1999

Mechanisms Mediating Social Enhancement of Alcohol Intake in the Rat

Jennifer L. Holloway
College of William & Mary - Arts & Sciences

MECHANISMS MEDIATING SOCIAL ENHANCEMENT OF ALCOHOL INTAKE IN THE RAT

A Thesis

Presented to

The Faculty of the Department of Psychology

The College of William & Mary in Virginia

In Partial Fulfillment

Of the Requirements for the Degree of

Master of Arts

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by

Jennifer L. Holloway

1999

APPROVAL SHEET

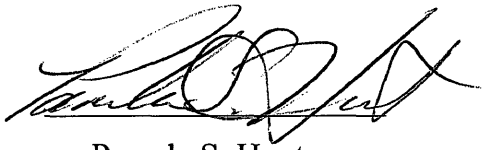
This thesis is submitted in partial fulfillment of

The requirements for the degree of

Master of Arts

Jennifer L. Holloway

Approved, May 1999



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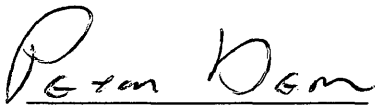

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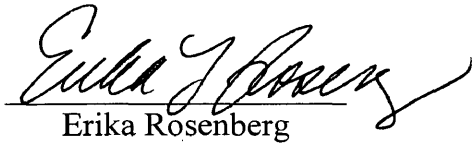

Erika Rosenberg

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ACKNOWLEDGEMENTS

I wish to express my appreciation to Pam Hunt, under whose guidance this investigation was conducted, for her patience and criticism throughout the investigation.

I would also like to thank my thesis committee, Professors Peter Derks and Erika Rosenberg, for their careful reading and criticism of the manuscript.

I am especially grateful to Elena Cuticelli and Tracy McCormick for their hard work and commitment during the many hours of conducting these experiments.

I thank my parents, Mike and Marcia Holloway, and my sister, Jessica Holloway, for their never ending support and encouragement. Their love and confidence in me has made this all possible.

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MECHANISMS MEDIATING SOCIAL ENHANCEMENT OF ALCOHOL INTAKE IN THE RAT

Abstract

Research has found that a rat's (referred to as the observer) food preference can be influenced by smelling diet cues on the breath of another rat (referred to as the demonstrator; Galef & Wigmore, 1983). More recent applications of this paradigm have demonstrated that a rat can learn to increase its alcohol preference after interacting with another rat that had been administered alcohol (Scordalakes, 1998). The purpose of this research was to examine the mechanisms underlying social learning of alcohol cues using a modification of Scordalakes' procedure. Experiment 1 examined whether an observer's alcohol preference would be dose-dependently related to the amount of ethanol given to the demonstrator. The results replicated the findings presented by Scordalakes and further indicated that the magnitude of the observer's alcohol preference was dependent upon the dose of alcohol administered to demonstrators. Experiment 2 examined whether rats would learn an alcohol preference when carbon disulfide (CS_2) was paired with alcohol odor. CS_2 and alcohol odor pairings were not shown to alter alcohol preferences in observers. Experiment 3 examined whether the transmission of alcohol odor cues through social interaction was activating the endogenous opioid system, by administering naloxone hydrochloride, a nonspecific opiate antagonist prior to the interaction phase. Naloxone injections were not shown to alter alcohol preference in observers, compared to saline injected controls. The present findings and their contributions to understanding social transmission of food preferences in rats are discussed.

Introduction

In the search for the cause and subsequent treatment of alcohol abuse, many researchers have begun to develop animal models in hopes of mimicking the characteristic features of alcohol abuse. Over the last decade, researchers have developed particular strains of alcohol preferring and nonpreferring rats in order to study the underlying genetic components of alcohol abuse (Lumeng, Waller, McBride, & Li, 1982; Waller, McBride, Lumeng, & Li, 1982). However, as is evident in human twin studies, not all alcohol abuse cases are the strict result of one's genetic makeup. There seems to be some type of interaction between genetic and environmental factors that influence an individual's vulnerability to alcohol abuse. Therefore, it is necessary to encompass both domains in order to develop a clear picture of alcoholism.

The ability to easily manipulate the environment in a laboratory setting makes the use of an animal model more advantageous when examining social influences on alcohol consumption. A rat is highly dependent upon social interactions to survive. In particular, researchers have shown that a rat's food preference can be affected by smelling the diet on the breath of another rat (Galef & Wigmore, 1983). This latter example is a particular type of social learning, and the laboratory paradigm used to study it is referred to as the demonstrator-observer procedure. The term demonstrator refers to the rat that is force fed a particular diet. The observer is a naïve conspecific that interacts with the demonstrator and will exhibit an enhanced preference for the diet that the demonstrator had consumed. This paradigm has been paramount for the understanding of the learned food preferences of adult rats. More recent applications of this paradigm have demonstrated that a rat can alter its alcohol preference after interacting with another rat

that had been intragastrically administered alcohol (Scordalakes, 1998). The implications that this paradigm might have for future research of alcohol abuse make it necessary to understand the associated mechanisms responsible for these socially learned food preferences.

As was stated earlier, a rat's food preference is highly modifiable by social interactions. There have been several studies demonstrating that early experiences of young rats affect their later food preference. Nursing and weanling pups are profoundly influenced by the feeding behavior patterns of the adult colony members. Nursing pups receive dietary cues transmitted through the mother's milk. At weaning, pups are able to distinguish between their mother's diet and the diet of another dam (Galef & Sherry, 1972), and will preferentially ingest the diet that the mother had consumed during nursing (Galef & Henderson, 1972). A weanling pup's diet is also influenced by the physical presence of adults and the residual odor cues deposited by the adult around the food site (Galef, 1977; Galef & Clark, 1971, 1972). These residual cues have been shown to direct the weanlings to their first meal of solid food (Galef & Heiber, 1976).

In the adult rat, social transmission of olfactory cues of a particular diet between a demonstrator and an observer enhances food preferences in the observer (Galef & Wigmore, 1983; Posadas-Andrews & Roper, 1983; Strupp & Levitsky, 1984). This social learning of food preferences has been deemed a "robust phenomenon" as evidenced by the fact that: first generation laboratory bred wild rats exhibit demonstrator-observer induced food preferences; observers will exhibit these food preferences regardless of whether or not they are food-deprived or nondeprived at the time of the interaction, and regardless of whether or not they are interacting with a familiar or an

unfamiliar demonstrator (Galef, Kennett, & Wigmore, 1984). Observer's food preferences can be altered following an interaction with a demonstrator lasting as little as two minutes (Galef & Stein, 1985). Studies have also found that the demonstrator does not need to be an active participant in the interaction with the observer. Observers that interacted with an anesthetized demonstrator that had its head powdered with a diet showed an enhanced preference for that diet (Galef et al., 1984).

Taken together, these studies illustrate the pervasiveness of social learning on an observer's food preference. Most studies, to date, have only studied this interaction by manipulating factors of one or both animals in the pair (i.e. having an observer interact with either an alert or anesthetized demonstrator). However, there has only been one study, to the author's knowledge, that has attempted to examine the mechanisms underlying social transmission of food preferences between rats. Galef, Mason, Preti and Bean (1988) identified a sulfur-based compound, carbon disulfide (CS_2), as being an important constituent in rat breath. In this experiment, they concluded that CS_2 was a critical component mediating social influences on diet selection in rats.

In effect, the olfactory cues specific to a given diet are perceived in combination with naturally occurring CS_2, promoting the transmission of diet choice to the observer. Although the procedures employed by Galef and colleagues (1988) allowed for only an indirect test of their hypothesis, their conclusion was that CS_2 was a necessary and sufficient condition for this type of social learning to occur. One purpose of the proposed experiments will be to more directly examine this hypothesized role for CS_2. A second question is: How is CS_2 inducing observers to alter their food preferences? In other words, how exactly is this compound exerting its reinforcing effects in the central

nervous system? These are questions that will also be addressed in the following experiments.

Previous research has laid down the foundation that there is a naturally occurring phenomenon rats exhibit in which social transmission of food cues between a demonstrator and an observer come to elicit changes in an observer's food preference. The next step is to determine the necessary components of this interaction, whether it is the presence of CS_2 or some other feature of the demonstrator, which enhances food preferences. Not only must the components be investigated, but where and how these components are evoking changes in neurochemistry needs to be examined as well.

As was stated earlier, a rat is highly dependent upon social interactions to survive. One behavior of the rat that is highly dependent on social interactions is the social transmission of food preferences. Because of the influential nature of the social interactions on learned food preferences, it is likely that some component of the demonstrator is activating the endogenous opioid system in the observers during the interaction. Research investigating the mechanisms underlying social attachment has implicated the endogenous opioids, along with oxytocin and norepinepherine, in mediating social behaviors (Nelson & Panksepp, 1998; Panksepp, Herman, Vilberg, Bishop, & DeEskinazi, 1980). In particular, researchers have proposed a brain opioid theory of social attachment in which 1) opioids decrease the effects of social separation, 2) social contact elicits opioid release; 3) the rewarding effects of opioids condition odor and place preferences; and 4) decreased opioid levels motivate animals to engage in social contact (Nelson & Panksepp, 1998). In addition to their important role in initiating and maintaining social contact, the endogenous opioids may also be involved in the social

behaviors mediating transmission of food preferences between animals. Here it is hypothesized that the social interaction may be causing the activation of the endogenous opioid system that may, in effect, be reinforcing for the acquisition of food preferences.

The purpose of this research was to examine the underlying chemical mechanisms mediating the association between social interaction and alcohol preference in the rat. The project consisted of three experiments. Experiment 1 implemented Galef's demonstrator-observer procedure to induce alcohol preferences in observers. This study was intended to replicate and expand upon the findings observed by Scordalakes (1998). Experiment 2 was conducted to examine whether this demonstrator-observer interaction can be explained in terms of simple Pavlovian conditioning. Specifically, whether there is some component of the demonstrator-observer interaction that is acting as an unconditioned stimulus (which can be specifically identified) that is being paired with the conditioned stimulus (alcohol odor cues). Based on the study conducted by Galef and colleagues (1988), we believe the unconditioned stimulus is CS_2. Experiment 3 was conducted to further examine whether the component (e.g. some feature of the demonstrator) mediating the demonstrator-observer interaction is activating the release of endogenous opioid neurotransmitters. This was accomplished through pharmacological blockade of the opioid system with the opioid antagonist, naloxone hydrochloride.

Experiment 1

Using a modification of the demonstrator-observer procedure, it has been shown that a rat's (observer) alcohol preference is altered after interacting with another rat (demonstrator) that had been intragastrically administered alcohol (Scordalakes, 1998). In this study, a dose of 1.5 g/kg of ethanol (EtOH) was administered to the demonstrator.

This dose was chosen based on studies indicating that it resulted in a sufficient and detectable amount of alcohol being eliminated on the breath (Molina & Chotro, 1989a). If there is a threshold amount of alcohol an observer must be exposed to in order to induce an alcohol preference, then decreasing or increasing the dose administered to the demonstrator should result in alterations in alcohol preference exhibited by the observer.

