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Social Transmission of Ethanol Preference in a Sign-Tracking Paradigm

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SOCIAL TRANSMISSION OF ETHANOL PREFERENCE IN A SIGN-TRACKING PARADIGM

By

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Abstract

The Observer/ Demonstrator Effect suggests that rats can socially obtain preferences for novel foods/drugs by interacting with experienced conspecifics. Sign-tracking is a paradigm which uses a conditioning procedure to evoke compulsive-like behaviors directed towards a tangible conditioned stimulus (CS) paired with a motivating unconditioned stimulus (US). The present study investigated the possibility that increased alcohol use results from the combination of socially transmitted cues and the kind of conditioning effect seen in sign-tracking procedures. Therefore, it was hypothesized that social exposure to an alcohol-experienced conspecific would summate with the conditioning experience and increase the sign-tracking of a bottle CS containing alcoholic beer. Subjects were exposed to social cues via conspecifics who consumed alcoholic- (AOG) or non-alcoholic beer (NAOG). Both groups experienced a sign-tracking procedure with alcoholic beer as the CS and a sugar pellet US. Sign- and goal-tracking were measured as licks on the bottle CS and nose pokes into the food receptacle US, respectively. The results showed no differences in sign tracking between groups across conditioning days. Preference tests outside of the conditioning paradigm indicated the social transmission was successful, as the AOG drank significantly more alcoholic beer than the NAOG. However, this difference between groups was not maintained once sign-tracking began. The hypothesis could not be supported as the two main groups, AOG and NAOG, did not behave differently during sign-tracking.

Social Transmission of Ethanol Preference in a Sign-Tracking Paradigm

The medical model of addiction is a widely known and accepted disease model of drug abuse that posits drug taking behaviors are initiated and maintained by the reinforcing effects of psychoactive drugs which eventually leads to permanent brain changes (American Psychiatric Association, *Diagnostic and statistical manual of mental disorders*, 2013; National Institute of Drug Abuse, 2014). These permanent brain changes do not happen rapidly, instead they can take months or years to develop. These changes in the brain result in drug tolerance and physiological dependence (DSM-5, 2013). Tolerance to a drug is indicated when there is a diminished response to a drug requiring an increase in the drug dose to obtain the original, desired effect. Repeated use of some drugs increases tolerance and may subsequently result in physiological dependence as indicated by withdrawal symptoms when the drug is withheld. Withdrawal symptoms are physiological reactions that can vary from mild (e.g., nicotine) to severe (e.g., heroin) and life threatening (e.g., alcohol). Decreases in dopamine and serotonin in the synapses of the nucleus accumbens, increases in the neurotransmitter NMDA broadly across the brain, and increases in stress hormones all play a part in creating these symptoms (Koob, 2006) that maintain drug-taking behaviors through negative reinforcement, i.e. by temporarily removing the withdrawal symptoms. Though physiological dependence and the associated brain changes are important to understanding addiction and drug abuse, there are other factors that play a role in initiating and maintaining these drug taking behaviors before they reach the level of abuse and irreversible brain alterations.

Positive reinforcement can maintain drug use behaviors as the drug itself can be rewarding without permanently re-wiring the brain. Drug use is frequently paired with other rewarding stimuli such as food, social activity, and entertainment. All of these stimuli can

contribute to the effectiveness of a drug as a positive reinforcer. Thus, a drug that, by itself, may have insufficient or weak reinforcing properties may become increasingly reinforcing by being paired with other rewarding stimuli. Even in the absence of physical dependence, the repeated use of a reinforcing drug can develop into drug-taking behavior that can be described as compulsive. For example, rats who have had access to cocaine via self-administration have been shown to continue seeking the drug even when experimenters begin applying a painful shock whenever cocaine is self-administered (Vanderschuren & Everitt, 2004). This shows that positive reinforcement from the drug resulted in the animal becoming compelled to seek it, regardless of the consequences and without physical dependence. Uncontrollable craving for a drug and compulsive drug seeking despite adverse consequences to oneself, or others, is typically described as a hallmark of addiction in humans (NIDA, 2014).

The withholding of some drugs, like cocaine, do not result in the physiological reactions (i.e., withdrawal symptoms) of drugs such as nicotine, heroin, or alcohol. Nevertheless, negative reinforcement may also play a role in the initiation of drug use by allowing the user to temporarily escape from pain, anxiety, and other negative situations. The successful decrease in negative affect can serve to increase these drug-use behaviors and if the source of the negative affect persists the opportunity for negative reinforcement may lead to repeated drug use. Eventually, repeated drug use can lead to abuse and addiction which is when the brain goes through unchangeable alterations and, depending on the drug, physiological dependence occurs. Understanding how conditioning, environmental factors, scheduled drug-taking, and other such variables influence drug-taking behavior is important for a better understanding of drug use as a whole and how it leads to abuse and addiction.

Environmental Factors in Drug Use

Environmental factors such as social situations/ norms, availability of alternative reinforcers, and various conditioning phenomena are all able to modulate drug-taking behaviors (Alexander et al., 1981; Tomie et al., 2004a; Ahmed, Lenoir, & Guillem, 2013). Alexander et al., (1981) examined a tangential variable to the typical medical model of drug addiction in animals by looking for differences in morphine-drinking behaviors when rats were either housed alone in small cages or housed with multiple cage mates in a larger home cage. The results indicated that the more space and cage mates there were, the less drug the animals would consume; that is, isolated animals consumed more morphine than rats in a social group. Though the researchers speculated on why this difference may have occurred, the results demonstrate that the initiation of drug-taking is a complex phenomenon that is not solely dependent on a drug's ability to produce positive reinforcement. The Alexander et al., study has been noted by several authors as problematic for the medical model of addiction (e.g., Ramsden, 2013), nevertheless it had little immediate impact on investigations of animal models of drug addiction.

More recently, however, studies of social influence on drug abuse and addiction have rekindled interest in the Alexander et al study (e.g., Ahmed et al., 2013) and spurred the development of animal models of social influence on drug use, abuse and addiction (Strickland & Smith, 2015).

For an animal model of social influence on drug use to be effective, it is important that the animal being studied (the observer) is able to interact with a conspecific (the demonstrator) as much and closely as possible and that information that influences choice behavior is transmitted in this interaction from the demonstrator to the observer. One study, which looked at the social transmission of food, by Galef et al., (1988) demonstrated that a rat can learn to prefer

a novel diet simply by interacting with a cage mate that had recently consumed the food. When a rat ingests a novel diet, the scent of that food on its breath can be perceived by other rats and this causes a socially transmitted preference for the novel food. A similar phenomenon can happen with ethanol where a demonstrator rat is intoxicated and an observing rat can develop an ethanol preference by being exposed to the intoxicated rat (Hunt et al., 2001). Although rats are sometimes intoxicated by ethanol injection in this model, rather than through the voluntary consumption of the drug, the ethanol appears to reach the oral cavity and be transmitted to the observing rat (Javors et al., 2005). Another model related to the social transmission of drug use involves two rats in the same cage but separated by a partition. Both have access to an intravenous drug via self-administration. It is seen that both animals have higher rates of self-administration when their cage mate has access to the drug as well. However, if they are isolated or paired with an animal that does not have access to the drug then the effect does not occur (Strickland & Smith, 2015).

These models of social influences are very useful for studying drug use but there are a variety of weaknesses related to external validity. Laboratory animals are housed in very specific, standardized cages and typically have only one cage mate. These small, limited environments suppress many of the animal's natural behaviors including a wide range of social behaviors (Alexander et al., 1981). These experiments are also typically done with only two rats where one is intoxicated, and the other is observing. Rats tend to live in colonies where they interact with many conspecifics in complex ways. By influencing their natural social situations there may be an alteration in their natural behaviors or their behaviors may be an artifact of the experimental situation. For example, rats appear not to consume morphine when housed in a social group because the drug interferes with the species-typical social interactions with others in

the group (Alexander et al., 1981). By removing conspecifics and abolishing social interaction, the animals increase their interaction with the morphine solution. Also, animals are often forcibly intoxicated (e.g., injections or force fed) during these studies or are allowed to self-administer a drug intravenously. Neither of these mimics a genuine drug-taking approach as both intoxicate the animal via direct intervention of the researcher. External validity is very important in social transmission experiments since there is little information on the precise ways in which rats interact with the members of their social group.

Availability of Alternative Reinforcers

The issue of forced consumption is a crucial one if animal models are to generalize to human examples of addiction. As seen in Alexander et al.'s (1981) study, isolated rats drank the morphine solution, the only stimulus in their limited environment. However, rats with environmental stimuli to engage and cage mates to interact with, chose these alternative rewarding stimuli over the drug. These results represent a complex, realistic situation where one has access to various stimuli. In a human situation, many people have the option to drink frequently, yet they choose to engage in more productive activities such as work, family, or exercise. These adaptive behaviors are reinforced financially, socially, or physically. Ahmed et al. (2013), make the point that the medical model generally states that a drug's positive reinforcing properties sustain drug-taking behaviors to the point of permanent brain changes. This may play a part but if it were the only factor influencing drug-use than everyone who consumes a drug will become an addict. This is obviously not true, only certain people show aberrant learning in response to drug-use. Having other positive stimuli in the environment, and continuing to use a drug regardless, is not a typical behavior and to understand how it arises researchers need to give subjects more than one reinforcing stimulus to choose from.

