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Enhanced Preference for Ethanol Odor and Increased Voluntary Intake Following Exposure to Ethanol During the Early Postnatal Period

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by

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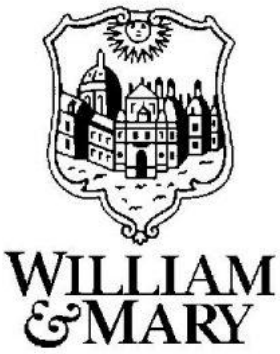

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Abstract

A focus on early development is critical for understanding the etiology of alcoholism. The observation that alcoholism runs in families has strengthened the idea of a genetic basis for the disease. It is also recognized, however, that growing up in a household where alcohol cues are omnipresent may provide critical experiences that could increase that risk. The effects of early experience with ethanol cues can be examined using the demonstrator-observer paradigm in rats. During the early postnatal period, experimental subjects (observers) were exposed to intoxicated siblings (demonstrators) in their home cage. Controls were exposed to sibling demonstrators that were administered water. Animals were tested for voluntary ethanol intake (Experiments 1, 2a, 3 and 4) or ethanol odor preference (Experiment 2b) when they reached an age corresponding to early adolescence in humans, postnatal day 30. Results revealed that infant experience with ethanol cues in the home environment resulted in increased ethanol intake and ethanol odor preference by adolescent-age subjects. These responses to alcohol were not only evident in observer subjects, but in the demonstrator animals as well (Experiments 2a and 2b). Thus, both direct (intoxication) and indirect (via siblings) exposure to ethanol cues in the home environment can increase animals' preferences for this drug. Experiment 3 was designed to evaluate whether a reactivation treatment (exposure to ethanol odor) prior to a test for ethanol ingestion would increase the expression of the ethanol preference. Results of this experiment failed to reveal such an effect. Finally, Experiment 4 evaluated the hypothesis that the endogenous opioid system is involved in this type of social learning. Observer subjects were injected with the mu opioid antagonist naltrexone prior

to interactions with intoxicated siblings. Results failed to support our hypothesis. Observers injected with naltrexone ingested just as much ethanol during the intake test as those that were injected with saline. Collectively, the results of these experiments indicate that social interactions with an intoxicated conspecific early in development are critical experiences impacting later ethanol ingestion. The findings from these experiments emphasize the important influence of early social experiences on later behavioral responses toward ethanol, and suggest that this type of ethanol exposure can have long-lasting consequences. Early learning about alcohol in the context of the home environment may result in increased risk for the later development of alcohol abuse and dependence.

Enhanced Preference for Ethanol Odor and Increased Voluntary Intake Following Exposure to Ethanol During the Early Postnatal Period

Background and relevance:

Alcohol abuse has become a heightened problem in today's society. A 2005 National Institute on Alcohol Abuse and Alcoholism (NIAAA) survey reported 51.5% of older adolescents (18-20 years) and 26.3% of younger adolescents (15-17 years) reported drinking within the past month (NIAAA, 2009). Another recent survey revealed that 23.7% of adolescents had their first drink before age 13 (NIAAA, 2006). Moreover, a 2005 Monitoring the Future (MTF) study reported that 11% of eighth graders, 22% of tenth graders, and 29% of twelfth graders reported binge drinking, defined as consuming more than five drinks on occasion, within the past two weeks (NIAAA, 2006). With that said, adolescents are beginning to consume alcohol at increasingly younger ages and such early onset patterns of ingestion are correlated with later emergence of alcohol and other drug abuse problems. Grant (1998) conducted a large survey of alcoholics and asked about their age of onset of drinking. A negative, linear correlation was obtained, indicating that a greater percentage of alcoholics began drinking at younger ages. Nearly half of the alcoholics reported drinking onset prior to 16 years of age. This was true for both men and women, and was observed in individuals who had either a positive or negative family history of alcoholism. It was concluded that the younger the age of onset of drinking, the more likely that the individual would experience alcohol abuse problems. It is therefore essential to investigate the causative factors involved in voluntary alcohol

consumption, especially in early adolescence. Such factors include genetic predisposition as well as social and environmental interactions present early in development.

Genetic determinants of alcoholism:

A major focus of research in this area has been to identify the genes underlying the development of alcoholism. The recognition that alcoholism runs in families has strengthened the hypothesis of a genetic basis for the disease. Historically speaking, animal models have been extensively used to investigate mechanisms underlying alcohol-seeking behavior and abuse (Li, Lumeng, McBride & Murphy, 1993). Several rodent lines have been selectively bred for alcohol intake, implying a genetic basis for drug ingestion. Specifically, alcohol preferring (P) and nonpreferring (NP) rats differ in their voluntary alcohol consumption (Crabbe, Belknap & Buck, 1994; Foroud, Bice, Castelluccio, Bo, Miller, Ritchotte, Lumeng, Li & Carr, 2000), with P rats ingesting much higher quantities of alcohol on their first exposure than NP rats. Genetically-mediated differences in the reward value of alcohol are seen in P rats in comparison to NP rats. Like the P and NP lines, high-alcohol-drinking (HAD) and low-alcohol-drinking (LAD) genetic lines differ in their alcohol consumption (Foroud et al., 2000), as do alcohol preferring (C57BL) and non-preferring (DBA) mouse lines (Randall & Lester, 1975). Non-selected lines tend to drink alcohol in volumes that fall between the pairs of selected lines, so it appears that genetic selection can act in both directions – leading to both alcohol preference and avoidance. Further distinctions between opposing genetic lines may include neurotransmitter concentrations, mainly differences in dopamine and serotonin, within the nucleus accumbens (Li et al., 1993). These distinctions further add

to arguments that genes play a chief role in alcoholism or other substance abuse (Crabbe et al., 1994; Li et al., 1993).

Effects of early experience with ethanol cues:

It is recognized that genes are not the only factor contributing to later substance abuse. Honey and Galef (2004) argued that maternal alcohol consumption during pregnancy can more accurately predict offspring's age of first intoxication, as well as its frequency, more than genetics alone. Because maternal consumption during pregnancy causes fetal blood-alcohol levels to be roughly equivalent to their mothers, exposure to alcohol may predispose offspring towards early alcohol consumption. Intrauterine ethanol exposure impedes proper physiological and neurological development of the fetus, sometimes resulting in Fetal Alcohol Syndrome (FAS; Jones & Smith, 1973). Fetal alcohol exposure may increase affinity for alcohol consumption as well as influence later substance abuse (Yates, Cadoret, Troughton, Stewart & Giunta, 1998). Chemosensory cues associated with the maternal diet are detected by the fetus in the amniotic fluid and appear to enhance consumption of similar foods during infancy (Mennella, Jagnow & Beauchamp, 2001). Baer et al. (1998) examined alcohol drinking patterns in adolescents with known prenatal exposure to alcohol, and reported significantly greater alcohol consumption in this group compared to a group not exposed to alcohol during gestation. Thus, prenatal exposure to ethanol may dispose an individual toward alcohol abuse in adolescence.

This effect has been examined in animal models as well. During the final days of gestation, the fetus is able to identify chemosensory properties of substances present in the amniotic fluid to subsequently increase palatability for these substances during

infancy (Arias & Chotro, 2006). Arias and Chotro (2005) found that fetal alcohol exposure during gestational days 17-20 of the prenatal period (total gestational period in the rat is 21 days) not only increases alcohol consumption during infancy, but pups also display more appetitive behaviors, including mouthing movements and paw licks, and fewer aversive responses than subjects with no prior exposure. From gestational day 17 onward, the fetus has the ability to form associative memories as well as acquire conditioned responses to chemosensory stimuli. Possibly mediated via activation of the opioid system, these conditioned responses and associations can be expressed throughout infancy and into the juvenile period (Arias & Chotro, 2005; 2006; Diaz-Cenzano & Chotro, 2010).