In Experiment 1, a modification of Scordalakes' procedure (Scordalakes, 1998) was used to determine if an alcohol dose-response curve would be obtained in terms of its effects on an observer's alcohol preference after the observer was allowed to socially interact with a demonstrator, administered either a 0.0, 1.0, 1.5, or 3.0 g/kg dose of EtOH. The question was whether an observer's alcohol preference could be altered by (1) lowering the amount of alcohol administered to demonstrators, resulting in an inability of the observer to perceive the stimulus on the demonstrator's breath, and (2) increasing the amount of alcohol administered to demonstrators, resulting in more salient odor cues that the observer would perceive. If 1.5 g/kg is the minimum dose of EtOH necessary for socially inducing alcohol preferences, then lowering the dose of administered EtOH to 0.0 or 1.0 g/kg should reduce the magnitude of the alcohol preference of the observer. If observers are unable to detect the alcohol odor resulting from the lower dose, then they should show no increase in preference for alcohol. Increasing the dose of administered EtOH to 3.0 g/kg should increase the magnitude of the observer's alcohol preference. If a higher dose of alcohol produces more salient odor cues on the demonstrator's breath, then observers should consume more alcohol than observers in the 1.5 g/kg group.

Method

Subjects

Ninety-four experimentally naïve, 26-35 day-old Sprague-Dawley-derived rats from 13 litters were used as subjects. Animals were born and maintained in the animal vivarium of the psychology department at the College of William & Mary. Animals were weaned from their mother on days 21-23 and group housed with littermates in standard opaque cages with wood chip bedding. Animals had free access to ProLab rat chow and water. The vivarium was maintained on a 12:12 hr light:dark cycle, with light onset at 0700 hrs. All procedures occurred during the light portion of the cycle.

Apparatus

Same-sex demonstrator-observer pairs were housed in hanging wire cages (24.2 x 17.8 x 17.9 cm).

Intragastric Administration

Demonstrators were intubated using 5 ml syringes with flexible polyethylene tubing (PE-50, Clay Adams) attached to a 23-gauge needle.

Intragastric Solution

The intragastric administration procedure was modified from that used in an experiment reported by Molina and Chotro (1989a). The volume of ethanol (EtOH) intragastrically administered to the demonstrators was calculated based on the dose of EtOH (0.0, 1.0, 1.5, or 3.0 g/kg) using a 12.0% v/v EtOH solution that was dissolved in a tap water vehicle. The dose of EtOH was calculated by multiplying the animal's body weight (measured in grams) by 0.01, 0.015, or 0.03 for the 1.0, 1.5, or 3.0 g/kg groups,

respectively. The 0.0 g/kg group was administered tap water in a volume equivalent to the 1.5 g/kg group.

Testing Solutions

The ethanol solution used for the measurement of observer's ethanol intake was the same as that used by Scordalakes (1998). Observers were given a choice between two solutions: 5.6% v/v ethanol that was dissolved in a tap water vehicle and 1.5% w/v decaffeinated coffee (Sanka) that was dissolved in a tap water vehicle. These solutions were chosen based on the observation that experimentally naïve rats of this age prefer them equally (Scordalakes & Hunt, 1998, unpublished data). Each solution was placed in 50 ml graduated drinking tubes and hung on the outside of the cages.

Procedure

The experimental procedure was as follows (see Figure 1):

(1) To familiarize animals to the cages, demonstrator and observer pairs were housed together with free access to Prolab rat chow and water for a 2-day period. Same-sex sibling, demonstrator-observer pairs were randomly assigned to the water control group (0.0 g/kg; $\underline{n} = 12$) or one of three ethanol groups, 1.0 g/kg ($\underline{n} = 12$), 1.5 ($\underline{n} = 12$), or 3.0 g/kg ($\underline{n} = 11$). Each animal was handled on both days for approximately 30 seconds.

(2) On day 3, demonstrators were moved to a separate cage and allowed free access to food and water. At that time, the observers were water-deprived, but given free access to food. This social deprivation has been found to increase the amount of time animals stay in physical contact when reunited (Panksepp & Beatty, 1980), ensuring that the pair engaged in the necessary

perioral contact (Galef & Stein, 1985). The water deprivation ensured that the observers would be motivated to drink detectable volumes of the novel solutions during the intake test.

(3) On day 4, 23 hrs following separation, demonstrators were intubated with the appropriate volume of solution (12% v/v ethanol for the 1.0, 1.5, and 3.0 g/kg groups or tap water for the 0.0 g/kg group). Intubation consisted of placing the tube over the tongue and passing it down the esophagus into the stomach. The solution was infused over a 15-20 second period. This procedure produced little struggling or other obvious signs of distress from the animals. Animals were intubated in order to minimize any traces of the solution on the facial area of the demonstrator. This reduced the potential of observers tasting the solution. Demonstrators were placed back into their cages for 30 minutes. This time period was chosen based on studies indicating that this interval resulted in a sufficient amount of alcohol being expired on the breath (Molina & Chotro, 1989b).

(4) Thirty minutes following intubation, demonstrators were placed into the observer's cage and were allowed to interact with the observer for 30 minutes.

(5) After this interaction period, demonstrators were moved to their holding cages and observers were offered two drinking bottles, one containing 5.6% v/v EtOH and one containing 1.5% w/v coffee. Coffee and EtOH bottles were randomly placed on the right or left side of the cage to eliminate any bias of side preferences when animals drank. Pretest measurements were taken to record the amount of solution initially present in the drinking bottles.

(6) On day 5, 24 hrs after bottle placement, post-test measurements were recorded for the amount of each solution remaining in the drinking tubes. The amount of each solution the observers ingested was calculated by subtracting the pretest measurements from the post-test measurements.

Data Analysis

Observers' alcohol preferences were calculated by converting the amount of EtOH ingested into percentage scores.

% EtOH preference = (amount of EtOH ingested/ total fluid intake) x 100

The % EtOH preference data was analyzed using a one-way analysis of variance (ANOVA). Separate planned comparisons were conducted with t-tests. In all cases, the α level was set at .05.

Results

Gender Differences

The total amount of fluid intake was found to differ as a function of gender, with male observers having a higher fluid consumption. An independent-samples t-test on total intake yielded a significant effect of gender, $t(45) = 2.13$, $p < 0.05$. However, the increased fluid consumption in males did not result in a greater ethanol (EtOH) preference. There were no differences in EtOH preferences between male and female observers, $t(45) = 0.02$. Therefore, the data were analyzed by collapsing across gender.

Ethanol Preference

A one-way ANOVA conducted on the data obtained during the testing phase revealed that there were no differences in EtOH preferences exhibited by the 0.0 g/kg, 1.0 g/kg, 1.5 g/kg, and the 3.0 g/kg groups, $F(3,43) = 1.68$. The mean percent EtOH

preferences of observers are shown in Figure 2. Separate planned comparisons, however, revealed a significant increase in EtOH preference for the 1.5 g/kg group, $t(22) = 2.22$, $p < .05$. Moreover, the 1.0 g/kg animals exhibited higher mean EtOH preferences than the control animals, although planned comparisons found these differences to be nonsignificant, $t(22) = .75$. The mean percent EtOH preference of the 3.0 g/kg group was equivalent to the 0.0 g/kg group, $t(21) = .08$. Additionally, there were no differences in the amounts of total fluid intake between the groups, $F(3,43) = .03$ (see Table 1).

Discussion

The primary findings of Experiment 1 indicate that a rat's alcohol preference can be altered after interacting with another rat that had been administered alcohol. These data replicate the findings presented by Scordalakes (1998) of increased alcohol preferences of observers that had interacted with demonstrators in the 1.5 g/kg group. Additionally, these findings indicate that the magnitude of the observer's alcohol preference was dependent upon the dose of alcohol administered to demonstrators. Of the doses tested, it appears that 1.5 g/kg is a critical dose for eliciting the increased alcohol preference.

Previous research by Molina and Chotro (1989a) investigated whether alcohol odor cues acted as conditioned stimuli when an appetitive reinforcer (sucrose infusion) was paired with varying doses of administered alcohol. The researchers intragastrically administered a dose of either 0.0, 0.37, 0.75, 1.5, or 3.0 g/kg ethanol to rats. Animals in the paired group received infusions of sucrose 30-60 minutes following ethanol administration, a time when animals were purportedly experiencing their own respired alcohol cues (Molina & Chotro, 1989b). Animals in the unpaired group received sucrose

infusions 60 minutes prior to ethanol administration. Twenty-four hours later, pups' subsequent alcohol preferences were tested by measuring voluntary intake of an alcohol solution. Among the doses tested, they found that doses of 0.37 and 0.75 g/kg paired with the appetitive reinforcer did not alter voluntary alcohol intake. Furthermore, a dose of 1.5 g/kg ethanol resulted in the greatest alcohol intake. Although different procedures were used, the present study similarly found that a dose of 1.0 g/kg ethanol given to demonstrators failed to significantly increase alcohol preference in the observers.

Although not statistically significant, the mean percent alcohol preference increased dose-dependently up to 1.5 g/kg. The apparent difference between the mean percent alcohol preference of the 1.0 g/kg (62.3%) and the water controls (52.6%) suggests that the alcohol odor was detected by animals in the 1.0 g/kg group, but was not salient enough to substantially change alcohol preference. Kamin and Schaub (1963) observed that associations between a conditioned stimulus (CS) and an unconditioned stimulus (US) were acquired faster, and that a greater conditioned response (CR) was exhibited, when more intense stimuli were used. According to this idea, therefore, the 1.5 g/kg group exhibited a stronger association between the alcohol odor (CS) and the demonstrator (US), as well as a greater alcohol preference (CR) than the 1.0 g/kg group because the dose of 1.5 g/kg resulted in a more intense conditioned stimulus.

Furthermore, Rescorla and Wagner (1972) developed a mathematical model of conditioning which stated that identical unconditioned stimuli can have varying effects on conditioning, depending upon how well available antecedent stimuli predict the US. They argued that changes in the conditioned value of a CS due to a CS-US pairing were a function of (1) the extent of prior learning about the CS, and (2) the extent of prior

learning about the entire stimulus complex in which the CS occurred. Rescorla and Wagner observed that differing CSs that were equally reinforced with the US might acquire differing associative strengths if the CSs differ in salience. In other words, if the CSs differ on some noticeable dimension (e.g. brightness) and are equally reinforced with the US, then different rates of conditioning to the CS can occur. Therefore, both the 1.5 and 1.0 g/kg groups were presumably receiving equal amounts of reinforcement with their demonstrators (US); however, the 1.0 g/kg exhibited less alcohol preference when compared with the 1.5 g/kg group because the alcohol odor cues were less salient on the 1.0 g/kg demonstrators' breath.

