Chem. Senses 32: 129–137, 2007
doi:10.1093/chemse/bjl038
Advance Access publication November 2, 2006

Taste Reactivity and Fos Expression in GAD1-EGFP Transgenic Mice

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Abstract

The central distribution of QHCl-elicited Fos-like immunoreactivity (FLI) suggests the location of a brain stem circuit that controls the oral rejection response. Although many species display an oral rejection response to bitter stimuli, the distribution of FLI associated with this response has been investigated only in rats. Fos data are minimal for the mouse, a species of increasing importance, due to its use in molecular and transgenic studies and taste-evoked oromotor responses are also only incompletely described in these rodents. We investigated these questions in FVB/NJ mice and a related transgenic strain (FVB-Tg(GadGFP)4507) that expresses green fluorescent protein in a subset of GAD1-containing neurons. QHCl, sucrose, or water delivered through intraoral cannulae yielded behavioral profiles that clearly differentiated QHCl from sucrose. Similar to rat, the number of neurons expressing FLI in the medial third of the solitary nucleus was elevated following QHCl compared with the other stimuli. In mice expressing green fluorescent protein, there was a pronounced distribution of GABAergic neurons in the ventral half of the solitary nucleus. Approximately 15% of solitary neurons expressing Fos were GABAergic, but this proportion did not differ according to stimulus.

Key words: brainstem, GABA, anatomy, double label

Introduction

The expression of Fos-like immunoreactivity (FLI) in the nucleus of the solitary tract (NST) and parabrachial nucleus show differential patterns following gustatory stimulation in the rat. In particular, QHCl and other bitter-tasting (to human) stimuli elicit a distinct pattern in the medial third of the NST, compared with stimulation with sucrose, NaCl, citric acid, or water (Harrer and Travers 1996; Hu et al. 1997; Travers 2002). This unique pattern of FLI has been postulated to reflect the substrate for oral rejection and visceral reflexes elicited by bitter stimuli (DiNardo and Travers 1997; King et al. 1999, 2000; Travers SP and Travers JB 2005). Decerebrate preparations, in which the "gape" (oral rejection) response remains viable, show similar patterns of FLI as intact preparations (Travers et al. 1999). Furthermore, transection of the IXth nerve that diminishes the distinct FLI pattern (King et al. 1999) is also associated with a reduction in the number of gape responses to QHCl, although the discriminability and rejection of QHCl presented in a bottle remains intact (Travers et al. 1987; St John and Spector 1998). Although many animals emit stereotyped rejection responses to QHCl, including rat (Grill and Norgren 1978), hamster (Brining et al. 1991), rabbit (Ganchrow et al. 1979), chick (Ganchrow et al. 1990), axolotl (Takeuchi et al. 1994), goldfish (Lamb and Finger 1995), mudpuppy, human (Steiner 1979) and nonhuman primate (Steiner and Glaser 1984), and some species, including humans, share a common gape response, the central topography of QHCl-elicited FLI has not been investigated in other species. Thus, one goal of the present study was to test the generality of the QHCl-induced FLI pattern in another rodent species, that is, the mouse.

Determining the pattern of FLI to taste stimuli in the awake mouse also afforded us the opportunity to observe behavioral responses. Previous reports of taste reactivity in mice to stimulation with QHCl have been somewhat equivocal. In one report using ICR outbred mice, stimulation with either preferred (0.1 M sucrose or NaCl) or aversive (0.0005 M QHCl) stimuli evoked gape responses (Kiefer et al. 1998). In another investigation using the ddY mouse, differential taste reactivity responses were obtained to preferred and aversive stimuli, but no specific mention of the frequency of gape responses was made (Manabe et al. 2001). Thus, a second goal of the present study was to contrast taste reactivity to sucrose and QHCl in another mouse

