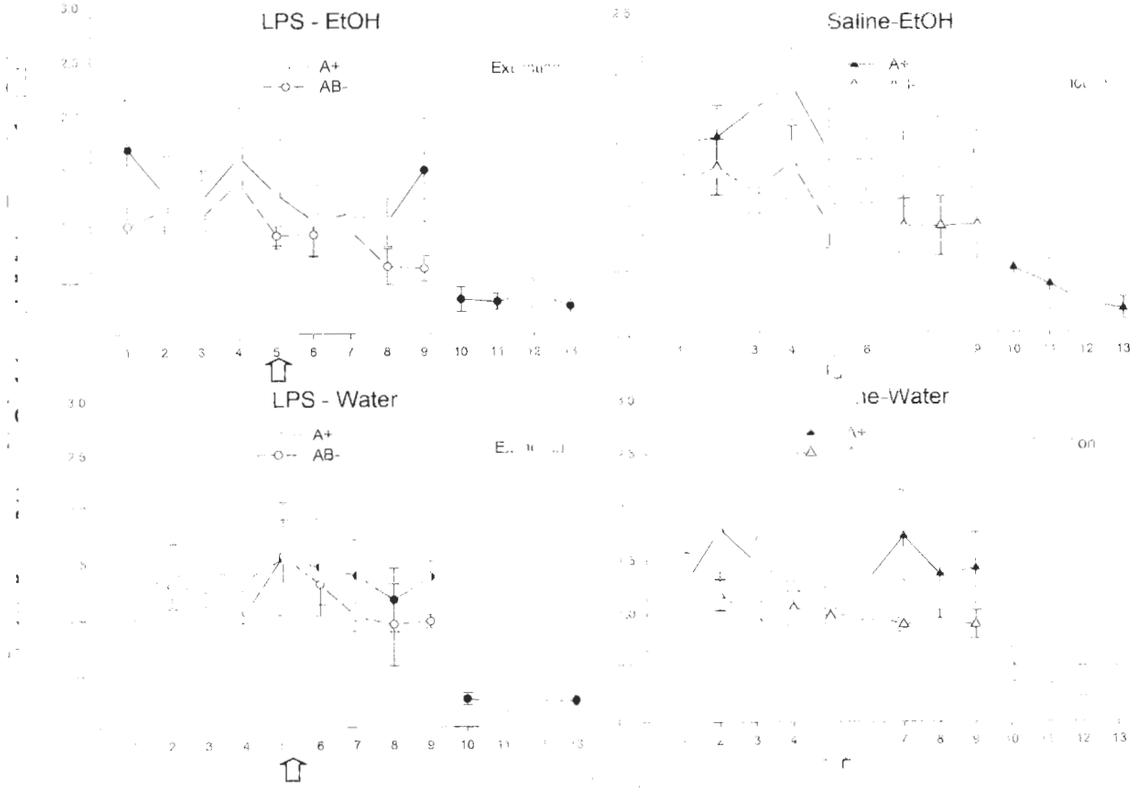
[illegible]ly, the results of the last 4 [illegible] (b[illegible]th sug[illegible] [illegible]t were compared against the 4 day[illegible] [illegible] to [illegible] being learned by the r[illegible] A[illegible]tor Al[illegible]A was [illegible]on [illegible] (2) x bottle [illegible]ation (2) [illegible] (2) [illegible] (4) [illegible] a si[illegible] [illegible] of Phase, $F(1, 20) = 81$. [illegible], [illegible] t[illegible] [illegible] was [illegible]ific to sign-t[illegible] furt[illegible]ortin[illegible] the hyp[illegible] [illegible] sign-tracking behavio[illegible] (but n[illegible] [illegible] sponding.



[illegible] 4 – [illegible]gat[illegible]-featu[illegible] [illegible] th[illegible] [illegible] by the sugar p[illegible]llet. A[illegible] [illegible] by t[illegible] [illegible]llet. Or[illegible] [illegible]

