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Chemosensory Factors Influencing Alcohol Perception, Preferences, and Consumption

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Abstract

This article presents the proceedings of a symposium at the 2002 RSA/ISBRA Meeting in San Francisco, California, co-organized by Julie A. Mennella and Alexander A. Bachmanov of the Monell Chemical Senses Center. The goal of this symposium was to review the role that chemosensory factors (taste, smell, and chemical irritation) play in the perception, preference, and consumption of alcohol. The presented research focused on both humans and laboratory animals and used a variety of approaches including genetic, developmental, pharmacological, behavioral, and psychophysical studies. The presentations were as follows: (1) Introduction and overview of the chemical senses (Julie A. Mennella and Alexander A. Bachmanov); (2) Taste reactivity as a measure of alcohol palatability and its relation to alcohol consumption in rats (Stephen W. Kiefer); (3) Early learning about the sensory properties of alcohol in laboratory animals (Juan Carlos Molina); (4) Early learning about the sensory properties of alcohol in humans (Julie A. Mennella); (5) Genetic dissection of the ethanol-sweet taste relationship in mice (Alexander A. Bachmanov and Michael Tordoff); and (6) Human genetic variation in taste: connections with alcohol sensation and intake (Valerie B. Duffy and Linda M. Bartoshuk). The symposium concluded with a general discussion.

Keywords

Alcohol; Ethanol; Taste; Olfaction; Smell; Drinking; Perception; Genetics; Learning; Development

INTRODUCTION AND OVERVIEW OF THE CHEMICAL SENSES

Julie A. Mennella and Alexander A. Bachmanov

The oral consumption of alcohol is accompanied by chemosensory perception of flavor, which plays an important role in its acceptance and rejection in both humans and laboratory animals. Three independent sensory systems— taste, olfaction, and chemosensory irritation—are involved in the perception of flavor. Humans perceive alcohol as a combination of sweet and bitter tastes, odors, and oral irritation (e.g., burning sensation), all of which vary as a function

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of concentration (Bartoshuk et al., 1993;Green, 1987;Mattes and DiMeglio, 2001). Likewise, rodents detect the sweet (sucrose-like) and bitter (quinine-like) taste (Kiefer and Lawrence, 1988;Kiefer and Mahadevan, 1993) and odor volatiles (Kiefer and Morrow, 1991;Molina et al., 1999) of alcohol and probably the other components detected by humans as well (Belknap et al., 1977;Nachman et al., 1971).

Many factors underlie the role that alcohol flavor plays in the development of alcohol preference and consumption patterns. Such factors include the activation of peripheral chemoreceptors by alcohol (Trevisani et al., 2002); central mechanisms that mediate the hedonic responses to alcohol flavor (Ferraro et al., 2002); learned associations of alcohol's sensory attributes and its postingestive effects and early postnatal exposure to alcohol flavor (Mennella, 1999;Molina et al., 1999); and genetically determined individual variation in chemosensation (Bartoshuk et al., 1993;Duffy et al., 2003;Pelchat and Danowski, 1992). The study of the role of chemosensory factors in alcohol intake and preferences is of special interest because the past decade has witnessed significant technical and scientific advances, which include identification of receptors and other key molecules involved in the transduction mechanisms of olfaction (Buck and Axel, 1991), chemosensory irritation (Caterina et al., 1997), and taste (Adler et al., 2000;Bachmanov et al., 2001b;Kitagawa et al., 2001;Li et al., 2002;Matsunami et al., 2000;Montmayeur et al., 2001;Nelson et al., 2001,2002;Sainz et al., 2001). The following presentations review the role that the chemosensory factors play in alcohol perception, preferences, and consumption in humans and laboratory animals throughout the lifespan.

TASTE REACTIVITY AS A MEASURE OF ALCOHOL PALATABILITY AND ITS RELATION TO ALCOHOL CONSUMPTION IN RATS

Stephen W. Kiefer

Given that ethanol is normally introduced to one's internal milieu only through the oral cavity, taste factors represent an important arbiter in an organism's decision to ingest or reject such a solution (Garcia et al., 1974). Frequently, ethanol's palatability is deduced from simple consumption measures. If an animal drinks a lot of ethanol, it must like the taste. And, conversely, if an animal avoids consumption of ethanol, it must dislike the taste. Such conclusions, although logical, are not supported by work indicating that taste palatability and consumption can be experimentally dissociated.

Taste palatability has been measured with the taste reactivity test, a quantitative measure of palatability that is reflected by orofacial responses to orally infused fluids (Grill and Norgren, 1978). The measure of ethanol palatability with the taste reactivity paradigm in rats has been used extensively in our laboratory (for a review, see Kiefer, 1995). In general, ethanol solutions elicit a complex combination of hedonic (positive or ingestive) and aversive responses. Early experiments focused on characterizing, in detail, the response of outbred rats to ethanol (Kiefer and Dopp, 1989). Simple consumption measures were made, but the correlations between ethanol reactivity measures and consumption were weak at best. In this first experiment and in all subsequent studies, care was taken to ensure that the videotapes of the rats' responses were scored with the scorer blind to the solution and the rat's experimental condition.

The majority of work with ethanol reactivity and consumption has taken advantage of the rat lines selectively bred for high (and low) levels of ethanol consumption. In our laboratory, three sets of rats have been tested extensively: the ethanol-preferring P rats and the nonpreferring NP rats from the Indiana University School of Medicine (Bice and Kiefer, 1990); the replicate line of HAD (high-alcohol-drinking) and LAD (low-alcohol-drinking) rats, also from Indiana University (Kiefer et al., 1995); and the high-ethanol-drinking AA rats and low-ethanol-

drinking ANA rats from Finland (Badia-Elder and Kiefer, 1999). Although these lines of rats were tested in separate experiments and reported in separate articles, the general findings were similar. Prior to experience with ethanol consumption (all rats were received as ethanol naïve), the high-drinking and low-drinking rats were not significantly different in their taste reactivity response to ethanol across a 5% to 40% range of concentrations. There was only one exception to this characterization and that was reflected by AA rats, which showed significantly more hedonic (ingestive) responses than ANA rats.

After 3 weeks of continuous access to 10% ethanol and water, during which time high- and low-drinking patterns were confirmed, consistent shifts in reactivity were found. For hedonic responding, all high-drinking rat lines exhibited a significant increase across all concentrations; conversely, the high-drinking rats demonstrated a significant decrease in aversive responding, particularly at the higher concentrations. Low-drinking rat lines did not alter their reactivity to ethanol after the experience with 10% ethanol. The decreases in aversive responding to ethanol were extremely consistent across the three sets of rats. The high-drinking rats made a small number of aversive responses to the lowest concentrations, but, at the higher concentrations, virtually no high-drinking rats made any aversive responses.

It is important to note that the changes in reactivity found in the high-drinking rat lines were consistent across the range of concentrations even though the rats only had experience consuming a single concentration: 10%. The fact that consumption of one ethanol concentration has significant impact on the reactivity response to other concentrations (the highest concentration was four times that of the concentration consumed) may be significant for any transition that high-risk humans might make from low concentrations of ethanol to more concentrated drinks.

Although reactivity and consumption appear to covary in a consistent manner with selectively bred lines, additional data from drug manipulations suggest that ethanol reactivity and consumption can be dissociated. Hill and Kiefer (1997) reported that naltrexone, a general opiate antagonist, caused a significant shift in ethanol reactivity to a more negative reaction. That is, outbred rats treated acutely with naltrexone showed a decrease in hedonic responding and a concomitant increase in aversive responding when tested for reactivity to 10% ethanol. The most consistent results were found with the 1 mg/kg and 3 mg/kg dosages (the 0.5 mg/kg dose produced inconsistent results). After the reactivity tests were completed, the effect of acute naltrexone treatment on 10% ethanol consumption was tested. Rats were placed on a schedule of restricted fluid access and, once acclimated to the drinking schedule, were injected with naltrexone 10 min before ethanol access. A consistent dose-dependent decrease in ethanol consumption was found.

Reductions in both ethanol palatability and ethanol consumption after naltrexone treatment in outbred rats have been confirmed in an additional experiment. Coonfield et al. (2002) found that relatively low doses of naltrexone (0.25-1.0 mg/kg) produced results similar to those reported earlier (Hill and Kiefer, 1997). Naltrexone reduced ingestive responding, increased aversive responding, and decreased ethanol consumption, the last measure again being dose dependent. An additional experiment (Ferraro et al., 2002), where only reactivity measures were made, found that naltrexone shifted the palatability of a variety of taste solutions (ethanol, sodium chloride, and quinine hydrochloride) across a range of concentrations. For each solution, rats made fewer ingestive and greater aversive responses. The pattern of data for sucrose concentrations was unique: naltrexone failed to alter ingestive responding to sucrose but consistently increased aversive responding, particularly at the low concentrations. These data support an earlier suggestion that the mechanisms mediating ingestive responding may be independent from those mediating aversive responding (Berridge and Grill, 1984).

