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Social Learning and Alcohol

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Social Learning and Alcohol

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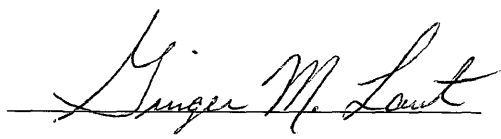

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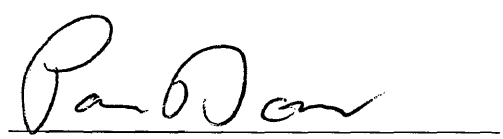

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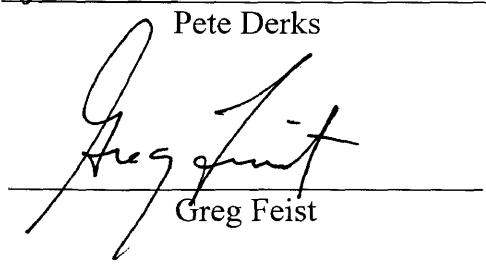

Greg Feist

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Social Learning and Alcohol in Preweanling Rats:

Development and Retention

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Abstract

Using Galef and Stein's (1985) demonstrator/observer paradigm, previous research has found that periadolescent rats were able to develop a preference for alcohol after exposure to the odor on a sibling's breath during social interaction (Scordalakes, 1998). The present experiments investigated whether a preference for alcohol would occur in preweanling rats after similar exposure. In the first experiment, 16-day-old rats were administered alcohol intragastrically and exposed to a sibling 30 minutes later to allow social interaction. Subsequent testing of the sibling observer revealed a significant preference for alcohol relative to controls. Experiment 2 investigated the development of this type of learning about alcohol in 8-, 12-, and 16-day-old rat pups. Animals in each age group with alcohol demonstrators ingested significantly more alcohol than controls. The retention of an alcohol preference was evaluated in observer animals on PD22 (Experiment 3). This was accomplished by administering alcohol to the demonstrators on PD12, 14, and 16. Four of the remaining littermates from each litter were given an alcohol ingestion test on PD22. Results suggest that animals are able to retain the learned preference for alcohol.

Social Learning and Alcohol in Preweanling Rats: Development and Retention

The decision about what is selected by different species and cultures as food and what is not is a complex issue that involves many aspects. Selecting a certain diet can influence the predicted physiology and appearance of an animal species, and, thereby, affect its evolution. The realm of food selection has other components, which include emotional aspects. Food can be viewed as a source of pleasure. Furthermore, the experience of food can be essentially social, which is especially true for humans. Social factors can influence food preferences in two ways, indirectly or directly (Rozin, 1977).

The indirect method refers to Indirect Social Action, which involves learning where the social agents are not present. There is an understanding of what is to be learned and under what conditions it will be learned. Learning of this nature does not necessarily involve social mediation. To accomplish this type of social influence one could control the availability of certain types of foods. In India, for example, there are rules about what foods can be eaten by whom. The decision is dependent upon who prepared the food and is used as a vehicle for the expression of their society's structure and family maintenance (Appadurai, 1981). In the Indian society, a person must eat food prepared by someone of the same social status as he/she is. This is because it is believed that the food contains the essence of its preparer, and therefore, eating food prepared by someone of an inferior rank could harm your own status.

The direct method, called Direct Social Effects or Social Learning, has two different categories. The first is Social Agency, where social learning about food occurs through a teaching process, such as showing a child which berries are poisonous and which one are not (Rozin, 1977). The other method of social learning is called Inadvertent

Social Agency (Rozin, 1977). In this category, preferences are established through social learning that occurs without teaching.

This also refers to the ability to obtain information from others in a social setting or through social interaction. This particular process is invaluable for allowing members of different species to transmit knowledge from one individual to another and the type of learning evaluated in this research (Rozin, 1977).

Learning of this nature is particularly important during development when massive amounts of information must be acquired and assimilated. The capacity to acquire knowledge from others enables the individual to better adapt to its environment. There are many methods by which information of this sort can be conveyed during social contact. Information can be transmitted about a mother's diet to her infant during nursing through her breast milk, direct observations can be made about food choice by watching the eating behaviors of others, and cues about ingested diets can also be detected through odors on another's breath (Galef & Clark, 1972; Galef & Wigmore, 1983; Mennella & Beauchamp, 1991a, 1993).

Direct observational learning about food preference in preschool children was examined by Birch (1980). The influence of socio-affective context on food preference formation in children was evaluated. Participant's vegetable preferences were established from a baseline done in a school lunchroom setting. After establishing this preference, the target child watched 3-4 peers choose a different vegetable than his/her favorite and then he/she was given an opportunity to choose a vegetable. The results showed a significant change in vegetable choice by the target child to a less preferred one by the fourth day of testing, which demonstrates that social learning had occurred.

Birch, Zimmerman and Hind (1980) conducted another study about modifying food preference in a social context. In this study, snack food preferences were manipulated using social cues. Once again baselines for initial preferences were established and social settings were assigned. A less-preferred snack food was given in one of four social contexts (1) as a reward for completing a task or engaging in a specific behavior, (2) non-contingently with behavior, but in the context of adult attention, (3) in a non-social context, or (4) during snack time without a contingent behavior or adult attention. A significant shift in snack food preference to the non-preferred item occurred for children in groups 1 and 2. Those social settings that were effective in shifting preferences were associated with adult attention or with reward. Preference shifts were maintained for at least six weeks following the manipulation. The findings of these two studies suggest social context and learning have an important role in determining dietary preferences.

Research has demonstrated that non-human animals transmit diet selection information by social learning mechanisms. Preferences have been established that confirm rats will approach conspecifics while they are feeding (Galef, 1978). Other research has discovered that rats will choose to eat in the same place as a conspecific as opposed to another location away from where the feeding animal has eaten (Galef & Clark, 1972).

Another method for the transmission of dietary preference information is to allow animals to socially interact after one animal has just eaten a distinct diet. Galef, Kennett and Wigmore (1984) found that same-sex pairs of rats showed evidence of dietary preference transmission through social cues. The study entailed one rat (called the

demonstrator) having to ingest either rat chow adulterated with 2% powdered cocoa or 1% ground cinnamon prior to interacting with the other rat (termed the observer). After the demonstrator consumed this adulterated food, the two animals were allowed to interact for 15 minutes. The observer was then offered both cocoa and cinnamon foods. The results showed a significant preference for the food consumed by the animal's demonstrator, indicating that information about diet preference was socially transmitted between the demonstrator and observer rats. Galef and his colleagues (Galef & Wigmore, 1983; Galef, Kennett & Wigmore, 1984; Galef, Kennett & Stein, 1985; Galef, 1989) have reported similar preferences across a variety of procedures. Furthermore, they have isolated the source of information transmission and determined that it occurs through olfactory cues. The observer animal must smell the diet on the breath and oral region of the demonstrator rat in order for a food preference to be observed.