Conditioned Induction of Behavior

One way to induce drug-taking in a realistic way is to condition animals to take a drug by pairing it with natural reinforcers in their environment. This creates a paradigm where the animal has access to multiple reinforcers concurrently and/or consecutively. By creating these conditioning schedules, researchers can examine if and how subjects begin exhibiting drug-taking behaviors as a factor of their environmental situation. Examining the initiation of drug-use as it relates to unconditioned stimuli can help delineate variables that compel a subset of the population toward consistent drug-use and abuse.

The sign-tracking model of drug addiction emphasizes the role of Pavlovian associations in addiction. In appetitive Pavlovian conditioning procedures, a rewarding unconditioned stimulus (US), such as a sugar pellet is paired with a conditioned stimulus (CS) such as an illuminated light. After several pairings of the CS and US the animal begins to approach the light CS even though this approach behavior has no effect on the delivery of the US reward (Tomie et al. 2002). This approach behavior has been described as sign tracking because bird subjects in the earliest studies appeared to be "tracking" the light CS as a predictive signal for food reward. If the light CS is replaced with the brief insertion of a lever the rat will consistently approach and manipulate the lever CS even though lever manipulation is a wasted effort; it does not result in any further reward (Tomie et al., 2008). If a brief presentation of a water bottle serves as the CS, rats will typically approach and lick the water bottle causing the animal to drink water even though it is not thirsty (Tomie et al., 2004b). The conditioned responding that is elicited toward these various CSs has been characterized as compulsive-like behavior; persistent, excessive, nonfunctional, and potentially maladaptive (Tomie et al., 2008). When alcohol is added to the water bottle the compulsive-like drinking behavior resembles alcohol addiction.

Sign tracking of an alcohol-containing bottle now appears functional because the animal is consuming a drug that contains calories and induces pharmacological effects, nevertheless the behavior in this context is still maintained by a Pavlovian association and the behavior is still persistent, excessive, and potentially maladaptive. Though any liquid can be used in the sipper bottle of a sign-tracking procedure, many sign-tracking studies show that an alcohol bottle will lead to higher levels of consumption than a water-filled bottle suggesting that a conditioned compulsive effect summates with the biological effects of ethanol (Tomie et al., 2005b). In other words, Tomie's sign-tracking model suggests that the addictive-like behavior of a rat involves the integration of compulsive-like conditioned responses of sign-tracking plus the pharmacological effects of alcohol and that a similar phenomenon may occur in people.

In the animal model of sign-tracking, everything is done via experimental manipulations and so the exact stimuli can be delivered on very specific schedules making any effect easy to observe. In a human example, it is much more complicated as alcohol consumption happens in a much broader context. It is possible to make connections in the human world that link to the animal model. The CS in a human example could be one's favorite drinking mug or a favorite alcohol brand/ bottle. Though untested in the sign-tracking paradigm, it is known that certain contexts, such as one's favorite bar or one's favorite spot on the sofa after a day at work can initiate drinking (Bouton, 2002). These contexts may serve as the CS as well, where one develops a habit of drinking in that same location because it is rewarded by some US. There are also several possible stimuli that could serve as a US. For example, alcohol consumption in and of itself is rewarding due to its caloric value and pharmacological effects, indicating alcohol itself may serve as a US that rewards a CS. Many people tend to consume alcohol while relaxing, enjoying entertainment, socializing, or eating a meal (such as having wine or beer with

dinner). All of these are rewarding and could serve as the US. Similarly, many alcoholic drinks are made to taste good, and so if the CS of one's favorite drinking mug was frequently paired with a good tasting alcohol, this could condition one to maintain these pairings. Alcohol is frequently consumed amongst peers and this social environment can be rewarding and may serve as the US as well. In fact, animal models of sign-tracking have used a social stimulus US in some studies (Tomie et al., 2004a; Tomie et al., 2004c; Tomie et al., 2006).

One of the most unusual findings related to the sign-tracking procedure is when a social stimulus is used as an US and ethanol is used as a CS (Tomie et al., 2004a; Tomie et al. 2004c; Tomie et al. 2005a). Pairings of the ethanol sipper followed by access to a same-sex conspecific (through a mesh wire cage) increases ethanol consumption suggesting that social activity is rewarding. However, increased consumption also happens at the same rate even when a pseudoconditioning group is used. Pseudoconditioning is a control group in the sign-tracking paradigm where the US and CS are presented randomly. So, access to the CS will not necessarily happen prior to the US, it could happen after or during too. In this group, increased ethanol consumption still occurs, indicating that intermittent access to a social peer increases drinking and does not necessarily require the establishment of Pavlovian associations. This result resembles another procedure that generates excessive, compulsive-like behavior known as schedule-induced adjunctive behavior (Falk, 1961). Intermittent access to a rewarding US (e.g., a food pellet administered every minute independent of any response) can lead to an increase in behaviors directed toward other objects in the environment. This phenomenon was first observed as unusually high levels of water intake in non-thirsty rats exposed to an intermittent schedule of food pellet delivery with free access to a water bottle. Falk (1966) named this result *schedule-induced polydipsia* and when it became clear that intermittent schedules of reward can

induce a variety of excessive behaviors depending on the objects available in the test chamber he introduced the term *schedule-induced adjunctive behavior*. Falk also argued that to understand drug addiction it was more important to recognize the role of the schedule induction of excessive behavior than the properties of the drug being abused and he argued that scheduled induced polydipsia of an alcohol solution is a valid model of alcohol abuse (Ramsden, 2013; Ford, 2015).

Schedule-induced behavior is very similar to sign-tracking in that both involve presentation of an intermittent rewarding stimulus (US). The difference is that sign-tracking introduces a Pavlovian association by administering an explicit CS before reception of a US. Whereas schedule-induced polydipsia does not administer the CS periodically and provides only the US based on a specific intermittent schedule. When the CS is an alcohol-containing bottle and the US is a social peer it appears that the intermittent presentation of the rewarding social US is the only factor inducing ethanol consumption (Tomie et al., 2004c). However, when food reward is administered intermittently to induce ethanol intake this intake is further enhanced when the ethanol-containing bottle acts as a reliable CS for the food reward (Tomie et al., 2004b). These experimental procedures demonstrate the power of reward schedules and Pavlovian associations in inducing drug use behavior.

The proposed study will shed light on the nature of the social transmission of alcohol consumption by examining how prior exposure to an alcohol-consuming peer influences sign-tracking of alcohol-containing bottle and choice consumption of alcohol outside of the sign-tracking procedure. It is known that alcohol consumption and choice in animals can be increased via social exposure to an alcoholic drink alone, however the influence of socially-obtained information about alcohol in a context that induces compulsive-like responding (the sign-tracking paradigm) has never been tested.

Evidence of Social Transmission of Information in Animals

Prior to there being social models of drug addiction, there were social models of food preference transmission. One such model is called the observer/ demonstrator model. Galef et al., (1983; 1984) systematically examined the social transmission of food preference for the first time. In these studies, the researchers discovered that rats that had eaten a novel diet could transmit information about the diet to an observer rat. These observer rats were given a choice between two novel diets and preferred the one that their conspecific peer had previously eaten. How was information about a new and safe food source transmitted between rats? One hint was that this observer/demonstrator effect was still present when the demonstrator was anesthetized. Galef et al., (1988) tested the hypothesis that this socially-acquired food preference could be happening via Pavlovian conditioning with some olfactory cue US endogenous to the demonstrator. Galef and his fellow researchers discovered that a carbon disulfide compound in rat breath can be perceived by other rats. The experimenters used demonstrators that were anesthetized rats, as well as cotton swabs serving as surrogate rats smelling of the diet alone, or of the diet and the carbon disulfide compound. The observer/ demonstrator effect was only seen for the anesthetized rat and the surrogate smelling of carbon disulfide plus the diet. This indicated that only an association between the carbon disulfide (the US) and some food stimulus (the CS) was required to transmit the food preference, there were no behavioral cues necessary. As long as the odor of carbon disulfide and diet A are contiguously paired and perceived together, whether it be via rat expiration or a cotton surrogate, then the rat will prefer diet A over diet B.

Hunt et al., (2001), were the first to apply this model to ethanol consumption and did so using same sex sibling pairs for the observer and demonstrator. They found the same effect with

ethanol as others had seen with food. However, Maldonado and Molina (2008) found a difference between food and alcohol transmission. The researchers found that males will find ethanol to be aversive if they are unfamiliar with their demonstrator. It is only when the demonstrator is familiar, such as a cage mate, that the social transmission occurs successfully. For females, the effect occurs regardless of their familiarity with the demonstrator. In the proposed study, this problem will be avoided by having the animals live together and simply removing the observers temporarily while the demonstrators consume beer or non-alcoholic beer. Fernandez-Vidal and Molina (2004) conducted a series of experiments that found another important difference between the social transmission of alcohol compared to food. Unlike Galef et al. (1988), they found that an anesthetized demonstrator who had been intoxicated was not sufficient to transmit an ethanol preference. This would indicate that there may be a necessary behavioral component to the social transmission of alcohol consumption.