The salience of ethanol odor cues eliminated on the demonstrator's breath is a function of blood alcohol levels (BALs). Research has found several factors that contribute to blood alcohol levels (BALs) resulting from intragastric administration. In particular, Molina, Chotro, and Spear (1989) demonstrated that peak BALs were dependent upon the dose of administered alcohol as well as the time of assessment in 11-day-old rats. They intragastrically administered alcohol in doses of 1.5 or 3.0 g/kg to 11-day-old rats, and also varied the time between administration and sacrifice. Their results showed that peak BALs for the 1.5 g/kg group (120-140mg%) and the 3.0 g/kg (greater than 250mg%) both occurred 30 minutes after intragastric administration. However, there has been a lack of consistent data in studies that assessed peak BALs across developmental ages. Research has found that peak BALs following intragastric alcohol administration were higher in 1-10 day-old rats than in 15-60 day-old rats (Kelly, Bonthius, & West, 1987). Additionally, Hollstedt and Rydberg (1985) found that younger rats (25 g) that had been injected with alcohol into the peritoneal cavity (i.p.) had

higher peak BALs than older animals. L. P. Spear, Moody, Frambes, & N. E. Spear (1991), however, have found that 16- and 26-day-old rats had lower BALs than 36- and 60-day-old rats at 30 minutes after alcohol administration. Zorzano and Herrera (1989) even reported seeing no differences in peak BALs at 30 and 60 minutes after alcohol administration among 10-, 20-, and 30-day-old rats.

What are these peak BALs measuring? Molina and colleagues (1989) argue that orosensory alcohol processing occurs during peak BALs. They found that a dose of 1.5 g/kg ethanol resulted in BALs that peak and remain stable, as measured by gas-liquid chromatography, 30-60 minutes after administration in 11-day-old rats. Additionally, 11-day-old rats were administered a dose of 1.5 g/kg ethanol and 30 minutes after administration, sufficient for reaching peak BALs, the rats experienced a footshock. Twenty-four hours later, pups were tested for alcohol preference, as assessed in terms of voluntary ethanol intake. They found that rats that had been administered alcohol and then given footshocks 30-60 minutes later exhibited decreases in the amount of alcohol consumed. They concluded that 11-day-old rats perceived the orosensory cues eliminated on their breath when BALs were at a peak and stable level, approximately 30-60 minutes after alcohol administration. Furthermore, these rats were able to associate the orosensory cues (CS) being respired with the footshock (US) and subsequently exhibited an aversion toward alcohol.

There has been a considerable amount of research examining the properties of alcohol eliminated through respiration. In particular, researchers interested in alcohol elimination have found that alcohol is mainly metabolized into inactive products, but that 10-12% of the administered alcohol is eliminated, unmetabolized, through respiration,

salivation, perspiration, and urination (Goldstein, 1983; Hollstedt, 1981). Moreover, infant rats, such as the 11-day-old rats in Molina's study (Molina et al, 1989), are presumed to eliminate unmetabolized alcohol at a higher rate than adult rats (Abel, 1984; Hollstedt & Rydberg, 1985; Kelly et al., 1987).

Due to the variability of the BAL findings, the higher elimination rates of unmetabolized alcohol in younger animals (e.g. 11 days old) than older animals (15-60 days old), and to the fact that these peak BALs at 30 minutes resulted in orosensory alcohol processing in 11-day-old rats (Molina et al., 1989), it is possible that age related differences in alcohol pharmacokinetics affected the alcohol odor cues eliminated from demonstrators in the 1.0g/kg group. In the present experiment, demonstrator-observer pairs were 31 days of age when they interacted. Perhaps the 30 minute post-administration time period used with the 11-day-olds (c.f. Molina & Chotro, 1989b) was not sufficient to reach peak BALs that produce more salient alcohol odor cues in the 31-day-olds. Due to 31-day-old rats not eliminating as much unmetabolized alcohol as the 11-day-olds, the 30 minute post-administration period may not be long enough, or conversely too long, to reach peak BALs which produce salient alcohol odor cues for the observers in the 1.0 g/kg group to detect. Future experiments could test this hypothesis by examining the time frame of peak BALs for varying doses of administered alcohol in 31-day-olds to more fully understand how the relationship between peak BALs and administered alcohol in the demonstrator influences subsequent alcohol preferences in the observers.

A second puzzling finding of the present experiment was that the mean percent alcohol preference of the 3.0 g/kg group (53.3%) was equivalent to the water controls

(52.3%). Had alcohol preference continued to increase dose-dependently, we would have expected the 3.0 g/kg group to exhibit the greatest alcohol preference. There are two possibilities as to why this finding might occur.

The first possibility is that observers were unable to detect the alcohol odor cues eliminated by demonstrators due to the sedating effects of the alcohol. Demonstrators administered a dose of 3.0 g/kg ethanol have been reported to be highly intoxicated (Molina et al., 1989), and experience motor impairments. During the interaction, the 3.0 g/kg demonstrators in our experiment were personally observed to display little movement, sprawling out in the cage, and did not display the age-typical play behavior (characterized primarily by chasing, pouncing, and wrestling; Panksepp & Beatty, 1980) seen in this age. Galef and Stein (1985) have reported that mouth to mouth contact between observers and demonstrators is critical for the rapid transmission and alterations of observers' diet preferences. The researchers analyzed videotapes of 2-minute demonstrator-observer interactions for behaviors between the pairs that might predict subsequent diet preference in the observers. They found that observers' contacts with demonstrators' mouths significantly predicted the observers' subsequent preferences for their demonstrators' diet.

Furthermore, Galef & Stein (1985) observed that even anesthetized demonstrators could influence observers' diet preference if appropriate perioral contact was made. Both the demonstrator and observer were placed in a cardboard bucket during the interaction. Anesthetized demonstrators, however, were placed in a cylinder constructed of screen and positioned into a circular opening in the bucket that was 12 cm above its floor. This placed the anesthetized demonstrator at the appropriate height for perioral contact with

the observer. The researchers found that anesthetized demonstrators were just as effective in altering observers' diet preference when perioral contact was made as were the awake demonstrators. In the present study, the sedative effects of the alcohol could have somehow affected the necessary perioral contact, therefore eliminating diet preference transmission between demonstrator-observer pairs. Procedural modifications are needed to ensure that observers are receiving the necessary amounts of perioral contact with their demonstrators to induce alterations in alcohol preference. We plan on conducting a future experiment that will use a modification of Galef and Stein's apparatus for testing demonstrators and observers to ensure the appropriate perioral contact between 3.0 g/kg demonstrators and their observers. It is predicted that if the 3.0 g/kg demonstrators are positioned at an appropriate height to enable perioral contact, we will see a further dose-dependent increase in observer alcohol preferences.

A second possibility for why the 3.0 g/kg observers showed no change in alcohol preference is that observers did detect the alcohol odor cues on the demonstrators' breaths; however, the demonstrators were communicating an alcohol aversion. There are two arguments against this hypothesis. First, research from Galef's laboratory (Galef, 1985; Galef et al., 1983) has consistently found that rats socially transmit diet preferences, they do not transmit odor cues which induce food aversions. Therefore, it is unlikely that the observers did not exhibit an increased alcohol preference because of the demonstrators transmitting alcohol avoidance. A second argument, inconsistent with the hypothesis that observers are learning an alcohol aversion, is that observers exhibited equal preferences for both the alcohol and coffee solutions. Had the demonstrators

transmitted an alcohol aversion, we would have expected the observers to consume more coffee.

Therefore, it appears that with modifications to the interaction procedure ensuring necessary perioral contact, observers may exhibit learned alcohol preferences that increase dose-dependently. Future plans to videotape the demonstrator-observer interactions will also be beneficial in quantitatively and qualitatively measuring appropriate contact. Future experiments might also examine how aversive the alcohol intoxication is to the demonstrators following administration of 3.0 g/kg alcohol. If the demonstrators do not exhibit an alcohol aversion, then the argument that 3.0 g/kg demonstrators were transmitting an alcohol aversion to their observers would be erroneous. Additionally, more research is needed to examine the nature of the interactions between the demonstrators and observers. Galef and Stein (1985) have reported that an interaction between demonstrators and observers as short as two minutes was sufficient to influence subsequent food preferences in the observers. Another interesting experiment would be to vary the time period animals are allowed to interact to see how this might affect observers' alcohol preference. Perhaps shorter or longer interactions would affect the amount of information observers could gain from odor cues being eliminated on demonstrators' breaths.

Taken together, these findings demonstrate the significance of the demonstrator-observer procedure for eliciting voluntary intake of alcohol. The next two experiments were conducted in order to understand the underlying mechanisms of this effect. Experiment 2 was conducted to determine whether there was a particular component of the breath (e.g. carbon disulfide, Galef et al., 1988), that when paired with alcohol odor

cues, comes to elicit an increased alcohol preference. Experiment 3 examined whether this social transmission of alcohol odor cues was activating the endogenous opioid system.

Experiment 2

Results of Experiment 1 replicated and expanded upon the parameters of the demonstrator-observer induced modifications of observer's alcohol preference observed by Scordalakes (1998). The data are providing further support for the existence of the phenomenon of socially mediated food preferences. Because social learning of food preferences has such a profound effect on the rat, it is of interest to study the mechanisms that are responsible for this socially learned food preference.

Galef and colleagues (Galef et al., 1984; Galef et al., 1985) have been interested in determining what aspects of the demonstrator-observer interaction are responsible for mediating food preference. This interaction is often discussed in terms of social learning. However, researchers have not yet fully examined the underlying mechanisms responsible for this alteration in food preference. In an initial attempt to do this, Galef and colleagues (1988) identified the presence of both carbon disulfide (CS_2) and carbonyl sulfide (COS) in rat breath. These researchers observed that a rat's response to exposure of CS_2 in combination with food was similar to the response obtained when a rat smells food on the breath of a conspecific. Observers were found to exhibit a preference for a diet associated with CS_2 that was similar to the preference of those observers who actually interacted with another rat.

Even though CS_2 is present in rat's breath and has been implicated in mediating social learning of food preference, it has yet to be determined what the role of CS_2 is

playing in this interaction between the demonstrator and the observer. It is possible that the effects of this demonstrator-observer interaction can be explained in terms of a simpler learning paradigm, such as Pavlovian conditioning. It could be that Pavlovian conditioning is the basis of social learning of a food preference. That is, the seemingly complex cues involved in the learning of a food preference might be characterized as a pairing of a conditioned stimulus (CS) with an unconditioned stimulus (US). Using this perspective, we suggest that the alcohol odor is the CS. Based on the findings that CS_2 has been shown to alter food preferences, we additionally hypothesize that CS_2 is acting as the US. During the interaction between the demonstrator and the observer, the observer is being exposed to (1) an alcohol odor (CS) that is being expired on the demonstrator's breath, and (2) to the CS_2 (US) which is also present on the breath. Both the stimuli needed to induce learning (the CS and US) are present in this situation. Therefore, it is possible that the observers are changing their food preferences as a result of simple Pavlovian conditioning. The alcohol odor (CS) is being paired with the CS_2 (US). As a result of this pairing, observers might then be conditioned to respond to the alcohol odor, which is seen as an alteration in its alcohol preference. If observers are altering their food preferences because of Pavlovian conditioning, then learning of food preferences should be subject to the principles underlying this conditioning paradigm.