Both the animal and children studies provide support for the idea that social learning can influence preferences for food. Besides the aforementioned studies, there are other parallels between food preference learning in humans and animals. Establishing an increased likelihood of ingestion of foods considered aversive or unpalatable has also been demonstrated. In some instances, social traditions make such preferences possible. Certain cultures advocate these types of food, which leads to repeated exposure. Although initially the substances are considered aversive, simple repeated exposure leads to a preference for this food. This is exemplified by the Mexican peasant populations, where members display a strong preference for spicy foods, like chili peppers (Rozin, 1977; Rozin & Schiller, 1980). The propensity for spicy foods is a result of gradually familiarizing themselves with the relevant flavorants. This process can also be seen with

the north Indian laborers who eat tamarind fruit, which is an extremely sour and slightly bitter fruit (Galef, 1981). Coffee is yet another example of a bitter substance that is initially perceived as unpalatable but can eventually become preferred.

Similarly, animals are also able to learn preferences for aversive or unpalatable substances through social exposure. Galef and Stein (1985) conducted a study that illustrated their propensity to acquire such preferences. In this experiment, a paradigm similar to the above demonstrator/observer methodology was used with a few modifications. The experimenters infused either a coffee or a cider vinegar solution directly into the stomach of the demonstrator rats instead of allowing them to consume it orally. Following the administration of one of these solutions, the demonstrator was placed with the observer to interact. Subsequently, the observer rat was presented with the choice of drinking either the coffee solution or the cider vinegar solution. The results revealed that observers drank significantly more of the demonstrator's solution than the other solution. This study confirms again the similarity in patterns of social learning between humans and animals.

Another substance initially considered aversive but after repeated exposure may become more palatable is alcohol, which was the focus of these studies. Alcohol is found in virtually all of the social domains in our culture, from religious ceremonies to cultural events to social gatherings. Its consumption in our society is encouraged and sometimes even positively reinforced. Nevertheless, repeated ingestion of alcohol has repercussions that can be potentially devastating, such as alcoholism and fatalities from drunk driving. Alcohol abuse is a widespread problem in today's society. The incidence of abuse is increasing, while the age at which large quantities of alcohol are being consumed is

decreasing. According to a health survey conducted by the National Institute on Alcohol Abuse and Alcoholism, binge drinking (defined as 5 or more drinks on occasion) is on the rise (NIAAA, 1997). This survey found that 30% of 12th graders and 16% of 8th graders acknowledged engaging in this behavior within the previous two weeks. This information exemplifies the necessity for understanding what social factors serve to initiate and maintain alcohol consumption.

Scordalakes (1998) examined the implications of social learning for alcohol consumption using an animal model. The age of the rats ranged from 29-40 days corresponding to the age of human adolescence. The same-sex demonstrator/observer paradigm utilized by Galef was implemented for this study. Demonstrators were stomach loaded with either a coffee solution, an alcohol solution, or tap water. After a 30-minute interval to ensure that the alcohol solution had been absorbed into the bloodstream, the demonstrator rat was allowed to interact with the observer for 30 minutes. The observer was then immediately offered both a coffee solution and an alcohol solution for a 24-hour period. Results indicated that the rats whose demonstrators were intubated with alcohol showed a significant increase in alcohol intake relative to controls. These findings supported the notion that social learning can influence preferences for alcohol and indicated that further research was necessary to explore further this relationship.

The focus of the research below was to establish the existence of social learning with respect to alcohol. Discovering that transmission of this preference was possible prior to adolescence could have important ramifications, such as suggesting that the development of a preference for alcohol can occur before adolescence. This could indicate that children are able to learn a preference for alcohol prior to the teen years

through social exposure to the substance by parents, siblings, or peers who have alcohol on their breath.

Experiment 1

Considering it has been established that adolescent rats have the ability to acquire a preference for alcohol by socially interacting with a demonstrator, the purpose of this study was to investigate whether this phenomenon occurred in 16-day-old animals. At this age, animals are still suckling from the dam and could be exposed to alcohol cues there. They are also beginning to venture away from the mother and start exploring, which would allow them to become socially exposed to alcohol cues from their peers. It was expected that observers who interacted with an alcohol demonstrator consume more alcohol than those do with tap water demonstrators. These results would further support the hypothesis that alcohol preferences are socially transmitted even in younger animals.

Method

Subjects

Forty, naive Sprague-Dawley rats from 5 litters were divided into 20 pairs of 16-day-old (+/– 1 day) same sex siblings to conduct this experiment. They were all born and reared in the Psychology Department vivarium at the College of William and Mary. The animals were housed in the maternal cage (36-cm x 46-cm x 21.5-cm) with pine shavings for bedding and maintained under a normal 12h light / 12h dark cycle with light onset at 0700h. Animals were maintained on ad lib ProLab chow and water. All testing was performed during the light phase. Equal numbers of males and females were randomly selected from the litters and randomly assigned to either an ethanol group (ETOH) or a

water group (W) to be demonstrators and observers. Subjects had not yet been weaned from their mother.

Apparatus

Animals were weighed using an Ohaus top-loading balance (model #E4000). A wire attached to 5-cm polyethylene (Clay Adams, PE-10) tubing was used to cannulate the observers. The end of the cannula was flared with a Weller soldering iron (model #SP23) to prevent the tubing from falling out of the cheek. Due to the immaturity of the thermoregulatory system, a small heating pad was used to maintain body temperature of the preweanling rats during testing. To infuse the ethanol solution, a Harvard Apparatus Compact Infusion Pump (model #975) was used with a flow rate of .14ml/min, which held a 5cc syringe and 30-cm of polyethylene tubing (Clay Adams, PE-50) attached to the end of the needle. The testing chamber consisted of clear Plexiglas that was 30-cm wide, 22-cm long and 40-cm high with an open top and bottom. Eight (36-cm x 22-cm x 18-cm) polypropylene cages were used to house the rats during the deprivation period. A 1cc syringe with a 7-cm length of PE 10 tubing was used for intubations.

Solutions

There were two solutions prepared for this experiment. One was an ethanol solution used for intubations. This solution was 12% v/v ethanol dissolved in a tap water vehicle (intubated in a dose of 1.5 g/kg). This dose was chosen because it allowed sufficient elimination of alcohol from respiration by the demonstrator (Molina & Chotro, 1989a). The other solution was a 5.6% v/v ethanol solution dissolved in a water vehicle and used with the infusion pump to test the observers for ethanol intake.

Procedure

This experiment was carried out in 5 steps.

Step 1. The preweanlings were separate from their dam and divided into same-sex pairs of demonstrators and observers. They were then numbered to mark the pairs and different colors were used to distinguish the observers from the demonstrators. The rats assigned to be observers were then cannulated. This procedure involved placing a small hook shaped guide wire with 7-cm of PE 10 tubing attached to it through the cheek of the rat. It took approximately 5 seconds per animal and has been shown to cause only minimal distress (Spear, Specht, Kirstein, & Kuhn, 1989). One end of the cannula was then flanged using a soldering iron.

Step 2. After cannulations were completed, animals were separated into their own individual holding cage and food and water deprived for 3 hours. No heating pad was used during this time in order to facilitate interaction between the pairs later during the interaction period. Rat pups are unable to completely thermoregulate by themselves at this age, and they tend to huddle together to keep warm (Albert, 1984). Animals isolated like this were found to huddle together when reunited, thus facilitating social contact and interaction. Results of pilot data revealed less interaction between the animals when they were kept warm.