strain. Lastly, a role for GABAergic neurons in both taste processing (Liu et al. 1993; Grabauskas and Bradley 1996; Bradley and Grabauskas 1998; Smith and Li 2000) and taste-reflex function has been postulated (Chen and Travers 2003). Based on the ability of bicuculline infusions into the reticular formation ventral to the rostral nucleus of the solitary tract (rNST) to potentiate sucrose-induced mouth openings such that they appeared "gape-like," we developed the hypothesis that natural QHCl-induced gapes are mediated, in part, by disinhibition through GABAergic projections originating in the rNST (Chen and Travers 2003). Thus, in the present study, we predicted that stimulation with QHCl would 1) produce a high proportion of double-labeled GABAergic neurons in the QHCl-induced FLI "hotspot" in the medial NST or 2) decrease the number of double-labeled neurons in the ventral NST if these neurons were inhibited by QHCl stimulation. Thus, we sought to evaluate a mouse strain that expresses green fluorescent protein in a subset of GABAergic neurons (Oliva et al. 2000) to determine whether FLI was differentially coexpressed following QHCl or sucrose stimulation.

Materials and methods

Surgery and Gustatory Stimulation

Female transgenic mice, 4–8 weeks old (18–24 g), expressing enhanced green fluorescent protein (EGFP) in a subset of GAD1-containing neurons (The Jackson Laboratory [Bar Harbor, ME], FVB-Tg(GadGFP)45704Swn/J: abbreviated "GIN" for GFP-expressing inhibitory neurons; see also [Oliva et al. 2000]) (n = 11) or their parent strain (The Jackson Laboratory: FVB/NJ mice, n = 10) were implanted bilaterally with intraoral cannulas (PE50). Mice were anesthetized with Nembutal (50 mg/kg) and placed supine in a stereotaxic device modified for mice. Placement of the intraoral cannulas was aided by propping the mouth open with a small piece of tubing between the mandibular and maxillary incisors and elevating the preparation so that the oral cavity could be viewed through an operating microscope. An intraoral cannula consisted of a 15-mm length of PE50, fitted at one end with a 10-mm length of 23 g SS tubing extending out 5 mm. The other end of the intraoral cannula was flared slightly to prevent it from being drawn into the oral mucosa.

Following surgery, mice were adapted to the testing chamber (Plexiglas: 11 inch high × 5 inch diameter) and to intraoral stimulus delivery using distilled water to minimize novelty-associated Fos expression during testing (Harrer and Travers 1996; Travers 2002). On the test day, mice were infused with 25 μl aliquots of fluid 20 times over a 30-min period with one of the following stimuli: distilled water (n = 6, 2FVB/4GIN), 0.3 M sucrose (n = 7, 4FVB/3GIN), or 0.003 M QHCl (n = 8, 4FVB/4GIN). Pilot studies were conducted to determine the appropriate flow rate for stimulus delivery. When the fluid was delivered too slowly, it was impossible to elicit an overt response to water and sucrose. Behavioral responses could be elicited, however, when the pump speed was increased to deliver a 25-μl bolus at a rate of 100 μl/min. These behavioral responses were videotaped for analysis. All procedures involving animals were approved by the Ohio State University Institutional Animal Care and Use Committee and conformed to National Institutes of Health guidelines on the care and use of laboratory animals.

Tissue processing

Seventy-five minutes after the start of stimulation, mice were deeply anesthetized (150 mg/kg Nembutal) and perfused with a mixture of 4% paraformaldehyde, 1.4% lysine, and 0.2% sodium metaperiodate in 0.1 M sodium phosphate (Travers 2000). Coronal sections through the hindbrain (30–40 μm) were collected into 3 series; tissue was either reacted immediately or stored in cryoprotectant at −20 °C (Hoffman et al. 1992) for later processing. For both transgenic mice and their parent strain, one series of sections was reacted using standard 3,3′-diaminobenzidine (DAB) immunohistochemistry for Fos, similar to earlier studies from this laboratory (Harrer and Travers 1996; Travers and Hu 2000; Travers 2002; Chan et al. 2004). Except where noted, all processing took place at room temperature, and each step was followed by rinses in phosphate buffer or phosphate-buffered saline, pH = 7.4, which also served as the diluents for the reagents. Briefly, sections were treated with 1% Na borohydride, endogenous peroxidase quenched with 0.5% H_2O_2, and nonspecific binding sites blocked with 10% sheep serum before incubation in the primary antibody (anti-Fos, Oncogene, Oncogene Science [Cambridge, MA], PC38, rabbit anti-c-fos; 1:25–30K) for 48–72 h at 4 °C. The anti-Fos antibody was detected with a biotinylated anti-rabbit antibody (1:600), followed by ABC reagent (Vector Laboratories, Burlington, CA), and then incubated in a mixture of DAB (0.05%) and $NiHSO_4$ (0.02%). The final oxidation step was initiated by addition of 0.003% H_2O_2. For the transgenic mice, a second series was processed to detect Fos by immunofluorescence in order to determine the relationship between neurons expressing GAD1 and Fos. The procedures were similar to those just described except that a lower dilution of the primary antibody was used (1:3K), and the secondary antibody was detected with streptavidin–Cy3 (1:1K, Jackson Laboratories).