We have recently combined naltrexone treatment and high-drinking rat lines to determine whether the pattern of decreased palatability and decreased consumption would result. In one experiment with AA rats (Coonfield et al., 2001), 1 mg/kg and 3 mg/kg naltrexone treatment failed to produce significant changes in taste reactivity to ethanol. There was a hint of the expected palatability shift, but the changes were not statistically significant. Despite the lack of effect of naltrexone on taste reactivity, similar treatment clearly produced the expected decreases in consumption of ethanol. Thus, these data suggested that ethanol reactivity and ethanol consumption could be dissociated. When P rats were tested, a decision was made to use a stronger dose, so both 3 mg/kg and 7.5 mg/kg doses were used in a group of naïve P rats. The pattern of the data was similar to that already described: the naltrexone dosages had no significant effect on taste reactivity to 10% ethanol, but the same treatments clearly produced significant reductions in ethanol consumption. We are currently analyzing a third experiment, again with AA rats, by using the higher 3 mg/kg and 7.5 mg/kg dosages. Although the taste reactivity data have yet to be completely analyzed, it has been confirmed that the naltrexone dosages significantly decrease consumption of 10% ethanol.

The ability of naltrexone to dissociate ethanol reactivity from ethanol consumption in selectively bred rats may provide an avenue for understanding the underlying mechanisms of the rat's response to ethanol. We believe that it is important to consider taste or palatability as an issue separate from the actual consumption of ethanol. Ultimately, however, attempts to eliminate ethanol consumption and abuse would bring to bear interventions that would recruit both taste factors (somehow render the taste of alcohol as unpalatable) and consumption factors (particularly postingestive consequences).

EARLY LEARNING ABOUT THE SENSORY PROPERTIES OF ALCOHOL IN LABORATORY ANIMALS

Juan Carlos Molina

The interaction comprising early development and alcohol exposure has been primarily analyzed from a teratological perspective. The incidence and prevalence of fetal alcohol syndrome and fetal alcohol-derived effects certainly justify this approach. This perspective also allows us to consider the possibility that initial exposure to alcohol's sensory and/or toxic properties can take place during prenatal life. After maternal alcohol ingestion, the levels of alcohol observed in the amniotic fluid closely parallel those encountered in fetal and maternal blood (Domínguez et al., 1996). In different mammalian species, the immaturity of the olfactory subsystems and of the gustatory sensory modality during late fetal life does not preclude functional detection, discrimination, and learning about chemosensory cues that are present in the amniotic fluid (Molina et al., 1999). Early alcohol-related experiences are also likely to take place whenever the nursing infant has access to maternal milk contaminated with the drug. In different cultures, moderate or even heavy maternal alcohol consumption is regarded as a means to promote milk production or rectify problems that lead to milk insufficiency (Mennella, 1999;Mennella, 2002). If alcohol consumption does not inhibit milk production and ejection from the mammary gland, the concentration of alcohol in maternal milk tends to be similar to that encountered in blood (Pepino et al., 1998). There are also other cultural uses of the drug related to its sedative, analgesic, and antiseptic properties. These later applications also imply the possibility of infantile processing of alcohol's sensory and/or postabsorptive effects (Choonara, 1994;Molina et al., 1999). If our purpose is to understand how alcohol acceptance patterns develop in a given organism, it seems logical not to neglect the likelihood of significant experiences with the drug during early ontogeny. Some of the hypotheses that are likely to be derived from this general statement are also difficult to test via epidemiological and clinical studies. Methodological and particularly ethical problems will inevitably arise whenever the research strategy requires controlled exposure to alcohol during

the course of early ontogeny. Animal studies have revealed that prenatal and/or early postnatal alcohol experiences can have profound effects on later alcohol detection, recognition, and acceptance patterns (Spear and Molina, 2001). We will now discuss some of our most recent findings related to these particular issues.

Near-term rat fetuses and neonates show specific behavioral responses to chemosensory cues experienced previously in the prenatal milieu (Robinson and Smotherman, 1995). When alcohol accumulates in the amniotic fluid, the rat perinate seems capable of encoding sensory information inherent to the drug and of retaining this information during considerable time intervals. A brief (10 min) exposure to alcohol diluted in the amniotic fluid (peak alcohol concentration: 100 mg%) prior to cesarean delivery is sufficient to (a) affect neonatal autonomic and behavioral components of orienting responses to alcohol odor, (b) increase infantile alcohol olfactory preferences and alcohol intake patterns, and (c) interact with postnatal learning experiences where alcohol odor is explicitly associated with different reinforcers (Molina et al., 1995). It is important tonote that subsequent studies indicated that the perinatal alcohol-related sensory memory was most likely established via an associative process (Domínguez et al., 1999). It became evident that the delay existing between alcohol contamination of the amniotic fluid and the consequences derived from cesarean delivery and/or perinatal manipulations was a critical factor in terms of allowing subsequent recognition and acceptance of alcohol odor and/or taste. Specifically, and as suggested by prior studies (Wilson and Sullivan, 1994), activating tactile stimulation applied by the experimenter during and immediately after cesarean delivery represented an effective unconditioned stimulus capable of supporting associative learning where alcohol's sensory properties act as a conditioned stimulus. Hence, we had been able to determine fetal detection and retention of alcohol-derived cues but only under very particular experimental circumstances.

What happens when fetal exposure to alcohol is derived from maternal administration of the drug? When moderate alcohol doses are used, will the accumulation of ethanol in the amniotic fluid or in fetal blood reach suprathreshold levels in terms of recruiting sensory processing? If so, will the state of fetal intoxication compete against sensory processing and/or acquisition and retention of the information?

Some of these questions were addressed through the examination of perinatal and infantile responsiveness to alcohol after relatively low daily alcohol doses (1 or 2 g/kg) were administered to pregnant rats through gestational days 17 to 20. Chemosensory processing of biological and artificial odorants, as well as nonassociative (habituation) and associative (Pavlovian conditioning) learning processes, have been observed during this late prenatal stage of development (Robinson and Smotherman, 1995;Ronca and Alberts, 1994).

Alcohol levels in fetal and maternal blood and in the amniotic fluid were determined through head-space chromatography 1 hr after pregnant females received the last 1 or 2 g/kg alcohol dose corresponding to gestational day 20. As could be expected, dose-dependent ethanol concentrations were observed across the different sites of assessment. Alcohol levels in the amniotic fluid resulting from maternal administration of 1 and 2 g/kg alcohol doses were equivalent to 50 and 120 mg%, respectively. Prenatal alcohol exposure did not affect placental or umbilical cord sizes and weights, fetal body weights, or weights and sizes of different central nervous system structures. When perinates pretreated with alcohol were subsequently exposed to an unscented air stream or to air scented with a novel odorant (lemon), motor activity scores were higher than those registered in saline-pretreated subjects. This hyperactive phenomenon, which has been described in different animal models related to fetal alcohol effects, was not a permanent behavioral condition. When newborns pretreated with alcohol were exposed to the smell of the drug, a dramatic decrease in their activity rate was evident (Domínguez et al., 1996;Molina et al., 1999). Subsequent experiments demonstrated that maternal alcohol

intoxication during late gestation generates in the progeny a specific memory of alcohol's chemosensory attributes (Domínguez et al., 1998). Immediately after birth, pups prenatally exposed to saline or to 1 or 2 g/kg alcohol doses were exposed to orosensory stimuli in gas phase (amniotic fluid, amniotic fluid compounded with alcohol, or alcohol alone). When the durations of either overall motor activity or head movements were the dependent variables, a consistent behavioral pattern emerged. Alcohol experience in utero, particularly with the higher alcohol dose, predisposed pups to react to the smell of the drug in a similar way, as did alcohol-naïve newborns when they were stimulated with the smell of the amniotic fluid. Subsequent infantile intake tests provided further evidence for alcohol-related memories established during fetal life. Consumption of five different palatable solutions was assessed. Prenatal alcohol treatment failed to modify ingestive responses elicited by the palatability of two basic tastants such as quinine and sucrose. Water intake scores were also similar across prenatal treatments. Nevertheless, pups subjected to intrauterine alcohol experiences were more likely to ingest an alcohol solution and a sucrose-quinine compound (a stimulus that represents a psychophysical equivalent of alcohol in the rat; Kiefer, 1995) when compared with infants with no prior alcohol experience (Domínguez et al., 1998).

