Alcohol Activates a Sucrose-Responsive Gustatory Neural Pathway

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Submitted 2 February 2004; accepted in final form 18 February 2004

Lemon, Christopher H., Susan M. Brasser, David V. Smith. Alcohol activates a sucrose-responsive gustatory neural pathway. J Neurophysiol 92: 536–544, 2004. First published February 25, 2004; 10.1152/jn.00097.2004. A strong positive association exists between the ingestion of alcohol and sweet-tasting solutions. The neural mechanisms underlying this relationship are unknown, although recent data suggest that gustatory substrates are involved. Here, we examined the role of sweet taste receptors and central neural circuits for sugar taste in the gustatory processing of ethanol. Taste responses to ethanol (3, 5, 10, 15, 25, and 40% vol/vol) and stimuli of different taste qualities (e.g., sucrose, NaCl, HCl, and quinine-HCl) were recorded from neurons of the nucleus of the solitary tract in anesthetized rats prior to and after oral application of the sweet receptor blocker gurmarin. The magnitude of ethanol-evoked activity was compared between sucrose-responsive (n = 21) and sucrose-unresponsive (n = 20) neurons and the central neural representation of ethanol taste was explored using multivariate analysis. Ethanol produced robust concentration-dependent responses in sucrose-responsive neurons that were dramatically larger than those in sucrose-unresponsive cells. Gurmarin selectively and similarly inhibited ethanol and sucrose responses, leaving NaCl, HCl, and quinine responses unaltered. Across-neuron patterns of response to ethanol were most similar to those evoked by sucrose, becoming increasingly more so as the ethanol concentration was raised. Results implicate taste receptors for sucrose as candidate receptors for ethanol and reveal that alcohol and sugar taste are represented similarly by gustatory activity in the CNS. These findings have important implications for the sensory and reward properties of alcohol.

INTRODUCTION

There is considerable interest in understanding how drugs of abuse interact with physiological substrates that mediate adaptive behaviors to natural reinforcers. The rationale behind this focus is that the nervous system did not evolve mechanisms to facilitate drug-taking behavior but rather that drugs of abuse co-opt pathways designed to promote appropriate responding to natural rewards (e.g., food and sex) that are beneficial to survival (Kelly and Berridge 2002). In the case of alcohol, it has been demonstrated that alcohol consumption both activates and is regulated by neural systems involved in mediating responses to naturally reinforcing ingestive stimuli (Reid 1996; Thiele et al. 1998; Zhang and Kelley 2002).

One of the most salient and consistent predictors of alcohol intake observed in animals is the consumption of sweet-tasting solutions.1 Ethanol-prefering C57BL mice (Fuller 1974; Lush 1989) and selectively bred lines of alcohol-prefering rats (Sinclair et al. 1992; Stewart et al. 1994; Woods et al. 2003) display greater intake of sweeteners (e.g., sucrose and saccharin) than their nonethanol-prefering counterparts. Direct positive correlations between alcohol and sweetener consumption are also observed in randomly bred rats (Kampov-Polevoy et al. 1990), inbred mice (Belknap et al. 1993) and the F2 progeny of ethanol-prefering and -avoiding lines/strains (Bachmanov et al. 1996; Overstreet et al. 1993). Further, human alcoholics prefer more highly concentrated sucrose solutions than nonalcoholic control subjects (Kampov-Polevoy et al. 1997), and abstinent alcoholics may substitute ingestion of sweets for alcohol (Yung et al. 1983).

Covariation in preference for alcohol and sweet-tasting substances suggests that common neural substrates are involved in mediating their ingestion. Behavioral and physiological evidence has implicated gustatory processes in this relationship, although precise mechanisms are not understood. Conditioned taste aversions generalize between ethanol and sucrose in C57BL mice (Blizard and McClearn 2000) and ethanol and sucrose mixtures in randomly bred rats (Di Lorenzo et al. 1986; Kiefer and Mahadevan 1993), suggesting that ethanol possesses a sweet taste component. Despite evidence for a behavioral association between ethanol and sweet taste in rodents, there are few electrophysiological data on the gustatory effects of ethanol in these species. Although peripheral gustatory nerve recordings in primates indicate that orally applied ethanol stimulates sucrose-sensitive fibers (Hellekant et al. 1997), only whole-nerve recordings have been done in rats, showing little sensitivity to ethanol (Hellekant 1965; Sako and Yamamoto 1999). Further, understanding the role of taste in ethanol-mediated behavior necessitates knowledge of the gustatory processing of ethanol in the CNS. Data from one study pertain to this issue, showing only a weak correlation between ethanol and sucrose responses in central gustatory neurons (Di Lorenzo et al. 1986). However, this study examined only two ethanol concentrations (6 and 9%), and only a few cells in this sample were strongly responsive to sucrose. Finally, there are no data currently available on the gustatory receptor mechanisms for ethanol and their relationship to the central processing of ethanol taste.