There are limitations with this paradigm because it's typically conducted in a small home cage with only one observer and one demonstrator. This sacrifices ecological validity because a) animals placed in smaller cages drink at a different rate than if they had an expansive environment (Alexander et al. 1981) and b) social contact between only one observer and demonstrator may not accurately parallel a realistic, complex social situation. The proposed study intends to examine alcohol preference in observers who have been exposed to multiple alcohol-consuming demonstrators at the same time. Furthermore, the cage used will be a larger cage than is standard, allowing the animals to have more space to behave compared with similar studies. The use of a large cage allows the animals to express natural behaviors that may be suppressed in a smaller home cage. This is especially important in this study because, as mentioned before, there seems to be a behavioral aspect to the social transmission of alcohol

preference. Any effects that are to be observed in this study will be more salient if the animals have ample space to behave normally.

Sign-Tracking as a Model of Compulsive-Like Behavior

Testing the observer/ demonstrator model has been done using a variety of paradigms such as a two-bottle choice test or an odor preference test (Hunt et al., 2001, Fernandez-Vidal et al., 2004). It has never been tested in a sign-tracking paradigm, a paradigm that explains how an association between a rewarding stimulus US and an initially neutral stimulus CS can lead to a compulsive-like CRs towards the CS even when interaction with the CS is not required to receive access to the US. In other words, operant conditioning is a type of learning where reward is contingent upon responding, one must interact with a stimulus in order to receive some form of reward. Therefore, it is not surprising when an animal interacts with the stimulus to repeatedly earn that reward. An animal continuously pressing a bar for a drug serves as an example of this. In Pavlovian or classical conditioning, an animal learns that an initially neutral stimulus can predict some forthcoming rewarding stimulus. Importantly, the animal does not need to interact with this neutral stimulus to earn the reward as it would with operant conditioning. Classical conditioning is interesting because the initially neutral stimulus can become a conditioned stimulus, meaning that stimulus itself can incite reflexive, physiological reactions that are akin to ones seen in the presence of the naturally rewarding unconditioned stimulus. In sign-tracking, classical conditioning takes place between a neutral stimulus, such as a lever or a sipper bottle full of some substance, and a rewarding stimulus such as a sugar pellet. One would expect the CS to elicit physiological reactions such as salivating or increased motor activity as the rat is expecting to soon receive a rewarding stimulus which is usually in a different location near the CS. One would not expect the animal to interact with the CS instead

of staying near the entrance where the US is dispensed. Such a behavior would be indicative of operant conditioning where the animal must perform some necessary behavior to earn the reward. Yet, some animals interact substantially with the CS and perform consummatory behaviors on it such as biting, licking, etc. The animals may even do this in favor of the actual rewarding stimuli. In other words, if a sipper bottle full of water, ethanol, or anything else is repeatedly presented for 10s prior to receiving a sugar pellet then the animal will start to drink from the bottle for those 10s and will drink more and more with repeated pairings. If a lever is repeatedly presented for 10s prior to receiving a sugar pellet, then the animal learns to press the bar for those 10s although it is an unnecessary behavior for reception of the reward.

There appear to be individual differences in the behaviors of rats in the sign tracking procedure (Robinson & Flagel, 2009; Robinson et al., 2014). Animals who learn to perform the unnecessary behaviors are referred to as sign-trackers while those who remain focused on the US are referred to as goal-trackers. While goal trackers are using the bottle CS to predict and anticipate the arrival of the food US, sign trackers appear to attach motivational or incentive properties to the bottle CS. Simply put, sign-tracking is a model of alcohol addiction that accounts not for the rewarding effects of the alcohol per se, but rather it describes how rewarding stimuli can transfer their rewarding properties to another object such as a bottle of alcohol or even a bottle of water. These associations can be considered to contribute to addiction by helping initiate and maintain these maladaptive behaviors (Tomie et al., 2008). Sign-tracking is often used in conjunction with food deprivation or a saccharin solution to sweeten the ethanol. However, it has been repeatedly shown that it is also able to occur in the absence of food deprivation and any sweetening solutions (Tomie et al., 2004b, 2004c). This is a further testament to the strength of this effect since ethanol is usually considered aversive at first due to

its taste. It also applies to rats of all ages (Tomie et al., 2005a) though adolescent rats tend to initiate sign-tracking more quickly and strongly than adult rats. Interestingly, Tomie et al., (2003) discovered that an ethanol and saccharin solution can be used as the US in a sign-tracking paradigm and a lever can be used as the CS. This will reliably lead to bar pressing prior to administration of the ethanol. This is most likely due to the fact that ethanol is rewarding in and of itself and may serve as a US. This idea is furthered by the fact that bar pressing greatly increased when the US was switched from water to ethanol.

Summary and Purpose of the Proposed Experiment

Overall, the question to be answered in this study is: does the prior social transmission of alcohol preference manifest itself in a sign-tracking paradigm? This is an interesting question because the social transmission of alcohol preference has not been tested as a variable affecting sign-tracking, it has only been examined under other models of alcohol use, abuse, and addiction. Understanding how drug use is initiated and maintained is vital for understanding how it leads to compulsive behavior that can be maladaptive and uncontrollable. It takes time for drug use to progress to a more serious stage of abuse and during this time compulsive behaviors are formed which support the drug-taking up to, and beyond, the point of permanent brain changes. The proposed study expects the social transmission of alcohol preference to increase the animal's vulnerability to these learning-related compulsions in a Pavlovian setting.

In the proposed study, rats will be caged in large groups and in large cages to improve ecological validity. This is important because Alexander et al., (1981) saw increased drug taking when animals were isolated in small cages compared to more ecologically accurate ones, suggesting that drug use in animals is partly an artificial result of social isolation and low environmental stimulation. Fernandez-Vidal et al., (2004) discovered that, unlike the social

transmission of food, the social transmission of alcohol preference requires an alert, behaving animal. The smell of carbon disulfide along with the smell of alcohol is not sufficient to create a preference and so a bigger cage with more animals is preferable because it allows the animals to behave normally compared to a standard home cage with a single cage mate. With all this in mind, I hypothesize that animals who are exposed to alcohol-consuming same-sex peers will have a preference towards alcohol consumption and animals who were exposed to sober, non-alcohol consuming same-sex peers will prefer non-alcoholic beer. This effect will be seen when the observers are given a two-bottle choice test between non-alcoholic beer and regular beer. In addition, I hypothesize that this socially-transmitted preference for alcohol will also increase vulnerability to alcohol consumption within the sign-tracking paradigm. Although alcohol consumption in the sign-tracking procedure is induced by the intermittent presentation of the food US and the Pavlovian association between the alcohol-containing bottle and the US, I predict that sign-tracking will be further enhanced in rats exposed to alcohol-drinking peers.

Method

Subjects

A total of 24 male Sprague-Dawley rats were used in this study. The animals were purchased from Envigo (Indianapolis, IN). Six animals were caged in each tower and there were a total of four towers. Four of the six animals in each tower served as observers and the other two as demonstrators. A total of eight observers made up the alcohol observing group (AOG) and the eight others made up the non-alcohol observing group (NAOG). Two AOGs and two NAOGs were housed in each tower. All animals were on ad lib food (Purina lab chow) and water except when experimental manipulations required food deprivation overnight (see procedure). IACUC approval was obtained prior to data collection.

Materials

Animals were housed in wire-mesh tower cages purchased from Martin Cages, Inc (Nanticoke, PA). The tower dimensions were 18" x 11" x 24. This housing provides a bottom, middle, and top floor as well as a small landing as the highest point. O'Doul's non-alcoholic beer was used. Commercial non-alcoholic beer has less than 0.5 % alcohol. 200 proof ethanol purchased from Koptec was used to alcoholize the beer to 4% for the appropriate group. The sign-tracking apparatus and the attached pellet dispenser were standard operant chambers (Ralph Gerbrands Co) modified to accept a retractable bottle and sipper tube device purchased from Med Associates, Inc. Licks on the water bottle, while sign tracking, were recorded with lickometers. Head pokes into the food tray while goal tracking were recorded with a photobeam detector. When a rat poked its head into the food tray it interrupted the photobeam, signaling a head poke. The lickometer and photobeam detectors were purchased from Med Associates. The equipment was controlled by programs written in Med PC.

Procedure

Adaptation to housing conditions. Upon arrival to Seton Hall the rats were randomly assigned to the 4 towers, 6 rats per tower. The rats were housed for 2 to 3 weeks with ad lib food and water. During this time the rats were acquainted with the home cages and standard diet. This phase allowed the animals to become acquainted with one another, a necessary condition for successful social transmission.

Social transmission of information about food. The purpose of this phase was to encourage observer rats to seek information about food from demonstrator rats. The observer/ demonstrator effect requires direct animal to animal contact. By implementing this procedure,

we provided an opportunity for the effect to occur before testing the major hypotheses. Once the major manipulations began, the observers already had experience with deriving food-related information from their cage mates. The procedure for this phase was continued for 6 days, adding to 6 sessions for each observer. The day prior to each testing, the rat chow was removed from the cages. This was done to ensure the demonstrators consumed more food, and at a faster rate, before testing began. The intention was to improve the saliency of the social transmission. On test days (Mon, Wed, and Fri), all 6 rats were removed from the tower and weighed. Weighing the demonstrators confirmed the food deprivation procedures were successful. The demonstrators were then replaced into their towers. The 4 observer rats, after being weighed, were temporarily placed in standard shoebox cages. Next, the 2 demonstrators were given access to a specific diet for 30 minutes. The type of food provided varied. Diets were made up of standard rat chow flavored with powdered seasonings. The first seasoning was 1g of ground cinnamon, this was used for the first week. 1g of ground nutmeg was used for the second week. After the 30-minute period of open access to the diet was complete, it was removed from the tower and the tower was double-checked for any remaining pellets. This prevented the observers from finding spare bits of the diet that fell from the receptacle. A metal sheet was then placed into the tower, locking the animals in the upper area of the tower where they could successfully interact. A single observer rat was returned to its home tower and allowed 10 min to interact with the demonstrator cage mates. Each day a different observer rat was chosen to be the first observer to return to the tower for social interaction with the demonstrators. The other three observers then went through the same procedure in their respective towers. After all the observers had gone through a trial, they were all put back into their towers. After 10 minutes the novel food of the day was returned to the tower for 30 minutes of additional open access so all

rats could consume the food. The standard rat chow was then made available ad lib for 30 minutes. The metal sheet was removed, restoring full access to the tower and ending data collection for that day.