Exposure to specific chemicals, such as alcohol, in breast milk during development impacts infants' olfactory preferences as well as subsequent behavior (Mennella & Beauchamp, 1998). This effect is seen in infants as early as six to twelve months in age. In addition to breastfeeding, infants of alcoholic parents, as determined by the Michigan Alcoholism Screening Test (MAST), are frequently exposed to alcohol's odor within the home environment. In this study, all mothers were also asked to complete a questionnaire regarding the number and type of alcoholic beverages consumed during pregnancy and while breastfeeding. Women consuming four or more beverages per week were classified as frequent consumers compared to occasional consumers with approximately one beverage per month. The control group consisted of mothers ingesting equivalent amounts of vanilla-flavored foods because like alcohol, it too can be easily detected within the breast milk (Mennella & Beauchamp, 1994, 1996). Mennella and Beauchamp (1998) found a correlation between odor preference and parental drinking

patterns in infants. When presented with both vanilla-scented and alcohol-scented toys, infants whose parents were alcoholics or frequent consumers preferentially chose to interact with the alcohol-scented toy. Such interaction included manipulation and even mouthing of the toy. This response was specific to the alcohol odor alone and not seen in infants whose parents were occasional consumers. These findings, combined with those obtained from testing animal models, suggest that olfactory preferences are learned early in development and may provide a greater understanding of the importance of early alcohol exposure within the home environment.

Experience with alcohol during nursing may also lead to increased acceptance of alcohol later on (Honey & Galef, 2003; 2004; Hunt, Kraebel, Rabine, Spear & Spear, 1993). Early rearing experience can even counter a genetic predisposition for low levels of alcohol intake (Randall & Lester, 1975). In fact, exposure to an ethanol-ingesting dam throughout gestation, lactation, and weaning increases voluntary alcohol consumption in offspring tested as adolescents (Honey & Galef, 2003). Hunt and colleagues (1993) demonstrated that preweanling exposure to ethanol in the nursing context can also modify later acceptance of the drug (see also Molina, Pepino, Johnson & Spear, 2000; Pepino, Lopez, Spear & Molina, 1999). Exposure to an ethanol-consuming dam during the preweanling period can increase affinity for later consumption during adolescence that persists for a minimum of six weeks (Honey & Galef, 2004). Results of these studies suggest that exposure to alcohol via maternal consumption has potentially long-lasting effects on pups' responsiveness to ethanol.

Role of social learning:

Considerable evidence suggests that experiential factors may play a chief role in the initiation of alcohol drinking behavior. Such factors are present during early postnatal development and may continue throughout adolescence. Prior to alcohol consumption, children have developed expectations and attitudes towards alcohol drinking and its related behaviors via socialization. Socialization is a fundamental process by which children learn about various expectations of their society primarily through agents that include, but are not limited to, family, friends, peers and the media (Velleman, 2009). Humans, primarily adolescents, are more inclined to ingest alcohol following repeated long-term or brief interactions with their intoxicated peers (Fernandez-Vidal & Molina, 2004). In fact, social learning and behaviors resulting from alcohol-related cues may first be established in early childhood. Noll, Zucker and Greenberg (1990) found that accurate odor identification of at least one alcoholic beverage directly correlated with parental drinking patterns. Preschoolers whose parents were categorized as heavy drinkers were able to correctly identify odors of various alcoholic beverages. This effect was not seen in preschoolers whose parents engaged in minimal alcohol consumption. Such learning is attributed to direct exposure within the home environment. Similar to the experiment described above, Mennella and Garcia (2000) found similar results. In this study, infants with frequent alcohol exposure, as determined from questionnaires regarding parental alcohol consumption, preferentially selected and subsequently mouthed toys that had been scented with alcohol over vanilla-scented toys. The opposite pattern was seen in infants with minimal prior alcohol exposure.

Research conducted in several laboratories, including ours, has focused on identifying experiential factors that may contribute to the onset of alcohol drinking behavior (Fernandez-Vidal & Molina, 2004; Hallmark & Hunt, 2004; Honey & Galef, 2003, 2004; Hunt et al., 1993). It has been repeatedly demonstrated that the propensity of rats to drink alcohol can be increased following social exposure to alcohol (Hunt & Hallmark, 2001; Hunt, Holloway & Scordalakes, 2001; Hunt, Lant & Carroll, 2000). Specifically, the Hunt lab has adapted the Demonstrator-Observer paradigm, originally developed by Strupp and Levitsky (1984), and utilized extensively by Galef and his colleagues (e.g. Galef, Mason, Preti & Bean, 1988; Galef & Stein, 1985), for studying social learning about alcohol. Galef and colleagues have shown that when an experimental animal, known as the observer, is allowed to interact with a conspecific that has ingested a novel food, known as the demonstrator, the observer animal will later voluntarily ingest much more of the demonstrator's diet than it would in the absence of such social experience. In fact, observers ingest a greater amount of the diet following interaction with a demonstrator than they do following direct exposure to the diet (Galef, Kennett & Stein, 1985). This general procedure has been applied to the social transmission of information about alcohol. With approximately 10-15% of a given dose of alcohol eliminated in its unmetabolized form through salivation and respiration (Hollstedt & Rydberg, 1985), rats are able to detect and transfer information regarding ethanol via oronasal contact. By doing so, the observer animal is able to detect the odor of alcohol on the breath of the demonstrator and thus, it is believed that the odor becomes associated with the rewarding aspects of the social interaction (Galef et al., 1985, 1988).

The developmental period of adolescence seems particularly important in the etiology of alcohol and other substance abuse (Grant, 1998). Hunt, Holloway and Scordalakes (2001) allowed adolescent observer rats to interact for a brief period of time (30 min) with a sibling demonstrator that had previously been administered alcohol. Immediately following this interaction the observers were given a choice between alcohol and an equally palatable decaffeinated coffee solution during a 24-h ingestion test. It was found that the observers that had interacted with ethanol demonstrators later ingested much more alcohol, relative to coffee, than control observers that had interacted with demonstrators administered coffee or water. Hunt, Lant and Carroll (2000) further showed that the ability of animals to exhibit this type of social learning about alcohol cues is present within the first week of life, and that with repeated exposures to intoxicated demonstrators the enhanced alcohol ingestion was evident when animals were tested as weanlings (postnatal day 23). These data indicate that early learning via exposure to alcohol-intoxicated siblings may have long-lasting consequences for alcohol acceptance (see also Hallmark & Hunt, 2004). Moreover, Fernandez-Vidal and Molina (2004) highlighted the importance of social influences on alcohol-related behaviors. Brief social interactions with an intoxicated littermate (*i.e.* alcohol-intoxicated demonstrator) are sufficient to facilitate alcohol recognition and mediate alcohol responsiveness in periadolescent rats. However, exposure to alcohol odor by an alcohol-scented cotton surrogate is insufficient to produce an alcohol preference. Similarly, alcohol odor preferences were not established in observers that interacted with an intoxicated, yet anesthetized, demonstrator (see also Musisca, 2004).

The purpose of the present experiments was to further explore the effects of early (preweanling) ethanol exposure in the home environment on voluntary intake of ethanol and ethanol odor preferences by animals that were tested as adolescents. The novelty of the experiments were that (1) animals were repeatedly exposed to ethanol during infancy, a younger age than previously examined, (2) ethanol odor preferences, in addition to ethanol intake, were measured, and (3) both demonstrator and observer subjects were tested in some experiments. Overall, the results demonstrate that ethanol preferences exhibited by adolescent animals are enhanced by experiences that occurred in infancy.

EXPERIMENT 1

This research design was intended to build upon previous procedures, like those from Strupp and Levitsky (1984) and later, Galef and colleagues (e.g. Galef et al., 1985), to better identify experiential factors mediating voluntary ethanol ingestion and preference by adolescents. Galef, Kennett, and Stein (1985) used the Demonstator-Observer paradigm to study the transmission of diet preferences in rats. This paradigm was designed to examine the effects of social interactions in learning without extrinsic reinforcement (Maldonado, Finkbeiner & Kirstein, 2008). Following periods of social interaction, the observer exhibited an increased preference for whichever diet their respective demonstrator had consumed, suggesting that olfactory cues transmitted from the demonstrator to the observer in a positive social context were sufficient to influence subsequent dietary preferences. Social interactions, especially during adolescence, are reinforcing and necessary to condition odor and dietary preferences as well (Fernandez-Vidal & Molina, 2004; Galef & Stein, 1985; Hunt & Hallmark, 2001). If, however, a

cotton surrogate scented with a particular odor is used in-lieu of a demonstrator rat, observers show no preference towards the dietary information (Fernandez-Vidal & Molina, 2004; Galef et al., 1985; Galef & Stein, 1985). This evidence suggests that diets presented on a rat demonstrator in a social context are more effective in influencing dietary preference than surrogates, and second, that exposure to the olfactory cues of the diet alone are not sufficient to alter an observer's preference. Research involving social learning and transmission on dietary preference can be applied to paradigms studying voluntary alcohol consumption.