How are these effects determined? Is prenatal exposure to alcohol's sensory attributes the sole determinant of subsequent differential responsiveness to alcohol odor and taste, or does this memory also depend on alcohol's unconditioned properties related to maternal-fetal intoxication? This second question implies the possibility of associative learning in utero based on the contingency between alcohol's sensory (conditioned stimulus, CS) and toxic (unconditioned stimulus, US) properties.

The main problem in terms of analyzing whether alcohol intoxication can support fetal associative learning is directly related to alcohol distribution processes. Maternal alcohol administration results in not only fetal intoxication but also an opportunity for the fetus to process the sensory characteristics of alcohol that enters the amniotic fluid. The difficulty is in establishing experimental procedures to evaluate potentially separable effects of (a) alcohol's orosensory features, (b) alcohol's pharmacological consequences, and (c) the association between both factors. Hence, we decided to use as a CS a nonalcohol chemosensory stimulus (cineole, main component of essential eucalyptus oil) that could be administered to the mother and traced through chromatography. The pharmacokinetic profiles related to the presence of cineole and alcohol in the amniotic fluid and in maternal blood allowed us to design experiments where prenatal temporal contiguity between peak levels of cineole in the amniotic fluid and the induction of maternal/and or fetal alcohol intoxication was systematically varied (Abate et al., 2000,2001). This strategy proved to be successful. For example, newborns that during prenatal lifeexperienced cineole shortly before commencement of alcohol's postabsorptive effects were more likely to attach to an artificial nipple scented with this olfactory cue than were neonates exposed to unpaired prenatal experiences involving cineole and alcohol intoxication or relative to completely naïve controls (Abate et al., 2003). This observation supports the possibility that prenatal alcohol exposure can result in chemosensory associative learning mediated by alcohol's postabsorptive effects.

As previously mentioned, the nursing environment provides alternative possibilities related to early alcohol chemosensory processing. Dams exposed to intragastric administrations of subnarcoleptic alcohol doses (0.5-3.0 g/kg) exhibit milk alcohol levels that range between 25 and 200 mg% (Pepino et al., 1998). Preweanling rats readily acquire sensory information about alcohol when suckling from a mother previously subjected to a moderate alcohol dose that results in milk alcohol levels approximately equivalent to 175 mg%. Furthermore, a relatively brief history of infantile interaction with ethanol-intoxicated dams seems to facilitate subsequent responsiveness to ethanol's orosensory cues and also to predispose the animal to express a negative affective component of the memory acquired through these interactions

(Pepino et al., 1999,2001). Interestingly, after interacting for the first time with a non-tolerant alcohol-intoxicated dam (postnatal day 3), socially isolated pups exhibit heightened distress ultrasonic vocalizations and abnormal motor patterns relative to conspecifics that interacted with an alcohol-free dam. The former animals rapidly learn to avoid a novel tactile stimulus that is explicitly associated with the smell of alcohol. When maternal care is disrupted by alcohol is when preweanlings are more likely to express behavioral signs of distress (Molina et al., 2000). Under these circumstances, it is conceivable that infants learn to associate alcohol's sensory cues present in the nursing context with disruptive effects of alcohol on maternal care. Later in life, when suffering social and nutritional deprivation, infants nursed by alcohol-intoxicated dams consume more alcohol than do control pups. This effect survives the passage of time and is likely to be observed in male rats during juvenile stages of development (Pepino et al., 2000). It seems paradoxical that an aversive memory related to sensory components of the drug will result in heightened alcohol intake. Nevertheless, we should not disregard the possibility that this heightened alcohol consumption can represent a behavioral strategy to obtain stress-relief through anxiolytic properties of the drug. This hypothesis remains to be tested.

Is it possible to expect an interaction between the consequences of pre- and early postnatal experiences with alcohol's sensory attributes? At least two studies directly endorse this possibility. Pups prenatally exposed to alcohol during late gestation and postnatally stimulated with the orosensory components of the drug exhibit exacerbated motor and heart rate responses when stimulated with the smell of alcohol relative to animals subjected only to pre- or postnatal alcohol experiences (Chotro et al., 1996). Similarly, in a recently conducted study, we observed that maternal administration of alcohol during late gestation followed by infantile interactions with an alcohol-intoxicated mother facilitates subsequent detection of relatively small concentrations of ethanol (175 mg%) in water (M. Pueta and J. C. Molina, unpublished data, 2002).

As a function of the studies summarized here, the following concluding remarks appear pertinent:

1. During late gestation and breastfeeding, the developing rat processes the presence of alcohol in the corresponding niche.
2. Alcohol-related memories arise from these early experiences and facilitate subsequent responsiveness to the drug's chemosensory cues.
3. A common denominator of these experiences is that they promote alcohol-self administration later in life.
4. Prenatal and early postnatal experiences with alcohol interact and appear to facilitate the discrimination of the sensory properties of the drug.
5. Some of these early memories are generated through associative processes that comprise alcohol's sensory properties and alcohol's postabsorptive effects on the organism itself or on other conspecifics.

EARLY LEARNING ABOUT THE SENSORY PROPERTIES OF ALCOHOL IN HUMANS

Julie A. Mennella

Research on humans and laboratory animals has revealed remarkable consistency in how young animals learn about the dietary choices of the mother (Mennella, 2001;Mennella and Beauchamp, 1998b). Flavors from the mothers' diet are transmitted to amniotic fluid and mothers' milk and permeate the ambient environment. Such early flavor learning may be a

fundamental feature of all mammals because it is presumably important for the preweanling infant to accept and be particularly (but not exclusively) attracted to the flavors consumed by the mother. All else being equal, these are the flavors that are preferred by the mother or, at least, foods the mother has access to. Under this hypothesis, exposure occurring in utero and during breastfeeding, when flavors mothers consume are transferred to the infants' chemosensory environment, would influence subsequent acceptance and preference. During the past decade, research in our laboratory has focused on the ontogeny of responsiveness to the sensory qualities of alcohol in humans (Mennella, 1999,2001). The goal of this research is to identify some of the developmental, experiential, and cultural factors that contribute to an individual's hedonic responses to the sensory properties of alcohol. Because of the olfactory system's intense and immediate access to the neurological substrates underlying emotion (Cahill et al., 1995), such investigations of the hedonic responses to sensory stimuli may provide a window into children's emotional responses and reveal information about contextual effects of learning and the role of early experience on the development of preferences and aversions.

The flavor changes resulting in human milk after the mother consumes an alcoholic beverage parallel the changing concentrations of ethanol in her milk (Mennella and Beauchamp, 1991). Moreover, the infant can detect the flavor of alcohol in the milk, and experience with the flavors modifies subsequent responsiveness in the context of milk feeding (Mennella, 1997), a finding that is consistent with the infants' response to a wide variety of other flavors (Mennella and Beauchamp, 1998a). In addition to the impact that maternal alcohol consumption has on infant nutrition, experience with the sensory qualities of ethanol in mothers' milk may affect infants in other important ways. Animal studies revealed that memories are formed as a result of orosensory experiences during nursing and retained for a considerable time span (Molina et al., 1999). This is especially relevant because the infant can detect the flavor of alcohol, and the context in which alcohol is experienced, with the mother and during breastfeeding, consists of a variety of elements that can be positive (e.g., tactile stimulation, warmth) reinforcers for early learning.

When breastfed infants (6-13 months) were exposed to toys that were identical in appearance but differed in their characteristic scent (e.g., vanilla-scented, ethanol-scented, or no scent), we found that those infants who had more exposure to ethanol, as inferred from questionnaires about maternal and paternal alcoholism and alcohol intake, behaved differently in the presence of an ethanol-scented toy compared with less exposed infants (Mennella and Beauchamp, 1998b). Of the four behaviors monitored in the study (mouthing, looking, manipulating the toy, and vocalizing), this differential response was manifested in mouthing the ethanol-scented toy more. This finding might be anticipated based on animal studies which indicated that pups that were exposed to the flavor of ethanol in milk increased their mouthing rates to the odor of ethanol and were more willing to ingest ethanol-flavored solutions (Hunt et al., 1993). Whether mouthing the ethanol-odorized toy more reflects their familiarity with the flavor of ethanol, which, in turn, leads to a greater willingness to accept ethanol-flavored substances, remains to be investigated. Nevertheless, these data provide circumstantial evidence that prior ethanol exposure in humans alters the willingness of infants to orally explore toys scented with this odor. Moreover, this learning appears to be keenly selective, as it allows for the discrimination of closely related aromas, vanilla and ethanol.