[illegible] findings of [illegible] in [illegible] [illegible]nge that contin[illegible]d for two [illegible]ght [illegible] [illegible]re [illegible] by the end [illegible] the tw[illegible] the [illegible] [illegible]ness. [illegible] [illegible] diff[illegible] betw[illegible] [illegible] with wa[illegible] [illegible]d th[illegible] [illegible]tion of [illegible] [illegible]ng behavio[illegible] [illegible] driv[illegible] [illegible]arily by th[illegible] [illegible] in t[illegible] [illegible]anol sign tracker [illegible] [illegible]d all rats [illegible] [illegible] [illegible]nce tests with 6% [illegible]% [illegible]. The [illegible] [illegible] a higher preference for [illegible] to [illegible] [illegible] sugg[illegible] that alt[illegible] [illegible]tion [illegible] during sig[illegible] [illegible] [illegible]d by daily ho[illegible]-cag[illegible] [illegible]

[illegible] results of the [illegible]gati[illegible] [illegible]ature t[illegible] so[illegible] [illegible] [illegible]ign tracking, especia[illegible]y wh[illegible] [illegible] [illegible]lly in w[illegible]hdrawal. [illegible] to be [illegible] [illegible]ons[illegible], but by head poke [illegible] Ev[illegible] [illegible]cking procedure s[illegible] [illegible] [illegible]d t[illegible] [illegible]prague [illegible] group. [illegible] to [illegible]inate suggests that sig[illegible] [illegible] [illegible]ns rats [illegible]ect [illegible]mp[illegible]

General [illegible]

[illegible] tracking procedure using a [illegible] approach to drug use and abuse. First, [illegible] drug [illegible] to respond to the [illegible] cues that guide [illegible] drug [illegible] Second, [illegible] nce of a [illegible] between [illegible] food [illegible] attentive salience to the [illegible] t [illegible] Further, [illegible] drug-directed behavior. Finally, [illegible] compulsive behavior in connection with the [illegible] to the [illegible] compulsive behavior si[illegible] in addiction of drug (e.g. alcohol). Per[illegible] type of [illegible] generated by [illegible]

[illegible], which [illegible] than sta[illegible] rats [illegible]. The negative-fe[illegible] to [illegible] ponding; dif[illegible]culty [illegible] of [illegible] negative-feature discrim[illegible] [illegible] readily reduced [illegible] ls. If the effect of enri[illegible] behavior, [illegible] ral profile would h[illegible]t to [illegible] ive-feature discrim[illegible] rats [illegible] t sign tracking [illegible] not re[illegible] als be[illegible]e [illegible] rats were [illegible]

sign tracking, [illegible] impulsive
is seen as a [illegible] behavior which is typic[illegible]
and [illegible] sugar pellets as the b[illegible]
[illegible] in the consumption [illegible]
at gr[illegible] of drug use. Although the em[illegible] have a longer
[illegible] within the sign tracking [illegible] have
[illegible] addictive propensities (i.e. [illegible]
[illegible]). Perhaps, this is due [illegible] or
[illegible] modulated. However, a [illegible]
[illegible] contributing factors. In [illegible] to the [illegible]
[illegible] impulsive intake m[illegible] factors to addiction but
is not [illegible] an addiction. Pe[illegible]
[illegible] with [illegible]
like [illegible] an addiction.

[illegible] drinking of the Lon[illegible]
[illegible] the sign tracking [illegible]
[illegible] sign tracking [illegible] failure to [illegible]
[illegible] ve-feature disc[illegible]
[illegible] possibility is that t[illegible] should [illegible]
be [illegible] able to support disc[illegible]. [illegible]
[illegible] discrimination of ge[illegible]

[illegible] results of Experiments 1 and 2 [illegible] that the Long Evans rats are [illegible] than the Sprague Dawley rats.

[illegible] debate concerning the [illegible] primarily focused on the role of the serotonin (5-HT) system in [illegible] It has been suggested that impulsivity [illegible] a function of its regulation by the serotonergic system (Winstanley, [illegible] 2004). Data from human volunteers with [illegible] (which is inversely related to brain functioning) found increased impulsive action, but not in [illegible] The data suggest that [illegible] more sensitive to alterations in serotonergic system [illegible] of the reduced discrimination seen in the present study may be an [illegible] of impulsivity.

[illegible] the Sprague Dawley rats [illegible] showed a preference for EtOH. This [illegible] not surprising given the [illegible] of EtOH in brief daily tests to establish a preference. Thus in Experiment 2 ethanol preference was induced by providing 24 hour [illegible] daily [illegible] concentrations of EtOH in the home cage. These [illegible] injections resulted in a [illegible] preference for EtOH. Not [illegible] other preparations [illegible] EtOH the [illegible] of EtOH was not increased in the [illegible] compared to the [illegible] that had not treated with saline.