Genetic mapping has recently revealed a chromosomal locus contributing to both alcohol intake and saccharin preference (Bachmanov et al. 2002) that contains the gene for a newly identified taste receptor, T1R3 (Max et al. 2001; Montmayeur et al. 2001; Nelson et al. 2001). T1R3 is critically involved with sweet taste reception (Damak et al. 2003; Max et al. 2001; Montmayeur et al. 2001; Nelson et al. 2001; Zhao et al. 2003). Here, we directly investigated the involvement of gustatory receptors and central neural circuits for sugar taste in the...
gustatory processing of ethanol. Taste responses to an ethanol concentration series and stimuli of different taste qualities were recorded from neurons of the nucleus of the solitary tract (NST) in anesthetized rats prior to and after oral application of the sweet receptor blocker gurmarin (Imoto et al. 1991). Data were evaluated with respect to the neural representation of ethanol taste information.

METHODOLOGY

Animals and preparation

Twenty-six adult male Sprague Dawley rats, weighing 350–450 g, were used. Rats were housed in a vivarium that maintained a 12-h light/dark cycle and an ambient temperature of ∼23°C. Food and water were available ad libitum. Animals were deeply anesthetized with urethan (1.5 g/kg ip) and prepared for electrophysiological recording. Each rat was tracheotomized and secured in a nontraumatic head holder that deflected its snout ∼27° downward; this configuration served to minimize brain stem movements associated with breathing. The occipital bone was removed and parts of the cerebellum were gently aspirated to expose the brain stem and allow vertical access to the NST, the first central synapse for taste information processing. Body temperature was maintained at ∼37°C by a heating pad.

Single-neuron electrophysiology

Etched tungsten microelectrodes, insulated except for the tip (impedance = 1–8 MΩ at 1 kHz, FHC, Bowdoinham, ME), were used to record extracellular action potentials from individual NST neurons. For each preparation, the area of the brain stem where the rostral pole of the solitary tract resided was visually located using vascular landmarks present on the dorsal surface of the exposed tissue. A hydraulic micromanipulator was then used to slowly advance the microelectrode through the brain stem. The portion of the NST that contained neurons responsive to lingual stimulation was initially identified by a change in neural activity associated with the passage of anodal current (10 μA/500 ms) across the anterior tongue; cells were then verified as taste-driven by application of various gustatory stimuli (see following text). The gustatory-responsive portion of the NST was encountered ∼1 mm ventral to the brain stem surface in each preparation.

Electrophysiological activity was band-pass filtered (bandwidth = 0.3–6 kHz), differentially amplified (Grass P511 with high-impedance probe) and subsequently routed to various monitors and analytic devices. Spikes that arose from single neurons were identified based on waveform consistency, which was continuously observed throughout each recording session using a storage oscilloscope and, after analog-to-digital conversion (sampling rate = 25 kHz), a spike waveform template-matching algorithm (Power 1401 RISC acquisition interface coupled with Spike 2 software, CED, Cambridge, UK). Trains of action potentials were pulse-code modulated and stored, along with voice and trial marker cues, on VHS tape. Digital records of aggregate electrophysiological activity, including template-matched spikes, were downloaded to storage media for off-line quantitative analysis.

Taste stimuli

All neurons were tested with prototypical taste stimuli categorized by humans as sweet, salty, sour, or bitter (referred to as the standard stimuli; Table 1) and an ascending ethanol concentration series. A random subset of cells was tested further using an extended stimulus array (Table 1) for the purpose of multivariate analysis of the neural representation of ethanol taste. All stimuli were dissolved in deionized water. Tastants were made from reagent grade stock (Sigma, St. Louis, MO). Ethanol solutions (3, 5, 10, 15, 25, and 40% vol/vol) were made from a 95% stock (Pharmco Products, Brookfield, CT).