In Experiment 1, demonstrator animals were administered ethanol every other day, beginning on postnatal day [PD] 12 and continuing through PD 18. These procedures were based on those described by Hunt and Hallmark (2004) who showed that this pattern of ethanol experience resulted in increased voluntary ethanol ingestion by weaning-age (PD 22) subjects. In Experiment 1 subjects were tested after a longer interval, on PD 30.

METHODS

Subjects. The subjects were 58, 12-day-old Sprague-Dawley-derived rats (24 demonstrators, 34 observers) from six different litters. Animals were offspring of breeders originally obtained from Charles River Laboratories (Wilmington, DE) and were reared in the Psychology Department vivarium at the College of William & Mary. The colony was maintained on a 14:10 h light:dark cycle with light onset at 0700 h. Male and female breeders were housed in 50.8 x 40.6 x 21.6-cm clear polycarbonate cages with pine-chip bedding wire lids. Subjects had free access to food and water and diet consisted

of Purina Rat Chow. Cages were checked daily for pups and the day of birth was designated as PD 0. Litters were culled to 8-10 pups on PD 2. Pups were weaned on PD 21 and were maintained in a separate cage with littermates until testing.

Apparatus and materials. Intragastric (i.g.) administration of ethanol and water solutions was accomplished via 15-cm lengths of polyethylene tubing (PE-10; Clay Adams, Parsippany, NJ) attached to 1 cc disposable syringes. The ethanol solution given to demonstrator animals was 12 % v/v, dissolved in tap water. Subjects were weighed using an Ohaus top-loading balance (Model GT8000), accurate to 0.1 g. For the ethanol ingestion test, 50 ml graduated drinking tubes, with rubber stoppers and curved sipper spouts, were used. Each subject was presented with two drinking tubes: one containing a 6% v/v ethanol solution and the other containing a 1 % w/v Sanka decaffeinated coffee solution. Both solutions were made in tap water.

***Procedure*.**

Ethanol exposure: Three litters were assigned, at random, to the ethanol (EtOH) group and the remaining three to the water (control) group. On PD 12, all animals in the litter were removed from the home cage and placed into a 35.3 x 21.9 x 13.0-cm opaque polyethylene holding cage with pine-chip bedding, maintained at 34°C by a heating pad placed beneath. Four pups (2 male and 2 female) were chosen at random from the litter of 8-10 animals to serve as demonstrators. The remaining animals served as observers. All animal were weighed, marked with magic marker for later identification, and ear clipped to designate treatment condition. Demonstrator pups were administered either 1.5 g/kg

ethanol or an equivalent volume of water. Following demonstrator intubations, all pups were returned to the home cage. The procedure was repeated on PD 14, 16, and 18.

Ethanol Ingestion Test. On PD 28, observers from each litter were individually housed in hanging wire cages with free access to food and water. Subjects were handled for two consecutive days (PD 28, 29). On PD 30, the animals were weighed and the 24-h ingestion test was started. Water was removed from the subjects' cages and two drinking tubes were hung on the front wall of each cage. One drinking tube contained the ethanol solution (6 %) and the other contained coffee (1%). The left-right position of the drinking tubes was balanced across subjects. Initial levels of coffee and ethanol solutions were recorded and subjects were left undisturbed for 24 h. On PD 31, the final fluid levels were recorded. The primary measure was a percent preference for ethanol, which was calculated using the formula: % Preference = amount of ethanol ingested (ml) / total fluid ingested (ml) x 100.

Results. The % Preference data were analyzed using a 2 (drug: ethanol vs. water) x 2 (sex: male vs. female) Analysis of Variance (ANOVA). The main effect of drug approached statistical significance, F (1, 30) = 3.70, p = .064. There was no effect of sex on ethanol preference scores. However, as can be seen in Figure 1, adolescent observers that had previously been exposed to intoxicated sibling demonstrators in the home cage tended to ingest more alcohol (nearly 70% preference) compared to the observers that were in the control group (45% preference). However, as mentioned, this difference did not reach statistical significance.

Discussion. These data are highly suggestive that early ethanol exposure can increase the likelihood of voluntary drug ingestion by young adolescent rats. The pattern of results is similar to those reported previously (Hallmark & Hunt, 2004). Hunt, Lant and Carroll (2001) also demonstrated that multiple social learning experiences in the home cage can promote long-term ethanol preferences. In both of these studies however, animals were tested one week after the final exposure treatment, on PD 22-23. The present results suggest that early experiences can impact voluntary ethanol intake into the adolescent period. Given the findings from human populations demonstrating a strong correlation between the age of onset of drinking and lifetime alcohol dependence rates (Grant, 1998), a further understanding of this early alcohol ingestion is warranted.

EXPERIMENT 2

Not only is social transmission of information regarding ethanol evident within preweanling rats (Hunt et al., 2000), but such learning can also impact long-term ingestion or dietary preferences (Honey & Galef, 2004; Hunt & Hallmark, 2001). Because early exposure to ethanol in the home environment is suggested to modify alcohol's responsiveness in childhood and early adolescence, repeated alcohol exposure may explain the high frequency of consumption and later abuse reported in adolescents (Hunt et al., 2000). Therefore, it is important to demonstrate retention within preweanling rats as a model for understanding patterns of learning within human subjects. Findings by Hunt and colleagues (2000, 2001) indicate that early learning does occur within the home environment, is retained, and may impact later initiation of alcohol consumption. In addition, unpublished data (Colona, 2003) has suggested that earlier exposure to higher

doses of ethanol can affect intake in adolescent animals. The Colona study was designed for a different purpose, so the data are only suggestive. Because data from the present Experiment 1 reflected a nonsignificant trend, in Experiment 2 the procedure was modified. Ethanol exposure occurred earlier in development, on consecutive days, and a higher dose of ethanol was administered to the demonstrator subjects (cf. Hunt et al., 2001). In Experiment 2a voluntary intake was examined in both demonstrator and observer animals, and in Experiment 2b ethanol odor preference was measured.

Experiment 2a: Ethanol Ingestion

METHODS

Subjects. The subjects were 66 Sprague-Dawley rats (30 demonstrators, 36 observers) that were 8 days old at the beginning of the experiment. Subjects were derived from 12 different litters. Animals were maintained under the same conditions as in Experiment 1.

Procedure.

Ethanol exposure. Six litters were assigned to the ethanol-exposed condition and six to the water (control) condition. On PD 8, four pups (2 male and 2 female) were chosen at random from each litter to serve as demonstrators. The remaining animals served as observers. All animals were removed from the home cage, marked with magic marker and ear marked to denote exposure condition, as in Experiment 1. Demonstrator animals were then administered either 2.5 g/kg ethanol (12% v/v) or an equivalent volume of the water vehicle. Immediately following demonstrator intubations, all pups were returned to the home cage. The procedure was repeated on PD 9, 10, 11, and 12. Thus, all

demonstrators were administered ethanol (or water) on five separate occasions. The test for ethanol vs. coffee ingestion occurred on PD 30, and was identical to that described in Experiment 1.