In 2 mice of the parent strain, in situ hybridization to detect the mRNA for GAD1 was employed to compare the pattern of GAD1 staining observed in the transgenic mice because the latter is known to occur only in a subset of GAD1-containing neurons (Oliva et al. 2000). Because the in situ procedure interfered with Fos immunostaining, double labeling was not possible. Detailed methods for production of the probe and fluorescent in situ hybridization follow the protocol described earlier by Travers et al. (2005) and will only be summarized here. Detection of GAD1 used an antisense digoxigenin-labeled cRNA probe transcribed from

a pBluescript II SK (+) vector containing a 3.2-kb rat GAD1 cDNA fragment subcloned into the *Eco*RI site (kindly supplied by Dr A.J. Tobin, University of California, Los Angeles, CA). Unless noted, subsequent steps took place at room temperature. Sections were pretreated with Proteinase K (Sigma, 5 μg/ml in 0.01 M Tris buffer, pH 8, 10 min), rinsed with ice-cold 2× standard saline citrate buffer (SSC), and soaked in hybridization buffer for 1–4 h before adding the riboprobe (final concentration = 1–25 pg/μl). Sections were incubated with the riboprobe at 50 °C for 16–20 h, followed by rinses through decreasing concentrations of SSC (2× to 0.1×, 50 °C, 20 min). The location of the riboprobe was revealed using a biotinylated anti-digoxigenin antibody, followed by amplification with streptavidin/biotinylated tyramide (TSA indirect kit, Perkin–Elmer Scientific, Wellesley, MA), with the final visualization accomplished using Cy2–streptavidin. Internal controls for the specificity of in situ hybridization included a lack of staining in oral motor nuclei (hypoglossal and facial) and preganglionic parasympathetic neurons in the dorsal motor nucleus of the vagus.

Data analysis

In order to quantify the number of FLI-positive neurons following gustatory stimulation, plots of the DAB-reacted material were made using the NeuroLucida System (Microbrightfield Inc, Williston, VT). One section per mouse was plotted at the level where the NST is just lateral to the fourth ventricle. In rat, this corresponds to the location where maximum FLI is observed following QHCl stimulation and where the clearest distinction between the distributions of QHCl-elicited FLI and FLI elicited by other stimuli occurs (Chan et al. 2004). Outlines of the NST were drawn under dark-field optics. Subsequently, an investigator unaware of the stimulus condition plotted Fos-positive cells using a 20× lens. In order to be counted, there had to be a clear outline of an oval or round nucleus that was darker than the background. To analyze the topography of the FLI distribution, the NST was divided into "subfields," by dividing the nucleus into mediolateral thirds and then into dorsal and ventral halves (King et al. 1999). To determine the incidence of double-labeled cells for Fos and GAD1, a confocal microscope (Zeiss LSM 510) was used to generate Z stacks (40×, optical section thickness = ~2 μm) at the standard NST level at 40×, using the appropriate filter settings (505–530 nm excitation; 488 nm detection, for EGFP; >560 nm excitation; and 543 detection for Cy3). Because we observed that most of the GAD1-positive neurons were in the ventral half of the NST, counting from the Z stack was restricted to this area. Fos data were analyzed by performing a 2-way analysis of variance (ANOVA) with stimulus as one factor and subfield as another factor, followed by separate ANOVAs and post hoc Fisher's least square tests for each subfield. The alpha level for significance was set at $P < 0.05$.