Step 3. Following the deprivation period, the demonstrator rats were intubated with either the ETOH solution or the W control. Animals were first weighed to determine the volume of solution to be given (a volume of .015 ml/g of body weight). To intubate the animal, 7-cm of PE 10 tubing was attached to 1cc syringe. The tubing was placed in the mouth of the demonstrator and fed to the back of the throat. The animal began to

actively swallow the tube. The tube was gently pushed until it reached the stomach. The liquid was then infused directly into the stomach. The demonstrators were then put back into their cages for a period of 30 minutes to allow the alcohol to be absorbed.

Step 4. Thirty minutes after intubation, the demonstrators were placed in the cage with their same-sex sibling observer. The animals were allowed to interact for 30 minutes.

Step 5. Immediately after the interaction, the observers were tested for alcohol ingestion. The animals' bladders were voided and they were weighed before testing and their weights were recorded. After the initial weighing, the animal's cannula was attached to PE 50 tubing that was connected to a 5cc syringe placed on the infusion pump. The animal was then put into a Plexiglas testing chamber and allowed a 2-minute adaptation period. The chamber had a heating pad placed beneath it that was covered by paper towels. The heating pad was used to promote ingestion of the solution. This was a concern because a study by Hall (1979) found that animals would not ingest unless they were kept warm. The heating pad was set to 28° C. After the 2-minute adaptation period, the infusion pump was turned on for a 5-minute testing period. The pump delivered the ETOH solution at a flow rate of .14ml/minute. Immediately following the test, the animal was weighed again. The towels were changed and the chamber was cleaned after testing each animal to remove all alcohol cues from previous observers. This ingestion procedure was used instead of the one employed by Scordalakes because preweanlings are not able to drink from sipper tubes at this age.

Data Analysis

Each animal's pre-infusion body weight was subtracted from its post-infusion body weight. The amount ingested was then converted to a percentage of body weight gained (%BWG) using the formula [post-pre/pre] x 100.

Results

For this experiment, a two way ANOVA [Condition x Gender] was used to analyze the %BWG data, with $p < .05$. There was a significant main effect of Condition, $F(1, 16) = 13.65$ (see Figure 1) and a medium effect size, $\eta^2 = 0.46$. There was no significant main effect of Gender, $F(1,16) = .09$ or interaction (Condition x Gender), $F(1,16) = 0.03$. The results suggest that interacting with an alcohol demonstrator influenced the alcohol intake of the observer rat.

Discussion

As was expected, the observers exposed to a conspecific ethanol demonstrator showed significantly higher ethanol ingestion than those that had a water demonstrator. The animals were exposed to alcohol cues from a sibling's breath during the social interaction period. When offered the ethanol solution after this interaction, the ethanol observers showed a greater preference by ingesting more of the solution than controls. Casual observations also revealed differences in the behaviors of the alcohol and water observers during the ingestion testing. Animals that had a water demonstrator engaged in more moving around the test chamber and wiping their chins on the floor and walls, where as, alcohol observers engaged in more grooming behaviors during the testing. The finding suggest that rats as young as 16-days-old have the ability to acquire a preference for alcohol through social interaction with a demonstrator, supporting the hypothesis that

social learning about alcohol preferences can occur at this age. This is not surprising when you consider other literature findings. Preweanlings exhibited an increased preference for foreign odors that were paired with their dam or siblings (Galef & Kaner, 1980). During this study, pups were exposed to either peppermint extract painted on the face of their dam for thirty-three days following their birth. They were tested on postnatal day 33 (PD33) using an airstream chamber that filtered odors in when the animal inserted their nose into the stimulus chamber. The preweanlings presented with the peppermint odor activated the flow of peppermint significantly more than control animals.

The above research illustrates that social factors have a strong impact on ingestive behaviors of animals, especially those associated with the home cage and adults (Galef, 1981). Research has also been conducted with human children to assess the transfer of information about alcohol from their families. A study conducted by Fossey (1993) established that Scottish and English children could correctly identify the odor of alcohol 80% of the time. In addition, research conducted in Michigan found that children of parents who were heavy drinkers correctly identified alcohol odor more often than those whose parents had moderate drinking habits or did not drink alcohol at all (Cornwell-Jones & Sobrian, 1977).

The combined data suggest that young animals may be "programmed" to pay attention to odors that they encounter in the home nest (Bannoura Kraebel, Spear, and Spear, 1998). If this is the case, then it raises questions about when this phenomenon would emerge. Gaining an understanding of this could be very beneficial in establishing when social learning about alcohol begins and how environmental exposure affects alcohol preferences at different ages prior to adolescence. Children may be exposed to

alcohol through social interaction with their relatives or friends. Exposure could come from the breast milk of their mother, since it has been shown that alcohol is transmitted directly into breast milk after its consumption (Mennella & Beauchamp, 1991a). It could also be detected on the breath of another while having a conversation.

Experiment 2

The results of Experiment 1 showed those rats as young as 16 days of age have the ability to use social cues provided by same-sex siblings to develop an alcohol preference. In the second experiment, we investigated the development of social learning about alcohol to discern when this ability emerges. A suckling study conducted by Hunt, Kraebal, Rabine, Spear, and Spear (1993) evaluated ethanol tainted breast milk's influence on developing an ethanol preference. This suckling study involved using anesthetized foster dams whose milk let down had been chemically blocked. The pups where then allowed to attach to the nipple and the alcohol solution was infused into their mouths through a cannula in their cheek in 8 one minute intervals every 5 minutes for a 45 minute period. Hunt et al. found that 12-day-olds, but not 8-day-olds showed an enhanced intake of ethanol following exposure through breast milk while suckling. It is possible that although 8-day-olds did not show the preference for alcohol, that this was not because they were not learning. This may have been a function of the underdeveloped taste receptors (Mistretta, 1981) and therefore, their sense of taste was not advanced enough to detect the alcohol in the test solution. This explanation could be evaluated using an odor preference test in place of the ingestion test because the rat's olfactory system is fully

developed by birth (Alberts, 1984). This method of testing has been commonly used for assessing olfactory preferences in animals (Kucharski & Spear, 1984).

Based on Hunt et al.'s (1993) findings, we conducted an experiment to assess whether 8 and 12 –day-olds' abilities to socially learn about ethanol had developed yet. It was predicted that this ability to detect and learn about alcohol would emerge around 8-12 days of age.

Method

Subjects

One hundred and two, naive Sprague-Dawley rats from 12 litters were divided into 51 pairs of same sex siblings to conduct this experiment. The sibling pairs were composed of 8-day-olds, 12-day-olds, and 16-day-olds (+/– 1 day). They were all born and reared in the Psychology Department vivarium at the College of William and Mary. The animals were housed in the maternal cage and maintained under a normal 12h light / 12h dark cycle with light onset at 0700h. Animals were maintained on ad lib ProLab chow and water. All testing was performed during the light phase. Equal numbers of males and females were randomly selected from the litters and randomly assigned to either an ethanol group (ETOH) or a water group (W) to be demonstrators and observers. Subjects were housed with both parents and littermates in a standard maternity cage. The bedding consisted of pine shavings. Subjects had not yet been weaned from their mother. All of the conditions were the same as in Experiment 1, except the following. The ages varied and consisted of rats postnatal day (PD) 8, 12 and 16 (+/– 1 day). The number of preweanlings in each age group was as follows: PD8 = 15, PD12 = 16, and PD16 = 20.