***Results*.** The data from two of the ethanol-exposed litters were discarded because of a mistake in mixing the coffee solution. The intake data from the remaining animals are shown in the left panel of Figure 2. The hatched bars represent the data obtained from the observer animals and the black bars are the data from the demonstrator animals. The data were analyzed using a 2 (drug; ethanol vs. water) x 2 (sex; male vs. female) x 2 (exposure; demonstrator vs. observer) between-groups ANOVA. The analysis yielded a significant main effect of drug, F (1, 38) = 4.84, $p < .05$. There was no effect of sex or exposure, and no interactions. Results indicated that both observers and demonstrators that were derived from ethanol-treated cages ingested significantly more ethanol than animals that had no prior exposure to ethanol (water cages). The main effect of drug is more clearly depicted in Figure 3 (left panel), in which the data shown are averaged across exposure conditions (demonstrator and observer).

Experiment 2b: Ethanol Odor Preference

METHODS

***Subjects*.** The subjects for this experiment were 43 Sprague-Dawley rats (22 demonstrators, 21 observers), from 11 separate litters. Animals were maintained under the conditions described previously.

***Procedure*.** Five of the litters were assigned to the ethanol condition and the other 6 were assigned to the water condition. Intragastric administration of 2.5 g/kg ethanol (12%) or water to demonstrator animals occurred on PD 8-12, and was identical to that described in Experiment 2a.

On PD 30 subjects were given a 3 min test of ethanol odor preference. A 43.8 x 17.3 x 25.7 cm chamber was used for this test. The walls and top were made of clear Plexiglas and the floor of the chamber was constructed of stainless steel bars. Gauze pads soaked with 1.5 ml 95% ethanol were placed beneath the floor at one end of the chamber, and gauze soaked with 0.5 ml lemon flavoring (McCormick™) was placed beneath the other end. Animals were placed into the middle of the chamber and the time spent on the ethanol-scented side of the chamber was recorded during the 3 min test. In order to be designated as being on the ethanol-scented side, the head and both forepaws had to be across the midline of the chamber. The measure of odor preference was a percent preference score, calculated using the formula: % Preference = time spent on the ethanol side (s) / total test duration (180 s) x 100.

Results. The odor preference data recorded from observers (hatched bars) and demonstrators (black bars) are shown in the right panel of Figure 2. The data were analyzed using a 2 (drug) x 2 (sex) x 2 (exposure) ANOVA. The analysis yielded a significant main effect of drug, F (1, 35) = 12.57, $p < .01$. There were no effects or interactions involving sex or exposure. Animals that were exposed to ethanol during the infant period, either directly (demonstrators) or indirectly (observers), exhibited greater preference for the ethanol-scented side of the test chamber than animals exposed to water.

The main effect of drug is shown in the right panel of Figure 3, where the data shown are collapsed across exposure (demonstrator and observer) condition.

Discussion. The results of these two experiments clearly show that infant experience with ethanol cues in the home cage can have a long-lasting impact on ethanol preference. When animals were exposed to ethanol on postnatal days 8-12, they learned about the ethanol cues and this information was retained into the adolescent period. As adolescents (postnatal day 30), these animals increased their voluntary intake of an ethanol solution (Experiment 2a) and showed a preference for ethanol odor (Experiment 2b). This is the first time that adolescent responding to ethanol has been enhanced in animals that were given ethanol exposure in infancy. Moreover, the resulting ethanol preferences were seen in animals that were directly exposed to ethanol (demonstrators) and indirectly exposed to ethanol cues (observers). These results suggest that early exposure to ethanol can set the stage for early-onset drinking patterns.

Of additional interest is that when relatively high doses of ethanol (3.0 g/kg) are administered i.g. to animals in isolation, even on a single occasion, the animals later express aversions to ethanol odor and taste (Molina & Chotro, 1989; Molina, Chotro & Spear, 1989). The present experiments, in contrast, suggest that when intoxication is experienced in a social context (the home cage) the state of intoxication may not be as aversive as when experienced in isolation. Gauvin, Briscoe, Goulden and Holloway (1994) reported that adult rats experience a state of intoxication as less aversive when experiencing that state in the presence of another animal. Specifically, Gauvin et al. used a place aversion procedure. Rats were given training trials on alternating days, such that

placement into one side of a black-white chamber occurred after administration of a high (4.0 g/kg) dose of ethanol, while placement in the opposite side occurred aftre a saline injection. Following several days of training, adult rats will avoid the ethanol-paired side of the chamber. However, if the animals were allowed to interact with another animal in the ethanol-paired side, their avoidance of that side was reduced. Gauvin et al. (1994) concluded that the aversiveness of the state of intoxication can be reduced when that state is experienced in the presence of another individual.

One reason for these effects could be ethanol-induced changes in body temperature that occur in animals that are kept isolated from one another. Ethanol disrupts the ability of animals to independently maintain body temperature and, when exposed to an ambient temperature that is below normal body temperature, will experience hypothermia. Cunningham, Hawks and Niehus (1988), working with adult rats, and Hunt, Spear and Spear (1991) working with preweanling rats, have both shown that decreases in body temperature that are commensurate with high dose ethanol administration contribute to the conditioning of an ethanol-mediated taste aversion. Although never explicitly studied, it is highly probable that the animals in the Molina studies cited above exhibited a large decrease in body temperature because they were maintained in isolation following i.g. administration of ethanol, and this contributed to the aversive state produced by ethanol and the subsequent expression of taste and odor aversions conditioned with ethanol. Alternatively, the demonstrators in the present Experiment 2 may indeed have acquired an aversion to ethanol, but this may have been counteracted by an acquired preference for ethanol transmitted via social learning from other, intoxicated demonstrators.

EXPERIMENT 3

Exposure to ethanol cues in the home environment is typically not unitary or discrete. If a child is exposed to ethanol-related cues (its sight, odor or taste), these experiences are likely to be intermittent throughout development. Thus, although the demonstrator-observer paradigm used previously was effective in promoting increased preference for ethanol three weeks after the final exposure, a more realistic analogy to early social learning in humans would also include periodic re-exposure to those cues. In order to evaluate the effectiveness of re-exposure to ethanol cues, a reactivation procedure was employed in Experiment 3.

The impact of early experiences on later behavior has been well documented (see Caza & Spear, 1984; Diaz-Cenzano & Chotro, 2010; Hunt & Hallmark, 2001; Hunt et al., 2000, 2001; Spear & Molina, 2005). In fact, early learning via exposure to alcohol-intoxicated siblings may have unintended, long-lasting consequences on choice behavior (Hunt & Hallmark, 2001; Miller, Jagielo & Spear, 1991). Interestingly, events occurring in the first years of life are rarely explicitly recalled, a phenomenon termed "infantile amnesia" (Campbell & Spear, 1972). The specific events that may be critical for later ethanol ingestion are, therefore, not always available to our conscious recollections (e.g. Rovee-Collier, Hayne & Colombo, 2001). If, however, environmental stimuli are presented in an appropriate context, then learning that appeared to have been forgotten can be retrieved and subsequently expressed in behavior (Spear & Parsons, 1976). Procedures intended to make earlier-acquired memories more accessible are known as reactivation or reinstatement procedures. A reactivation treatment is one in which a

memory can be brought back into an active and accessible state by presenting some aspect of the learning episode. Reactivation treatments are effective in the maintenance of memories established via classical conditioning procedures. Reactivation treatments typically involve brief exposure to the conditioned stimulus (CS), the unconditioned stimulus (US) or the contextual cues that were present throughout the learning episode (Spear, 1973).

Miller, Jagielo and Spear (1991) examined the effects of various treatments in the reactivation of a conditioned odor aversion memory. For training, preweanling rats were exposed to a lemon odor (CS-) followed by a pairing of methyl salicylate odor (CS+) with footshock (US). In this classical discrimination conditioning procedure, the lemon odor is designated as a CS- because its presentation is never followed by the US (footshock). After a number of trials, the rats learned to distinguish between the CS+ and CS- odors and engage in the conditioned response, avoidance of the CS+ odor. Although this learning was readily acquired, the memory was forgotten rapidly. Preweanling rats failed to engage in odor avoidance following a 3-hour retention interval. Reactivation treatments, however, proved effective in promoting memory retrieval for odor aversion following a 3-hour retention interval. In fact, presentation of the footshock or CS- odor just prior to test was sufficient for reactivation of the previously-conditioned, but forgotten, odor aversion. This experiment along with several others suggests that reactivation treatments are beneficial for maintenance of a memory (Campbell & Jaynes, 1966; Miller et al., 1991). Rovee-Collier, Sullivan, Enright, Lucas and Fagen (1980) have applied reactivation treatments toward the maintenance of human infant memories as well. Early experiences appear to influence one's behavior throughout development,

suggesting that reactivation of the experience may successfully maintain a memory for a longer period of time (Rovee-Collier & Hayne, 1987). In fact, Hildreth and Rovee-Collier (2002) reported that reactivated memories were maintained for longer than original memories – that is, reactivated memories were less susceptible to forgetting than initial memories. Perhaps early learning and subsequent opportunities for reactivation of an early ethanol memory could increase the likelihood of alcohol or other drug-related behaviors among the adolescent population.