Behavioral analysis was based on inspection of digital video clips of the 15 s following each stimulation trial. We did not score the number or length of each of 9 different behaviors, but only their incidence for a given trial. Eight of the behaviors could be seen in "real time," and were sufficiently clear to allow for little ambiguity. Thus, "grooming" (rat on 2 legs licking ventral surface, e.g., genitals or twisting to lick dorsal side), "paws-to-mouth" (paws positioned over the mouth with possible, but usually unobservable licking of paws), "rearing" (standing on 2 legs without other behavior), "chin rubs" (pushing chin to substrate surface), "paw flailing" (rapid paw shaking), "jumps" (vertical "popcorn" jumping (Dewey 1986), "headshakes" (rapid lateral head shaking), and "mouth movements" (rhythmic oral movements) could all be observed readily. Observing and scoring gapes (large prolonged mouth openings) was more problematic and had to be done frame by frame. This was accomplished by 2 investigators blind to the stimulus conditions for the first 5 trials of each session. As explained in Results, mice became highly agitated after 5 trials of QHCl stimulation, and observing the mouth systematically was virtually impossible. Thus, we report the proportion of 20 trials displaying each of 8 behaviors and the proportion of gapes for the first 5 trials. The frame by frame analysis of the first 5 trials also afforded us the opportunity to score lateral tongue protrusions. None were observed in response to any of the stimuli and are therefore not included as a behavioral category. Differences between stimuli were assessed with separate ANOVAs for each behavioral measure, followed by Fisher's least significant difference (LSD) post hoc tests. The alpha level for significance was set at $P < 0.05$.

Results

Taste reactivity and behavior

The responses to sucrose and water consisted primarily of mouth movements, paws-to-mouth, grooming, and rearing. Figure 1 shows the mean proportion of occurrences for each behavior over 20 trials, for example, on average, intraoral sucrose stimulation produced mouth movements on 40% of the trials across the 7 cases. ANOVAs for each behavior with stimulus as a factor, followed by post hoc tests, indicated that only the occurrence of gapes differentiated between the responses to sucrose and water ($F(2,18) = 10.8$, $P = 0.027$). Paws-to-mouth, mouth movements, grooming, and rearing were all observed relatively frequently during both water and sucrose stimulation, but headshakes, jumps, paw flails, or chin rubs were not associated with either of these stimuli. Water also produced a relatively high incidence of gaping, but gaping rarely occurred with sucrose stimulation. The responses to QHCl, however, differed dramatically from both sucrose and water and produced a significant increase in chin rubbing ($F(2,18) = 48.2$, $P < 0.01$), paw flailing ($F(2,18) = 18.8$, $P = 0.01$), and jumping ($F(2,18) = 10.7$, $P < 0.01$). Gapes were observed in approximately 60% of the trials compared with 30% for water ($F(2,18) = 10.8$, $P = 0.042$)