[illegible] that the activation of the [illegible] is [illegible] preference for EtOH extends Bleda [illegible] (2011) findings in mice to rats [illegible]

that immune system regulation imp ... It is ... increased E ... did not compare Long- ... cage E...

... tion (L...) may induce ... to ... inf... in adulthood. This ... such as the ... training ... (memory ...) ... age (Bilbo, Levkoff ... 200...). ... increases hypotha... y–ad... influences ... tracl... in ... K... activity and impairs tumor ... ability ... attenua... fever in response ... infected ... oli, ... displayed im... in adulthood (... et al, 200...). ..., this ... ly after ... (such as, interleukin [IL-1β]) ... in the cou... of an ... significant influence on mem...

... is ... distribut... throug... with ... which makes it vulnerable to ... may lead to ... ments. ... her... -1β ... and IL-1β is ... As such, ... normal memory ...

[illegible] as may occur as a result of i[illegible] development [illegible] processes [illegible] (2005). [illegible] use are also [illegible] (ed) [illegible] the development of th[illegible] this [illegible] ng [illegible] ance [illegible] sa[illegible] and [illegible] g was fo[illegible]

[illegible] found to in[illegible] opioid [illegible] (in [illegible]) [illegible] dorph[illegible] are [illegible] on [illegible] d with [illegible] (20[illegible]). [illegible] gnant [illegible] male [illegible] ts [illegible] w [illegible] ted in the mal[illegible] ng a [illegible] ol in a [illegible] test ([illegible] vs. [illegible]) [illegible] Yee, [illegible] & [illegible], 20[illegible]) [illegible], heavy [illegible] or [illegible] al, [illegible]

[illegible] ver, [illegible] studies have shown [illegible] in the nucleus accumbens [illegible] d [illegible] high [illegible] sumption in rats. M[illegible] in rats wi[illegible] m [illegible] ly [illegible] rats consume E[illegible] to [illegible] ol [illegible] ase has also been re[illegible] d al[illegible] et al, [illegible]

[illegible] negatively impacts th[illegible] amine release may be [illegible]

[illegible] ating ([illegible] sign [illegible] [illegible] [illegible] pulsive sign tracking [illegible]

[illegible] the presen [illegible] in compuls[illegible]-like [illegible] are consistent with st[illegible] [illegible] performance and high [illegible] [illegible] ite [illegible] us [illegible] umb[illegible] [illegible] iden[illegible] [illegible] the [illegible] drug-taking and that sig[illegible] late [illegible] rchers ha[illegible] noted [illegible] ilariti[illegible] [illegible] g procedu[illegible] [illegible] with sign tracking [illegible] ng [illegible] recovery ([illegible] ach, [illegible] [illegible] eff[illegible] [illegible]. The e[illegible]ts of sig[illegible] [illegible] pa[illegible] ways r[illegible] mble[illegible] [illegible] of ab[illegible] [illegible] cal pro[illegible] of subje[illegible] to [illegible] king [illegible] profile of vuln[illegible]ability [illegible] vulr[illegible] to [illegible] lf- [illegible] et al, 2008) [illegible] [illegible] drug abuse may [illegible] na ([illegible] [illegible] [illegible]3).

[illegible] of the sign tracking [illegible]

[illegible] underlying mechanisms [illegible] contribute to [illegible]

[illegible] function. But before th[illegible] to

[illegible] present [illegible]

[illegible] strain it would be a good [illegible] Sprague [illegible] and Long-

Evan[illegible] in the same [illegible] with the [illegible] It

[illegible] compare groups in [illegible]

[illegible] determine how [illegible]

[illegible] in [illegible] to

[illegible] dependent [illegible] be [illegible] to

[illegible] individual effects that [illegible]

[illegible] may increase [illegible] ethanol ([illegible]

[illegible] A+ trials of the discrimi[illegible] a couple of days). [illegible]

[illegible] sign tracking model [illegible] to [illegible] effects of

[illegible] ethanol consumption.

[illegible]

[illegible] is primarily [illegible]

[illegible] (e.g. preference [illegible]) [illegible]

[illegible] i.e., drinking to [illegible]

[illegible] periods of withdrawal and [illegible] on [illegible]

Et[illegible] informative. Finally, although [illegible] focus of [illegible]

[illegible] investigated by mixing [illegible] water [illegible]

[illegible] produce sensitization in mice, [illegible] demonstrate

[illegible] sensitization in rats. [illegible] amphetamine may be [illegible]

[illegible] sign tracking, have [illegible]

[illegible], S.L., [illegible]ucher, M.H. (2009). [illegible] and [illegible]
[illegible] and [illegible]
[illegible]ience and [illegible]behavio[illegible] [illegible] 51–524.