Experiment 2 examined whether the pairing of CS_2 with alcohol odor was sufficient to influence an observer's ingestion of ethanol. The question was whether observers can be conditioned to respond to alcohol odor after it has been paired with CS_2. The simple pairing of alcohol and CS_2 should result in observers exhibiting an increased preference for alcohol.

Method

Subjects

Subjects were 36 experimentally naïve, male and female, 26-35 day-old Sprague-Dawley-derived rats from 5 litters. Animals were reared and maintained as described in Experiment 1.

Apparatus

Animal housing was the same as that in Experiment 1.

Odor Stimulus

The volume of ethanol (EtOH) deposited on cotton balls was 5 ml of a 12.0% (v/v) EtOH solution dissolved in a tap water vehicle. The volume of carbon disulfide (CS_2) placed on the cotton ball was 6 drops of 1 part-per-million (ppm) solution of CS_2 (Galef et al., 1988) dissolved in a tap water vehicle. Four cotton balls were placed in a plastic weighing dish (Fisher Scientific) that was placed directly beneath the rat's cage.

Testing Solutions

The ethanol and coffee solutions used to measure observer ethanol intake were the same as those of Experiment 1.

Procedure

The experimental procedure was the same as Experiment 1 with the following modifications (see Figure 3):

(1) Observers were individually housed with free access to Prolab rat chow and water for a 2-day period. Observers were randomly assigned to one of three groups. The experimental group (EtOH+CS_2) was exposed to 5ml of ethanol and 6 drops of the CS_2 simultaneously presented on cotton balls beneath their

cages. The control groups consisted of a CS-only control group (EtOH+H_2O), in which observers were exposed to the 5ml of ethanol and 6 drops of water, and a US-only control group (H_2O+CS_2), in which observers were exposed to 5ml of water and 6 drops of the CS_2 (all ns = 12). To ensure that the observers were only exposed to the odors present in their condition, only one group was run at a time, with two groups being represented per litter. Therefore, animals were also randomly assigned to a young group, in which animals were tested when they were 26-30 days of age, and an old group, in which animals were tested when they were 30-34 days of age. Animals were handled on both days for approximately 30 seconds.

(2) On day 3, the observers were water-deprived for a 24 hr period, but were given free access to food.

(3) On day 4, 23 ½ hrs after water-deprivation, the EtOH+CS_2 group received pairings of EtOH with CS_2, the EtOH+H_2O group received exposure to EtOH odor only, and the H_2O+CS_2 group received exposure to CS_2 only. Four cotton balls were saturated with the appropriate solution (EtOH+CS_2, EtOH+H_2O, or H_2O+CS_2) and were placed under the cages in plastic dishes. Special precautions were taken to ensure that the observer's only smelled and could not taste the solutions.

(4) The observers were exposed to the saturated cotton balls for a 30 minute period.

(5) After this 30 minute period, observers were tested for ethanol intake. The testing procedure was the same as that of Experiment 1. Pretest

measurements recorded the amount of solution initially present in the drinking bottles.

(6) On day 5, 24 hrs after bottle placement, posttest measurements recorded of the amount of solution remaining. The amount of solution the observers ingested was calculated by subtracting the pretest measurements from the posttest measurements.

Data Analysis

Observer's alcohol preference was determined by converting the amount of EtOH ingested into percentage scores. The % EtOH preference data was analyzed using a one-way analysis of variance (ANOVA). Separate planned comparisons were conducted with t-tests. In all cases, the α level was set at .05.

Results

Gender Differences

The total amount of fluid intake was found to differ as a function of gender, with male observers having a higher fluid consumption. An independent-samples t-test on total intake yielded a significant effect of gender, $t(34) = 2.22$, $p < .05$. The increased fluid consumption in males, however, did not result in a greater ethanol (EtOH) preference. There were no differences in EtOH preferences between male and female observers, $t(34) = .56$. Therefore, the data were analyzed by collapsing across gender.

Age Differences

The total amount of fluid intake was not found to significantly differ as a function of age, $t(34) = 1.43$. Additionally, there were no differences in EtOH preferences

between young and old observers, $t(34) = .56$. Therefore, the data were subsequently analyzed by collapsing across age.

Ethanol Preference

A one-way ANOVA conducted on the data obtained during the testing phase revealed that there were no differences in EtOH preferences exhibited by the EtOH+CS2, EtOH+H20, and H20+CS2 groups, $F(2,33) = .58$. The mean percent EtOH preferences of observers are shown in Figure 4. Nevertheless, the EtOH+CS2 animals appeared to exhibit slightly higher mean EtOH preferences than the control animals; although planned comparisons found these differences to be nonsignificant, $t(33) = 1.07$. No differences were found in EtOH preferences between the EtOH+H20 and H20+CS2 control groups, $t(33) = .10$. Additionally, there were no differences in the amounts of total fluid intake between the groups, $F(2,33) = 1.29$ (see Table 2).

Discussion

In Experiment 2, we were interested in whether the pairing of CS_2 with alcohol odor was sufficient to influence an observer's ingestion of alcohol. It was hypothesized that the CS_2 normally present in the demonstrator's breath was acting as the unconditioned stimulus that was being paired with the conditioned stimulus, alcohol odor cues, also on the demonstrator's breath. When observers in the EtOH+CS_2 group were exposed to the CS-US pairing, the observers were expected to learn an increased preference for alcohol. Control animals (EtOH+H_20 and H_20+CS_2) that did not have CS-US pairings were not expected to learn an alcohol preference, and were expected to exhibit an equal preference for both alcohol and coffee solutions. These predictions were not confirmed. The present findings suggest that the particular volumes of the ethanol

and CS_2 solution utilized were not sufficient for producing increased alcohol preferences in the EtOH+CS_2 group, compared to the control EtOH+H_2O and H_2O+ CS_2 groups (cf. Galef et al., 1988).

Procedures in Experiment 2 were modified after an experiment conducted by Galef and colleagues (1988). Galef's experiment was designed to examine whether CS_2 plays a role in the social transmission of diet preferences in rats. They had observers interact in the classic demonstrator-observer fashion; however, the demonstrators were one of three conditions: (1) an anesthetized rat, with the diet placed in its mouth, (2) a tube wrapped with cotton-batting (referred to as a surrogate rat), with a diet and 6 drops of distilled water placed on one end (surr+H_2O group), or (3) a surrogate rat with the diet and 6 drops of 1 part-per-million solution of CS_2 placed on one end (surr+CS_2 group). When observers were given a choice between their demonstrator's diet and another diet, they found that observers that had interacted with the surr+CS_2 exhibited a similar increase in preference for their demonstrator's diet as the observers that had interacted with the anesthetized demonstrators. Moreover, observers that had interacted with the surr+H_2O did not exhibit an increased preference for their demonstrator's diet. Although, in essence, this experiment and Galef's experiment were investigating learning of a diet preference that resulted from CS-US pairings, several factors differed between the two experiments that might account for why Galef and colleagues reported that their observers exhibited greater diet preferences than was found in our observers.

One factor that might account for the differing results between experiments is the nature of the interaction. Although artificial conditions were used to represent the natural social interaction in both studies, Galef and colleague's study (1988) provided observers

with a surrogate demonstrator in the presence of the CS_2 for the 30 minute interaction, which may have more closely approximated the natural social interactions of learned diet preferences between rats. Our experiment, on the other hand, completely eliminated the common visual and tactile stimuli present in the social interaction; more specifically, we eliminated the visual and tactile cues of the demonstrator. If we assume that there are no differences in associative strength, meaning the strength of the acquired association between the CS (diet odor cues) and US (CS_2) between the two experiments, then differences in the learning of the alcohol preference in our experiment might be due to the amount of time observers were exposed to the pairing. Even though the associative strengths might be equal in both experiments, the fact that observers in Galef's experiment received both visual and olfactory cues might have made the context, or the US itself, more salient. This salience, therefore, may have allowed observers a sufficient amount of time to extract valuable information about the diet cues during the 30 minutes. Even observers in our Experiment 1 were given olfactory cues in the presence of two sensory modalities (e.g. sight of the demonstrators and odor cues respired on the demonstrator's breath). In contrast, observers in this experiment were only presented with olfactory cues, which might not have sufficiently made the context salient during the 30 minute interaction. Had the observers been given a longer exposure time to the olfactory cues, they might have been able to learn the alcohol preference.

We have been presuming that the US in Galef's experiment and in our own experiment was equally reinforcing. However, the nature of the US presentation was different in the two experiments, which might differentially affect the value of the US in the two experiments. Galef presented observers with CS_2 placed on the surrogate

demonstrator, whereas in our experiment the CS_2 was placed on cotton balls. By using the surrogate demonstrators, Galef might have produced a more ecologically appropriate context that positively affected observers' diet preferences. The combination of visual cues from the surrogate and odor cues of the CS_2 may have sufficiently captured the US that occurs naturally in the demonstrator-observer interactions. In our experiment, we were lacking the visual stimuli that might be needed in combination with the CS_2 to make the US complex more salient. Rescorla and Wagner (1972) observed that differences in the conditioned value of a CS were not only a function of CS salience, but also were a function of US salience as well. Had we used a surrogate demonstrator in combination with the CS_2, we may have been able to produce a more salient US, that when paired with the EtOH odor, would have enabled observers in our experiment to exhibit similar increases in diet preferences as were seen in Galef's observers.

If we assume that the USs in both experiments were providing equal amounts of reinforcement, then another potential factor responsible for the differences in observers' diet preferences between the two experiments would be due to the salience of the CS (Rescorla & Wagner, 1972). In Experiment 1, our demonstrators were intragastrically administered a dose of ethanol that was derived from a 12% concentration. This concentration was sufficient for producing detectable amounts of alcohol odor cues when eliminated on the breath of 1.5 g/kg demonstrators. In the present study, a 5 ml solution of 12% ethanol was placed on cotton balls instead of being intragastrically administered. The EtOH cues on the cotton balls might present less salient orosensory cues than the EtOH cues that are eliminated on the demonstrator's breath due to evaporation. Future experiments are necessary to determine the duration of EtOH odor cues from EtOH

placed on cotton balls. If the EtOH is evaporating during the 30 minute exposure period, thus making the odor cues less salient, then increasing the amount of the 12% concentration placed on the cotton balls might make these odor cues more salient in the presence of the CS_2; thus enabling the observers to learn the association between the CS and US and exhibit an EtOH preference that resembles the observer's increased diet preference in Galef's experiment.