### TABLE 1. Taste stimuli and concentrations

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>Concentration, M</th>
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<tbody>
<tr>
<td><strong>Standard taste stimuli</strong></td>
<td></td>
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<tr>
<td>Sucrose</td>
<td>0.5</td>
</tr>
<tr>
<td>Sodium chloride (NaCl)</td>
<td>0.1</td>
</tr>
<tr>
<td>Hydrochloric acid (HCl)</td>
<td>0.01</td>
</tr>
<tr>
<td>Quinine hydrochloride</td>
<td>0.01</td>
</tr>
<tr>
<td><strong>Extended stimulus array</strong></td>
<td></td>
</tr>
<tr>
<td>Sucrose</td>
<td>0.1</td>
</tr>
<tr>
<td>Sucrose</td>
<td>1.0</td>
</tr>
<tr>
<td>D-fructose</td>
<td>1.0</td>
</tr>
<tr>
<td>D-glucose</td>
<td>1.0</td>
</tr>
<tr>
<td>Sodium nitrate (NaNO₃)</td>
<td>0.1</td>
</tr>
<tr>
<td>Citric acid (H-citric)</td>
<td>0.01</td>
</tr>
<tr>
<td>Quinine hydrochloride</td>
<td>0.0001</td>
</tr>
<tr>
<td>Nicotine</td>
<td>0.01</td>
</tr>
<tr>
<td>Potassium chloride (KCl)</td>
<td>0.2</td>
</tr>
<tr>
<td>Magnesium chloride (MgCl₂)</td>
<td>0.01</td>
</tr>
<tr>
<td>Sucrose + quinine hydrochloride</td>
<td>0.1 + 0.0001</td>
</tr>
</tbody>
</table>

Chemical abbreviation, if applicable, is denoted in parentheses.

Solutions were delivered at room temperature to the anterior tongue and palate via a gravity flow system at a rate of ∼2.5 ml/s. A three-way solenoid fluid valve, which was controlled by the acquisition system, regulated solution delivery. A curved, polyethylene tube extended from the outlet port of this valve and was directed toward the palate of each subject. Visual inspection revealed that this configuration allowed solutions to bathe both the palate and anterior tongue, as solutions were deflected downward on encountering the palate. Moreover, independent tests using methylene blue dye verified that our delivery system effectively bathed the entire anterior tongue and palate, including the nasoincisor ducts (NIDs). Studies have shown that gustatory input from the VIIth cranial nerve, which innervates the anterior tongue and palate, is critical for taste discrimination (Spector and Grill 1992; St. John and Spector 1998).

During data acquisition, stimuli were presented to each subject using the following protocol. The tongue and palate were first rinsed with deionized water for 10 s, followed immediately by a stimulus for 10 s. The tongue and palate were then rinsed with ≥50 ml of deionized water, and ≥2 min were allowed to elapse between trials. The stimulus delivery system was thoroughly rinsed with deionized water between stimulus presentations. Excluding the ascending ethanol concentration series, the stimulus presentation order was randomized for each data acquisition period. For each neuron, stimulus trials of interest were replicated as many times as possible during a single recording session.

Gurmarin testing

A random sample of neurons that exhibited strong responses to sucrose was tested with the sweet receptor blocker gurmarin (Imoto et al. 1991) to determine if ethanol and sucrose stimulate a common taste receptor. Gurmarin selectively antagonizes a subset of receptors for sweeteners (Ninomiya et al. 1997, 1999). The inhibitory effect of gurmarin lasts for several hours (Imoto et al. 1991) and is not easily reversible within the time frame of our experiments (Ninomiya et al. 1999). Therefore we chose a simple pre-/post-gurmarin design, with responses to NaCl, HCl, and quinine-HCl serving as controls for the viability of the neurons from which we recorded, as peripheral (Imoto et al. 1991; Ninomiya and Imoto 1995; Ninomiya et al. 1997) and central (Lemon et al. 2003) neural responses to these stimuli are not altered by gurmarin treatment.

After acquisition of control responses to ethanol and the standard stimuli, gurmarin (15 μg/ml, dissolved in deionized water; ∼3.6 μM) was applied to both the tongue and palate of each subject using a blunt-tipped syringe (2–4 ml total volume). This concentration max-
imally inhibits responses to 0.5 M sucrose in rat NST neurons, producing ~50% suppression on average relative to control (Lemon et al. 2003). This partial rather than complete reduction of responding to sucrose in NST neurons by gurmarin is attributable to the convergence of gurmarin-sensitive and -insensitive inputs onto these cells (Lemon et al. 2003). For each preparation, the application of gurmarin to the tongue and palate was visually verified. Moreover, independent tests using methylene blue dye verified that our gurmarin-application procedure adequately bathed both the anterior tongue and palate, including the NIDs. After gurmarin application, 10–15 min were allowed to elapse before proceeding with experimentation, which allowed the inhibitory effect of gurmarin to fully develop (Nimomiya et al. 1999). After this time, ethanol and the standard stimuli were presented as previously described and neural responses were recorded.