Apparatus

Similar equipment was used as that described in Experiment 1, except for the changes noted. A different testing chamber was used. This clear Plexiglas chamber was 30-cm x 22-cm x 40 cm, but was divided in half lengthwise and the divider was opaque to allow for the testing of two animals at a time. The same infusion pump was used, but the infusion rates were modified for the younger animals. The 8-day-olds were infused at a rate of .07-ml/minute and the 12-day-olds at a rate of 0.09-ml/minute, and the 16-day-olds at 0.14-ml/minute.

Solutions

The same solutions were used as in Experiment 1 for intubation of the demonstrators and testing the observers.

Procedure

This experiment was carried out in 5 steps.

Step 1. The preweanlings were separate and cannulated using the same methodology as in Experiment 1.

Step 2. The same procedure previously explained for the deprivation period was used for this experiment with a slight modification. Heating pads were used with the younger (8 and 12 day-olds) animals to help alleviate the drastic drop in body temperature that occurs when animals are maintained at room temperature (Hunt et al., 1991). The temperatures in the holding cages were kept at 28°C for the 12-day-olds and 32°C for the 8-day-olds.

Step 3. The same method was used here for intubation procedure as Experiment 1.

Step 4. The interaction technique remained the same. The chambers used during the interaction phase were not heated in order to facilitate behavioral contact.

Step 5. The same procedure for testing ingestion was used.

Data Analysis

The percent body weight gained scores were calculated using the same method as in Experiment 1.

Results

A 3-way ANOVA [Condition x Age x Gender] was used to analyze the data, with $p < .05$. There was a significant main effect of Condition, F (1,39) = 32.83 (see Figure 2) with a medium effect size, $\eta^2 = 0.44$. There was no significant main effect of Age, F (1,39) = 1.33, nor Gender, F (1,39) = 2.78, and no significant interactions. The largest value for the interactions was Condition x Age, F (2, 39) = 1.20. The ANOVA revealed that all three age groups of preweanlings exposed to an ethanol demonstrator ingested significantly more ethanol than controls.

Discussion

Findings in the present study illustrate that rats as young as postnatal day 8 have the ability to take in odor cues they are exposed to during social interaction, process these cues, and translate the information into a learned preference for alcohol. In all three of the age groups, those animals with ethanol demonstrators ingested significantly more of the ethanol solution than did controls. The 16-day-olds tested in this experiment replicated the findings of Experiment 1. The data on 12-day-olds supported the findings of Hunt et al. (1993). The findings on 8-day-olds suggest that they were able to develop a preference for alcohol after socially interacting with a sibling alcohol demonstrator. Hunt et al.'s

study found that PD8 rats did not develop this preference after previous exposure to ethanol during suckling. The present experiment contradicted Hunt et al's findings.

Differences in the abilities of PD8 preweanlings in these studies could be explained by the method of initial exposure. The techniques used to introduce ethanol cues to the animals differed. The disparity in presentation methods required the animal to use different sensory systems in order to detect the ethanol. The current experiment only required olfaction for the ethanol to be perceived where as in the suckling experiment taste was necessary. Rat pups are not born with a fully developed gustatory system (Mistretta, 1981). Preweanlings do not achieve adult configuration of their taste buds until postnatal day 12 (Farbman, 1965). Therefore, it is possible that the pups did not detect the ethanol because it was presented in a half-and-half vehicle. Their taste buds may not have adequately developed enough to allow detection of the ethanol due to the complexity of the solution. The current study presented the EtOH cue on the breath of a sibling, so the rats would use their fully developed olfactory systems to detect the EtOH. Discerning the presence of EtOH during the test procedure was not a factor either, because water was used as the vehicle instead of half-and-half. Therefore, the under-developed gustatory system might have been able to process the simple taste cue effectively and a preference was expressed.

Other research supports the above conclusion that the ability to learn preferences for EtOH has developed by PD8. It actually indicates that an ability to learn from social cues is present even before birth. Detection of cues about maternal ingestion can occur prenatally via the amniotic fluid. An unborn organism can perceive chemosensory stimuli present in the amniotic fluid, including alcohol (Smotherman, 1982). Fetal processing of

chemosensory stimuli (i.e. apple juice, mint, citral, and almond) has been observed in rat fetuses as early as gestational day 17 (Smotherman, 1982). Therefore, tainting the amniotic fluid with substances that had chemosensory properties promotes subsequent increased acceptance of those sensory stimuli, suggesting that learning about preferences is occurring.

Additionally, research on the development of taste may offer an explanation for why social learning of this nature was possible, especially prenatally. According to Mistretta (1981), mature taste response characteristics are acquired gradually. The slow acquisition process during development makes it possible for this system to be modified by early taste stimuli. Information from the maternal diet obtained from the amniotic fluid and/or breast milk reach the gustatory system during its development because the fetus or infant ingests the amniotic fluid or the breast milk (Mistretta, 1981). This exposure occurs while the structure and function of the taste system is changing. The presence of these substances may stimulate the taste system, thereby influencing its development. These physiological changes could then lead to alterations in taste preferences or aversions.

A study done by Chotro and Spear (1997) lends credence to this idea. Responsiveness to alcohol was measured in gestational day 20 (GD20) rat fetuses after receiving exposure on GD17-19. The exposure was accomplished by administering the alcohol solution intragastrically to the mother. The fetuses were delivered cesarean into an isotonic saline bath 30 minutes after the last ethanol exposure. The placenta was left intact to ensure viability of the animals by the umbilical cord still attached to each fetus. While in the saline bath, the rats were administered 9, 2-minute intraoral solution infusions of ethanol, lemon, or saline. Fetal behavior was videotaped and scored for

movement during the duration of the trials. Behavioral analysis revealed animals previously experienced with the ethanol odor emitted mouthing movements higher than baseline after repeated presentations of alcohol during the ingestion testing than were exhibited by the lemon or the saline injected animals. The researchers concluded that prenatal exposure to alcohol evidently sensitizes fetuses to alcohol's orosensory and pharmacological effects. The experiment also illustrates that fetuses have the ability to learn information about odor cues and express preferences prenatally.