Another possible explanation to account for the discrepancies in learned diet preferences between the two experiments might be due to the testing procedures. The initial stages of Galef's procedure were similar to ours (although see above). First, observers and demonstrators were acclimated to their cages, observers were then food deprived, and then observers interacted with demonstrators. However, instead of testing observers for diet preference after the interaction, as was done in our experiment, Galef's experiment had several intervening steps. Following the 30 minute interaction, Galef's observers were fed two diets in rapid succession, one that they had been previously exposed to on their surrogate and one that was novel. The observers were then poisoned with an injection of lithium chloride (LiCl). The observers were allowed 24 hours to recover from illness and then were offered the simultaneous choice of the two diets. They found that observers exhibited a preference for the diet they had experienced in association with CS_2 on their surrogate demonstrators. They concluded that CS_2 could just as effectively influence observer's diet preference as the presence of demonstrator rats. Galef's procedure, however, indirectly tested the observer's learned diet preference. According to theories on taste aversion (see Garcia, Lasiter, Bermudez-Rattoni, & Deems, 1985; see also Nachman, Rauschenberger, & Ashe, 1977), the observers had

been exposed to both diets prior to LiCl injection and, therefore, should have exhibited a learned taste aversion to both diets. In contrast, Galef and colleagues (1988) found that observers only avoided the diet that was not previously experienced with their demonstrators. Therefore, the researchers concluded that the observers had learned a preference for their demonstrators' diets, countering the subsequently acquired taste aversion. The present experiment attempted to more directly test the motivation to ingest a diet that was associated with CS_2. Had Galef and colleagues tested the observers after the interaction, they might have found that their observers exhibited similar diet preferences as our observers were found to exhibit. Once sufficient parameters are found for reliably conditioning diet preferences, our procedure will be a more powerful and convincing way of demonstrating the processes underlying social transmission of diet preferences.

Although not statistically significant, observers in the EtOH+CS_2 group did exhibit increased mean percent alcohol preferences (73.2%), compared to EtOH+H_20 (63.2%) and H_20+CS_2 (62.0%) control animals. The apparent difference between the mean percent alcohol preference of the EtOH+CS_2 and control animals suggests that this procedure may be tapping into the mechanisms underlying social transmission of learned diet preferences; however, modifications are needed to make either the US or CS, or both, more salient in order to substantially change observer's alcohol preference.

Taken together, the findings of Experiment 1 and 2 indicate that some component of the interaction, whether it is social contact between the demonstrator-observer pairs or CS_2 cues being respired on the demonstrator's breath, is rewarding to the observer and subsequently causing an increased preference for their demonstrator's diet. Because this

social interaction is having a rewarding effect, some or all of the components of the opioid system are possibly being activated. The opioid system appears to be one of the three neurochemical systems, in addition to oxytocin and norepinephrine, important in regulating the rewarding components of social interactions (Nelson & Panksepp, 1998). Experiment 3 examined whether the social transmission of alcohol odor cues was activating the endogenous opioid system. Specifically, we examined the effects of naloxone hydrochloride, a nonspecific opiate antagonist, on observer's alcohol intake after interacting with demonstrators that had been administered alcohol.

Experiment 3

It appears that the social learning of a food preference involves some rewarding consequences for rats. There have been vast amounts of research showing that the activation of the opioid system produces hedonic effects that are rewarding to an organism. Heroin activates the endogenous opioid system and produces feelings of euphoria when it binds to the receptors (Schenk & Nawiesniak, 1985). These feelings are so intense and desirable that the user becomes rapidly addicted to the drug. Rats will continue to press a lever to receive infusions of morphine, another opioid agonist, at the expense of other important behaviors, such as feeding (Van-Ree, Slangen, & de-Wied, 1978).

In the demonstrator-observer paradigm, it appears that there is some factor of the interaction that is influencing the observer's food preference. This interaction, whether it is with CS_2 or with a conspecific, could be evoking changes in neurochemistry that are somehow reinforcing to the observer. Because this social interaction is having a

rewarding effect, some or all of the components of the opioid system are possibly being activated.

There is indirect support that CS_2 acts on the opioid system. Smotherman and Robinson (1992a) found that another endogenous sulfur-based compound, dimethyl disulfide (DMDS), was present in pup saliva. DMDS was found to mimic the behavioral effects of milk; specifically, it was able to activate the kappa opioid system. If CS_2, being similar to DMDS, also somehow activates opioid receptors, then this could be the neurochemical basis for at least some social learning effects. When the CS_2 is paired with a food odor, a conditioned food preference could develop through the CS_2 activating the opioid system.

Additional studies have demonstrated that other behaviorally relevant cues also activate the opioid system. Milk activates the endogenous opioid system in both prenatal (Smotherman & Robinson, 1992b) and neonatal rats (Blass & Fitzgerald, 1988). During the juvenile period, rats engage in rough and tumble play (Panksepp, 1980) that involves a great deal of social contact. This play behavior appears to be mediated by the release of endogenous opioids, with naloxone reducing play in a dose-related fashion (Siegel, Jensen, & Panksepp, 1985). If the social interaction between demonstrators and observers is mainly in the form of play behavior, then this could be the neurochemical basis for socially learned diet preferences. When the alcohol odors are present during play behavior, a conditioned alcohol preference could develop through the social contact activating the opioid system. Furthermore, researchers have reported that stimuli that are present in the environment prior to or during opioid activation can result in conditioned taste and odor preferences (Blass & Kehoe, 1987; Lett & Grant, 1989). This can

subsequently lead to a conditioned activation of the opioid system (Siegel, 1979). Therefore, observers can learn an alcohol preference because the alcohol odor eliminated on the demonstrator's breath was present when the social contact was causing the release of opioids.

If some component of the demonstrator paired with an odor cue activates the release of endogenous opioid neurotransmitters, then administration of an opioid antagonist should, therefore, block an observer's ability to learn a food preference. Experiment 3 was conducted to examine whether social interactions with a demonstrator administered alcohol activated the release of endogenous opioid neurotransmitters in the observer. Specifically, we examined the effects of naloxone hydrochloride, a nonspecific opiate antagonist that was administered prior to the behavioral interaction, would block learning of the alcohol preference established by interacting with a demonstrator administered alcohol.

Method

Subjects

Forty experimentally naïve, 26-35 day-old Sprague-Dawley-derived rats from 6 litters were used as subjects. Animals were maintained as described in Experiment 1.

Apparatus

The apparatus was the same as that in Experiment 1.

Intragastric Administration

The intragastric administration was the same as in Experiment 1.

Injection Administration

Observers were injected using a 30-gauge needle attached to a 1ml syringe.

Intragastric Solution

The volume of ethanol (EtOH) intragastrically administered to the demonstrators was a dose of 1.5 g/kg of a 12.0% v/v EtOH solution that was dissolved in a tap water vehicle. The dose of EtOH was calculated by multiplying the animal's body weight (measured in grams) by 0.015.

Injected Solutions

The dose of naloxone hydrochloride administered to the observers was 5 mg/kg that was dissolved in a saline vehicle. The selected dose was based on previous dose-response studies of naloxone on play behavior in juvenile rats (Siegel et al., 1985). Control animals were injected with saline. The dose of the appropriate solution injected was calculated by multiplying the animal's body weight (measured in grams) by 0.001.

Testing Solutions

The ethanol and coffee solutions used for the measurement of observer's ethanol intake were the same as that used in Experiment 1.

Procedure

The experimental procedure was the same as Experiment 1 with the following modifications (see Figure 5):

(1) Demonstrators and observers were housed together with free access to Prolab rat chow and water on day 1. Same-sex sibling, demonstrator-observer pairs were randomly assigned to the naloxone group (EtOH+NAL) or the saline control group (EtOH+SAL; all ns = 10). Animals were handled for approximately 30 seconds.

(2) On day 2, demonstrators were moved to a separate cage. All demonstrators and observers were allowed free access to food and water.

(3) On day 3, 23 hrs following separation, demonstrators were intubated with a dose of 1.5 g/kg ethanol. Demonstrators were placed back into their cages for 30 minutes. Ten minutes after demonstrator intubation, observers received an intraperitoneal (i.p.) injection of either naloxone (EtOH+NAL) or saline (EtOH+SAL).

(4) Twenty minutes after injection, demonstrators were placed into the observer's cage and were allowed to interact with the observer for 30 minutes.

(5) After this interaction period, demonstrators were moved to their holding cages and observers were water-deprived for a 24 hr period.

(6) After this 24 hr period, observers were measured for ethanol intake. The testing procedure was the same as that of Experiment 1. Pretest measurements recorded the amount of solution initially present in the drinking bottles.

(7) On day 5, 24 hrs after bottle placement, post-test measurements were recorded for the amount of solution remaining. The amount of solution the observers ingested was calculated by subtracting the pretest measurements from the post-test measurements.

Data Analysis

Observers' alcohol preferences were calculated by converting the amount of EtOH ingested into percentage scores. The % EtOH preference data were analyzed using an independent-samples t-test.

Results

Gender Differences

The total amount of fluid intake was found to differ as a function of gender, with male observers having a higher fluid consumption. An independent-samples t-test on total intake yielded a significant effect of gender, $t(18) = 3.80$, $p < .001$. The increased fluid consumption, however, did not result in a greater ethanol (EtOH) preference. There were no differences in EtOH preferences between male and female observers, $t(18) = .76$. Therefore, the data were further analyzed by collapsing across gender.

Ethanol Preference

An independent-samples t-test conducted on the data obtained during the testing phase revealed that there were no differences in EtOH preferences exhibited by the EtOH+NAL and EtOH+SAL groups, $t(18) = .17$. The mean percent EtOH preferences of observers are shown in Figure 6. Additionally, there were no differences in the amounts of total fluid intake between the groups, $t(18) = .28$ (see Table 3).

Discussion

In Experiment 3, we were interested in whether the demonstrator-observer interaction in the presence of alcohol odors was activating the release of endogenous opioid neurotransmitters in the observer. We had hypothesized that some component of this interaction, whether it be the social interaction with the conspecific or the CS2 on the demonstrator's breath, was activating the release of opioids (US) in the observer that were being paired with the respired alcohol odors (CS) from the demonstrator. This CS-US pairing would subsequently condition the observers to learn an alcohol preference. Furthermore, we hypothesized that the administration of naloxone hydrochloride, a

nonspecific opiate antagonist, administered prior to the behavioral interaction, would block learning of the alcohol preference established in observers. Observers would, therefore, only be exposed to the alcohol odor cues without the corresponding activation of the opioid system. Without the CS-US pairings, observers were not expected to learn an alcohol preference, and were expected to exhibit an equal preference for both alcohol and coffee solutions. These predictions were not confirmed. The results of Experiment 3 indicate that there were no differences in alcohol preference between observers in the EtOH+NAL and EtOH+SAL groups, with observers in both groups exhibiting an increased preference for alcohol.

There are several possibilities as to why the naloxone-treated observers did not exhibit a marked decrease in alcohol preference in comparison to control animals injected with saline. The first possibility is that the opioid system is not involved in the social transmission of alcohol preferences in rats. Inconsistent with this argument are the number of studies demonstrating the profound influence of the opioid system on the rat's social behavior (cf. Panksepp et al., 1980). Therefore, it is more likely that the experimental manipulations (e.g. inadequate dose of naloxone) were not sufficient in blocking the observer's learned alcohol preference.