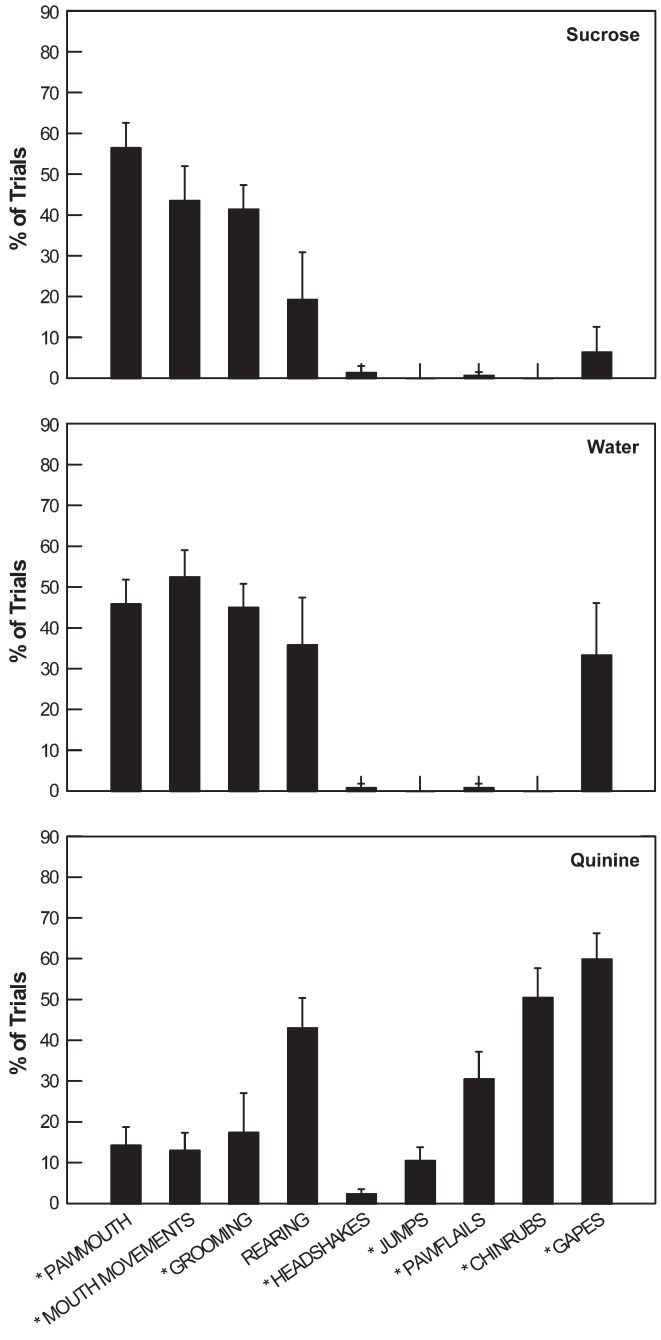

Figure 1 The mean percent expression of each of 9 behaviors over 20 trials for sucrose, water, and QHCl stimulation. Askerisks next to behavioral category indicate a significant ANOVA. See text for individual significant post hoc tests.

and just 8% for sucrose ($F(2,18) = 10.8$, $P < 0.001$). In addition, compared with sucrose and water stimulation, QHCl resulted in a significantly lower incidence of paws-to-mouth ($F(2,18) = 20.7$, P's < 0.01), mouth movements ($F(2,18) = 12.7$, P's < 0.01), and grooming behavior ($F(2,18) = 4.6$; sucrose, $P < 0.02$; water, $P < 0.01$). There was a trend towards more rearing in response to QHCl compared with sucrose stimulation ($F(2,18) = 1.9$, $P < 0.07$).

In general, these behavioral responses were distributed evenly across the 20 trials. Exceptions to this occurred in response to QHCl where mouth movements and paws-to-mouth occurred maximally in the early trials and subsequently decreased. Similarly, jumping behavior in response to QHCl did not begin immediately but rather developed over time. In comparison, mouth movements and paws-to-mouth in response to sucrose were evenly distributed over the 20 trials, and there were no occurrences of jumping (Figure 2).

Fos expression

Fos expression in the NST was clearly different for QHCl compared with sucrose and water. As evident in the photomicrographs of Figure 3, stimulation with QHCl (Figure 3A) elicited more FLI medially compared with stimulation with either water (Figure 3B) or sucrose (Figure 3C). Quantification of FLI in the rNST confirmed the pattern evident in the photomicrographs (Figure 4). An ANOVA for stimulus X subfield revealed no significant main effect for either stimulus or subfield, but the interaction between these 2 variables was significant ($F(10,90) = 2.6$, $P < 0.008$). Subsequent ANOVAs for both the dorsal-medial and ventral-medial subfields yielded significant effects of stimulus (dorsal-medial subfield: $F(2,18) = 10.9$, $P = 0.001$ and ventral-medial subfield: $F(2,18) = 6.9$, $P = 0.006$). Post hoc Fisher's LSD tests indicated that these effects were due to the fact that quinine was associated with significantly more FLI than either sucrose or water in both subfields (all P's < 0.05). There were no significant differences, however, between sucrose and water (all P's > 0.1).

Visual inspection further suggested that the number of FLI neurons in the reticular formation (RF) ventral to the NST was greater following QHCl stimulation compared with either water or sucrose. In fact, the pattern looked remarkably like that of the rat, with a well-defined arc of FLI extending from the NST through the intermediate zone of the reticular formation (IRt) (Figure 3A), past nucleus ambiguus into the ventrolateral RF. Adding contrast to the pattern of FLI was a notable lack of label in the more lateral parvocellular RF (PCRt) or the more medial nucleus gigantocellularis (Gi). Stimulation with sucrose and water produced a less defined pattern in the RF (not shown). The label for sucrose was more equally distributed between IRt and PCRt and did not extend as far ventrally, that is, there was less of an "arc" pattern. Water stimulation produced an inconsistent pattern in the RF with label variably distributed across PCRt, IRt, and Gi but, again, not extending ventrally in the RF.