That early experiences can also generate aversive memories about alcohol was evident in a study on older children (3-6 years). Here the children's hedonic response to the odor of alcohol was related to the emotional context in which parents experience alcohol and the parents' frequency of drinking (Mennella and Garcia, 2000). That is, children of a parent or parents who drank alcohol to change their state of mind or reduce dysphoric feelings (hereafter referred to as "escape drinking") were significantly more likely to judge the odor of beer as unpleasant

compared with similarly aged children whose parents did not drink to escape. This difference between the groups was odor specific. That is, the children in the two groups were similar in their preference for the bubble gum and rejection of the pyridine odors. These findings concur with previous studies on elementary school-aged children of alcoholic parents (Noll et al., 1990) and are consistent with animal studies which demonstrate that pups exposed to an intoxicated mother develop aversive memories toward the odor of alcohol (Molina et al., 2000;Pepino et al., 2001).

Early childhood represents a critical period for the development of expectancies about, and the affective disposition toward, alcohol, which may affect alcohol use during adolescence. These findings, together with those presented by Juan Molina in this symposium, suggest that at least some of the early learning about alcohol is based on sensory experiences and clearly anchor it to children's experiences at home and the frequency and the emotional context in which their parents experience alcohol. These data support the hypothesis that associative learning in the context of emotionally salient conditions is a powerful mechanism by which odors acquire personal significance and that the emotional context in which children experience an odor can influence subsequent behaviors. Whether the emotional response to the scent of alcohol conditioned during early childhood persists or can explain behaviors during later childhood and adolescence is not known. One of our goals is to tap into the sensory and emotional world of the child in an attempt to learn what the child becomes.

GENETIC DISSECTION OF THE ETHANOL-SWEET TASTE RELATIONSHIP IN MICE

Alexander A. Bachmanov and Michael G. Tordoff

Ethanol preference is associated with the liking for sweet taste. The relationship between ethanol and sweetener perception and consumption is supported by several lines of evidence. Electrophysiological recordings indicate that lingual application of ethanol activates sweetener-responsive neural fibers in the gustatory nerves (Hellekant et al., 1997;Sako and Yamamoto, 1999) and sweetener-responsive units in the nucleus of the tractus solitarius (Di Lorenzo et al., 1986). Conditioned taste aversions generalize between ethanol and sucrose (Blizard and McClearn, 2000;Kiefer and Lawrence, 1988;Kiefer and Mahadevan, 1993;Lawrence and Kiefer, 1987), suggesting that ethanol and sucrose share the same taste property, most likely sweetness. Human alcoholics were shown to have a stronger liking of concentrated sucrose compared with nonalcoholics (Kampov-Polevoy et al., 1998,1997). Genetic associations between preferences for ethanol and sweeteners are found among many rat and mouse strains and their segregating crosses (reviewed in Kampov-Polevoy et al., 1999;Overstreet et al., 1999).

We analyzed the genetic association between ethanol consumption and taste by using two mouse strains, C57BL/6ByJ (B6) and 129P3/J (129). In two-bottle choice tests, intake of ethanol and more than a dozen sweeteners was higher in B6 than 129 mice (Bachmanov et al., 1996b,2001d). Because both ethanol and sugars have caloric value for animals, we investigated the possibility that the higher intakes of sucrose and ethanol by the B6 mice are due to a greater appetite for calories in this strain compared with the 129 strain. We found that consumption of chow, complex carbohydrates (cornstarch and Polycose), and fats (soybean oil and margarine) was similar in the two strains (Bachmanov et al., 2001c). Although there may be common signals related to the caloric value of ethanol and sugars (Freed, 1972;Gentry and Dole, 1987;McMillen and Williams, 1998;Richter, 1941;Rodgers, 1966;Rodgers et al., 1963), our data suggest that the B6 and 129 mice have similar avidity for calories, and that the appetite for calories does not contribute to the strain differences in ethanol or sucrose consumption.

The B6 and 129 mice also have different responses to salty, sour, and bitter stimuli (Bachmanov et al., 2002a,1996b;Beauchamp and Fisher, 1993), which makes them a useful model to study the relationship between ethanol intake and taste. To examine whether differences between the B6 and 129 mice in ethanol and taste preferences are due to common genetic mechanisms or to independent genes fortuitously fixed during strain inbreeding, we produced the second filial generation (*F*2) of hybrids between these two strains. The *F*2 mice were offered solutions of ethanol, sucrose, citric acid, quinine hydrochloride, and NaCl in two-bottle choice tests. Analysis of genetic correlations showed that ethanol consumption positively correlated with sucrose intake, but it did not correlate with intakes of the other taste stimuli (citric acid, quinine, and NaCl). The differences in ethanol and sweetener intake between B6 and 129 strains depend on relatively small and partially overlapping sets of genes (Bachmanov et al., 1996a). Results of this and other (Belknap et al., 1993;Overstreet et al., 1993;Phillips et al., 1994;Stewart et al., 1994) studies support the presence of a genetically determined link between the consumption of ethanol and sweet solutions, which is consistent with the hypothesis that the higher ethanol intake by B6 mice depends, in part, on higher hedonic attractiveness of its sweet taste component. Previous work suggested a relationship between excess ethanol consumption and lower sensitivity to bitterness or aversion to NaCl (Grupp et al., 1991;Pelchat and Danowski, 1992). However, we found no cosegregation in the *F*2 generation between intakes of ethanol and quinine or NaCl, suggesting that independent genes fixed during inbreeding of the parental strains are responsible for these associations in the 129 and B6 mice.

Our most recent studies have been directed toward identifying the genetic loci (quantitative trait loci, QTL) underlying the association between ethanol and sweetener preferences. QTL locations were determined using linkage analysis. The B6 × 129 *F*2 mice were tested by using two-bottle tests with ethanol and the four sweeteners (glycine, D-phenylalanine, saccharin, and sucrose). In each *F*2 mouse, we tested DNA markers on different chromosomes to find out from which parental strain linked chromosomal regions were inherited. A genome screen that used these *F*2 mice identified three significant linkages for indexes of ethanol consumption. Two loci, on distal chromosome 4 (*Ap3q*) and proximal chromosome 7 (*Ap7q*), strongly affected 10% ethanol intake and weakly affected 3% ethanol intake. A male-specific locus on proximal chromosome 8 (*Ap8q*) affected 3% ethanol preference but not indexes of 10% ethanol consumption. In addition, six suggestive linkages (on chromosomes 2, 9, 12, 13, 17, and 18) affecting indexes of 3% and/or 10% ethanol consumption were detected. The loci with significant and suggestive linkages accounted for 35% to 44% of the genetic variation in ethanol consumption phenotypes. No additive-by-additive epistatic interactions were detected for the primary loci with significant and suggestive linkages. However, there were a few modifiers of the primary linkages and a number of interactions among unlinked loci. This demonstrates a significant role of the genetic background in the variation of ethanol consumption (Bachmanov et al., 2002b). Several linkages for ethanol consumption were also associated with sweetener preferences. In some cases, linkages for ethanol and sweeteners had distinct peaks. This suggests that each trait is linked to its own QTL, but because of the QTL proximity, they tend to be inherited as a linked group, which contributes to the genetic correlation between ethanol and sweetener intakes. However, in some other cases, linkage intervals for ethanol and sweetener consumption overlapped, suggesting that the same QTL may have pleiotropic effect on both traits.

One such locus that is likely to pleiotropically affect ethanol and sweetener consumption is *Ap3q* on distal chromosome 4. This region also contains the saccharin preference (*Sac*) locus (Bachmanov et al., 1997,Blizard, et al., 1999;Li et al., 2001;Lush et al., 1995;Phillips et al., 1994), which has been recently positionally cloned (Bachmanov et al., 2001b) and corresponds to a sweet taste receptor gene, *Tas1r3* (Kitagawa et al., 2001;Max et al., 2001;Montmayeur et al., 2001;Nelson et al., 2001;Sainz et al., 2001). Thus, the *Sac* and *Ap3q* loci are probably identical, suggesting that the *Tas1r3* gene is a candidate for the *Ap3q* locus and that genetic

differences in perception of the sweet taste component of ethanol flavor can affect ethanol consumption (Bachmanov et al., 2001a).

Because of evolutionary conservation between mouse and human genomes, genes positionally cloned in the mouse are likely to have human orthologs. For example, mouse distal chromosome 4 (including the *Ap3q* and *Sac/Tas1r3* loci) has conserved synteny with a subtelomeric region of the short arm of human chromosome 1 (1p36). This human region harbors the *TAS1R3* gene, an ortholog of mouse *Tas1r3* (Reed et al., 2001). Moreover, a locus influencing vulnerability to alcoholism has been mapped to human chromosome 1 (1p13-35) (Nurnberger et al., 2001); however, the linkage peak for this locus is substantially more centromeric than the region of conserved synteny for the *Ap3q* locus.