Results of Experiment 2 revealed that the procedures used for social learning were effective for promoting the subsequent preference for ethanol taste and odor. However, the effects were not particularly strong. Ethanol-exposed animals on average showed a 20 % increase in ethanol intake (Experiment 2a) and a 10 % increase in preference for ethanol odor (Experiment 2b). The purpose of the present experiment was to evaluate whether a reactivation treatment given prior to test would produce a stronger preference for ethanol.

Experiment 3a: Three Minute Reactivation Treatment

METHODS

Subjects. The subjects were 47 Sprague-Dawley rats (20 demonstrators and 27 observers) from 5 different litters. The animals were 8 days old at the beginning of the experiment and were maintained as in previous experiments.

Procedure. All five cages were assigned to the ethanol-exposed condition. Four demonstrators (2 male and 2 female) from each cage were administered 2.5 g/kg ethanol

on PD 8-12. Observer subjects were assigned to one of two reactivation treatments: ethanol or lemon odor. The reactivation treatment was given on PD 29, 24 h prior to the beginning of the ingestion test. Animals were placed into a novel holding cage in pairs and were presented with 1.5 ml of 95% ethanol or 0.5 ml of lemon extract (McCormick™). Odorants were placed on gauze pads that were contained in a small Tupperware container. The lid of the container had holes punched into it to allow odor dissipation into the holding cage. Reactivation treatments lasted for 3 min. Ethanol and lemon odorants were replaced every third animal to maintain potency. Immediately following the reactivation odor exposure the animals were returned to their individual hanging cages. The test for ethanol vs. coffee intake began 24 h later.

***Results and Discussion*.** The % Preference data were analyzed using a 2 (reactivation: ethanol or lemon) x 2 (sex) ANOVA. The analysis indicated a significant main effect of reactivation, F (1, 23) = 9.35, $p < .01$. Surprisingly, the effects of the reminder treatments were opposite to that predicted. Observers from ethanol-treated cages showed higher percent preference scores following the lemon odor reactivation treatment (M = 69.26 +/- 7.9%) than following the ethanol odor reactivation treatment (M = 38.02 +/- 6.49%). The discrepancy in these results could be attributed to two different processes. First, exposure to the novel lemon odor may have increased preference for the ethanol solution. Alternatively, exposure to ethanol odor may have resulted in extinction of the previously-acquired ethanol preference.

The reason that exposure to a novel lemon odor 24 h prior to the ingestion test resulted in greater ethanol intake is not clear. However, after the experiment was

completed, it was noticed that the lemon extract that was used did contain a substantial amount of ethanol (80%). It could be that exposure to the ethanol component of the lemon extract was sufficient to promote later ethanol intake. But if this were the case, then why did exposure to ethanol odor itself not have the same effect? This also is not clear. However, one possible explanation is that the long duration of exposure during reactivation (3 min) resulted in extinction of the previously acquired ethanol preference. In fact, the percent preference scores of the ethanol reactivated animals were quite low (35% preference). This level of ethanol ingestion is similar to that of control animals in previous experiments, so extinction of the learned ethanol preference is a possibility. Because of these issues, the experiment was conducted again using (1) a non-ethanol containing lemon odorant and (2) a shorter reactivation exposure. Water-exposed control cages were also included to better understand the effects of novel odor exposure on ethanol intake.

Experiment 3b: Fifteen Second Reactivation Treatment

METHODS

Subjects. The subjects were 78 Sprague-Dawley rats (32 demonstrators, 46 observers) that were 8 days old at the beginning of the experiment. Subjects were derived from 8 different litters and animals were maintained under the conditions described previously.

Procedure. Four litters were assigned to the ethanol-exposed condition and four litters to the water (control) condition. The ethanol and water administration procedures to demonstrator subjects were identical to those of Experiment 2.

Approximately half of the observer subjects received a 15 s ethanol reactivation treatment and the remaining observers received a 15 s lemon reactivation treatment. The reactivation treatment was given in the olfactory preference chamber used in Experiment 2b. A Plexiglas door was inserted to divide the olfactory testing chamber in half, and two animals were given the reactivation treatment at a time. Gauze pads soaked with 1.5 ml of 95% ethanol or 1.5 ml lemon juice (ReaLemon™) were placed beneath each side of the chamber. The two animals treated together were given the same odor reactivation treatment. These treatments were given on PD 29, 24 hr prior to ingestion testing. Ethanol or lemon odorant was replaced after every third animal to maintain potency. Following the reactivation treatment, subjects were returned to their individual hanging cage. The test for ethanol ingestion began on PD 30 and was as described previously.

Results. The % Preference data were analyzed using a 2 (drug: ethanol vs. water) x 2 (sex) x 2 (reminder: ethanol vs. water) ANOVA. The analysis revealed a significant Drug x Reminder interaction, F (1, 38) = 4.40, $p < .05$. No other effects were significant. The data are shown in Figure 4. The ethanol and lemon reminder treatments had different effects on intake of observer animals exposed to ethanol or water demonstrators. Post hoc comparisons were conducted with Newman-Keuls tests ($p < .05$). Observers from the ethanol-treated cages showed greater ethanol intake following the ethanol odor reminder than following the lemon odor reminder. The opposite pattern was seen in observers from the water-control cages. For these subjects, ethanol intake was higher following exposure to the lemon reactivation treatment than following the ethanol reactivation treatment.

Discussion. The results of this experiment are difficult to interpret, and should be replicated to see if the effect is reliable. While the effects of the reactivation treatments in ethanol-exposed subjects were in the predicted direction, the effects in the control subjects are difficult to explain.

Reactivation treatments involve presentations of some aspect of a prior learning episode, and have been shown to increase memory (Spear & Parsons, 1976; Rovee-Collier & Hayne, 1987). Observers in this experiment that were derived from ethanol-treated cages exhibited voluntary ethanol intake that was enhanced by prior exposure to ethanol odor. What is interesting is that the observers derived from water-exposed (control) cages exhibited the opposite effect. For these subjects, exposure to lemon odor increased ethanol consumption, while exposure to ethanol odor did not. The finding that exposure to a novel ethanol odor had no effect on ethanol intake is in line with the studies of Galef and Stein (1985) who showed that exposure to dietary cues does not result in increased intake of that diet, unless the cues were experienced in a social context. However, the fact that exposure to the lemon odor had a positive effect on ethanol intake is not clear. If exposure to a novel odorant enhances later intake of another solution, then why did the ethanol-exposed observers not show the same effect? Why did the novel lemon odor increase ethanol, but not coffee, in take? These questions are difficult to answer. Nonetheless, the effect of the ethanol odor reactivation treatment on intake of ethanol-exposed observers does suggest that periodic exposure to aspects of a prior learning episode can increase memory expression in this social learning procedure.

EXPERIMENT 4

The neurochemistry of social learning has been examined and may be applicable to studies involving social learning of ethanol preferences. In particular, there is evidence linking endogenous opioids and social reinforcement. Because opioids are released during periods of social interaction, they may reinforce subsequently generated odor preferences in the context of social encounters (Nelson & Panksepp, 1998; Varlinskaya & Spear, 2009). Stated differently, opioid activation may reinforce conditioning of stimuli, such as ethanol cues, present during social contact. The role of endogenous opioids in influencing social behavior has been found in several studies involving avian and mammalian species, and can be generalized across developmental stages in which various social contexts influence behavioral demands (Nelson & Panksepp, 1998).