Social transmission of information about beer. In this phase, beer served as the novel food. It was either 4% or about .5% depending on which group was being examined. The demonstrators were given access to alcoholic or non-alcoholic beer in a standard sipper bottle on alternate days. This means the AOG and NAOG were run on different days. Unlike similar studies, this experiment did not force alcohol on the subjects and instead allowed them to drink on their own. Demonstrators were food deprived as in the previous phase to produce strong, reliable rates of drinking and possibly intoxication (on days the 4% beer was served). Before collecting data on social transmissions, demonstrators were placed in standard cages with 24 hour access to 4% beer to further increase drinking rates. This procedure occurred for two days, by which time demonstrators had become accustomed to drinking beer. Testing of the observer/ demonstrator model began next. Observers were removed from their home cages to temporary cages and the demonstrators were moved to holding cages in a separate room. There the demonstrators were given access to the non-alcoholic or alcoholic beer for 30 minutes before being returned to their home cages. Then, an observer was placed in the home cage with the two demonstrators to allow social transmission to occur for 10 minutes. After the first trial, the first observer was removed, and the second observer was placed into the tower for 10 minutes. The other two observers remained in the standard housing overnight, preventing them from perceiving that day's diet on the demonstrator's breath. This is vital, if the NAOG smelled alcoholic beer on the demonstrator post-data collection, it would be a fatal confound. Standard rat chow was given ad lib to all the animals for one hour after the trials end. Unlike the previous

phase the novel diet was not reintroduced after testing ended. The impact of socially transmitted information about beer was measured by the two-bottle choice test and sign-tracking procedures (see below). Two rats from each tower served as the observers on days alcoholic beer was provided to the demonstrator rats (AOG), and two rats from each tower were observers on days the non-alcoholic beer was provided (NAOG). The 4% beer was created by adding 100% ethanol into the non-alcoholic beer. Two observers were used a day, since each trial is only 10 minutes it is not expected the demonstrators' intoxication levels will fluctuate between trials. To be sure, the two observers a day were counterbalanced allowing each to sometimes go first and sometimes second. This process continued until each observer has been grouped with the demonstrators 2 times. Numerous studies show that social transmission about food information happens very quickly. Gold et al., (2011) and Thapa et al., (2014) both used the observer demonstrator model to examine the abolishment of hippocampus-dependent memories and needed only one 30-minute trial to produce a food preference memory.

Sign tracking and two bottle choice test phase. Once the observers completed the prior social transmission phase the sign-tracking procedure began. After being placed into the apparatus, a pre-programmed mechanical arm pushed the 4% beer sipper bottle into the apparatus so the animal had access to the alcoholic beer inside. All observers were given 4% beer as their CS, regardless of their earlier group. After a duration of 10s, the mechanical arm retracted itself and the sipper bottle was no longer accessible. As soon as this was finished, a sugar pellet was dispensed into the food tray on the immediately adjacent wall. Twenty-five bottle-food pellet pairings were given per daily session with a 60s inter-trial interval. The animals were not be moved, touched, or interfered with in any way during the session. Once the 25 trials were complete, the animal was removed from the sign-tracking apparatus and placed

back in their home tower. Prior to being placed in the sign-tracking apparatus the bottles containing alcoholic beer were weighed. The sipper bottle was weighed a second time after the sign tracking session. Sign tracking behavior usually takes several weeks to develop fully. Therefore, the sign tracking phase was conducted for 10 sessions per subject, Monday-Thursday.

Once per week on Fridays the sign tracking procedure was replaced by a 20-minute two bottle choice test between the 4% beer solution and non-alcoholic beer. The rats were tested in individual stainless-steel cages in the same room as the sign tracking chambers. The first test took place after social transmission but before sign-tracking. Whereas the sign tracking procedure determined if socially-transmitted information about beer increased compulsive-like behavior directed towards a bottle containing alcoholic beer, the two-bottle test determined if socially-transmitted information about beer affected voluntary choice preference between alcoholic and non-alcoholic beer. After sign-tracking behaviors stabilized, the CS was replaced with water for 4 day. This allowed the experimenters to measure pure sign-tracking without the beer's intrinsic reinforcing properties. After this, beer was restored for another 4 days of data collection totaling 18 days of sign-tracking. Once sign-tracking with the observers was complete, one final phase occurred. The demonstrators were placed in the same sign-tracking procedure as the observers, except their sipper bottle CS contained water. This phase was useful similarly to when the observes were switched to a water CS; because it allows the researchers to examine a group that has pure sign-tracking exposure without the addition of a pharmacologically active, caloric CS. Furthermore, this group had no previous exposure to a beer CS in a sign-tracking paradigm, allowing for cleaner results when compared to the observers.

Statistics

Data was analyzed as a one factor or two factor Analysis of Variance (ANOVA) with Days (sign-tracking behavior) and CS interval (goal-tracking behavior) as a within-subjects factors and Treatment Group as a between-subjects factor. Partial eta-squared (η^2_p) from the SPSS analysis are reported as a measure of effect, with effect sizes labeled as small (η^2_p = 0.009), medium (η^2_p = 0.09), or large (η^2_p = 0.25) (Field, 2013). Calculated Cohen's D are reported as a measure of effect for paired t-tests, with effect sizes labeled as small (d= .2), medium (d=.5), or large (d=.8) (Field, 2013).

Results

Demonstrator rats: Sign tracking and goal tracking performance

To confirm that the training procedures used in the current experiment results in sign tracking behavior in at least some animals the Demonstrator rats were trained daily in the sign tracking procedure with water in the bottle after the social transmission phase was completed and the demonstrator rats had completed their sign tracking training. The demonstrator rats sign tracking data are presented first before presenting the performance of the observer rats. The food dispensers were discovered not to be working on Day 10, thus the data from this day was omitted from the analysis. Figure 1 shows that the average licks per trial increased over days and reached asymptote by day 8. A one-factor repeated measures ANOVA confirmed a statistically significant main effect of days, $F(13, 91) = 4.71$, $p = .000$, $\eta^2_p = .402$, indicating a large effect size.

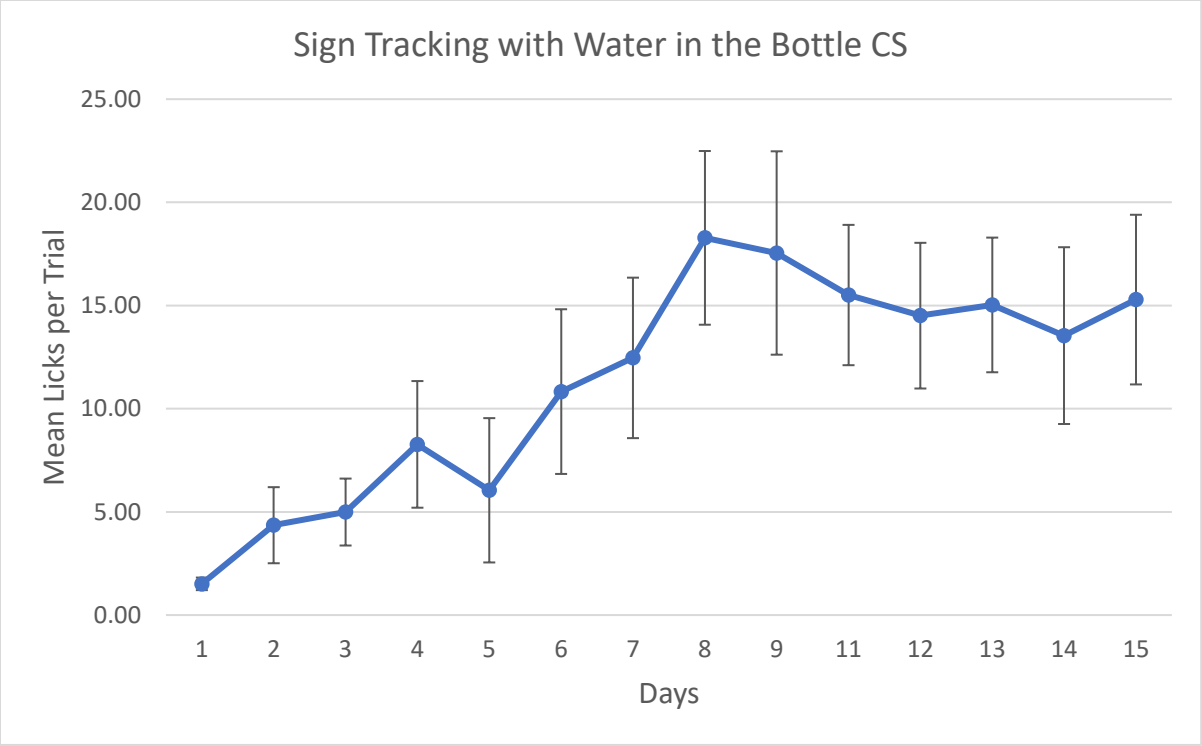

Figure 1. Mean licks per trial (sign tracking) of Demonstrator rats over 15 days of training in the sign-tracking procedure with water in the bottle CS. Day 10 was omitted from the analysis because the food dispensers were not working on this day.