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[illegible], A., [illegible], J.M., [illegible], H. [illegible], [illegible]
(2[illegible]) [illegible]on of inf[illegible]ory s[illegible]
[illegible], [illegible] Proof, [illegible]ine 23 [illegible]

[illegible], P., [illegible], J. (200[illegible]). Effe[illegible]
[illegible]on sucr[illegible] consum[illegible]on an[illegible]
[illegible] *[illegible]ic[illegible] 136*, 27[illegible]

[illegible], [illegible].M. (1[illegible]) [illegible]Auto[illegible] *[illegible] of*
[illegible]per[illegible]ental Analysis of [illegible]behav[illegible], *11*([illegible])[illegible]

Chr[illegible] (20[illegible]) [illegible]hol, neu[illegible] [illegible] *The [illegible] of*
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[illegible] Ac[illegible]my of Sciences of th[illegible] [illegible] of Am[illegible]a, *[illegible]*(1[illegible]), 61[illegible]
61[illegible]4.

de[illegible], [illegible] (2[illegible]). [illegible]ulsivity as a [illegible] [illegible] [illegible] [illegible]ce o[illegible] [illegible] A [illegible]
[illegible] [illegible]ying [illegible]ses. *Addiction* [illegible], [illegible]1), 22-31.

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[illegible] *12*(2), 172-185.

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... *...neurology, 99*, 99-111.

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[illegible]

[illegible] M., W[illegible]ss, S., [illegible] (20[illegible]). A [illegible] str[illegible] on [illegible] (...) [illegible] reve[illegible] [illegible] ...tive automaintenan[illegible], *169*(2), 1[illegible]-20[illegible]

Ki[illegible] (20[illegible]). Co[illegible] pl[illegible] amic proc[illegible] [illegible] ...ative automainten[illegible] *...f Expe...* [illegible] *...mal ...havior Processes, 2*[illegible](1), 4[illegible]

[illegible] A., Jorgensen, H., Garb[illegible] C., [illegible] (19[illegible]). ...ole [illegible] hypothalam[illegible] histam[illegible] in [illegible] of [illegible] [illegible] ...hine responses to [illegible]-S[illegible] *...roen...crinol...y* [illegible]

[illegible] F. (20[illegible]). A[illegible]nal mod[illegible]s in cravi[illegible] [illegible] mo[illegible]ls of [illegible]ving for [illegible] *...n, 9*[illegible] [illegible]73-[illegible]

[illegible] (20[illegible]). ...ovian conditioni[illegible] [illegible] [illegible], and ethanol se[illegible] [illegible]ion. *Al[illegible] Clin[illegible] ...ental Research, 27*(10), 1[illegible]

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Liu [illegible], S. K., B[illegible], C. J., [illegible] (20[illegible]).

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...ping. ...port: ... Rapid ... of Ne... ...
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...and, ... Bauer ...
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...P, Va... ...L.J., ...ries, T... ..., F., ...
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...*...ec...gy, 133*, 69–76.
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...*...y, 6*, 810–...4.
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...ivity in ... animal m... *Alco...* ...
...L., ...ker, J.L., & Le, A.... (199... ... ...ater alcohol consu...tion in ... ...
...k ... individual dif...ces. ... ...*...ce, 112*, ...
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...ol... ... *Pharmacology*, *3*, ...

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...ol... ...sisted dr...g-taking in h... ... ...*...*, *15*(3),
14...

...(20...) Loc...ing reward... at r... ... (C...), ...
... ... of drug abuse. ... ... *20*(...) ...-
...

[illegible] A.S., Pol[illegible] L.A., [illegible] (1[illegible]). [illegible] induc[illegible] [illegible] responding in a delay-[illegible] [illegible] predicts aut[illegible]shaping. *[illegible]*, *139*, 3[illegible]-382.

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[illegible] A., [illegible] r, [illegible], Sal[illegible]on, F[illegible] [illegible] A. (2[illegible]). [illegible] [illegible]king in nondepri[illegible] [illegible] of [illegible] [illegible]tion of ethanol pref[illegible]ence. *[illegible]*, *77*, 79[illegible]-[illegible]4.

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