Chotro and Molina (1991) extended this work by showing that knowledge gained in utero could be expressed postnatally. In one study, Gestational day 20 (GD20) rat fetuses were exposed to either an alcohol solution, a lemon solution, or saline that was injected into the uterine horn, proximal to the rostral area (snout) of the fetus. Ten minutes after this injection, the experimenters performed cesarean sections to deliver the fetuses. Shortly after birth (within 90-250 minutes), the newborns' baseline heart rates were taken. The infants were then presented with a cotton swab soaked in alcohol. Their heart rate changes were examined in response to the odor presentations. Chotro and Molina found a slight and stable decrease in heart rate (bradycardiac) in response to the odor from all three preexposed groups. However, the rats prenatally exposed to the ethanol solution exhibited a significantly larger change in heart rate than did the lemon odor or saline animals. These results suggest that the animals were able to retain the information gained prenatally and at least recognize the odor when encountering it postnatally. The implication here is that learning about alcohol-derived cues is likely to occur in utero and to be remembered.

Early in life, there are also research findings that indicate that rats tend to process olfactory and gustatory components of substances configurally rather than separately (Kucharski & Spear, 1985; Molina, Serwatka, Spear, & Spear, 1985). Spear and Molina (1987) went on to further state that the preweanling period is an ontogenetic stage where information is likely to be transferred across different sensory systems. Developmentally this makes sense because during this period massive amounts of dietary information are being taken in. Sharing information between the systems would make the acquisition of preferences more efficient.

This transferring of information was demonstrated in intoxicated infant rats (Molina & Chotro, 1989a). They intragastrically administered a mildly intoxicating dose of ethanol (1.5g/kg). After an absorption period, the animal received an oral infusion of sucrose, thus pairing the alcohol with sucrose. This pairing was sufficient to promote a significant ethanol preference. They believe this occurred because of the elimination of ethanol cues via respiration and salivation. A second experiment used the same pairing again with a higher dose of ethanol (3.0g/kg) and added an unpaired group. The paired group again showed the alcohol preference. The unpaired group, however, exhibited an ethanol aversion. In their third experiment, they found that preexposing animals to ethanol eliminated the sucrose conditioned ethanol preference. These experiments showed that orosensory processing of alcohol might act as a conditioned stimulus when an appetitive reinforcer is paired with the state of intoxication.

There is some indication that this does not occur after the animal is mature. Adult rats do not seem to pair cues and make cross senses relationships about alcohol as preweanlings do. Molina, Sewatka, Spear, and Spear (1985) exposed both preweanlings

(PD21) and adults (PD60-80) rats to either EtOH odor paired with the early stages of apomorphine-induced toxicosis or EtOH odor with recovery from toxicosis. A control group with no pairings was also used. Twenty four hours later, an odor preference test (EtOH vs lemon) or a two bottle ingestion test (5.6% v/v EtOH vs 0.25% w/v citric acid solution) was given to assess EtOH learning. They found both ages expressed substantial odor aversions during the odor preference test if the previous ethanol exposure was paired with toxicosis. However, the ingestion test yielded mixed results. Only the PD21 rats changed their EtOH intake. If alcohol was paired with illness, then they drank significantly less than controls. If ethanol was paired with recovery, then rats drank significantly more than controls. Adults exposed to either of the pairing conditions drank similar amounts of ethanol as controls did. These results indicted that exposure to EtOH odor during adulthood only affected avoidance of the odor, and did not impact drinking behavior. Whereas, in PD21 rats both odor and ingestion preference tests were impacted. Therefore, memory about ethanol odors must be stored as preweanlings in order for the animals to use information in terms of gustatory behavior in adulthood. The findings imply that it is at the time of initial exposure that memory information is stored. If this occurs during the preweanling period, olfactory information can be transferred to the gustatory system resulting in both odor and taste preferences and aversions. Adult rats seem to have lost the ability to transfer learning from one sense to another.

These findings suggest that the gestational and preweanling periods may be an optimal period for developing dietary preferences. This is a time when dietary information is being formed, so being able to share information between the senses would be adaptive. This theory of the transference of information is certainly supported by our research.

Once this preference is established, this information should be able to be retained for later usage. A study assessing the impact of gestational exposure looks at precisely this issue. Dominguez, Chotro and Molina (1993) evaluated whether preweanlings would be able to use in utero information about alcohol weeks after birth when encountering the substance again. Prenatal exposure was administered 10 minutes prior to delivery in a similar fashion to the procedure of Chotro and Molina (1990) and an ethanol ingestion test was conducted on postnatal day 11 (PD11) via an intraoral cannula. The procedure was similar to the one followed in Experiment 1 of this paper, except the deprivation period was 22 hours. Rats prenatally exposed to the ethanol odor showed higher percent body weight increases than those with prenatal exposure to saline. The research indicates that not only did the rats remember the information over time, but also the animals were actually able to translate this knowledge into a measurable preference.

Combining the above findings with the results in Experiment 2 raised the question of whether information encountered in the sibling social interactions would be utilized in the same manner as the in utero experiences were. It was also the question of whether or not the preweanlings would remember the social cues over time and express them in the form of an alcohol preference during adolescence. No research had directly assessed the impact of social exposure during the preweanling period on alcohol preferences in adolescence. Understanding adolescent preferences could be very important. It is during this developmental stage, autonomy is being established and the knowledge acquired earlier in life also is being assimilated to help form adult preferences. Gaining an understanding about what prior experiences are utilized in making these choices about alcohol could be very beneficial. Based on the developmental findings above, we

hypothesized that the important factors in acquiring an alcohol preference through social learning mechanisms are the context in which the information is expressed and the individual transmitting it.

Experiment 3

The third experiment was conducted to assess the maintenance of a socially induced EtOH preference. The purpose of this study was to determine whether the information about a preference for alcohol could be retained into adolescence. Repeated exposures to alcohol were given to the rats, in order to approximate more closely the fashion in children of alcoholics are exposed. Knowing how the frequency of exposure affects alcohol preferences could help us to understand the influence of social learning on adolescent alcohol preferences in these children. We expected to find that early and frequent exposure in rats would lead to a strong and robust preference for alcohol that was maintained at least into adolescence.

Method

Subjects

A total of 8 litters of Sprague-Dawley rats with 8-10 pups per litter were used to complete this experiment. Four of the pups from each litter were randomly chosen to be demonstrators, and of the remaining animals, four were randomly selected to be observers. Equal numbers of males and females were used from each litter when possible. They were all born and reared in the Psychology Department vivarium at the College of William and Mary. The animals were housed in the maternal cage and maintained under a normal 12h light / 12h dark cycle with light onset at 0700h. Animals were maintained on ad lib ProLab chow and water. All testing was performed during the light phase. The

groups varied in age depending on whether they are demonstrators or observers. Demonstrators were intubated on postnatal days12, 14, and 16. The observers were tested for ingestion on postnatal day 22 (+/– 1day). Animals were weaned 1-2 days prior to testing.

Apparatus

Similar equipment was used as that described in Experiment 1, except for the changes noted. The testing chamber used was the same as in Experiment 2. The same infusion pump was used, but the infusion rate was modified for the older animals. The infusion rate was .17-ml/minute for the 22 day-olds (+/- 1).

Solutions

The same solutions were used as in Experiment 1 for intubation of the demonstrators and testing of the observers.

Procedure

This experiment was carried out in 5 steps.

Step 1. On postnatal day 12, the pups were separated from the home cage and four demonstrator animals were randomly selected from the litter. Equal numbers of males and females were chosen as litters permitted.