Goal tracking is demonstrated when the rats head poke into the food tray more frequently during the presentation of the bottle (CS) than in the equivalent period just prior to the bottle presentation (Pre-CS). Figure 2 shows the mean head pokes per trial during the Pre-CS and CS across training days (day 10 was excluded because of food dispenser malfunction on this day). Head pokes were consistently higher during the bottle presentation (CS) compared to the equivalent amount of time just before the bottle entered the chamber (Pre-CS). This difference, which indicates goal tracking behavior, appeared to increase further by the end of training. A 2 (CS interval: CS, Pre-CS) x 15 (Days) repeated measures ANOVA revealed a statistically significant main effect of CS interval, $F(1,7) = 11.28$, $p = .01$, $\eta^2_p = .617$, a large effect size.

However, the CS Interval x Days interaction did not reach statistical significance, $F(13,91) = 1.51$, $p = .13$, $\eta^2_p = .177$. These results confirm that the experimental procedure used in this laboratory induces sign-tracking and goal-tracking behavior in Sprague-Dawley rats.

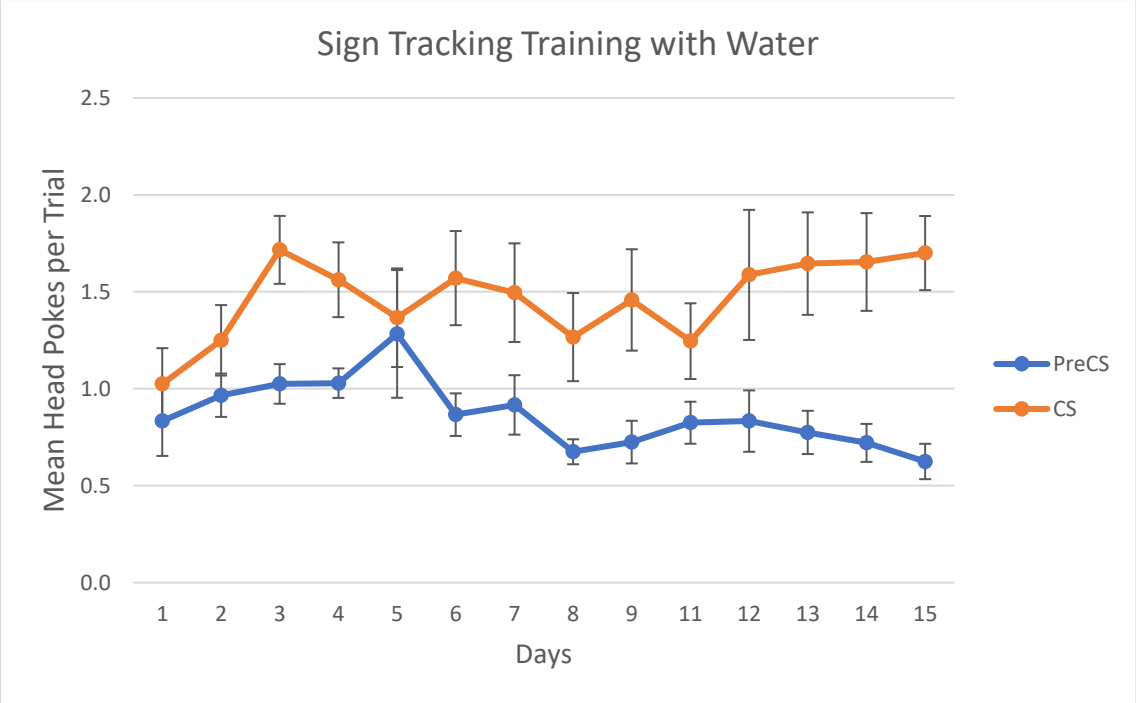

Figure 2. Mean head pokes per trial during the Pre-CS and CS period of Demonstrator rats over 15 days of training in the sign-tracking procedure with water in the bottle CS. Day 10 was omitted from the analysis because the food dispensers were not working on this day. Greater head pokes during the CS relative to the Pre-CS period indicates goal tracking behavior.

Observer Rats

Baseline Beer Preference Test After Social Transmission. After the social transmission procedure, the Observers were given a two-bottle choice test between alcoholic and non-alcoholic beer. This test verified the development of a social-based, enhancement of alcohol consumption in the experimental group relative to the control. Figure 3 shows this baseline beer preference in the AOG and NAOG subjects. A 50% preference would indicate the equal

consumption of alcoholic and nonalcoholic beer (no preference). A preference for alcoholic beer would result in a value greater than 50% whereas a preference for non-alcoholic beer would yield a value less than 50%. Although the preference scores were below 50% the AOG group showed a higher consumption for alcoholic beer in comparison to the NAOG group. An independent samples t-test statistically confirmed this difference in preference scores between the AOG (M= 24.1, SD= 9.5) versus the NAOG group (M= 15.6, SD= 2.5); $t(14)= 2.42$, $p= .021$, d= 1.224. Cohen's D demonstrates a large effect size here.

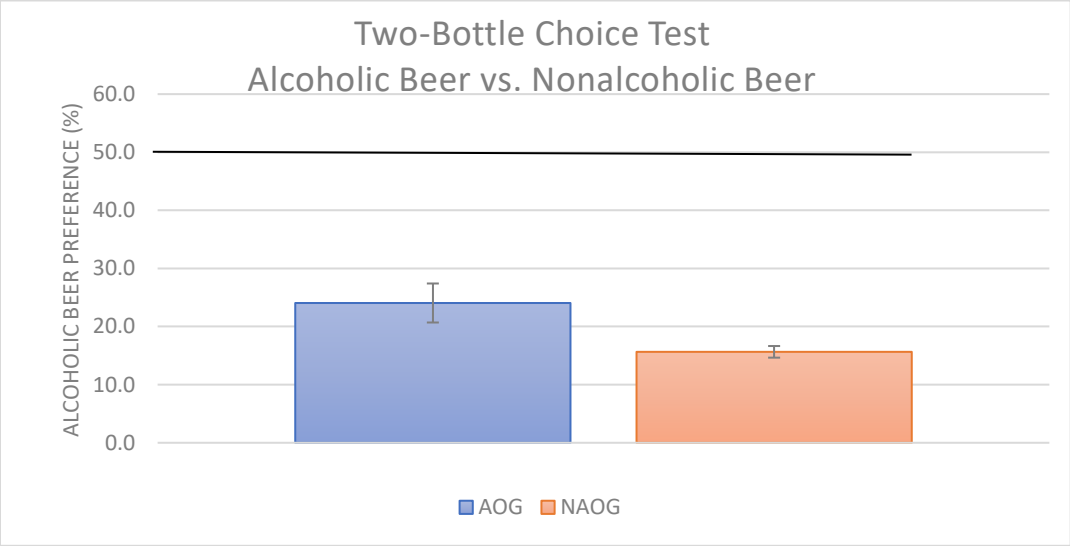

Figure 3. Alcoholic beer preference, prior to sign-tracking, in the AOG and NAOG subjects.

The sign-tracking paradigm can create two separate behaviors, goal- and sign-tracking (Figures 1 & 2). Goal-tracking is functionally sounder as it involves using the CS to make a prediction about the appearance of the US, a basic predicate of classical conditioning. Sign-tracking may be considered aberrant since it has no value for the organism in the sign tracking paradigm. Individual organisms may show a natural affinity towards sign-tracking by interacting with the CS early on. Others may goal-track more readily. Though subjects may have an orientation toward one of these separate behaviors, either behavior can be present in any given trial. This

means an organism has the potential to perform both types of behaviors regardless of their natural disposition. The general pattern seen when applying this procedure is that animals tend to start by learning to head poke for the US by using the CS as a predictor. Head poking may increase to a certain extent but eventually sign-tracking behaviors develop and this diminishes head poking to the US in favor of interacting with the CS. This general pattern is variable, with goal-trackers being more resilient to being conditioned toward sign-tracking and vice versa. The co-occurrence of these behaviors can be seen in the forthcoming graphs for mean head pokes and mean licks per trial. The general pattern can be seen to emerge; head pokes gradually decrease as licks emerge. Even though the quantity of both behaviors may change, the subjects continue to exhibit head pokes and licks throughout conditioning.

Sign Tracking Training After Social Transmission

The observers began the sign-tracking procedure after receiving the first Two-bottle choice test. Sign-tracking with alcoholic beer in the sipper bottle lasted for 10 days, followed by 4 days with water replacing beer and another 4 days with alcoholic beer returned to the bottle for a total of 18 days of sign tracking training. Here we will examine only the first 10 days of training, the other manipulations and results will be addressed later in the results when the logic for the manipulations are provided. Mean licks per trial increased as a function of Days (Figure 4). A 2 (Group; AOG, NAOG) x 10 (Days 1-10) Mixed Design ANOVA revealed a main effect of Days, $F(9,72)=4.05$, $p= .0001$, $\eta^2_p = .336$, indicating an increase in sign-tracking behaviors over the first 10 days, with a large effect size.