A second possibility is that naloxone might have been able to prevent learning the alcohol preference had naloxone been injected in a different manner. In the present experiment, the dose of naloxone (5 mg/kg), as well as the time administered (20 minutes before interaction), were based on a study by Siegel and colleagues (1985). In Siegel's experiment, they examined the effects of naloxone injected subcutaneously (s.c.) on social behavior in 26-day-old rats. In the present experiment, however, naloxone was

administered into the peritoneal cavity (i.p.). It is possible that the differing injection procedures resulted in differing levels of naloxone during the interaction period. The i.p. injections could have been insufficient for producing the necessary blood levels of naloxone during the 20 minute period prior to interaction, that were reached with the s.c. injection. Lower levels of naloxone might have been unable to completely block the social transmission of alcohol preference during the interaction, therefore, resulting in increased observer alcohol preferences. Inconsistent with this argument, however, are experiments that reliably found that a dose of 4 mg/kg naloxone injected i.p. blocked the conditioned analgesic effects to a footshock tested 1 minute after injection (Fanselow, 1984; Young & Fanselow, 1992).

Another possibility as to why naloxone did not have an effect on observer's learned alcohol preference is that naloxone has inconsistent effects. Researchers have reported that naloxone is not as effective if the animal is satiated as when it is food deprived (Panksepp et al., 1980). In the present experiment, the animals were given free access to food and water prior to naloxone administration. Future experiments can control for this potential confound by having observers food deprived prior to naloxone injection. Furthermore, Panksepp, Najam, and Soares (1979) found no consistent effect of naloxone on social motivation, as measured by the amount of time paired rats maintained physical proximity with each other. Naloxone was not found to increase social contact between naloxone-treated rats and their pair as they had hypothesized. In the present study, naloxone may have had inconsistent effects in the observers that were insufficient in blocking the learning of an alcohol preference.

There are a number of studies, however, that have reported consist effects of naloxone. In particular, Siegel and colleagues (1985) found that naloxone reduced play, as measured by one animal rolling onto its back with the other animal on top (this behavior is referred to as pinning), in a dose-related fashion. Thus, the naloxone administered to observers in our experiment might have affected the play behavior, but was not sufficient for eliminating the approach behavior. The approach behavior, furthermore, might have resulted in the critical perioral contact necessary for social transmission of diet cues; thus the observers would have still learned the alcohol preference. Future experiments are needed to determine whether the behaviors occurring during the interaction between naloxone-treated observers and their demonstrators is the same behavior occurring during normal social transmission of diet cues in the demonstrator-observer interaction.

An alternative explanation is that naloxone is having an effect on alcohol intake; however, it might not be affecting the learned alcohol preferences as originally believed. There are two explanations as to why this finding might occur. The first explanation for the reported increases in observer's alcohol preference may be the result of naloxone, and not social learning of diet cues, that were causing observers to consume more alcohol. Consistent with this explanation are preliminary data from a pilot study we conducted examining the effects of naloxone injections on subsequent alcohol preference, compared to saline controls. Rats that had not previously interacted with a demonstrator were either injected (i.p.) with naloxone (n = 4) or saline (n = 4), and were then tested for alcohol preference using the two-choice bottle procedure. We hypothesized that naloxone would not affect alcohol preference. Surprisingly, we found that rats injected with naloxone

exhibited a 74% preference for alcohol, compared to the 61% preference found in saline-treated animals. Although more animals need to be tested in order to reliably examine the effects of naloxone on subsequent alcohol consumption, the initial findings indicate that naloxone may have altered baseline alcohol preferences. This suggests that the increased alcohol preferences reported after the naloxone-treated observers that interacted with demonstrators may have resulted from an increased baseline alcohol preference due to the properties of naloxone. Had we used another diet choice in place of the ethanol, such as the cinnamon and cocoa diets frequently used by Galef (e.g. Galef and Stein, 1985), we might have found the predicted decreases in observer's diet preference resulting from the effects of naloxone on the social transmission of diet cues in the demonstrator-observer interaction.

A second explanation for why naloxone-treated observers exhibited increased alcohol preferences comes from studies examining the brain opioid theory of social attachment (Nelson & Panksepp, 1998). This theory suggests that endogenous opioids, oxytocin, and norepinephrine are interconnected within a neural circuit that mediates affiliative and attachment behaviors across mammalian species and development. Of particular interest to the present experiment is how this theory accounts for the role of endogenous opioids in social motivation. It is hypothesized that social isolation results in reduced basal opioid levels and that social stimuli act to increase endogenous opioid release. This theory further predicts that reduced basal opioid levels will motivate animals to seek out social contact, whereas increased opioid levels will lead to decreased motivation. Nelson and Panksepp reported two experiments that supported this prediction. The first experiment found that naltrexone-treated monkeys made more social

contact with their mothers than control animals (Martel, Nevison, Simpson, & Keverne, 1995). Similarly, opioid antagonists were found to increase motivation to receive grooming, whereas opioid agonists were found to decrease such motivation (Keverne, Martensz, & Tuite, 1989).

If the results of the present experiment are viewed in terms of the brain opioid theory of social attachment, then the increased alcohol preferences seen in naloxone-treated observers after interacting with demonstrators may be due to a motivation to increase endogenous opioid levels. There are several indirect lines of research that support this explanation. In the present study, the demonstrators and observers were separated 24 hours prior to the interaction period. Not only has this deprivation period been found to markedly increase social contact (Panksepp & Beatty, 1980), thus ensuring the appropriate perioral contact between pairs necessary for the social transmission of diet cues, but it also decreases endogenous opioid levels as well (Panksepp et al., 1980). In addition to the decreased opioid levels that were a function of social deprivation, some observers were given injections of naloxone that might have further reduced opioid levels. It is possible that the 30 minute interaction period was not sufficient for elevating endogenous opioid levels back up to predrug and predeprivation levels. In addition to the deprivation period prior to demonstrator-observer interaction, animals were also socially deprived during the testing phase. Research on preweanling rats has reported that social isolation followed by a brief reunion with the dam and then subsequent isolation resulted in substantially more distress, as measured by ultrasonic vocalizations, than the sum of the effects of each alone (Hofer, Brunelli, & Shair, 1994; Hofer, Masmela, Brunelli, & Shair, 1998). In the present study, this deprivation-reunion-deprivation effect the

observers experienced might have had similar distressing consequences as was found in preweanling rats. This potentiation effect may have extended to endogenous opioid release, with the observers potentially experiencing greater reductions in endogenous opioids than would have resulted from any of those conditions separately.

Therefore, it is likely that naloxone-treated observers had suppressed endogenous opioid levels during the testing phase of the experiments. These lower levels might have motivated the observers to drink more alcohol in order to increase endogenous opioid levels. Research examining the effects of alcohol on endogenous opioids has found that acute doses of alcohol increase dopamine concentrations in the extracellular compartment of the nucleus accumbens (Wozniak, Pert, Mele, & Linnoila, 1991). Low doses of alcohol have also been found to activate opioid and dopamine transmission (Di Chiara, Acquas, & Tanda, 1996). Thus, the naloxone might have blocked the social learning of alcohol preference, but the depleted endogenous opioid levels due to the drug and social deprivation increased the observer's motivation to seek out social contact. The 30 minute interaction, however, was insufficient for increasing the levels back to baseline. Moreover, the observers were again socially deprived, further decreasing endogenous opioids; therefore, the observers had reduced opioid levels at testing. After initially sampling both alcohol and coffee solutions, the activation of the opioid system after ingesting the alcohol would reinforce the observers to consume more alcohol in order to increase endogenous opioid levels. This hypothesis could account for why observers exhibited increased alcohol preferences.

Although the naloxone-treated observers' alcohol preferences were not found to significantly differ from saline-treated observers' alcohol preferences, a confound in the

procedure may have resulted in these findings. In light of the possible confounding effects of naloxone and social deprivation on subsequent alcohol consumption, it might be necessary to examine the effects of endogenous opioid activation and social learning of alcohol preferences. If social transmission of diet cues is dependent upon the functioning of the opioid system, then exogenous activation of this system during demonstrator-observer interaction will result in learned diet preferences. Future experiments will examine if morphine-treated observers that are exposed to alcohol odor cues will exhibit increases in alcohol preference. With the proper procedural modifications, we are likely to tap into the endogenous opioid mechanisms underlying social transmission of learned diet preferences.

General Discussion

The purpose of the research was to examine the underlying chemical and molecular mechanisms mediating the association between social interaction and alcohol preference in the rat. The primary findings of Experiment 1 indicate that a rat's alcohol preference can be altered after interacting with another rat that had been administered alcohol. These results replicate the findings presented by Scordalakes (1998). Furthermore, the results indicate that the magnitude of the observer's alcohol preference increased dose-dependently, with 1.5 g/kg appearing to be the critical dose for conditioning the alcohol preference. Overall, the results of Experiment 1 suggest that the social transmission of alcohol preferences in rats is a real phenomenon.

In Experiment 2, we were interested in whether the pairing of CS_2 with alcohol odor was sufficient to influence an observer's ingestion of alcohol. Based on experiments by Galef and colleagues (1998), we had hypothesized that the CS_2 normally

present on the demonstrator's breath was acting as the unconditioned stimulus that was being paired with the conditioned stimulus, alcohol odor cues, also present on the demonstrator's breath. Although our predictions were not confirmed statistically, the results suggest that observers in the EtOH+CS_2 group exposed to the CS-US pairings exhibited at least a slight increase in alcohol preference compared with the EtOH+H_20 and H_20+CS_2 control groups.

Experiment 3 was conducted to examine whether the demonstrator-observer interaction in the presence of alcohol odors was activating the release of endogenous opioid neurotransmitters in the observer. We hypothesized that the administration of naloxone hydrochloride, a nonspecific opiate antagonist, would block the opioid system in observers, preventing them from learning an alcohol preference. This prediction was not confirmed, with observers in the EtOH+NAL group consuming as much alcohol as observers in the EtOH+SAL group. Although it could be argued that these results suggest that the opioid system was not involved in the social transmission of alcohol preference, we would like to argue otherwise. There have been a number of studies conducted that have found that many of the social behaviors of the rat are dependent on the opioid system (c.f. Panksepp et al., 1980). Because the activation of the opioid system has such a profound influence on the rat's social behavior, we believe that the social transmission of food preference is also mediated by the opioid system. However, the present experimental manipulations were not sufficient for elucidating the effects of the pharmacological blockade of the opioid system on social transmission of alcohol preferences.

The pervasiveness of social transmission of food preferences for a rat's survival makes the demonstrator-observer procedure an ideal model for studying the social factors that influence alcohol consumption despite some of the obtained results. Due to the limitations previously discussed, such as stimulus salience and potential interactions between naloxone injections and deprivation, we might have been unable to sufficiently detect the role of the hypothesized mechanisms underlying alcohol preference in observers. Although we were unable to statistically confirm that CS_2 was acting as the unconditioned stimulus and that the activation of the opioid system results in learned alcohol preferences, we are still confident that the proper procedural modifications will reveal that these hypothesized mechanisms are indeed underlying the increased alcohol preferences in observers. Therefore, we feel that the procedural modifications proposed previously might more sufficiently test our hypotheses. By understanding more fully the behavioral and neurochemical mechanisms responsible for the socially learned food preferences, the demonstrator-observer procedure will undoubtedly contribute to important future research on the initiation and maintenance of adolescent alcohol abuse.