In conclusion, our data suggest that the genetic correlation between ethanol and sweetener consumption is due to two factors: linkage of distinct loci for each trait, and presence of loci with pleiotropic effects on both traits. The locus on distal chromosome 4 (*Tas1r3/Sac/Ap3q*) appears to have a pleiotropic effect on ethanol and sweetener consumption. The *Tas1r3* gene encodes a sweet taste receptor protein, T1R3, which suggests that genetic differences in perception of the sweet taste component of ethanol flavor can affect ethanol consumption. Other loci with pleiotropic effects on ethanol and sweetener consumption may be involved in common brain mechanisms, because the regulation of ingestive responses to ethanol and sweeteners appears to involve common opioidergic, serotonergic, and dopaminergic brain neurotransmitter systems (George et al., 1991;Gosnell and Majchrzak, 1989;Hubell et al., 1991;Pucilowski et al., 1992). These mechanisms could be responsible for the emotional response to the pleasantness of ethanol or sweeteners and the motivational mechanisms driving their intakes. The mouse genes involved in association of ethanol consumption and sweet taste are likely to have human orthologs. Thus, using the mouse as a model organism will help to understand the genetic determination of human alcohol intake.

HUMAN GENETIC VARIATION IN TASTE: CONNECTIONS WITH ALCOHOL SENSATION AND INTAKE

Valerie B. Duffy and Linda M. Bartoshuk

Humans show genetic variation in taste and oral sensation. One marker of genetic variation is the ability to taste the bitterness of 6-*n*-propylthiouracil (PROP) or phenylthiocarbamide. Individuals who taste PROP as intensely bitter also taste alcohol as more bitter as well as irritating. The question of interest is if alcohol presents enough of a noxious experience to individuals who taste PROP as intensely bitter to act as a sensory hindrance for overconsumption of alcohol. The validity of psychophysical methods measuring variation in PROP bitterness is the key to evaluating a link between PROP bitterness and alcohol behaviors.

Genetic variation in the ability to taste was discovered accidentally (Fox, 1931). We now know that PROP threshold procedures produce a bimodal distribution of nontasters (individual with high thresholds) and tasters (those with low thresholds). Bartoshuk and colleagues reported that tasters showed great variability in the perceived bitterness of concentrated PROP (e.g., 0.0032 M; Bartoshuk et al., 1994). Furthermore, threshold performance did not always reflect the ability to taste more concentrated PROP (Bartoshuk et al., 1986). Through continued study of PROP tasting, it is evident that (1) threshold procedures do not fully characterize variability in PROP tasting and (2) valid measures of perceived intensity are required to identify individuals who vary most in PROP bitterness (Bartoshuk, 2000). Through improved psychophysical methodology, the ability to see associations between PROP tasting and behaviors toward alcohol is enhanced.

Alcohol can present a more noxious experience to individuals who taste PROP as more bitter. Healthy adults reported the bitterness and irritation of 10% ethanol applied to the tongue tip as well as 10% to 50% ethanol sampled with the whole mouth and expectorated. These adults were also characterized for PROP tasting (Bartoshuk et al., 1993). The alcohol and PROP ratings were measured with magnitude estimation and normalized to the saltiness of NaCl as a sensory standard. Those who tasted greater PROP bitterness rated the alcohol as more bitter and irritating than those who reported PROP as only weakly bitter. (The use of NaCl as a sensory standard may have minimized the PROP effects, as subsequent study showed that NaCl is saltier to those who taste PROP as more bitter.) Prescott and Swain-Campbell (2000) also showed that ethanol was more irritating to PROP tasters, and Intranuovo and Powers (1998) reported that some beer is more bitter to those who taste PROP as very strongly bitter.

Differences in tongue anatomy associated with PROP tasting could explain oral sensory differences in alcohol sensation. Miller and Reedy (1990) first suggested an association between number of fungiform papillae taste buds (indicated by taste pores) and bitterness of PROP. In collaboration with them (Bartoshuk et al., 1994), we showed that those who taste PROP as the most bitter had, on average, the greatest number of fungiform papillae and taste buds. The relationship between PROP tasting and density of fungiform papillae has also been reported by others (e.g., Tepper and Nurse, 1997). Fungiform papillae receive innervation from taste (chorda tympani nerve, CN VII) and somatosensory (trigeminal nerve, CN V) nerve fibers (Whitehead et al., 1985). Chorda tympani nerve fibers synapse with cells in taste buds; trigeminal nerve fibers surround taste buds without synaptic contact.

Those who perceive the most bitterness from PROP also taste more saltiness from NaCl, more sweetness from sucrose, more bitterness from quinine hydrochloride, and more intense sensations from oral irritants such as alcohol and capsaicin and creamy/viscous stimuli (e.g., dairy fat, oil; see Prutkin et al., 2000, for a review).

PROP tasting can also associate with alcohol preference and behavior. Intranuovo and Powers (1998) reported that those least able to taste PROP bitterness consumed a greater number of beers during their first year of drinking than those who tasted PROP as intensely bitter. High consumers of beer (e.g., >3.6 liters/week) were also more likely to be nontasters of PROP/phenylthiocarbamide than low consumers (<720 ml/week) according to findings by Guinard et al. (1996). This study further found that low consumers reported the most dislike for the beer that was reported most bitter.

Findings from our laboratory support associations between PROP bitterness and reported intake of alcoholic beverages. In one study (Duffy et al., 2003), healthy adults (n = 80; mean age = 26 years) rated oral irritation from, as well as the degree of liking/disliking of, 50% ethyl alcohol. To assess frequency of alcohol intake, subjects reported how frequently they consumed beer, wine/wine coolers, and liquor/mixed drinks on The Block Food Questionnaire (version 98.1). Subjects who had a high level of cognitive restraint over eating were not included in the study for fear they might underreport the consumption of alcoholic beverages. The responses were coded as yearly intake to calculate a composite frequency of alcoholic beverages over 1 year. Those who tasted PROP as most bitter also reported the alcohol as most intense, most disliked alcoholic beverages, and consumed alcoholic beverages significantly less frequently than did those who tasted PROP as least bitter. Greater than 60% of those individuals who tasted the least bitterness from PROP reported consuming at least 1 alcoholic beverage every day.

Sex differences in PROP tasting can challenge studies assessing the relationship between PROP tasting and dietary behaviors, especially related to alcohol consumption. Many past and current studies show sex differences in the distribution of PROP tasting, with females showing greater

variance and skew toward greater PROP bitterness (see Bartoshuk et al., 1994, for a review). Duffy et al. (2003) found that the distribution in PROP tasting as well as the association between PROP and frequency of consuming alcoholic beverages did not vary across men and women.

The literature does not show clear support for associations between PROP and risk for alcoholism. Some studies report greater numbers of nontasters among alcoholics (e.g., DiCarlo and Powers, 1998;Pelchat and Danowski, 1992), whereas others do not (e.g., Kranzler et al., 1996,1998). For example, Pelchat and Danowski (1992) reported greater numbers of nontasters among children of alcoholics, whether or not the children were alcoholic themselves. Conversely, Kranzler et al. did not find that PROP nontasters were more likely to have a parental history of alcohol dependence whether (Kranzler et al., 1998) or not (Kranzler et al., 1996) they were alcoholic themselves.

The inconsistencies in the literature may be explained, in part, by differences in characterizing PROP tasting. Pelchat and Danowski (1992) reviewed some of the methodological procedures. Because PROP thresholds cannot consistently identify supertasters of PROP, those studies that rely on thresholds to characterize PROP tasting may miss the effect on dietary behaviors toward alcohol. Studies that scale the bitterness of PROP but do so with category scales may also miss PROP effects, as explained subsequently.

Studying PROP effects on alcohol behaviors requires scaling methods that can be compared across individuals. The challenges in studying PROP tasting have been reviewed previously (Bartoshuk, 2000); here they are briefly summarized. Adjective-labeled scales (e.g., Likert and category) are frequently used to make across-subject or group comparison. These scales are only valid for within-subject comparisons. The reason for this is that those intensity adjectives denote different absolute intensities depending on the domain in which they are applied. A “strong” burn from grain alcohol would reflect a greater perceived intensity than a “strong” flavor of oak in a chardonnay. Intensity adjectives also have different meaning based on the subject’s experience with that domain. The oral sensory world of an individual who tastes PROP as intensely strong is much different than that of a nontaster. A “strong” bitter refers to a greater absolute intensity to the individual who tastes PROP as intensely bitter than to a person for which PROP is tasteless. Assuming that the adjectives applied to oral sensation mean the same to these two individuals can diminish the PROP effects, hide the PROP effects completely, or even produce effects that are in the wrong direction (Bartoshuk et al., 2003).