Data analysis

Neural responses to chemical stimuli were quantified as the number of action potentials that arose during stimulus presentation minus the number of action potentials that spontaneously occurred during the 10-s period prior to stimulus onset. Multiple responses to a given stimulus were averaged. The 10-s period prior to stimulus onset. Multiple responses to a given number of action potentials that spontaneously occurred during the 10-s period prior to stimulus onset. Multiple responses to a given number of action potentials that arose during stimulus presentation minus the number of action potentials that spontaneously occurred during the 10-s period prior to stimulus onset. Multiple responses to a given number of action potentials that arose during stimulus presentation minus the number of action potentials that spontaneously occurred during the 10-s period prior to stimulus onset.

Overall, individual cells were sensitive to multiple tastants including the NIDs. After gurmarin application, 10 min were used to determine whether the gurmarin application adequately bathed both the anterior tongue and palate, as well as the gurmarin-application procedure adequately bathed both the anterior tongue and palate, including the NIDs. After gurmarin application, 10 min were allowed to elapse before proceeding with experimentation, which allowed the inhibitory effect of gurmarin to fully develop (Nimomiya et al. 1999). After this time, ethanol and the standard stimuli were presented as previously described and neural responses were recorded. Data analysis

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A measure of response profile entropy (Smith and Travers 1979) was calculated for each neuron to quantify its breadth of responsiveness to the standard stimuli. Entropy is defined as

$$H = -K \sum_{i=1}^{n} p_i \log p_i$$

where $p_i$ represents the response to the $i$th stimulus expressed as a proportion of the total response to $n$ stimuli and $K$ is a scaling constant. For four stimuli, $K = 1.661$, which results in $H$ ranging from a minimum of 0 (i.e., neuron responds to only 1 stimulus) to a maximum of 1 (i.e., neuron responds equally to all stimuli).

Multidimensional scaling (MDS) was used to examine the neural representation of alcohol taste information in the CNS. This analysis placed taste stimuli into a hypothetical “space” based on the degree of similarity (measured, in this case, using the Pearson product-moment correlation) of their evoked responses across all neurons included in the analysis. Across-neuron patterns of activity underlie perceptual similarities and differences among taste stimuli (for reviews see Scott and Giza 2000; Smith and Scott 2003; Smith and St. John 1999); patterns generated by stimuli that taste similar correlate strongly, whereas those that taste different correlate poorly. Stimuli that generate similar across-neuron patterns of response are proximally situated in the stimulus space whereas those with dissimilar patterns are placed apart, with distance proportional to degree of dissimilarity along each dimension of the scaling space. MDS analysis was performed using the Guttagman-Lingoes model. The dimensionality of the final scaling solution was determined by a scree test, which plots stress, an index of lack of fit, against the number of dimensions.

RESULTS

Central neural responses to ethanol and standard tastants

Trains of action potentials were recorded from 41 taste-responsive neurons in the NST. All neurons significantly responded to 0.1 M NaCl, 38 responded to 0.01 M HCl, 25 responded to 0.01 M quinine-HCl, and 21 responded to 0.5 M sucrose. Regarding the breadth of responsiveness of individual neurons, 9 cells significantly responded to all four standard tastants, 26 responded to three, 5 responded to two, and 1 neuron responded to only one standard stimulus. Overall, individual cells were sensitive to multiple tastants ($H = 0.76 \pm 0.02 SE$), which is representative of neuronal gustatory response profiles observed in the rodent NST (see Smith and St. John 1999).