Endogenous opioids may serve as neurochemical mediators of social transmission of food preferences. It is well established that endogenous opioid peptides are important in influencing social behaviors as well as ingestive responses (Moles, Valsecchi & Cooper, 1999). However, nonspecific opioid receptor antagonists, like naloxone and naltrexone, can reduce social behaviors/interactions, and also impair learning and expression of socially transmitted food preferences (Herz, 1997). Moles and Cooper (1993) reported that naltrexone is effective in reducing palatable food consumption in nondeprived adult mice. In their study, naltrexone modulated ingestion of sucrose solution via reducing the reward value of the solution itself. Similarly, Moles et al. (1999) injected observer mice with naltrexone prior to a 15-minute period of social interaction with demonstrator mice fed a specific diet. Following a choice test, results indicated that the observer animals treated with naltrexone failed to demonstrate the

typical shift in preference towards the demonstrator's diet. Similar findings were reported using the opioid receptor antagonist naloxone, therefore suggesting that blockade of opioid activity is sufficient to impair the social acquisition of food preferences (Herz, 1997; Moles & Cooper, 1993; Moles et al., 1999).

In addition to modulating dietary food preferences, the endogenous opioid system may play a role in social learning about alcohol. Hallmark and Hunt (2004) investigated this hypothesis in their demonstrator-observer procedure. In their study, demonstrator rats were intragastrically administered a 1.5g/kg dose of ethanol on PD 12, 14 and 16. At the same time, observers received either saline or naloxone injections. The ingestion test conducted on PD 17 revealed that naloxone was ineffective in preventing socially mediated ethanol preferences within observer subjects. This suggested that the opioid system is not involved in this social learning procedure. However, naloxone's half-life is relatively brief (approximately 40 minutes in rats) in comparison to the rate of ethanol elimination (several hours; Hollstedt & Rydberg, 1985) and it is possible that social learning continued to occur after naloxone's effects had worn off (Ngai, Berkowitz, Yang, Hempstead & Spector, 1976). In Experiment 4, naltrexone was used. Naltrexone has a half-life of approximately 4 hours, and therefore would produce longer-lasting opioid receptor blockade.

METHODS

Subjects. The subjects were 50 Sprague-Dawley rats (20 demonstrators, 30 observers) that were 8 days old at the beginning of the experiment. Subjects were derived from 5 different litters and were maintained as previously described.

Procedure.

Ethanol and naltrexone administration. All cages were assigned to the ethanol-treated condition. On PD 8, four pups (2 male and 2 female) were chosen at random from each litter to serve as demonstrators. The remaining animals (5-6 subjects) served as observers. Prior to demonstrator ethanol administration, half of the observers were injected with 0.5 mg/kg of naltrexone hydrochloride (NTX; Sigma-Aldrich) intraperitoneally, and the remaining observers received an equal volume of the saline vehicle (SAL). Naltrexone dose was chosen from Kehoe and Blass (1986). Intraperitoneal (i.p.) injections were delivered using 30-gauge ½" needles attached to 1 cc disposable syringes. Immediately following demonstrator intubations, all pups were returned to the home cage. The procedure was repeated on PD 9, 10, 11, and 12. Thus, all demonstrators were administered ethanol on five separate occasions. Similarly, all observers were injected with NTX (or SAL) on five separate occasions. Observer subjects from each litter were tested for ethanol ingestion. Testing occurred on PD 30.

Results. An independent groups t-test was used to compare the % preference data obtained from observer animals that were previously treated with saline or naltrexone. The t-test revealed a nonsignificant effect, t (27) = 0.04. Naltrexone-treated observers did not differ from saline-treated observers in their % preference scores (M_{SAL} = 68.73 +/- 7.9%; M_{NTX} = 68.25 +/- 8.2%).

Discussion. These results indicate that blocking endogenous opioid receptors does not prevent social learning about ethanol from intoxicated demonstrators. These data confirm

results previously published by Hallmark and Hunt (2004). In that study, opioid receptor blockade was accomplished with naloxone and, as discussed previously, the failure of naloxone to block social learning may have been due to its relatively short duration of action (half-life of approximately 40 min). In this study, a longer-lasting opioid receptor blockade was accomplished with naltrexone, which has a half-life of about 4 hours. Nevertheless, opioid receptor blockade here also failed to block this type of social learning. Together these findings and those of Hallmark and Hunt (2004) indicate that the endogenous opioid system is not involved in the social transmission of ethanol preference.

Other research, however, has obtained positive effects of opioid receptor blockade in animals' learning about diet cues from other animals. Using adult mice, Moles et al. (1999) found that naloxone and naltrexone both prevented observer animals from learning about a novel diet through interaction with a demonstrator animal. In this study however, the mice were socially deprived prior to the interaction phase and the interaction was relatively brief (15 min). It is likely that the social deprivation decreased endogenous opioid activity (Nelson & Panksepp, 1998) and the brief period of interaction increased endorphin release. If this is the case, then opioid receptor blockade in the observer subjects would be expected to have an effect. In our experiments, not only were the animals younger, but they were not socially deprived prior to the social learning procedure. It might be that, because of this, the opioid system was not compromised prior to interactions with the demonstrator, and therefore these interactions did not result in any noticeable increase in opioid release.

Recent data from Diaz-Cenzano and Chotro (2010) also indicate that opioid receptor blockade can interfere with learning about ethanol. Arias and Chotro (2005, 2006) had reported that fetal rats learn about ethanol cues if the dam was given ethanol. Here, pregnant dams were administered ethanol on gestational days (GD) 17-21. The offspring were tested two weeks after birth for responding to ethanol, and it was found that the pups with maternal ethanol exposure ingested more ethanol and exhibited more appetitive behaviors toward ethanol than control animals. Diaz-Cenzano and Chotro (2010) asked whether the opioid system was involved in this fetal learning. Dams were injected with naloxone (which crosses the placental barrier, and thus blocked opioid receptors in the fetus) prior to ethanol administration. The offspring of naloxone-treated dams failed to show the expected increase in ethanol consumption, as was evident in the offspring of dams injected with saline. These results indicate that endogenous opioid activity is responsible for fetal learning about ethanol cues provided by the dam's intoxication. These results, as well as those reported by Moles et al. (1999) contrast with those of the present experiment. At this time, it is not clear why naltrexone failed to have an effect in this variation of the demonstrator-observer paradigm.

CONCLUSIONS

Several procedures have been used to induce voluntary ethanol intake in rats. Several studies have used selectively bred rodent lines to study possible genetic bases of alcohol preference. However, genetics alone do not account for the high prevalence of alcohol consumption and/or abuse during adolescence. Experiential factors, including early exposure to ethanol cues within the home environment, as well as social

interactions with peers, play a substantial role in promoting ethanol consumption (Spear & Molina, 2005). The present results indicate that early ethanol exposure via interactions with intoxicated conspecifics can increase the likelihood of voluntary drug ingestion and approach to ethanol odor in adolescent rats. Our findings indicate that both the observers and demonstrators from the ethanol-treated cages showed a greater preference for ethanol in both types of tests than animals from the water-exposed cages. These data confirm and extend the research conducted previously in our lab and by other researchers. The propensity to consume alcohol can be increased in adolescent-aged rats following social exposure to alcohol during the early postnatal period (Eade & Youngentob, 2009; Fernandez-Vidal & Molina, 2004; Hunt et al., 2001). The ability of animals to exhibit this social learning is present early in life (Hunt et al., 2000), perhaps even in the fetus (Arias & Chotro, 2005, 2006; Diaz-Cenzano & Chotro, 2010). Hunt, Holloway and Scordalakes (2001) showed that a single 30-min exposure to an intoxicated sibling resulted in increased voluntary ethanol ingestion by adolescent rats when observers were tested immediately following the exposure. Hunt, Lant and Carroll (2000) showed that animals as young as 8 days of age had the capacity for increased voluntary ingestion of an ethanol solution following a brief interaction with an intoxicated sibling. It was further demonstrated that multiple social learning experiences in the home cage can promote relatively long-term ethanol preferences, assessed at the time of weaning (PD 23; see also Colona, 2003). That effect was replicated here and, in addition, the present results indicate that the retention of this information can guide behavior for a longer period of time, into the adolescent period.