Step 2. The demonstrator animals were then numbered, weighed, ear marked, and intubated with either the EtOH solution or water. The demonstrators were returned to the home cage immediately following the intubation procedure to interact with their sibling observers. This procedure was repeated on postnatal days 14 and 16. The EtOH and water demonstrators were from separate litters to prevent the control animals from being exposed to the ethanol odor.

Step 3. The preweanlings designated as observers were removed from the home cage on PD22 and cannulated using the same methodology as in Experiment 1.

Step 4. The same procedure previously explained for the deprivation period was used for this experiment, but only the observer rats were deprived.

Step 5. The same procedure for testing ingestion was used as in Experiment 1, except the infusion rate was altered to accommodate the older subjects.

Data Analysis

The percent body weight gained scores were calculated as in Experiment 1.

Results

For this experiment, a two way ANOVA [Condition x Gender] was used to analyze the %BWG data, $p < .05$. There was a significant main effect of Condition (ETOH and H2O), $F(1,26) = 205.05$ (see Figure 3) and a large effect size, $\eta^2 = 0.89$. There was no significant main effect Gender $F(1,26) = 0.28$ or interaction $F(1,26) = 1.57$. The results showed that preweanlings that were exposed to ethanol demonstrators on PD12, 14, 16 ingested significantly more ethanol on PD22.

Discussion

The data from the above experiment support the hypothesis that early and frequent exposure leads to a strong and robust preference for alcohol. The preference learned about during the preweanling period was maintained into the beginning of adolescence. Observer rats exposed to an ethanol demonstrator exhibited a strong preference for alcohol on postnatal day 22 when compared to controls with a water demonstrator. Thus supporting our predictions that alcohol preferences gained early through social interaction would exert an influence during adolescence.

The ethanol exposure procedure used here incorporated two factors that were previously proposed as important for transmitting dietary preferences. These were the environment where the exposure occurred and the relationship of the demonstrator to the observer. Ethanol was presented in the home cage. According to Brown (1982) preweanling Long-Evans rats prefer the odor of their home cage to either clean bedding or bedding soiled by other conspecifics. Bannoura, Kraebel, Spear, and Spear (1998) looked at alcohol ingestion of ethanol in 23-day-old rats. They exposed the animals to either ethanol or clove oil odors in their home cage from PD1-PD22 and tested them for an odor preference. Animals exposed to the ethanol odor in their home cage ingestion significantly more ethanol during an ingestion test than those exposed to the clove oil odor. Research also indicates that pups reared artificially away from conspecifics do not display a preference for eating at a sight marked by the feces of a conspecific. However, pups reared with a dam and siblings do show this preference (Galef, 1981). The demonstrator/observer relationship is also important. Galef and Kaner (1980) found that preweanlings exhibit an increased preference for foreign odors that are paired with their dam and/or siblings.

Animals were exposed to peppermint extract painted on the face of their dam or the dam and the dorsal surface of their siblings for a period lasting from birth to PD14. They were tested using an airstream apparatus for a peppermint odor preference and compared to a control group not exposed to the odor prior to testing. Significantly, more time was spent releasing the peppermint odor by the groups with the dam and the dam and sibling demonstrators than the control animals. Thus, establishing that family members play an important role in the transmission of dietary preferences (Rozin, 1977). The

methodology utilized paired the home environment with family members during the odor exposure. By doing this, the odor cues were doubly reinforced and produced a stronger odor preference. Repeating the exposure also enhanced the preference and its retention by giving the animals more learning trials to strengthen the memory. Experiment 3 of this paper supports the proposed explanation that pairing relevant social stimuli (home and family) with the ethanol odor cue produces a strong preference for alcohol.

General Discussion

The results of these experiments indicate that alcohol preferences were affected during a period of social interaction. After only a brief exposure to alcohol from a sibling, preweanling rats' later alcohol ingestion was increased (Experiment 1). This suggests that conspecifics play an important role in the transmission of alcohol preferences. The capacity to socially learn about alcohol was present very early in life (Experiment 2), and finally, this established preference for alcohol was retained and present in adolescence (Experiment 3). Collectively, the knowledge gained about how preferences for alcohol develop and are maintained have important implications for the social learning that may occur in human families. The literature presented earlier that established the similar ways other dietary preferences were transmitted in humans and rats suggests that we may develop preferences for alcohol in a comparable fashion.

It seems just as likely that human fetuses, which encounter alcohol in the amniotic fluid while their gustatory system is maturing, may have their taste system stimulated. The early stimulation may cause the system to be modified, because mature taste response characteristics are acquired gradually. The physiological changes that occur could promote the formation of a taste preferences for alcohol. This physiologically based taste

preference might make us more likely to drink alcohol, which may contribute in part to the biological/genetic link there is with alcoholism.

The postnatal period may differ a little because human infants' gustatory systems are fully developed before birth (Mistretta, 1981). Therefore, when infants are exposed to alcohol in the breast milk, they will be able to detect its presence. Research conducted by Mennella and Beauchamp (1991) evaluated this. They assessed the odor of the mothers' breast milk after she consumed alcohol. A blind panel of adults evaluated the odor of the milk and determined that it "smelled like alcohol." Furthering the above work, Mennella and Beauchamp (1993) found indications that human infants were also detecting cues about alcohol while breast-feeding. Babies whose mothers consumed a single beer drank less milk than controls, suggesting they noticed alcohol's presence in the breast milk.

Research with human infants has evaluated whether learned information could be translated into a preference and have the preference be retained. Beauchamp and Moran (1984) conducted an experiment with human infants to assess whether they had the ability to retain information gained about a food preference over time. Infants were presented with sugar water within the first months of life. At six months and two years, the babies were tested for a sugar water preference. Indeed the six-month-olds showed a preference for sugar water, the preference was demonstrated again at two years. These studies suggest that in both humans and animals the effects of early odor exposure have a long lasting impact on food preferences. If sugar water preferences can be established and remembered, it is possible that alcohol preferences could be, too. There has also been research that directly assessed children's ability to detect alcohol odors. Fossey (1993) found Scottish and English children were able to correctly identify the odor of alcohol

80% of the time. Another study conducted by Noll, Zucker and Greenberg (1990) discovered that Michigan preschoolers whose parents were heavy drinkers correctly identified alcohol odor more accurately than those whose parents drank moderately or not at all. Both studies provide support for the idea that children can learn to identify the odor of alcohol, so it is possible that transferring of this knowledge to a preference could occur.

As children get older, the most powerful force in the acquisition of culture, both food and other domains, is direct social "effects" (Rozin, 1977). The "effect" generally refers to situations when children are exposed to objects or attitudes that are valued by others (parents, sibling, other adults, certain peers), and this causes them to value the object or attitude more. Family (especially parents) are the most likely sources of social cue fostering exposure to food. Evidence suggest food preference are acquired by perceiving what others value.