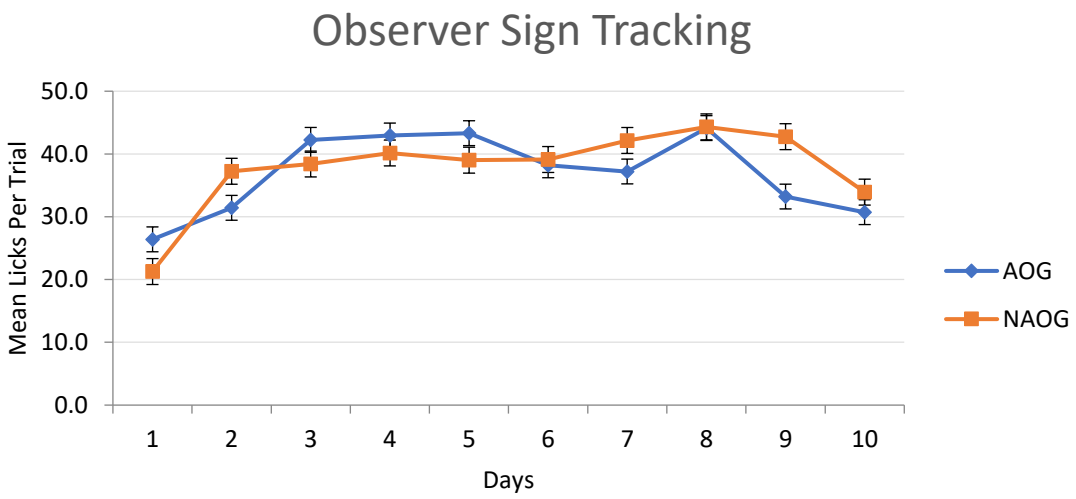

Figure 4. Sign-tracking measured as mean licks per trial during the first 10 days of sign tracking with alcoholic beer in the bottle CS.

Figures 5 show mean head pokes into the receptacle over all 18 days of training. Head-poking during the CS and Pre-CS periods decreased over the first 10 days. The decline in head poking during the CS is likely due to the emergence of sign tracking behavior because the animals cannot goal- and sign-track simultaneously, as sign-tracking increases goal-tracking must decrease. This suggests the subjects were sign-tracking when the CS was accessible. However, head poking during the CS never increased to levels above head poking during the Pre-CS period indicating that little if any goal tracking was occurring during CS presentation (for comparison see goal tracking behavior of the Demonstrator rats in Figure 1). A 2 (Group; AOG and NAOG) x 2 (CS Period; Pre and During) x 10 (Days; 1-10) Mixed Design ANOVA confirmed that there were was an overall decline in head pokes over days, $F(1,14)= 5.91$, $p=.01$, $\eta^2_p= .297$. Despite the decrease in head pokes over days, the same statistical analysis showed a small effect size and no main effect of CS Period, $F(1,14)=1.18$, $p=.296$, $\eta^2_p= .078$. The absence of goal tracking may be related to the palatable taste or caloric value of the alcoholic

beer in the bottle CS. Since the beer CS has some incentive value per se, it may drive licking behaviors outside of the current conditioning procedure. That is, the palatable taste of the beer may have kept the rats at the bottle, interfering with goal-tracking behavior and mimicking sign-tracking behaviors. To determine if these animals had acquired any conditioned goal-tracking behavior during the procedure the beer was replaced with water on Days 11-14 (Figure 5, middle panels). A 2 (Group; AOG and NAOG) x 2 (CS Period; Pre and During) x 4 (Days; 11-14) Mixed Design ANOVA produced a main effect of CS period where the average head pokes were higher during the CS compared to pre CS, $F(1,14)= 17.56$, $p=.001$, $\eta^2_p= .556$. This finding shows goal-tracking when the bottle CS is present and contains water which has little incentive value in non-thirsty rats. When water was present the animals would goal-track more during the CS than the Pre CS. When beer was replaced back into the sipper bottles from Days 15-18, the sign-tracking behaviors re-emerged in favor of goal-tracking behaviors, supporting the idea of beer's incentive value influencing the paradigm. This was shown with a 2 (Group; AOG and NAOG) x 2 (CS Period; Pre and During) x 4 (Days; 15-18) Mixed Design ANOVA which produced no main effect of CS Period, $F(1,14)= .138$, $p=.716$, $\eta^2_p= .01$. This indicates there were no differences across the Pre-CS and CS periods, meaning goal-tracking was interrupted when beer was re-introduced.

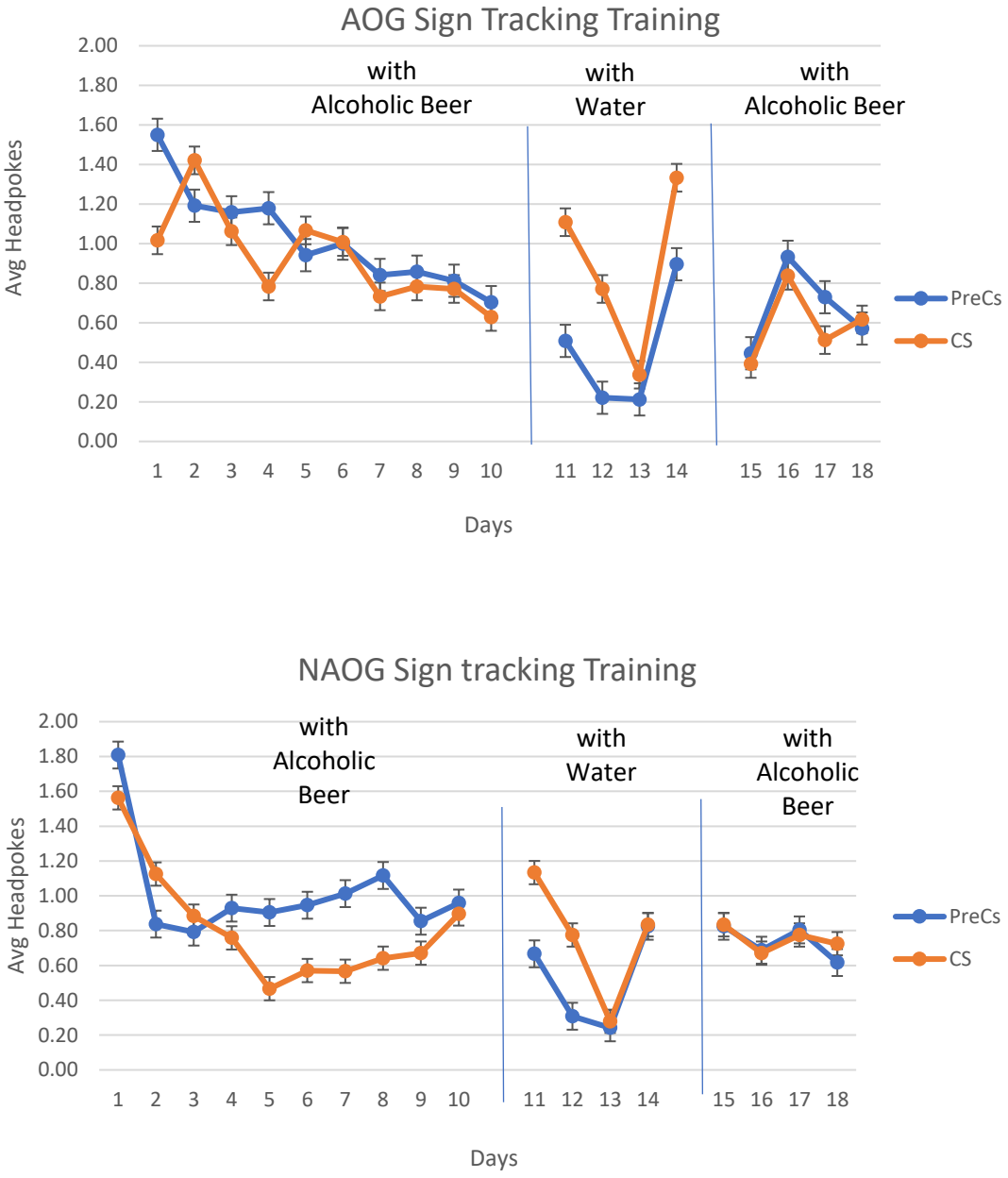

Figure 5. Goal-tracking behaviors were measured as head pokes into the receptacle for 18 days of training for the AOG (top panel) and NAOG (bottom panel) groups. Days 11-14 used a water CS to compare goal-tracking behaviors with and without 4% beer.