The finding that animals exposed to ethanol in the home cage exhibit a significantly greater preference for ethanol odor is also of interest. A propensity to approach ethanol odor could contribute to the animal coming into more direct contact with alcohol. For humans, increased approach toward the odor of ethanol would bring an adolescent into close contact with alcohol, and may be one of the initiating factors in taking that first drink. It is recognized that alcohol abuse problems often begin with alcohol ingestion during late childhood or early adolescence, and the earlier the age of onset of drinking, the higher the likelihood of later alcohol abuse disorders (Grant, 1998). The present findings, indicating that all animals in the cages exposed to ethanol, both the animals that received direct exposure to ethanol (demonstrators) and those that received exposure via social interactions with intoxicated siblings (observers), exhibited enhanced preference for ethanol odor and, more importantly, increased voluntary ingestion of ethanol is an important finding for understanding the causes of adolescent-onset alcohol use disorders. Social experiences in the home environment, in conjunction with a genetic predisposition for drug abuse, may help to explain adolescent onset of drinking behavior. Clearly, a further understanding of the mechanisms underlying this early learning about alcohol is warranted.

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FIGURE CAPTIONS

Figure 1. Mean (+/- SEM) percent preference for ethanol exhibited by observers from experiment 1. Subjects interacted with siblings that were administered either 1.5 g/kg ethanol (EtOH) or water (H_2O) on postnatal days 12, 14, 16 and 18. Animals were given a 24-h ingestion test with a choice between 6% ethanol and 1% Sanka decaffeinated coffee.

Figure 2. *Left*: Mean (+/- SEM) percent preference for ethanol recorded during the 24 h ingestion test. *Right*: Mean (+/- SEM) time spent on the ethanol-scented side of the odor preference chamber during a 3 min test. Observers interacted with sibling demonstrators that were administered either 2.5 g/kg ethanol (EtOH) or Water on Postnatal Days 8-12. The ingestion test began on PD 30 and consisted of a choice between 6% ethanol and 1% Sanka decaffeinated coffee. The odor preference test (EtOH vs. lemon) was given on PD 30.

Figure 3. Same results that are shown in Figure 2, but averaged across exposure condition (demonstrator/observer). These data more clearly show that all animals given exposure to ethanol (EtOH, black bars) in the home cage displayed greater voluntary ethanol ingestion (left) and greater preference for ethanol odor (right) than animals in the control (Water, white bars) condition.

Figure 4. Mean (+/- SEM) percent preference for ethanol recorded during the 24 h ethanol ingestion test of Experiment 3. Observer subjects were derived from cages in which demonstrators were administered ethanol (EtOH) or Water. Twenty-four hours prior to the beginning of the ingestion test the subjects were exposed to either ethanol odor or lemon odor for 15 s as a reactivation treatment.