Marinho (1942) demonstrated that the effects of social influence on food choice was greater and more persistent in children who had no strong initial preference for the novel food, and those induced changes that could last for months. This is long enough for children to have this preference reinforced and perpetuate its existence. Even if children did express a strong initial preference to alcohol, repeatedly exposing them to it could alter their feelings about alcohol. Rozin and Schiller (1980) found Mexican parents were able to influence their children into developing a preference for chili pepper. Initially, the youngsters disliked the hot burn of these peppers. The parents accepted their refusal of the food, but continued to offer the pepper at meals. This produced gradual acceptance of the hot sauce on their food. By about age five to seven, the children began adding the chili pepper to their food by themselves.

A relationship like this could work to promote an alcohol preference. Repeated exposure to the odor of alcohol on their parents creates a pairing of the two. The children may smell the aversive odor on their parents' breath or observe their drinking habits, but the context of their interaction is positive. Over repeated occurrences of situations like this, the children could come to associate the odor of alcohol with something they like and develop a preference for it. The opposite could just as easily be true. If the interaction were with an abusive alcoholic parent, these kids would receive negative parings with alcohol cues that could develop into an aversion to alcohol (I do not like this situation and the alcohol odor is associated with it, so it is bad, too). Making these associations could explain why some children of alcoholics follow their example and some do not. These implications do not claim that alcohol ingestion is completely determined by these factors. The claims made here only suggest that early and repeated exposure of the nature mentioned in the research that might lay the foundation for later alcohol preferences. We are not disputing that other factors influence people's relationship with alcohol. However, we do suggest that the role of the environment in this process may have been underestimated or overlooked. Future research should evaluate how social learning about alcohol develops. Alcohol preference retention also needs to be investigated further to establish whether preferences developed from early exposure are retained past adolescence and into adulthood.

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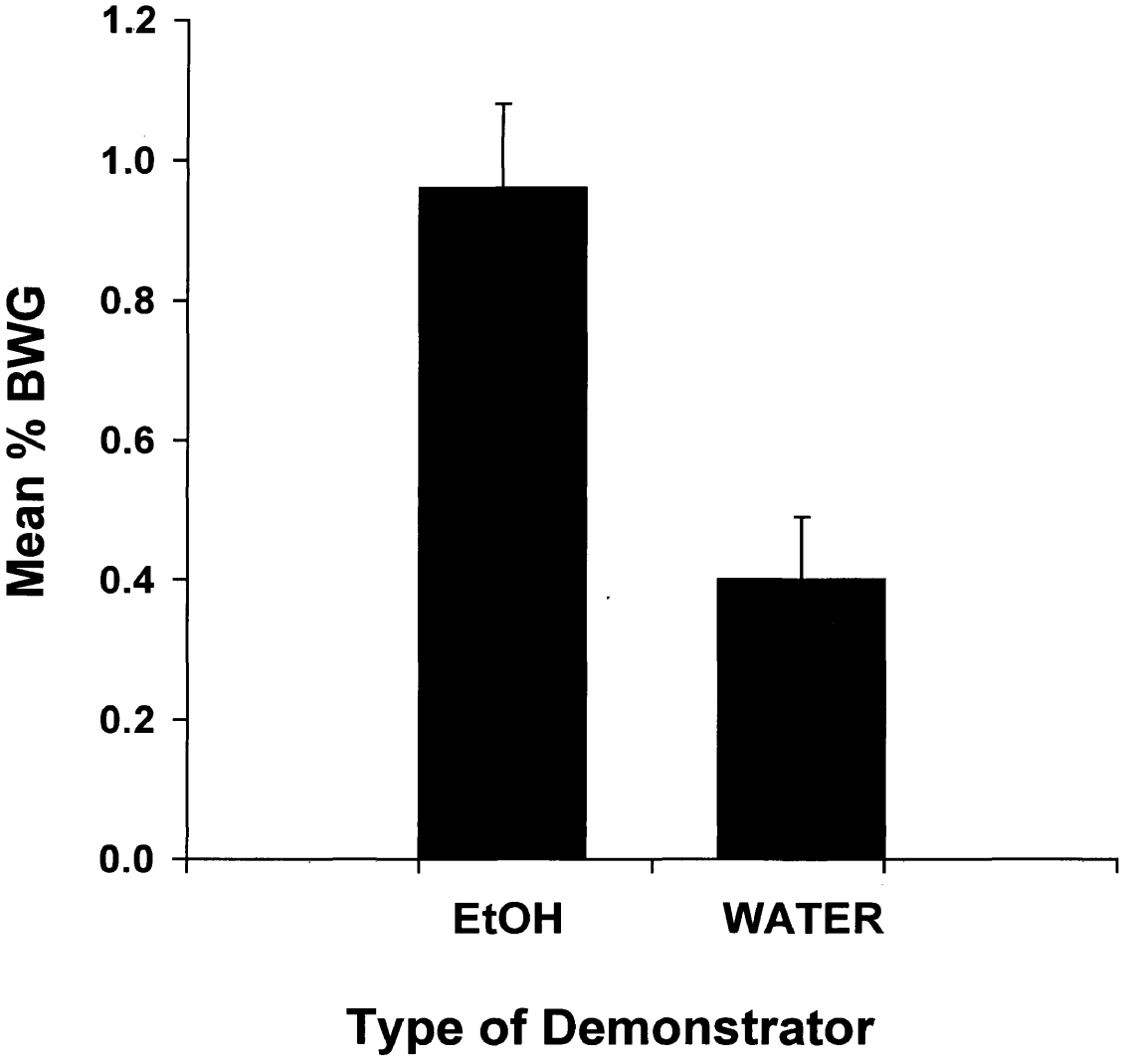
Figure Captions

Figure 1. Mean (+/- SEM) percent body weight gained (%BWG) of PD16 observers during 5 minute ethanol ingestion testing period after 30 minute interaction with ETOH or Water demonstrators.

Figure 2. Mean (+/- SEM) percent body weight gained (%BWG) of PD8, 12, 16 observers during 5 minute ethanol ingestion testing period after 30 minute interaction with ETOH or Water demonstrators.