The goal tracking data indicates that we cannot conclude that sign-tracking behavior during the first 10 days of training (as shown in Figure 4) actually occurred when alcoholic beer was the solution in the bottle CS. Rather, the licking behavior (our operational definition of sign tracking) may have been driven by a CS that was not intrinsically neutral. One way to determine how much of the licking behavior is due to classically conditioned sign tracking and how much is due to the taste or caloric content of the beer is to examine licking during days when beer is replaced with water. Figure 6 shows mean licks per trial on the CS for each daily session, over all 18 days of sign tracking, and between both groups. The first 10 days repeats the data from Figure 4 followed by 4 days with water replacing beer and another 4 days with alcoholic beer returned to the bottle. When the 4% beer was replaced with water, sign-tracking behaviors decreased but were not eradicated. When alcoholic beer was replaced in the sipper bottle, for Days 15-18, sign-tracking behaviors were restored to their earlier levels. Notably, the quantity of sign-tracking with water for the Observers is similar to that of the Demonstrators, seen in Figure 1. A 2 (Group; AOG, NAOG) x 3 (Blocks; Days 7-10, Days 11-14, Days 15-18) Mixed Design ANOVA revealed a main effect of blocks where blocks 1 and 3 were significantly greater than block 2 but not significantly different from one another, $F(2, 28)= 46.147$, $p= .000$, $\eta^2_p = .767$. The same analysis showed no main effect of Group between the AOG and NAOG, $F(1,14)= .009$, $p=.927$, $\eta^2_p = .001$. Similarly, no Group x Block interaction was found $F(2,28)= .263$, $p= .771$, $\eta^2_p = .018$, indicating the two groups did not differ at any point throughout sign-tracking. This suggests that sign tracking was present in at least some rats as licks persisted after the abolition of beer. Also, the social transmission procedure did not influence sign-tracking behaviors differentially between the AOG and NAOG.

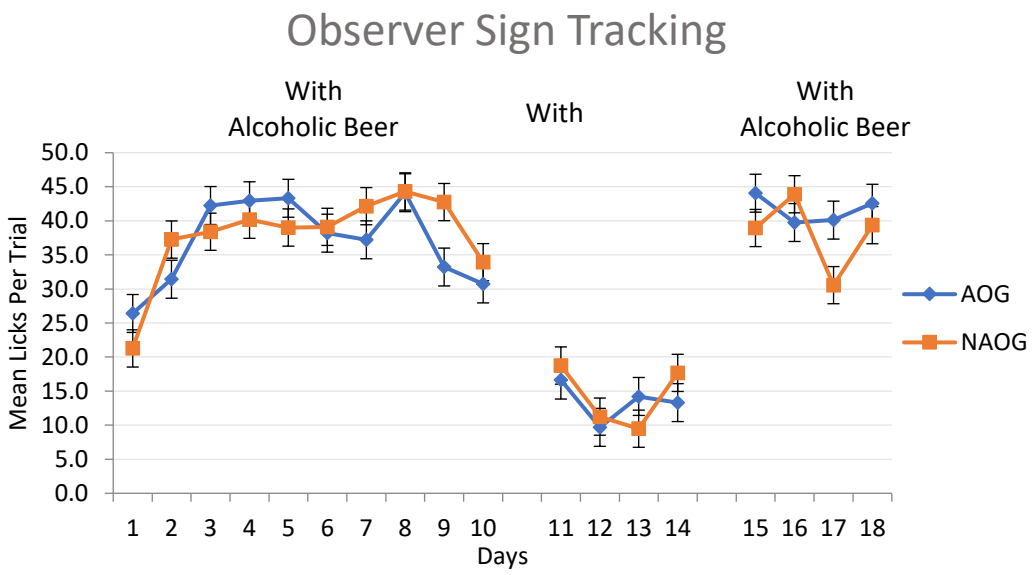

Figure 6. Sign-tracking measured as mean licks per trial for each day. There was a total of 18 days where Days 1-10 and 15-18 used 4% beer in the CS and Days 11-14 used water. Water was used to see if sign-tracking behavior was preserved when the taste and caloric value of the beer was removed. The first 10 days shows the same data as in Figure 4.

Preference Tests

Although sign tracking behavior was not enhanced by the social transmission procedure it is possible that the combination of sign tracking of a palatable alcoholic beer and the experience of social transmission together may have increased consumption of alcoholic beer to a point where there is a preference for alcoholic beer over non-alcoholic beer. Figure 7 shows five preference tests that were given during the course of the experiment. The first one took place before sign-tracking yet after social transmission. Four weekly beer preference tests were also conducted after sign-tracking had begun. A 4 (Tests; 1-4) x 2(Group; AOG and NAOG) Mixed Design ANOVA did not yield any differences between groups $F(1,14)= .238$, $p=.633$, $\eta^2_p= .017$, and no Group x Tests interaction $F(3,42)= .094$, $p= .963$, $\eta^2_p= .007$ These results differ when

compared to the initial two-bottle choice test which showed the AOG having a stronger preference after social exposure. Also, the same analysis showed no differences in preference between the 4 tests, $F(3,42)= 1.57$, $p=.211$, $\eta^2_p= .101$. After just 1 week of exposure to alcoholic beer in the sign tracking procedure both groups increased their consumption of alcoholic beer during the two-bottle choice test. This was supported by a paired-sample t-test where the AOG's consumption at baseline ($M= 24.1$, SD= 9.5) was lower than their first test after conditioning began ($M= 54.9$, SD= 15.0); $t(7)= -4.50$, $p= .003$, d= 2.458. The same pattern was observed in the NAOG where baseline ($M= 15.7$, SD= 2.5) showed less consumption than the first post-test ($M= 47.7$, SD= 10.9); $t(7)= -8.46$, $p=.000$, d=4.063. Additional weeks of training in the sign tracking procedure failed to increase further the consumption of alcoholic beer. Neither group developed a preference for alcoholic beer over non-alcoholic beer.

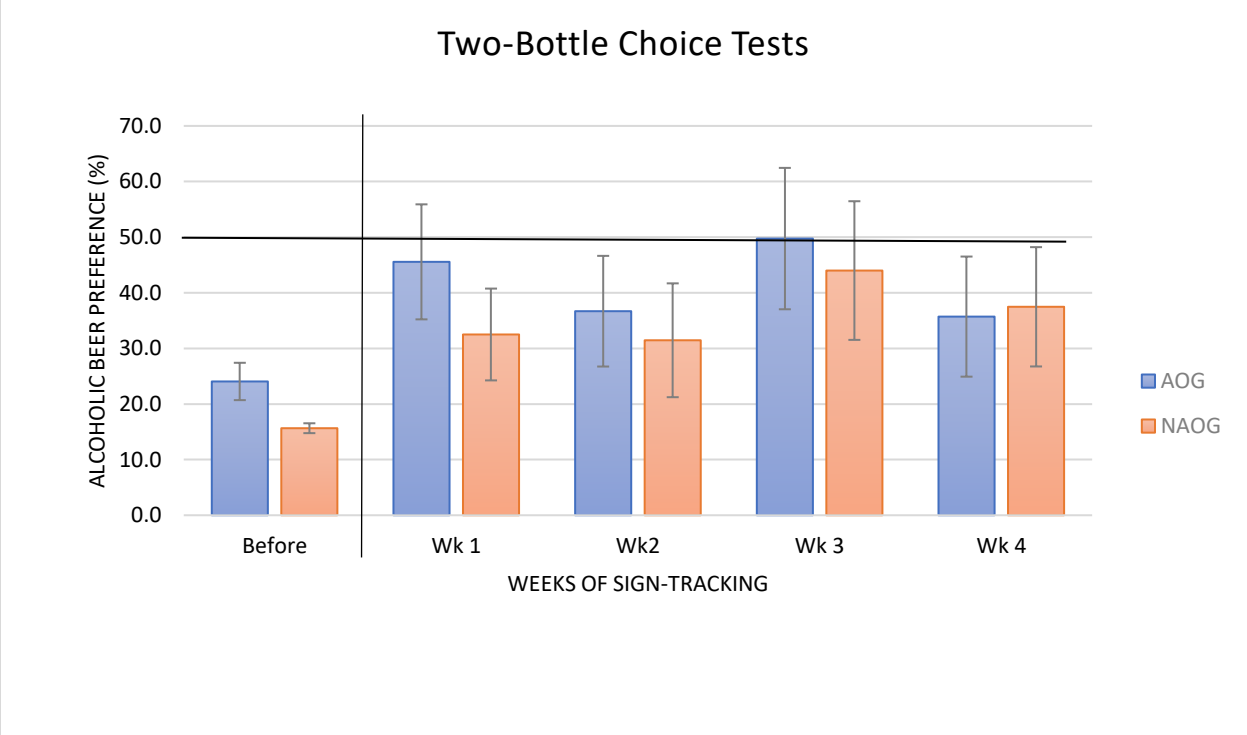

Figure 7. Preference was measured as the percentage of alcoholic beer drank compared to total consumption of both alcoholic and non-alcoholic beer.

Discussion

Addiction to alcohol is a gradual process often taking many months or years before permanently influencing the structure and function of the brain (American Psychiatric Association, *Diagnostic and statistical manual of mental disorders*, 2013; National Institute of Drug Abuse, 2014). Understanding these earlier stages informs later stages of addiction, potentially leading to preventative interventions on a clinical level. The early stages are complex and many different psychological, biological, and genetic variables play a role (Ahmed et al., 2013). The direct positive reinforcement experienced via alcohol's intrinsic incentive value may be one factor that increases alcohol consumption. However, behaviors related to drug reward tend to be overshadowed in favor of normal, adaptive behaviors such as self-grooming or social interaction, in animals (Alexander et al., 1981). This creates a need to investigate variables and situations that can de-rail these adaptive behaviors and eventually exacerbate to the point of addiction. Environmental variables drive these early behaviors via social cues, schedules of reinforcement, and other various stimuli (Ahmed et al., 2013). Though social interaction often serves as an alternative to consuming a drug, it can also influence the establishment of a preference for drugs or other consumables. This means social cues may help initiate maladaptive behaviors and pave the way for later problems. Conditioning phenomena resulting from personal experiences with repetitive environmental stimuli can also influence a multitude of behaviors, including those related to drugs. Sign-tracking is an example of an experimental paradigm that is capable of compelling behaviors toward a stimulus, whether it be a drug or not (Tomie et al., 2008). An addicted person or animal may end up conditioned toward a drug if it is often paired with reinforcing, species-specific cues. These different kinds of variables have been discretely

examined in previous studies and their influence inferred. However, the present study aimed to combine two of these paradigms in a novel way. The two (social preference and sign-tracking) were conducted consecutively, attempting to build a simple, early chronology that mimics the nascent path to addiction.