Figure 1

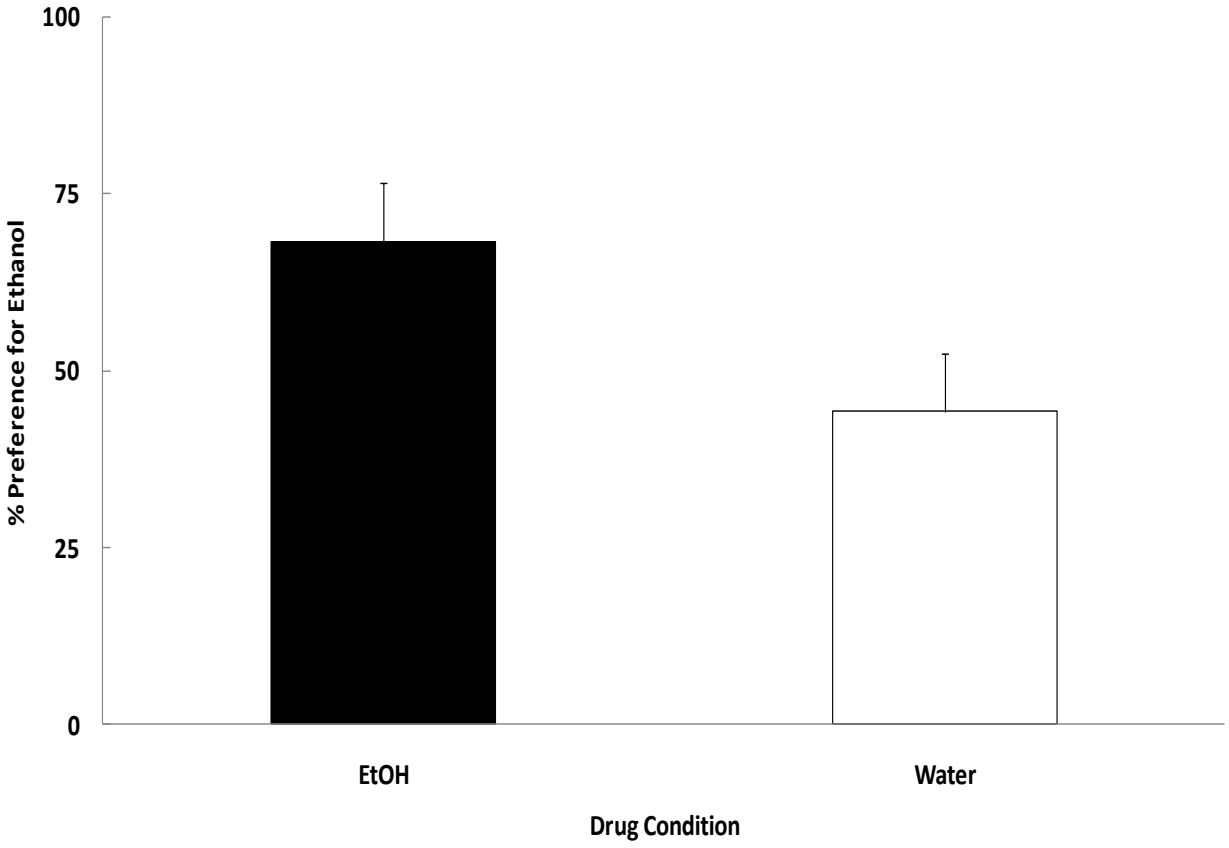

100
75
50
25
0
% Preference for Ethanol
EtOH
Water
Drug Condition

Figure 2

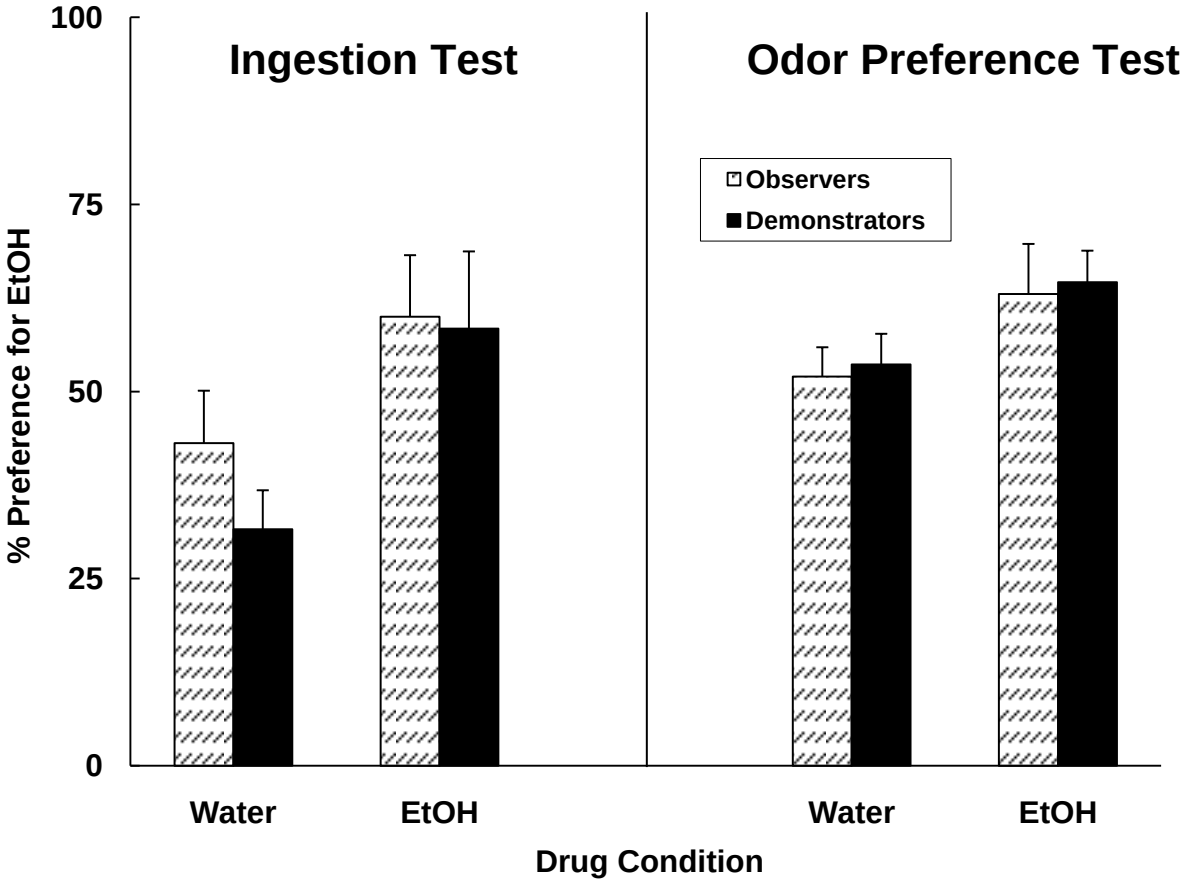

Ingestion Test
Odor Preference Test
Observers
Demonstrators
% Preference for EtOH
100
75
50
25
0
Water
EtOH
Water
EtOH
Drug Condition

Figure 3

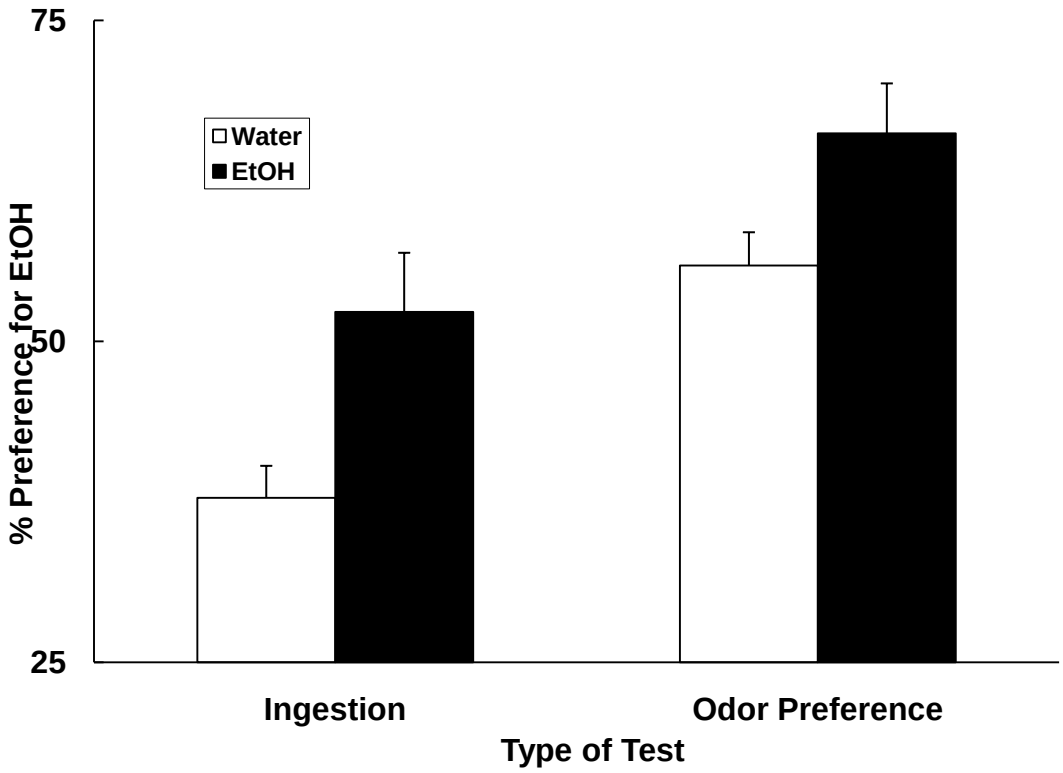
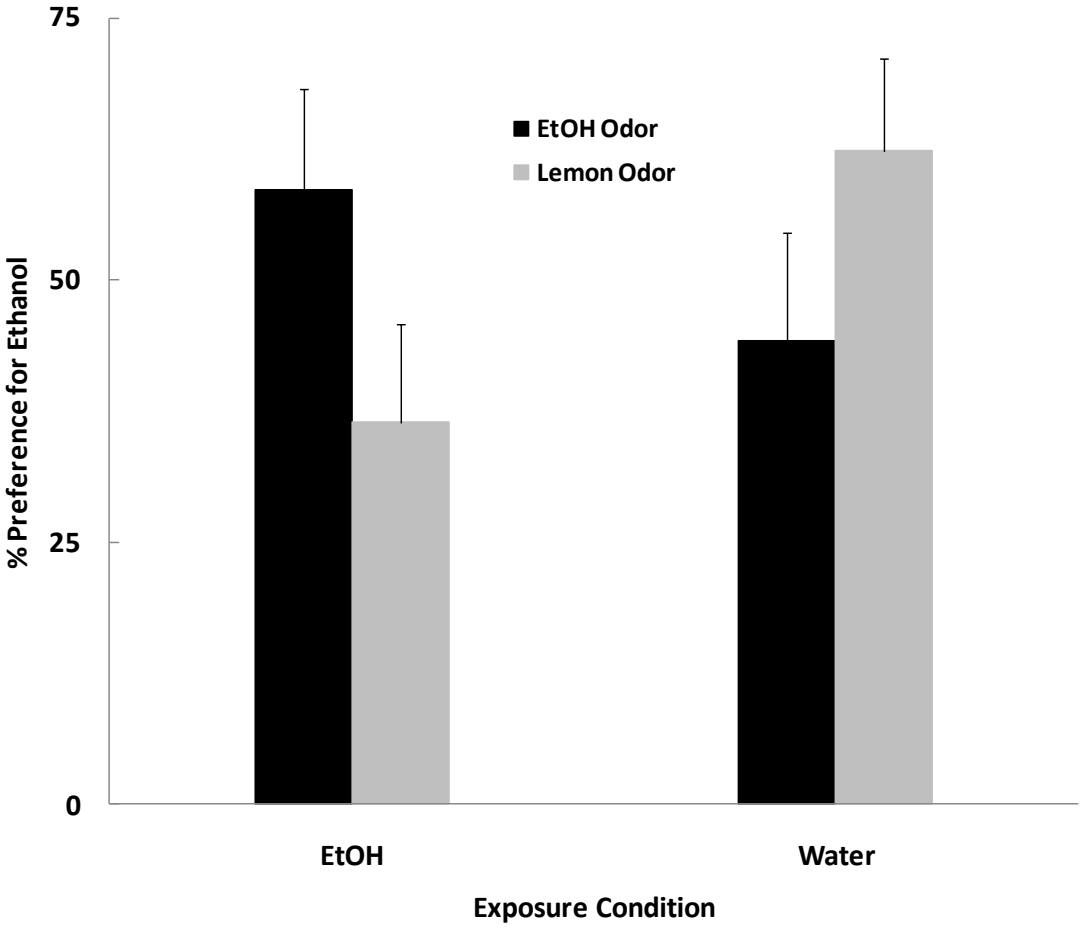

75
50
25
% Preference for EtOH
Water
EtOH
Ingestion
Odor Preference
Type of Test

Figure 4


75
50
25
0
% Preference for Ethanol
EtOH Odor
Lemon Odor
EtOH
Water
Exposure Condition