Figure 3. After repeated exposure to alcohol and water sibling demonstrators on postnatal days 12, 14, and 16, observer animals were given a 5-minute ethanol ingestion testing period. Mean (+/- SEM) percent body weight gained (%BWG) by PD22 observers with ethanol or water demonstrators.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0.4
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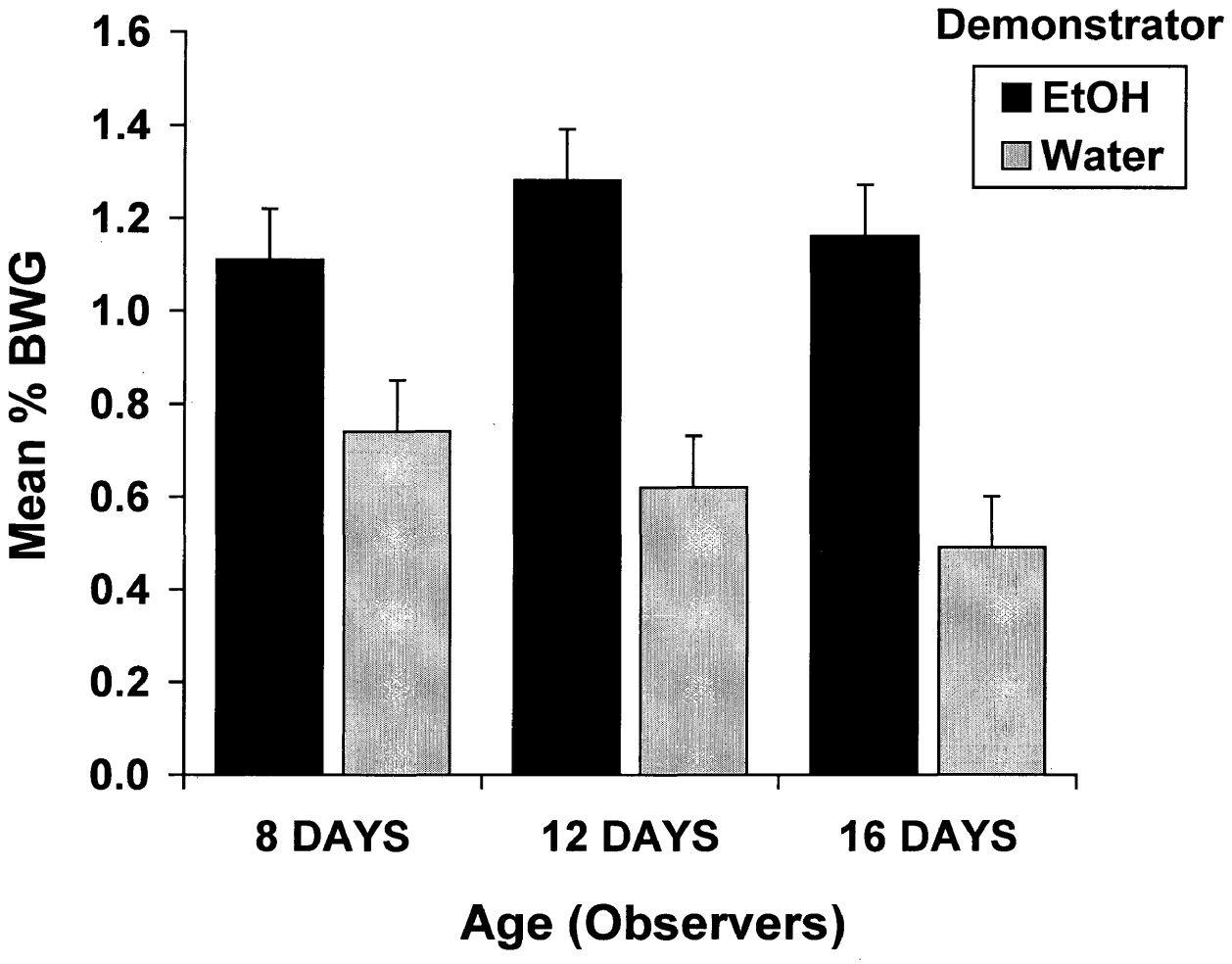
Mean % BWG
EtOH
WATER
Type of Demonstrator


Demonstrator
EtOH
Water
Mean % BWG
1.6
1.4
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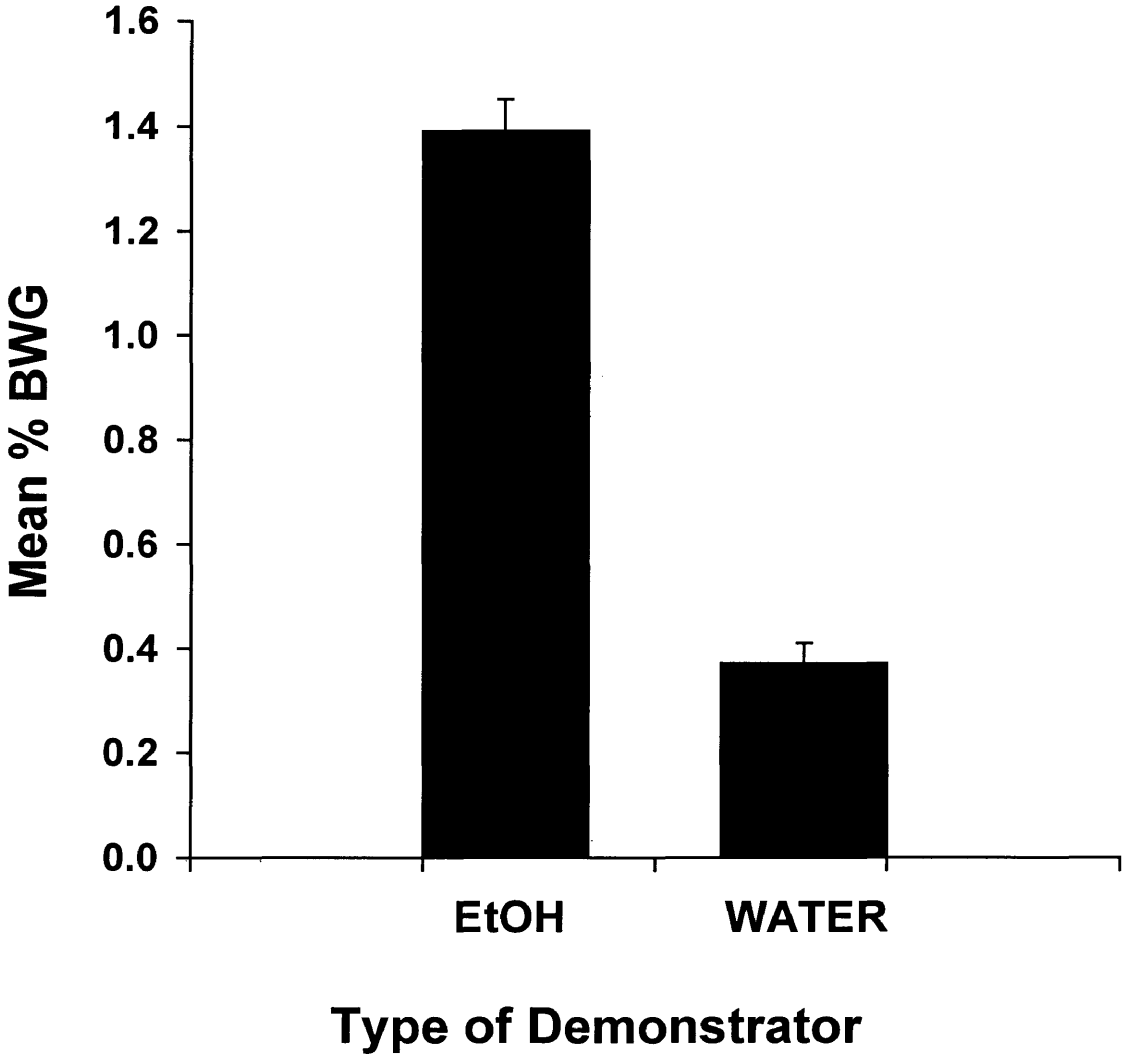
8 DAYS
12 DAYS
16 DAYS
Age (Observers)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Mean % BWG
EtOH
WATER
Type of Demonstrator

VITA

Ginger M. Lant

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