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Table 1

Mean Amount (ml) Ingested of Each Solution by Observer Group in Experiment 1 (± SEM)

	Solution	
Observer Group	EtOH	Coffee
0.0 g/kg	13.2 (1.4)	13.6 (2.5)
1.0 g/kg	16.2 (2.9)	10.8 (3.4)
1.5 g/kg	19.4 (1.6)	7.2 (2.1)
3.0 g/kg	14.2 (1.7)	13.0 (2.1)

Table 2

Mean Amount (ml) Ingested of Each Solution by Observer Group in Experiment 2 (± SEM)

	Solution	
Observer Group	EtOH	Coffee
EtOH+CS_2	20.0 (2.3)	8.3 (2.6)
EtOH+H_20	17.1 (2.6)	10.7 (2.8)
H_20+CS_2	19.4 (2.3)	12.2 (1.5)

Table 3

Mean Amount (ml) Ingested of Each Solution by Observer Group in Experiment 3 (± SEM)

	Solution	
Observer Group	EtOH	Coffee
EtOH+NAL	17.6 (2.7)	5.6 (2.3)
EtOH+SAL	17.9 (2.9)	4.3 (2.0)

Figure Caption

Figure 1. Demonstrator-observer paradigm utilized in Experiment 1. (D = demonstrator, O = observer, F = food, W = water, E = alcohol solution, C = coffee solution, → = interaction between D & O, INT = intubated with alcohol)

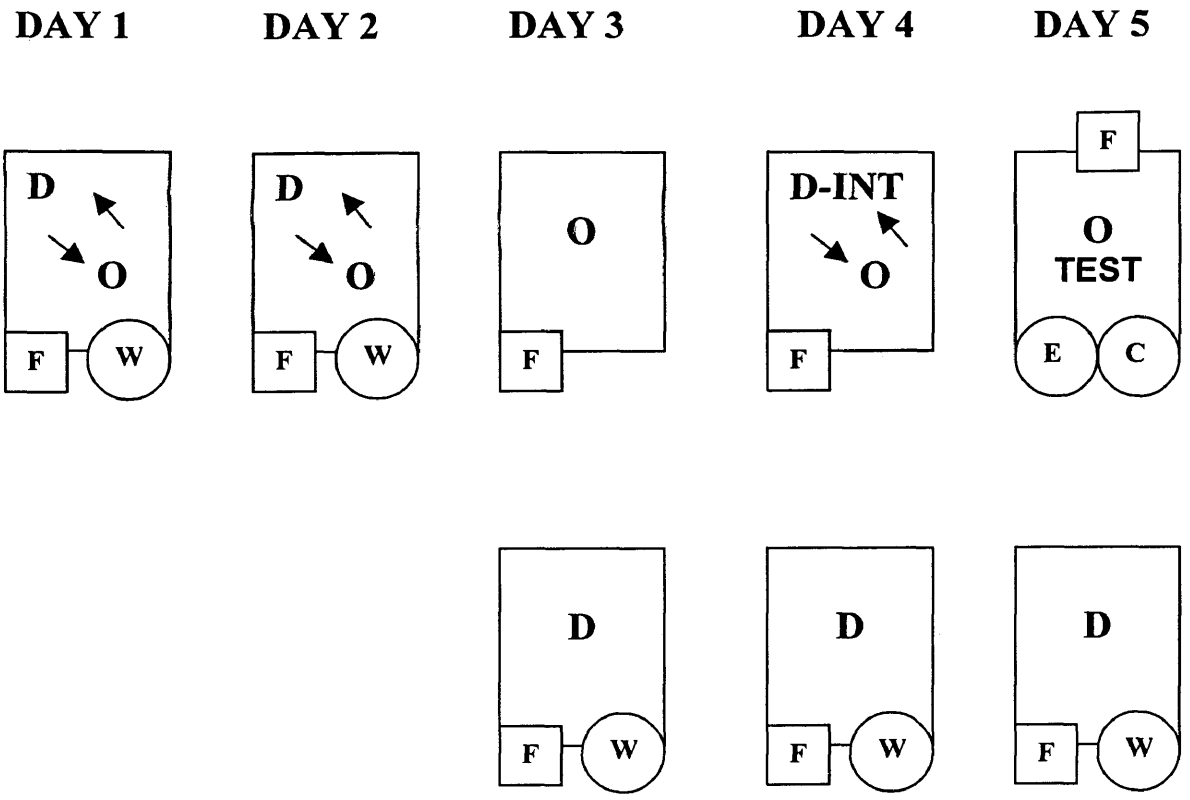
Figure 2. Mean (±SEM) percent ethanol (EtOH) preference of observers with demonstrators administered 0.0, 1.0, 1.5, or 3.0 g/kg EtOH in Experiment 1.

Figure 3. Demonstrator-observer paradigm utilized in Experiment 2. (O = observer, F = food, W = water, CB = cotton ball, E = alcohol solution, C = coffee solution)

Figure 4. Mean (±SEM) percent ethanol (EtOH) preference of observers exposed to water and carbon disulfide (H_2O+CS_2), ethanol and water (EtOH+H_2O), and ethanol and carbon disulfide (EtOH+CS_2) in Experiment 2.

Figure 5. Demonstrator-observer paradigm utilized in Experiment 3. (D = demonstrator, O = observer, F = food, W = water, E = alcohol solution, C = coffee solution, → = interaction between D & O, INT = intubated with alcohol, INJ = injected with naloxone or saline)