To examine the hypothesis that the central neural circuits underlying sucrose and ethanol taste overlap, we compared the magnitude of ethanol-evoked activity between neurons identified as significantly responsive or unresponsive to 0.5 M sucrose. Sucrose-responsive ($S_1$, $n = 21$) and sucrose-unresponsive ($S_0$, $n = 20$) neurons were differentially activated by the standard stimuli [group × stimulus interaction: $F(3,117) = 10.51, P < 0.001, 2 \times 4$ mixed ANOVA, Fig. 1A]. Responses to sucrose were greater in $S_1$ neurons, whereas responses to HCl and quinine-HCl were larger in $S_0$ cells (planned comparisons, $P$’s < 0.01). No difference ($\alpha = 0.05$) was detected for NaCl. Moreover, $S_1$ and $S_0$ neurons were found to differentially respond to ethanol [group × ethanol concentration interaction: $F(5,195) = 18.41, P < 0.001, 2 \times 6$ mixed ANOVA, Fig. 1B]. Ethanol responses observed in $S_1$ neurons were consistently and dramatically

![Fig. 1. Mean responses of sucrose-responsive ($S_1$) and sucrose-unresponsive ($S_0$) NST neurons to the standard taste stimuli and ethanol concentration series. A: mean ± SE responses to 0.5 M sucrose (S), 0.1 M NaCl (N), 0.01 M HCl (H), and 0.01 M quinine-HCl (Q) for both classes of neurons (*$P < 0.01$). B: average ± SE ethanol concentration response functions for $S_1$ and $S_0$ neurons ($*P < 0.02$).](http://jn.physiology.org/doi/abs/10.1152/jn.00275.2003)
greater than those in S0 cells for all concentrations except 3% (planned comparisons, P's ≤ 0.02). Moreover, ethanol activated S1 neurons in a concentration-dependent manner [simple effect of concentration: F(1,39) = 52.01, P < 0.001, Figs. 1B and 2]. Analysis of percentage of significant ethanol responses within each class also revealed that ethanol was an effective taste stimulus primarily for S1 neurons. Specifically, only 6% of responses to ethanol recorded from S0 cells (120 total responses) were significant versus 61% in S1 neurons (126 total responses). When considering only the strongest concentrations (15%, 25%, and 40%) of ethanol, 11% of these responses in S0 neurons exceeded criterion versus 91% in S1 neurons. All S1 cells significantly responded to 40% ethanol. Thus robust differences in taste responses to ethanol were observed between sucrose-responsive and -unresponsive neurons.

Linear regression was performed to determine the degree of correlation between responses evoked by each standard tastant and 40% ethanol, the concentration that produced the most salient responses. A strong and significant correlation between ethanol and sucrose responses emerged (r = +0.80, P < 0.001, Fig. 3), accounting for 64% of response variability. This result indicates that the across-neuron pattern of response to 40% ethanol is strongly correlated with that evoked by 0.5 M sucrose. A moderate correlation existed between responses to ethanol and NaCl (r = +0.41, P < 0.05), but this relationship explained only 17% of data variability. Responses to ethanol were uncorrelated (α = 0.05) with those to HCl (r = +0.04) or quinine (r = −0.04). Thus the response of central gustatory neurons to 0.5 M sucrose was a robust predictor of their sensitivity to 40% ethanol. The relationship between gustatory responses to ethanol and sucrose was even more pronounced using multiple regression to predict responses to 0.5 M sucrose from those evoked by all six concentrations of ethanol (multiple r = +0.89, P < 0.001).

**Effects of gurmarin on responses to ethanol and standard tastants**

The present finding that the gustatory signal for alcohol is conveyed primarily by central circuits that contribute prominently to sucrose taste suggests that ethanol and sucrose may activate overlapping taste receptor mechanisms. This hypothesis is consistent with data showing that ethanol stimulates sucrose-sensitive peripheral nerve fibers (Hellekant et al. 1997) and evidence from QTL analysis that the chromosomal region containing the gene for the T1R3 receptor strongly influences both ethanol and sweetener consumption (Bachmanov et al. 2002). To investigate whether ethanol and sucrose are acting on a common gustatory receptor, we recorded responses to the standard stimuli and ascending ethanol concentration series from a subset of S1 neurons (n = 9) prior to and after oral application of the sweet receptor blocker gurmarin. Across all neurons tested, gurmarin treatment suppressed ethanol responses to 50% of control values [main effect of treatment: F(1,8) = 15.53, P = 0.004, 2 × 6 repeated-measures ANOVA, Fig. 4A] and halved the magnitude of the ethanol concentration-response function (ratio of area under curves, area\textsubscript{postgurmarin}/area\textsubscript{control} = 0.51, Fig. 4B). These results parallel recent data showing that gurmarin inhibits responses to sucrose by ~50% and halves the magnitude of the sucrose-concentration response function in NST neurons that are strongly activated by sucrose (ratio of area under curves, area\textsubscript{postgurmarin}/area\textsubscript{control} = 0.53, Fig. 4C) (Lemon et al. 2002).
2003). The inhibition of sucrose responses by gurmarin is partial rather than complete as NST neurons receive convergent input from gurmarin-sensitive and -insensitive receptor mechanisms (Lemon et al. 2003). The effect of gurmarin on ethanol responses was differential across ethanol concentration interaction: $F(5,40) = 19.77, P < 0.001$, with responses to 15, 25, and 40% ethanol significantly inhibited after gurmarin treatment (planned comparisons, $P$'s $\leq 0.01$, Fig. 4B). Gurmarin selectively attenuated responses to ethanol and sucrose only, leaving NaCl, HCl, and quinine responses unaltered [treatment $\times$ stimulus interaction: $F(3,24) = 24.16, P < 0.001, 2 \times 4$ repeated-measures ANOVA; sucrose comparison: $P < 0.001$; Fig. 4D]. These results imply that ethanol and sucrose stimulate a common gustatory receptor mechanism as the effects of orally applied gurmarin on taste responses to these stimuli are similar.