Observer-Demonstrator Effect. The first component of this study attempted to create an ethanol preference in the test group via social exposure to an ethanol exposed conspecific, an effect seen in previous experiments (Galef et al., 1988; Hunt et al., 2001). The intention was to see if the success of this procedure would summate with a conditioning procedure that also used ethanol as a variable. This paradigm has been used as a model for initiating ethanol consumption in previously ethanol naïve subjects, similar to the conditioning procedure used in this study (Tomie et al. 2002). A two-bottle choice test, using non-alcoholic and 4% beer, was used to confirm the successful transfer of an ethanol preference before the conditioning procedure began. While neither group showed an overall preference to the 4% beer over the non-alcoholic beer, the group exposed to the alcoholic-beer-drinking conspecifics did show significantly higher consumption. This indicates that the observer-demonstrator procedure created a differential preference to ethanol between the two groups, as predicted by the hypothesis.

Sign-tracking. The next stage of the experiment was conducted to ascertain if the initial ethanol preference in the AOG (compared to the NAOG) would affect the subjects' behaviors in a classical conditioning paradigm. By interacting with the bottle CS, instead of seeking the sugar pellet US, the subjects are thought to be expressing sign-tracking behaviors. Sign-tracking assumes the animal is performing an unproductive behavior considering consummatory behaviors about the bottle CS do not influence the rate of US reception (Tomie et al., 2008). Typically the solution in the bottle CS has little or no intrinsic value and therefore the animal

should only interact with the bottle CS due to a conditioned compulsion, making it maladaptive. However, the solution in the bottle CS in the present experiment contained 4% beer which was not intrinsically neutral. Using a beer solution as a CS allowed us to examine how these conditioned compulsions can apply to drug-taking when the drug has some initial intrinsic value. Drugs of abuse often have intrinsic value, and so this study has a high level of external validity. However, this also decreases internal validity and creates a situation where it is difficult to extricate the variables' individual influence on sign-tracking. In the present study sign-tracking behaviors gradually emerged, and significantly increased over days, which seems to suggest that sign-tracking behaviors occurred as is expected in this paradigm (Tomie et al., 2002). Differences between the NAOG and AOG were not found, which suggests the social transmission was ineffectual toward sign-tracking. On Days 11-14 water replaced the 4% beer in the bottle CS to assess how much of the sign-tracking behavior was due to intrinsic value of the beer or due to the compulsive drinking directed toward the bottle CS. If sign-tracking were to continue despite the absence of alcoholic beer, this would confirm the data was a result of pure conditioning. This adjustment in procedure brought about a statistically significant drop in sign-tracking. This can be taken to mean some of the sign-tracking behaviors were not indicative of actual sign-tracking; the animals were less compelled to interact with the bottle CS when it contained water than when it contained alcoholic beer. When 4% beer was restored on Days 15-18, mean licks returned to their previous magnitude. Overall, it appears that the beer and/or ethanol has its own value outside of the conditioning procedure. Presently, it cannot be said if the value stems from calories, taste, or some other unknown factor. It is noteworthy, however, that the sign-tracking behaviors did not drop to zero when water was in the bottle CS, so one can suggest sign-tracking did develop to some extent. Moreover, the level of sign tracking was

similar to sign-tracking observed in the demonstrators exposed to 15 days of the sign-tracking procedure with water only. This suggests that actual sign-tracking may have developed but was exaggerated by the beer's intrinsic value. It has been concluded that the successful induction of a demonstrator-observer ethanol preference does not have any influence in a sign-tracking paradigm. This was exemplified as a lack of statistical differences between the AOG and NAOG throughout sign-tracking. This is not in line with the hypothesis as it was thought the two procedures would summate in the AOG group. Also, it was found that even when a CS is not neutral sign-tracking will still develop but cannot be measured until the intrinsic value is removed.

Goal-tracking. Goal-tracking behaviors are suggestive of adaptive conditioning. Subjects who successfully use the CS to predict the US exemplify normative classical conditioning, as compared to the more compulsory, potentially maladaptive sign-tracking behaviors. In this study, one can clearly see the animals were performing head pokes during the CS from the beginning but the quantity slowly decreased over days. Looking at the face value of this, one may interpret this as a decrease in goal-tracking when sign-tracking is initiated. However, as seen in the previous section, much of the lick data was not quantifying legitimate sign-tracking. This creates the need for an explanation of the changes in goal-tracking. Though head pokes were occurring, this is not enough to assume goal-tracking behaviors were present. As stated previously, head pokes when the CS is present must significantly exceed head pokes during the pre-CS period. If the subjects head poke similarly during pre-CS and CS, then one cannot say the CS is being used to predict the US any better than when there is no CS. Therefore, it appears goal-tracking was not truly happening when the beer CS was administered as head pokes during and before the CS were statistically similar. A better explanation for the

decrease in "goal-tracking" is to say that the animals gradually decreased their seeking of the US because the CS was a US in itself. The appetitive behaviors toward the sugar pellet were redirected to another tasty/caloric stimulus; beer. Subjects learned there was a reinforcer in the sipper bottle they could access. By consuming it, the rate of reception for the sugar pellets did not diminish; they could drink the beer while it was present and still get the sugar pellet after. Overall, the subjects quickly began to head poke to find the US. As time went on, the animals decreased head poking when the CS was present. This was not due to goal- and sign- tracking behaviors emerging and then competing with one another. The animals simply had two US' in their paradigm and preferred to interact with the transient stimulus.

Once the beer was replaced with water, head pokes during the bottle CS significantly increased. This further supports that licking behavior during the sign tracking procedure with beer was due to the value of the 4% beer in the bottle CS for both groups. Next, we looked to see how the sign-tracking procedure affected the two-bottle preference tests. Although group differences were not apparent in sign-tracking performance, it is possible that the sign-tracking experience may have affected preference differentially in the two groups.

Two-Bottle Choice Tests. The initial preference test yielded significant differences between the NAOG and AOG where the AOG developed a larger preference than the NAOG group. It is important to note that neither group had an overall preference for alcoholic over non-alcoholic beer. This confirmed the social transfer of a preference from the demonstrators to the observers. Since the procedure was successful, a failure to find differences between the groups during sign-tracking confirms the social procedure does not influence the conditioning procedure. In other words, the lack of significant results is not due to the social procedure being unsuccessful but because the hypothesis is not supported, so far as this study can demonstrate.

The first test to take place during the conditioning procedure, showed an overall significant increase in preference for alcoholic beer compared to the baseline test but the group differences were abolished. These results suggest that though social exposure to alcoholic beer creates a preference when compared to a control group, this differential preference is washed out when the control group is exposed to alcoholic beer outside of a social paradigm. All four of the two bottle choice tests to take place during conditioning showed statistically similar levels of alcoholic beer consumption. In other words, once all groups were exposed to the beer via sign-tracking, their preferences stabilized. This means the conditioning procedure did not have any effect on alcoholic beer preference outside of the sign-tracking apparatus. Though overall 4% beer drinking did increase during sign-tracking, this did not create actual statistical preferences in either group. Other studies have found similar results where sign-tracking did not influence ethanol preferences, however this was measured as overall ethanol intake during conditioning, not as an outside two-bottle choice test (Tomie et al., 2004b). This may be because sign-tracking is a discrete paradigm and a preference test outside of this context will not change due to more conditioning. This makes sense considering sign-tracking is thought to create a compulsion; something that is hard to abolish or even influence, similar to a habit (Tomie et al., 2008). The compulsion is most likely contained within its own paradigm and loses power outside the context. It is important to note that the difficulties in measuring sign-tracking (i.e. the intrinsic value of beer) may have interfered with the relationship between the preference tests and the conditioning in some unknown way. Future studies may benefit from higher concentrations of ethanol, as the aversive taste may buffer any incentive value.

Addiction is a complex, multi-faceted issue that relies heavily on compulsions to drive behavior. Before one is compelled to take a drug, other factors drive these behaviors including

reinforcement, context, associations, etc. These factors eventually become a part of the compulsion; acting as initiators and drivers of drug-seeking and drug-taking behaviors. This study looked at a closed behavioral circuit (sign-tracking paradigm), where the behaviors were thought to be due to a compulsion. It was found that these behaviors were not influenced by prior manipulations (Observer/ Demonstrator) and did not have an influence in alternate contexts (Preference tests) which exemplifies the powerful and discrete nature of stimulus-driven behaviors. Stimuli related to a compulsion attain the ability to direct behaviors regardless of consequences or outside influence. Understanding the relationships between stimuli, drugs, and the addicted organism's physiology creates a comprehensive picture of addiction. Influencing these connections to break-down maladaptive behaviors is a goal that will become attainable once all the variables involved become better understood.

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