Figure 6. Mean (±SEM) percent ethanol (EtOH) preference of observers that had been injected with either naloxone hydrochloride (EtOH+NAL) or saline (EtOH+SAL) prior to interacting with demonstrators administered alcohol in Experiment 3.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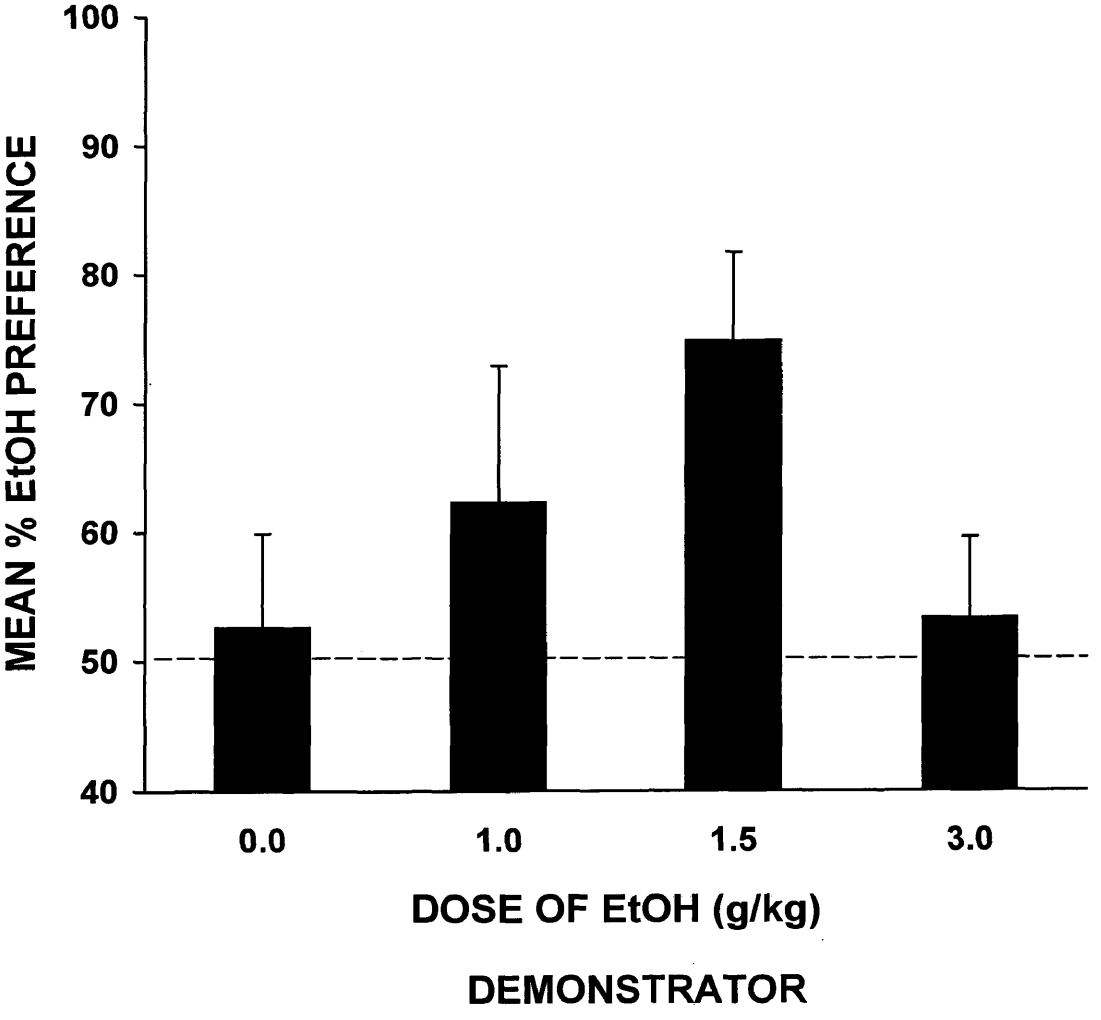
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MEAN % EtOH PREFERENCE
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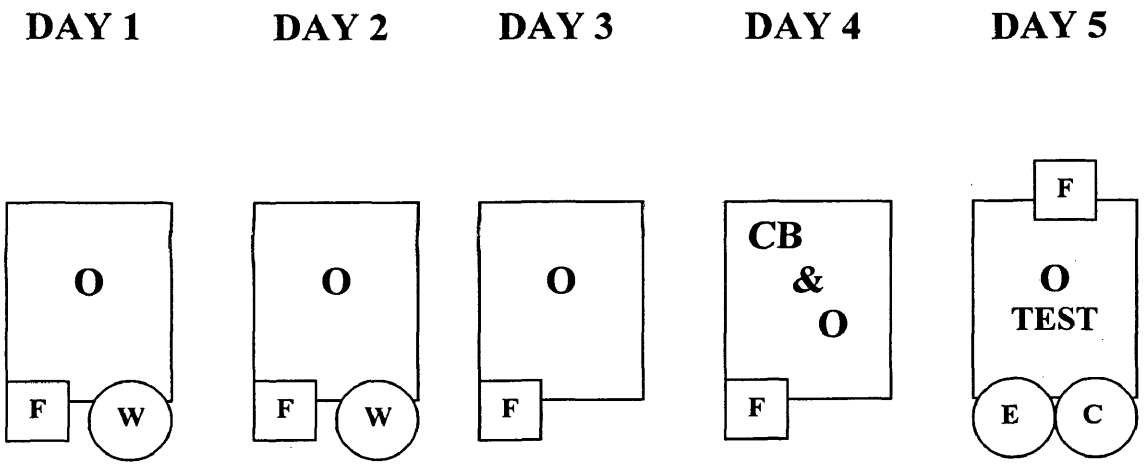
DOSE OF EtOH (g/kg)
DEMONSTRATOR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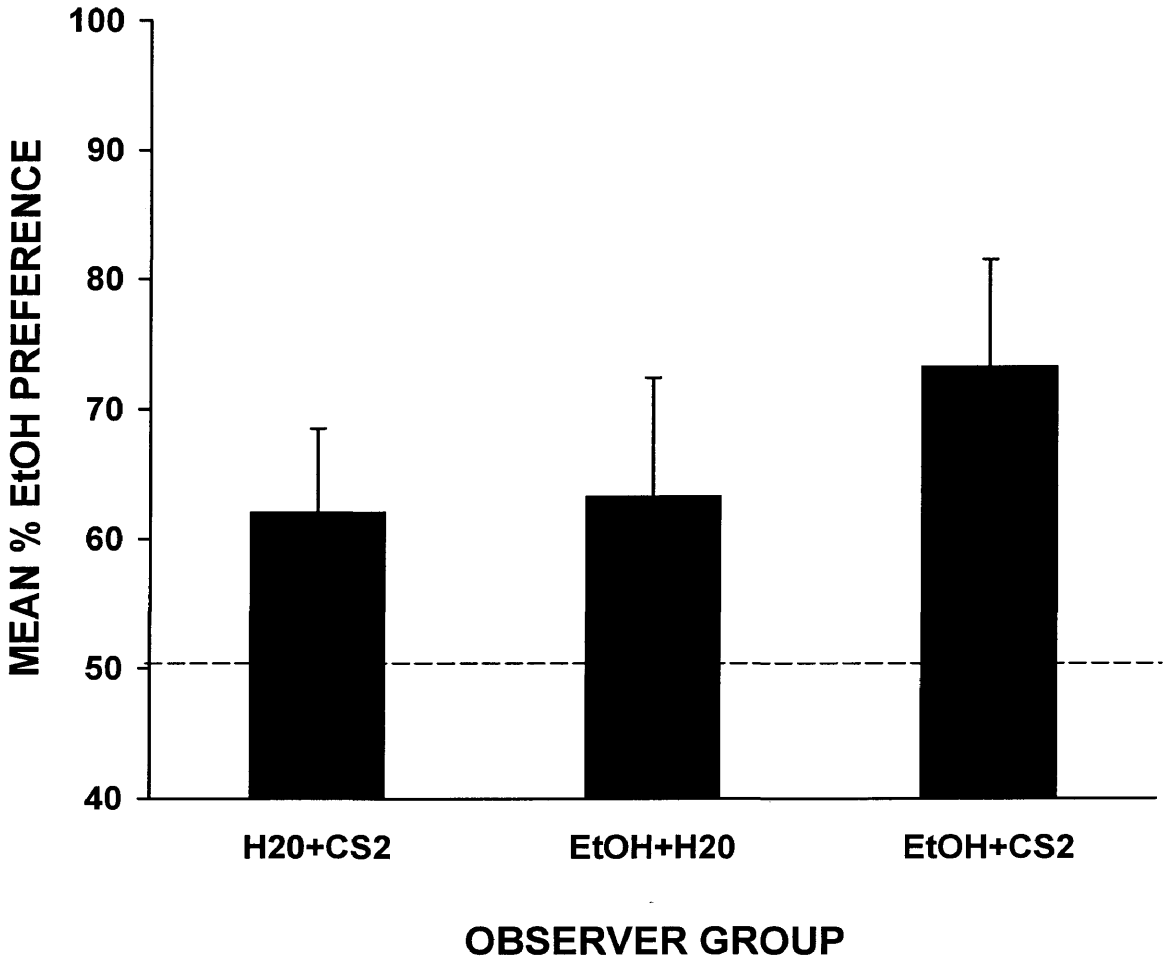
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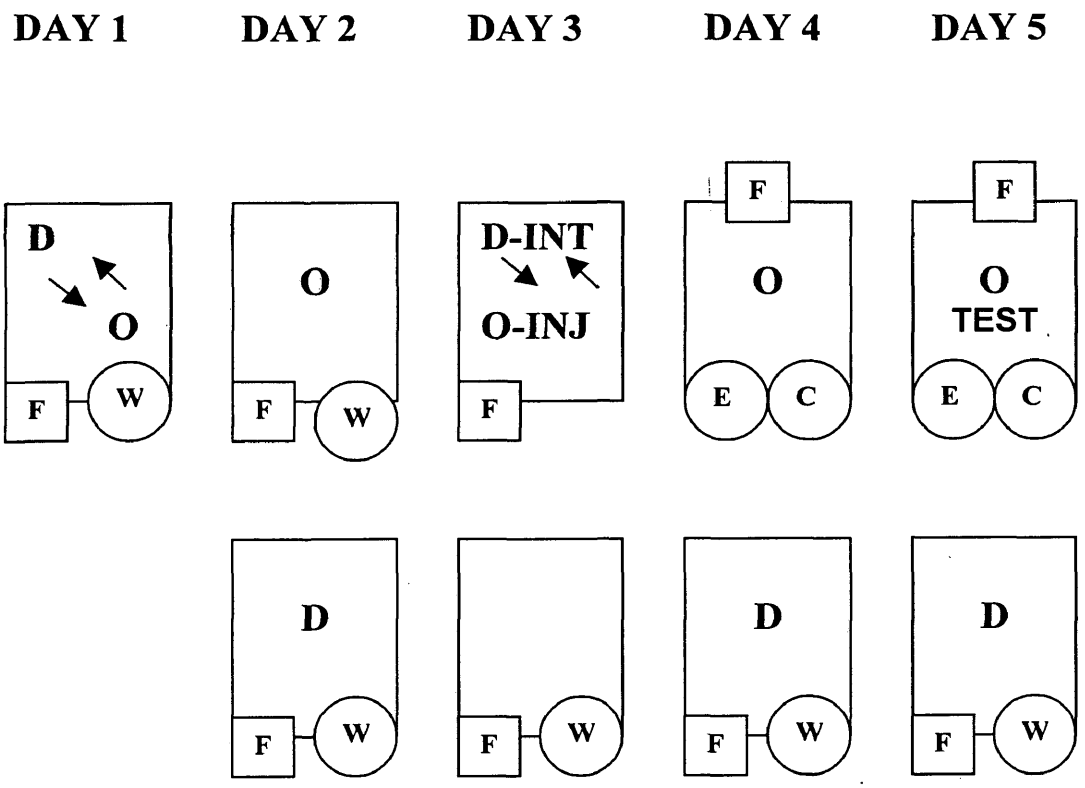
MEAN % EtOH PREFERENCE
H20+CS2
EtOH+H20
EtOH+CS2
OBSERVER GROUP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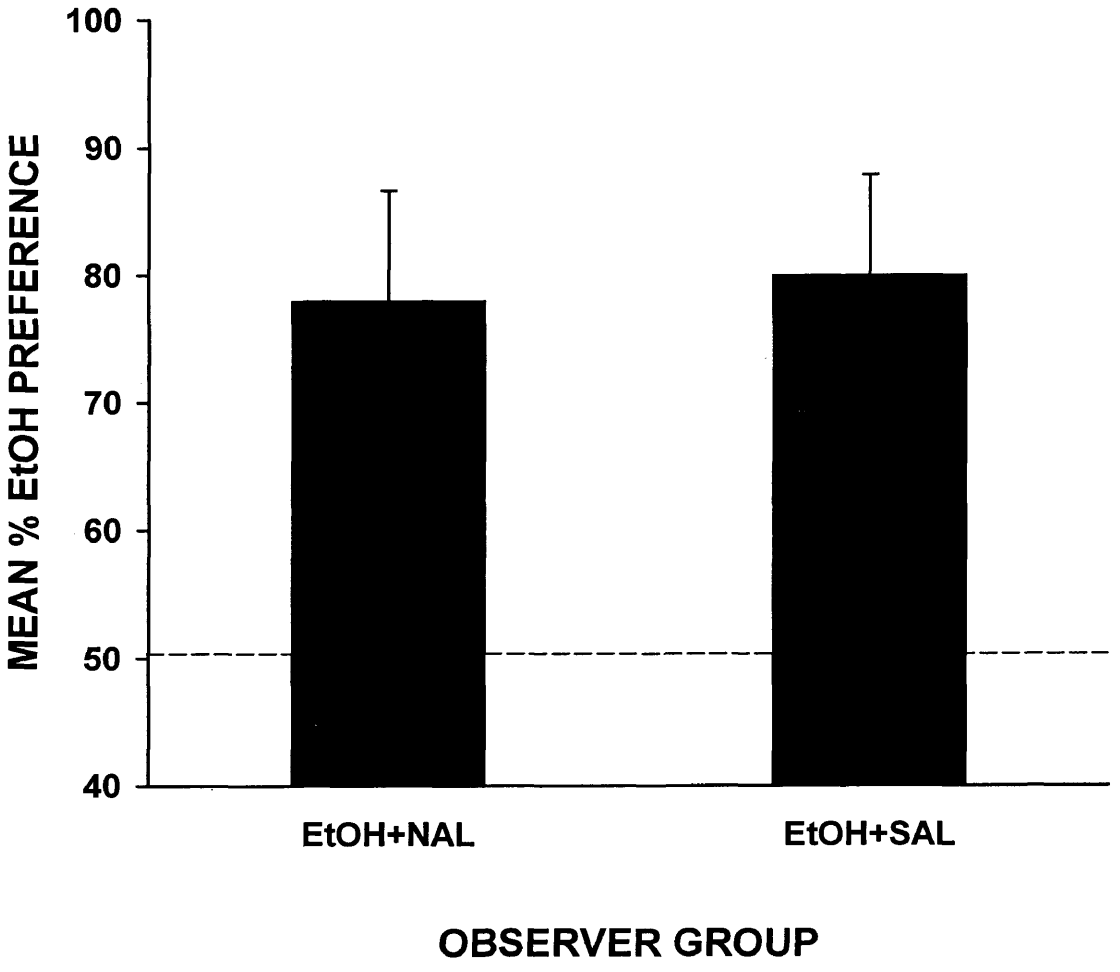
100
90
80
70
60
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MEAN % EtOH PREFERENCE
EtOH+NAL
EtOH+SAL
OBSERVER GROUP

VITA

Jennifer Leigh Holloway

Born in Lafayette, Indiana on September 27, 1974. She graduated from McCutcheon High School in that city, June 1993. She graduated from Indiana University in Bloomington, Indiana with a B.S. in Psychology and a minor in Biology from Indiana University, May 1997. She entered The College of William & Mary in Williamsburg, Virginia in August 1997 and graduated with a Master of Arts in Psychology in May, 1999.