Neural representation of alcohol taste information

To specifically address how ethanol taste information is represented by gustatory neural activity in the NST, MDS was applied to data obtained from 13 S$_1$ and 13 S$_0$ neurons in our sample that were tested using the standard stimuli and ethanol concentration series plus an extended stimulus array (Table 1). This extended stimulus battery included additional sweet-tasting compounds, bitter-tasting chemicals, sodium and nonso-dium salts, an additional acid, and a mixture of sucrose and quinine known to generalize to ethanol in behavioral experiments (Di Lorenzo et al. 1986; Kiefer and Mahadevan 1993).

The outcome of this analysis is represented in Fig. 5. A scree test indicated that a solution of greater than three dimensions produced no substantial further reduction in Kruskal stress. Dimension 1 of the scaling space reflected stimulus palatability, whereas dimension 3 inversely described response magnitude. The MDS solution revealed that across-neuron patterns of response to different concentrations of ethanol are highly similar to those evoked by sweeteners (sucrose, d-fructose, and d-glucose) and unlike patterns generated by salts (NaCl, NaNO$_3$, KCl, and MgCl$_2$), acids (HCl and citric acid) and bitter-tasting stimuli (quinine and nicotine) as evidenced by the clustering of ethanol near sweet compounds and away from other stimuli along each scaling dimension. In fact, patterns evoked by increasing concentrations of ethanol became more and more similar to those produced by sucrose and other sugars. Moreover, the across-neuron pattern evoked by the sucrose-quinine mixture was found to be most similar to patterns produced by sugars and ethanol as opposed to other tastants. These data indicate that ethanol stimulation of the anterior tongue and palate evokes a pattern of activity in gustatory circuits in the NST that resembles that produced by sweeteners.

DISCUSSION

These results demonstrate that orally applied ethanol produces a concentration-dependent activation of central gustatory neural circuits that underlie sugar taste. Further, oral application of the sweet receptor blocker gurmarin inhibited taste

![Figure 5](http://jn.physiology.org/doi/abs/10.1152/jn.00000.2004)

**FIG. 5.** Three-dimensional space representing the results of multidimensional scaling analysis of across-neuron patterns of response evoked by 20 individual taste stimuli and one binary mixture. The Kruskal stress value for this solution is 0.025. The axes are labeled in arbitrary units. Points in the space representing the standard taste stimuli are indicated by large type. Where applicable, stimulus concentration (% or M) is indicated.
responses to ethanol in sucrose-responsive NST neurons to 50% of control values, paralleling that recently shown for responses to sucrose (Lemon et al. 2003). In the peripheral nervous system, lingual gurmarin treatment blocks responses to sweeteners in a subset of sucrose-responsive chorda tympani (CT) nerve fibers, leaving sweeter responses in other sucrose-sensitive fibers unaffected (Ninomiya et al. 1999). The ability of gurmarin to partially rather than fully block sucrose responses in individual NST neurons is attributable to the convergence of input derived from gurmarin-sensitive and -insensitive receptor mechanisms onto these cells (Lemon et al. 2003). Moreover, the degree of suppression observed in the CNS is predicted given that gurmarin halves taste responses to sucrose and other sweeteners in whole-nerve CT recordings (Ninomiya and Imoto 1995; Ninomiya et al. 1997), which include activity generated by gurmarin-sensitive and -insensitive fibers (Ninomiya et al. 1999). Thus the present finding that gurmarin inhibits taste responses to ethanol in NST neurons by 50% would be expected if ethanol and sucrose activate common gustatory receptors.