We attempted to solve the problems with existing labeled scales (Bartoshuk et al., 2003). In our studies, we also ask subjects to judge a nonoral standard as well as to judge oral sensations such as alcohol (e.g., Duffy et al., 2003). The nonoral standard can be used to normalize the data and ensure that the subjects are using the adjective scales consistently. Continued study of genetic variation in taste will clarify associations between PROP tasting and alcohol sensations. This should contribute to our understanding of the association between oral sensations and ingestion of alcohol.

REFERENCES

Abate P, Pepino MY, Dominguez HD, Spear NE, Molina JC. Fetal associative learning mediated through maternal alcohol administration. Alcohol Clin Exp Res 2000;24:39–47. [PubMed: 10656191]

Abate P, Spear NE, Molina JC. Fetal and infantile alcohol-mediated associative learning in the rat. Alcohol Clin Exp Res 2001;25:989–998. [PubMed: 11505023]

Abate P, Varlinskaya EI, Cheslock SJ, Spear NE, Molina JC. Neonatal activation of alcohol-related prenatal memories: impact upon the first suckling response. Alcohol Clin Exp Res. 2003 in press.

Adler E, Hoon MA, Mueller KL, Chandrashekar J, Ryba NJP, Zuker CS. A novel family of mammalian taste receptors. Cell 2000;100:693–702. [PubMed: 10761934]

Bachmanov AA, Beauchamp GK, Tordoff MG. Voluntary consumption of NaCl, KCl, $CaCl_2$ and NH_4Cl solutions by 28 mouse strains. Behav Genet 2002a;32:445–457. [PubMed: 12467342]

Bachmanov AA, Li X, Reed DR, Li S, Chen Z, de Jong PJ, Wu C, West DB, Chatterjee A, Ross DA, Ohmen JD, Beauchamp GK, Tordoff MG. *Sac* (saccharin preference) locus: positional cloning and effects on ethanol consumption (abstract). Alcohol Clin Exp Res 2001;25(a):118A.

Bachmanov AA, Li X, Reed DR, Ohmen JD, Li S, Chen Z, Tordoff MG, de Jong PJ, Wu C, West DB, Chatterjee A, Ross DA, Beauchamp GK. Positional cloning of the mouse saccharin preference (*Sac*) locus. Chem Senses 2001;26(b):925–933. [PubMed: 11555487]

Bachmanov AA, Reed DR, Li X, Li S, Beauchamp GK, Tordoff MG. Voluntary ethanol consumption by mice: genome-wide analysis of quantitative trait loci and their interactions in a C57BL/6ByJ × 129P3/J F2 intercross. Genome Res 2002;12(b):1257–1268. [PubMed: 12176933]

Bachmanov AA, Reed DR, Ninomiya Y, Inoue M, Tordoff MG, Price RA, Beauchamp GK. Sucrose consumption in mice: major influence of two genetic loci affecting peripheral sensory responses. Mamm Genome 1997;8:545–548. [PubMed: 9250857]

Bachmanov AA, Reed DR, Tordoff MG, Price RA, Beauchamp GK. Intake of ethanol, sodium chloride, sucrose, citric acid, and quinine hydrochloride solutions by mice: a genetic analysis. Behav Genet 1996;26(a):563–573. [PubMed: 8990535]

Bachmanov AA, Reed DR, Tordoff MG, Price RA, Beauchamp GK. Nutrient preference and diet-induced adiposity in C57BL/6ByJ and 129P3/J mice. Physiol Behav 2001;72(c):603–613. [PubMed: 11282146]

Bachmanov AA, Tordoff MG, Beauchamp GK. Ethanol consumption and taste preferences in C57BL/6ByJ and 129/J mice. Alcohol Clin Exp Res 1996;20(b):201–206. [PubMed: 8730208]

Bachmanov AA, Tordoff MG, Beauchamp GK. Sweetener preference of C57BL/6ByJ and 129P3/J mice. Chem Senses 2001;26(d):905–913. [PubMed: 11555485]

Badia-Elder NE, Kiefer SW. Taste reactivity in alcohol-preferring AA and alcohol-avoiding ANA rats. Alcohol 1999;18:159–163. [PubMed: 10456567]

Bartoshuk L. Comparing sensory experiences across individuals: recent psychophysical advances illuminate genetic variation in taste perception. Chem Senses 2000;25:447–460. [PubMed: 10944509]

Bartoshuk LM, Conner E, Grubin D, Karrer T, Kochenbach K, Palsco M, Snow D, Pelchat M, Danovski S. PROP supertasters and the perception of ethyl alcohol. Chem Senses 1993;18:526–527.

Bartoshuk LM, Duffy VB, Fast K, Green BG, Snyder DJ. Labeled scales (e.g., category, Likert, VAS) and invalid across-group comparisons: what we have learned from genetic variation in taste. J Food Quality Preference. 2003 in press.

Bartoshuk LM, Duffy VB, Miller IJ. PTC/PROP tasting: anatomy, psychophysics, and sex effects. Physiol Behav 1994;56:1165–1171. [PubMed: 7878086]

Bartoshuk LM, Rifkin B, Marks L, Bars P. Taste and aging. J Gerontol 1986;41:51–57. [PubMed: 3941256]

Beauchamp GK, Fisher AS. Strain differences in consumption of saline solutions by mice. Physiol Behav 1993;54:179–184. [PubMed: 8327600]

Belknap JK, Belknap ND, Berg JH, Coleman R. Preabsorbtive vs. postabsorbtive control of ethanol intake in C57BL/6J and DBA/2J mice. Behav Genet 1977;7:413–425. [PubMed: 603475]

Belknap JK, Crabbe JC, Young ER. Voluntary consumption of alcohol in 15 inbred mouse strains. Psychopharmacology 1993;112:503–510. [PubMed: 7871064]

Berridge KC, Grill HJ. Isohedonic tastes support a two-dimensional hypothesis of palatability. Appetite 1984;5:221–231. [PubMed: 6524918]

Bice PJ, Kiefer SW. Taste reactivity in alcohol preferring and nonpreferring rats. Alcohol Clin Exp Res 1990;14:721–727. [PubMed: 2264601]

Blizard DA, Kotlus B, Frank ME. Quantitative trait loci associated with short-term intake of sucrose, saccharin and quinine solutions in laboratory mice. Chem Senses 1999;24:373–385. [PubMed: 10480673]

Blizard DA, McClearn GE. Association between ethanol and sucrose intake in the laboratory mouse: exploration via congenic strains and conditioned taste aversion. Alcohol Clin Exp Res 2000;24:253–258. [PubMed: 10776660]

Buck L, Axel R. A novel multigene family may encode odorant receptors: a molecular basis for odor recognition. Cell 1991;65:175–187. [PubMed: 1840504]

Cahill L, Babinsky R, Markowitsch HJ, McGaugh JL. The amygdala and emotional memory. Nature (Lond.) 1995;377:295–296. [PubMed: 7566084]

Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D. The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature (Lond.) 1997;389:816–824. [PubMed: 9349813]

Choonara Y. Percutaneous drug absorption and administration. Arch Dis Childhood 1994;71:F73–F74. [PubMed: 7979481]

Chotro MG, Kraebel KS, McKinzie DL, Molina JC, Spear N. Prenatal and postnatal ethanol exposure influences preweanling rats' behavioral and autonomic responding to ethanol odor. Alcohol 1996;13:377–385. [PubMed: 8836327]

Coonfield DL, Hill KG, Ferraro FM III, Kiefer SW, Sinclair JD. Naltrexone alters alcohol consumption but not palatability in alcohol preferring (AA) rats (abstract). Alcohol Clin Exp Res 2001;25:19.A.

Coonfield DL, Hill KG, Kaczmarek HJ, Ferraro FM III, Kiefer SW. Low doses of naltrexone reduce palatability and consumption of ethanol in outbred rats. Alcohol 2002;26:43–47. [PubMed: 11958946]

Di Lorenzo PM, Kiefer SW, Rice AG, Garcia J. Neural and behavioral responsivity to ethyl alcohol as a tastant. Alcohol 1986;3:55–61. [PubMed: 3964438]

DiCarlo S, Powers A. Propylthiouracil tasting as a possible genetic association marker for two types of alcoholism. Physiol Behav 1998;64:147–152. [PubMed: 9662078]

Domínguez HD, López MF, Chotro MG, Molina JC. Perinatal responsiveness to alcohol's chemosensory cues as a function of prenatal alcohol administration during gestational days 17-20 in the rat. Neurobiol Learn Mem 1996;65:103–112. [PubMed: 8833099]

Domínguez HD, López MF, Molina JC. Neonatal responsiveness to alcohol odor and infant alcohol intake as a function of alcohol experience during late gestation. Alcohol 1998;16:109–117. [PubMed: 9665312]

Domínguez HD, López MF, Molina JC. Interactions between perinatal and neonatal associative learning defined by contiguous olfactory and tactile stimulation. Neurobiol Learn Mem 1999;71:272–288. [PubMed: 10196106]

Duffy VB, Peterson JM, Bartoshuk LM. Bitterness of 6-n-propylthiouracil (PROP) associates with alcohol sensation and intake. Physiol Behav. 2003 in press.