Although the present data indicate that ethanol taste information is distributed predominantly to sucrose-responsive rather than sucrose-unresponsive neurons in the brain, the overall pattern of activation produced by ethanol across a population of gustatory neurons may more critically define how ethanol taste is represented in the CNS. All $S_1$ and $S_0$ neurons in the present study significantly responded to NaCl and many also responded to HCl and quinine-HCl. Stimuli of different taste qualities sometimes produce near equivalent responses within individual cells, and thus it may prove difficult for the discharge of a gustatory neuron to distinguish between all stimuli that activate it (Scott and Giza 2000). This breadth of responsiveness argues that it is the pattern of relative activity across gustatory neurons that constitutes the basis of the neural message for taste (for reviews see Scott and Giza 2000; Smith and Scott 2003; Smith and St. John 1999). The present data indicate that the neural representation of ethanol taste in the CNS, as indexed by the across-nerve pattern of gustatory activity, is most similar to that of sugars (see Figs. 3 and 5).

It has previously been shown that conditioned taste aversions to 0.2 M sucrose generalize to 10% ethanol in C57BL mice (Blizard and McClean 2000). Although rats generalize conditioned taste aversions between alcohol (6 and 9%) and sucrose (0.1 M) only when sucrose is mixed with an palatable stimulus such as quinine (0.0001 M) (Di Lorenzo et al. 1986; Kiefer and Mahadevan 1993), the sucrose component of the mixture is critical for generalization to occur (Kiefer and Mahadevan 1993). Rat behavioral data suggest that the sucrose component of this mixture is stronger than the quinine component (Spector and Kopka 2002; Spector et al. 1997). Accordingly, in the present study central across-nerve patterns of response produced by ethanol, 0.1 M sucrose, and a mixture of 0.1 M sucrose and 0.0001 M quinine were found to be similar. However, the present finding that central taste responses to increasing concentrations of ethanol become increasingly more similar to those produced by palatable sugars is somewhat unexpected, as randomly-bred rats typically avoid ethanol at concentrations >6% (e.g., Richter and Campbell 1940). Such limited consumption has been attributed to an aversive orosensory property of alcohol at high concentrations, generally assumed to be mediated by gustation.

In the present study, taste stimulation was limited to the anterior tongue and palate, which are innervated respectively by the CT and greater superficial petrosal (GSP) branches of the VIIth (facial) nerve. Gustatory stimuli did not reach receptor cells situated on the posterior tongue, innervated by the lingual-tonsillar branch of the IXth (glossopharyngeal) nerve. The VIIth nerve, particularly the GSP branch, responds strongly to sugars and modestly to bitter stimuli (Harada et al. 1997; Nejad 1986), whereas the IXth nerve responds more robustly to bitter-tasting substances (Dahl et al. 1997; Frank 1991). The present study cannot address how input from the IXth nerve contributes to the central gustatory processing of ethanol. However, in primates taste responses to ethanol recorded from the glossopharyngeal nerve are similar to those produced by sucrose (Danilova and Hellekant 2000). If ethanol does impart a bitter or aversive taste sensation in rats, the present findings suggest that input from the VIIth nerve does not contribute to this phenomenon as ethanol taste information conveyed by this nerve is represented in the CNS as similar to sugars. Moreover, nerve transection studies have revealed that input arising from the VIIth but not the IXth nerve is necessary for gustatory discrimination in rodents (Spector and Grill 1992; Spector et al. 1997; St. John and Spector 1998), which suggests that the VIIth nerve input is coupled with specialized central neural circuits that serve to identify taste stimuli (St. John and Spector 1998).

It is possible that the aversive orosensory component of alcohol is contributed by a modality other than taste. In addition to stimulating the gustatory system, orally applied ethanol produces a robust concentration-dependent activation of neurons in the trigeminal subnucleus caudalis (Carstens et al. 1998). These neurons receive afferent input from chemosensitive nociceptors in the oral epithelium and respond to noxious stimulation of intraoral tissue (see Carstens et al. 1998). Together with the present work, these findings indicate that oral ethanol consumption simultaneously activates disparate sensory inputs that serve either appetitive or protective functions. This observation is consistent with orosensory reactivity data showing that ethanol produces a combination of ingestive and aversive behavioral responses in rodents (Kiefer 1995). The gustatory, somatosensory, and also the olfactory (Kiefer et al. 1988) attributes of ethanol likely interact in a complicated manner during consumption to influence behavioral output. The present findings indicate that an important component of the overall sensory signal for ethanol is the stimulation of receptors and central neural pathways involved in the processing of sugar taste.