Ferraro FM, Hill KG, Kaczmarek HJ, Coonfield DL, Kiefer SW. Naltrexone modifies the palatability of basic tastes and alcohol in outbred male rats. Alcohol 2002;27:107–114. [PubMed: 12106830]

Fox AL. Six in ten "tasteblind" to bitter chemical. Sci New Lett 1931;9:249.

Freed EX. Alcohol polydipsia in the rat as a function of caloric need. Quart J Stud Alcohol 1972;33:504–507. [PubMed: 5064204]

Garcia J, Hankins WG, Rusiniak KW. Behavioral regulation of the milieu interne in man and rat. Science (Wash. DC) 1974;185:824–831.

Gentry RT, Dole VP. Why does a sucrose choice reduce the consumption of alcohol in C57BL/6J mice? Life Sci 1987;40:2191–2194. [PubMed: 3586856]

George SR, Roldan L, Lui A, Naranjo CA. Endogenous opioids are involved in the genetically determined high preference for ethanol consumption. Alcohol Clin Exp Res 1991;15:668–672. [PubMed: 1656811]

Gosnell BA, Majchrzak MJ. Centrally administered opioid peptides stimulate saccharin intake in nondeprived rats. Pharmacol Biochem Behav 1989;33:805–810. [PubMed: 2616599]

Green BG. The sensitivity of the tongue to ethanol. Ann NY Acad Sci 1987;510:315–317.

Grill HJ, Norgren R. The taste reactivity test. I. Mimetic responses to gustatory stimuli in neurologically normal rats. Brain Res 1978;143:263–279. [PubMed: 630409]

Grupp LA, Perlanski E, Stewart RB. Regulation of alcohol consumption by the renin-angiotensin system: a review of recent findings and a possible mechanism of action. Neurosci Biobehav Rev 1991;15:265–275. [PubMed: 1852316]

Guinard J-X, Zoumas-Morse C, Dietz J, Goldberg S, Holz M, Heck E, Amoros A. Does consumption of beer, alcohol, and bitter substances affect bitterness perception? Physiol Behav 1996;59:625–631. [PubMed: 8778845]

Hellekant G, Danilova V, Roberts T, Ninomiya Y. The taste of ethanol in a primate model: I. Chorda tympani nerve response in Macaca mulatta. Alcohol 1997;14:473–484. [PubMed: 9305463]

Hill KG, Kiefer SW. Naltrexone treatment increases the aversiveness of alcohol for outbred rats. Alcohol Clin Exp Res 1997;21:637–641. [PubMed: 9194917]

Hubell CL, Marglin SH, Spitalnic SJ, Abelson ML, Wild KD, Reid LD. Opioidergic, serotoninergic, and dopaminergic manipulations and rats' intake of a sweetened alcoholic beverage. Alcohol 1991;8:355–367. [PubMed: 1797032]

Hunt PS, Kraebel KS, Rabine H, Spear SP, Spear NE. Enhanced ethanol intake in preweanling rats following exposure to ethanol in a nursing context. Develop Psychobiol 1993;26:133–153.

Intranuovo LR, Powers AS. The perceived bitterness of beer and 6-n-propylthiouracil (PROP) taste sensitivity. Ann NY Acad Sci 1998;855:813–815. [PubMed: 9929691]

Kampov-Polevoy AB, Garbutt JC, Davis CE, Janowsky DS. Preference for higher sugar concentrations and Tridimensional Personality Questionnaire scores in alcoholic and nonalcoholic men. Alcohol Clin Exp Res 1998;22:610–614. [PubMed: 9622439]

Kampov-Polevoy AB, Garbutt JC, Janowsky D. Evidence of preference for a higher concentration sucrose solution in alcoholic men. Am J Psychiatry 1997;154:269–270. [PubMed: 9016281]

Kampov-Polevoy AB, Garbutt JC, Janowsky DS. Association between preference for sweets and excessive alcohol intake: a review of animal and human studies. Alcohol Alcohol 1999;34:386–395. [PubMed: 10414615]

Kiefer SW. Alcohol, palatability, and taste reactivity. Neurosci Biobehav Rev 1995;19:133–141. [PubMed: 7770193]

Kiefer SW, Badia-Elder N, Bice PJ. Taste reactivity in high alcohol drinking and low alcohol drinking rats. Alcohol Clin Exp Res 1995;19:279–284. [PubMed: 7625558]

Kiefer SW, Dopp JM. Taste reactivity to alcohol in rats. Behav Neurosci 1989;103:1318–1326. [PubMed: 2610924]

Kiefer SW, Lawrence GJ. The sweet-bitter taste of alcohol: aversion generalization to various sweet-quinine mixtures in the rat. Chem Senses 1988;13:633–641.

Kiefer SW, Mahadevan RS. The taste of alcohol for rats as revealed by aversion generalization tests. Chem Senses 1993;18:509–522.

Kiefer SW, Morrow NS. Odor cue mediation of alcohol aversion learning in rats lacking gustatory neocortex. Behav Neurosci 1991;105:25–32. [PubMed: 1851018]

Kitagawa M, Kusakabe Y, Miura H, Ninomiya Y, Hino A. Molecular genetic identification of a candidate receptor gene for sweet taste. Biochem Biophys Res Commun 2001;283:236–242. [PubMed: 11322794]

Kranzler H, Moore P, Hesselbrock V. No association of PROP taster status and parental history of alcohol dependence. Alcohol Clin Exp Res 1996;20:1495–1500.

Kranzler H, Skipsey K, Modesto-Lowe V. PROP taster status and parental history of alcohol dependence. Drug Alcohol Depend 1998;52:109–113. [PubMed: 9800140]

Lawrence GJ, Kiefer SW. Generalization of specific taste aversions to alcohol in the rat. Chem Senses 1987;12:591–599.

Li X, Inoue M, Reed DR, Huque T, Puchalski RB, Tordoff MG, Ninomiya Y, Beauchamp GK, Bachmanov AA. High-resolution genetic mapping of the saccharin preference locus (*Sac*) and the putative sweet taste receptor (T1R1) gene (*Gpr70*) to mouse distal Chromosome 4. Mamm Genome 2001;12:13–16. [PubMed: 11178737]

Li X, Staszewski L, Xu H, Durick K, Zoller M, Adler E. Human receptors for sweet and umami taste. Proc Natl Acad Sci USA 2002;99:4692–4696. [PubMed: 11917125]

Lush IE, Hornigold N, King P, Stoye JP. The genetics of tasting in mice. VII. Glycine revisited, and the chromosomal location of *Sac* and *Soa*. Genet Res 1995;66:167–174. [PubMed: 8522158]

Matsunami H, Montmayeur JP, Buck LB. A family of candidate taste receptors in human and mouse. Nature (Lond.) 2000;404:601–604. [PubMed: 10766242]

Mattes RD, DiMeglio D. Ethanol perception and ingestion. Physiol Behav 2001;72:217–229. [PubMed: 11240000]

Max M, Shanker YG, Huang L, Rong M, Liu Z, Campagne F, Weinstein H, Damak S, Margolskee RF. *Tas1r3*, encoding a new candidate taste receptor, is allelic to the sweet responsiveness locus Sac. Nat Genet 2001;28:58–63. [PubMed: 11326277]

McMillen BA, Williams HL. Role of taste and calories in the selection of ethanol by C57BL/6NHsd and Hsd:ICR mice. Alcohol 1998;15:193–198. [PubMed: 9539375]

Mennella JA. The human infants' suckling responses to the flavor of alcohol in mother's milk. Alcohol Clin Exp Res 1997;21:581–585. [PubMed: 9194908]

Mennella, JA. The transfer of alcohol to human milk: sensory implications and effects on mother-infant interaction. In: Spear, NE.; Spear, LP.; Hanningan, JH.; Goodlett, CR., editors. Alcohol and Alcoholism: Brain and Development. Erlbaum; Hillsdale, NJ: 1999. p. 177-198.

Mennella JA. Alcohol's effect on lactation. Alcohol Res Health 2001;25:230–234. [PubMed: 11810962]

Mennella, JA. Alcohol use during lactation: The folklore versus the science. In: Auerbach, KG., editor. Current Issues in Clinical Lactation. Jones and Bartlett; Sudbury, MA: 2002. p. 3-9.