**Genetic predisposition to consume alcohol and sweet substances**

Genetic variation in neural substrates for sweet taste may influence individual susceptibility to consume alcohol. Although alcoholism is a complex disorder involving multiple genetic and environmental factors, there is a robust literature supporting the presence of a genetically determined link between preference for ethanol and sweet-tasting substances.
Implications for alcohol consumption

The ability of ethanol to activate sucrose-responsive gustatory pathways in the CNS may have important consequences for the downstream hedonic processing of alcohol. It is known that substances that taste sweet activate orosensory substrates linked to central dopaminergic and opioidergic systems, which play a critical role in ingestive motivation and reward (Kelley and Berridge 2002; Kelley et al. 2002; Schneider 1989; Zhang and Kelley 2002). For example, sham-feeding of sucrose produces an immediate concentration-dependent rise in dopamine levels in the nucleus accumbens (Hajnal et al. 2004) and in rats. Furthermore, opioid agonists infused into the nucleus accumbens increase the intake of noncaloric saccharin (Zhang and Kelley 2002) and facilitate appetitive responses to sweeteners in C57BL mice (Murata et al. 2003).

References

(Bachmanov et al. 1996; Belknap et al. 1993; Blizard and McClearn 2000; Dess et al. 1998; Forgie et al. 1988; Kamrov-Polevoy et al. 1990, 1997, 1998, 2003a,b; Overstreet et al. 1993; Sinclair et al. 1992; Stewart et al. 1994; Woods et al. 2003). Common chromosomal loci with pleiotropic effects on alcohol and sweetener consumption have now been identified in different rodent species (Bachmanov et al. 2002; Foroud et al. 2002). In mice, the Ap3y locus, which strongly influences ethanol intake (Bachmanov et al. 2002), overlaps with the Sac locus controlling sensitivity to certain sweet tastants (Fuller 1974; Lush et al. 1995). This locus corresponds to the Tas1r3 gene, encoding the sweet taste receptor T1R3 (Max et al. 2001; Montmayeur et al. 2001; Nelson et al. 2001).

The present data raise the possibility that inherited variation in sweet taste mechanisms may confer differential alcohol sensitivity and influence individual alcohol preference (also see Bachmanov et al. 2003). Indeed, genetic polymorphisms in Tas1r3 are known to correlate with the sweetener preference phenotypes of C57BL (sweet “taster”) and DBA (sweet “nontaster”) mice (Montmayeur et al. 2001), which also display corresponding differential ethanol preference (McClearn and Rodgers 1959). Moreover, ethanol- and sweetener-prefering taster mice possess gurmarin-sensitive taste receptors for sweeteners, whereas nontasters do not (Ninomiya and Imoto 1995); these receptors were shown here to mediate CNS taste responses to ethanol. It remains to be determined whether the neural systems that underlie the taste of sweeteners in ethanol-prefering and -nontpreerring animals are differentially activated by ethanol and if antagonism of sweet taste receptors attenuates genetically mediated alcohol preference and consumption. Relevant to the latter hypothesis, it has recently been shown that gurmarin is effective in reducing behavioral responses to various sweeteners in C57BL mice (Murata et al. 2003).

The ability of ethanol to activate sucrose-responsive gustatory pathways in the CNS may have important consequences for the downstream hedonic processing of alcohol. It is known that substances that taste sweet activate orosensory substrates linked to central dopaminergic and opioidergic systems, which play a critical role in ingestive motivation and reward (Kelley and Berridge 2002; Kelley et al. 2002; Schneider 1989; Zhang and Kelley 2002). For example, sham-feeding of sucrose produces an immediate concentration-dependent rise in dopamine levels in the nucleus accumbens (Hajnal et al. 2004) and in rats. Furthermore, opioid agonists infused into the nucleus accumbens increase the intake of noncaloric saccharin (Zhang and Kelley 2002) and facilitate appetitive responses to sweeteners in C57BL mice (Murata et al. 2003).