Mennella JA, Beauchamp GK. The transfer of alcohol to human milk: effects on flavor and the infant's behavior. N Engl J Med 1991;325:981–985. [PubMed: 1886634]

Mennella JA, Beauchamp GK. Early flavor experiences: research update. Nutr Rev 1998;56(a):205–211. [PubMed: 9697386]

Mennella JA, Beauchamp GK. The infant's response to scented toys: effects of exposure. Chem Senses 1998;23(b):11–17. [PubMed: 9530965]

Mennella JA, Garcia PJ. The child's hedonic response to the smell of alcohol: effects of parental drinking habits. Alcohol Clin Exp Res 2000;24:1167–1171. [PubMed: 10968653]

Miller IJ, Reedy FE. Variations in human taste bud density and taste intensity perception. Physiol Behav 1990;47:1213–1219. [PubMed: 2395927]

Molina, JC.; Chotro, MG.; Domínguez, HD. Fetal alcohol learning derived from ethanol contamination of the prenatal environment. In: Lecanuet, JP.; Fifer, WP.; Krasnegor, NA.; Smotherman, WP., editors. Fetal Development: A Psychobiological Perspective. Erlbaum; Hillsdale, NJ: 1995. p. 419-438.

Molina, JC.; Domínguez, HD.; López, MF.; Pepino, MY.; Faas, AE. The role of fetal and infantile experience with alcohol in later recognition and acceptance patterns of the drug. In: Spear, NE.; Spear, LP.; Hanningan, JH.; Goodlett, CR., editors. Alcohol and Alcoholism: Brain and Development. Erlbaum; Hillsdale, NJ: 1999. p. 199-228.

Molina JC, Pepino MY, Johnson J, Spear NE. The infant rat learns about alcohol through interaction with an intoxicated mother. Alcohol Clin Exp Res 2000;24:428–437. [PubMed: 10798578]

Montmayeur JP, Liberles SD, Matsunami H, Buck LB. A candidate taste receptor gene near a sweet taste locus. Nat Neurosci 2001;4:492–498. [PubMed: 11319557]

Nachman M, Larue C, Le Magnen J. The role of olfactory and orosensory factors in the alcohol preference of inbred strains of mice. Physiol Behav 1971;6:53–95. [PubMed: 5125457]

Nelson G, Chandrashekar J, Hoon MA, Feng L, Zhao G, Ryba NJ, Zuker CS. An amino-acid taste receptor. Nature (Lond.) 2002;416:199–202. [PubMed: 11894099]

Nelson G, Hoon MA, Chandrashekar J, Zhang Y, Ryba NJ, Zuker CS. Mammalian sweet taste receptors. Cell 2001;106:381–390. [PubMed: 11509186]

Noll RB, Zucker RA, Greenberg GS. Identification of alcohol by smell among preschoolers: evidence for early socialization about drugs in the home. Child Dev 1990;61:1520–1527. [PubMed: 2245743]

Nurnberger JI Jr, Foroud T, Flury L, Su J, Meyer ET, Hu K, Crowe R, Edenberg H, Goate A, Bierut L, Reich T, Schuckit M, Reich W. Evidence for a locus on chromosome 1 that influences vulnerability to alcoholism and affective disorder. Am J Psychiatry 2001;158:718–724. [PubMed: 11329392]

Overstreet DH, Kampov-Polevoy AB, Rezvani AH, Murelle L, Halikas JA, Janowsky DS. Saccharin intake predicts ethanol intake in genetically heterogeneous rats as well as different rat strains. Alcohol Clin Exp Res 1993;17:366–369. [PubMed: 8488981]

Overstreet DH, Rezvani AH, Parsian A. Behavioural features of alcohol-preferring rats: focus on inbred strains. Alcohol Alcohol 1999;34:378–385. [PubMed: 10414614]

Pelchat ML, Danowski S. A possible genetic association between PROP-tasting and alcoholism. Physiol Behav 1992;51:1261–1266. [PubMed: 1641428]

Pepino MY, Abate P, Spear NE, Molina JC. Infant rats breastfed by alcohol-intoxicated dams: impact upon preweanling and periadolescent patterns of alcohol consumption. Alcohol Clin Exp Res (Suppl) 2000;24:15.A.

Pepino MY, Kraebel KS, López MF, Spear NE, Molina JC. Behavioral detection of low concentrations of ethanol in the preweanling rat. Alcohol 1998;15:337–353. [PubMed: 9590520]

Pepino MY, Lopez MF, Spear NE, Molina JC. Infant rats respond differentially to alcohol after nursing from an alcohol intoxicated dam. Alcohol 1999;18:189–201. [PubMed: 10456571]

Pepino MY, Spear NE, Molina JC. Nursing experiences with an alcohol-intoxicated rat dam counteract appetitive conditioned responses toward alcohol. Alcohol Clin Exp Res 2001;25:18–24. [PubMed: 11198710]

Phillips TJ, Crabbe JC, Metten P, Belknap JK. Localization of genes affecting alcohol drinking in mice. Alcohol Clin Exp Res 1994;18:931–941. [PubMed: 7978106]

Prescott J, Swain-Campbell N. Responses to repeated oral irritation by capsaicin, cinnamaldehyde and ethanol in PROP tasters and non-tasters. Chem Senses 2000;25:239–246. [PubMed: 10866983]

Prutkin J, Duffy V, Etter L, Fast K, Lucchina L, Snyder D, Tie K, Bartoshuk L. Genetic variation and inferences about perceived taste intensity in mice and men. Physiol Behav 2000;61:161–173. [PubMed: 10854927]

Pucilowski O, Rezvani AH, Janowsky DS. Suppression of alcohol and saccharin preference in rats by a novel Ca^{2+} channel inhibitor, Goe 5438. Psychopharmacology 1992;107:447–452. [PubMed: 1615142]

Reed DR, Li X, Chen Z, Mascioli K, Bachmanov AA, Beauchamp GK, Tordoff MG, Max M, Margolskee R, Bartoshuk LM, Duffy V. Alleles of the taste receptor gene *TAS1R3* may influence the pleasantness of sucrose and aspartame in human subjects (abstract). Obes Res 2001;10:67.

Richter CP. Alcohol as a food. Quart J Stud Alcohol 1941;1:650–662.

Robinson, SR.; Smotherman, WP. Habituation and classical conditioning in the rat fetus: opioid involvements. In: Lecanuet, JP.; Fifer, WP.; Krasnegor, NA.; Smotherman, WP., editors. Fetal Development: A Psychobiological Perspective. Erlbaum; Hillsdale, NJ: 1995. p. 315-330.

Rodgers DA. Factors underlying differences in alcohol preference among inbred strains of mice. Psychosom Med 1966;28:498–513.

Rodgers DA, McClearn GE, Bennett EL, Hebert M. Alcohol preference as a function of its caloric utility in mice. J Comp Physiol Psychol 1963;56:666–672. [PubMed: 13974620]

Ronca AE, Alberts JR. Sensory stimuli associated with gestation and parturation evoke cardiac and behavioral responses in fetal rats. Psychobiology 1994;22:270–282.

Sainz E, Korley JN, Battey JF, Sullivan SL. Identification of a novel member of the T1R family of putative taste receptors. J Neurochem 2001;77:896–903. [PubMed: 11331418]

Sako N, Yamamoto T. Electrophysiological and behavioral studies on taste effectiveness of alcohols in rats. Am J Physiol 1999;276:R388–R396. [PubMed: 9950916]

Spear, NE.; Molina, JC. Consequences of early exposure to alcohol: how animal studies reveal later patterns of use and abuse in humans. In: Carroll, ME.; Overmier, JB., editors. Animal Research and Human Health. American Psychological Association; Washington, DC: 2001. p. 85-99.

Stewart RB, Russell RN, Lumeng L, Li T-K, Murphy JM. Consumptions of sweet, salty, sour, and bitter solutions by selectively bred alcohol-preferring and alcohol-nonpreferring lines of rats. Alcohol Clin Exp Res 1994;18:375–381. [PubMed: 8048741]

Tepper B, Nurse R. Fat perception is related to PROP taster status. Physiol Behav 1997;61:949–954. [PubMed: 9177570]

Trevisani M, Smart D, Gunthorpe MJ, Tognetto M, Barbieri M, Campi B, Amadesi S, Gray J, Jerman JC, Brough SJ, Owen D, Smith GD, Randall AD, Harrison S, Bianchi A, Davis JB, Geppetti P. Ethanol elicits and potentiates nociceptor responses via the vanilloid receptor-1. Nat Neurosci 2002;5:546–551. [PubMed: 11992116]

Whitehead MC, Beeman CS, Kinsella BA. Distribution of taste and general sensory nerve endings in fungiform papillae of the hamster. Am J Anat 1985;173:185–201.

Wilson DA, Sullivan RM. Neurobiology of associative learning in the neonate: early olfactory learning. Behav Neur Biol 1994;61:1–18.