Although FLI in the parabrachial nucleus was not quantified, the pattern of label appeared stimulus related (not shown). Overall, there was more FLI in the lateral subdivision of the PBN compared with the medial subdivision. QHCl stimulation produced the most FLI, followed by

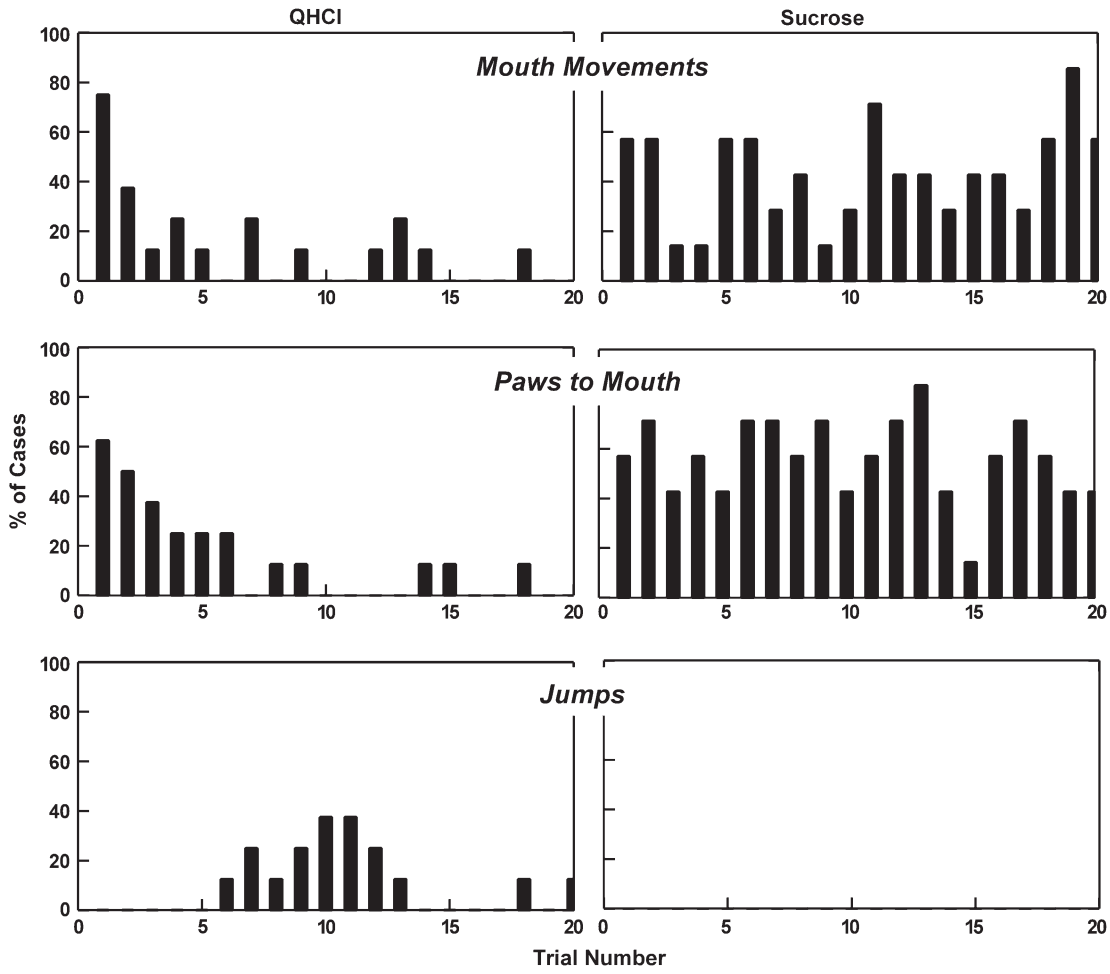

Figure 2 The percentage of mice displaying each of 3 different behaviors over 20 trials in response to sucrose and QHCl. In response to sucrose, mouth movements and paws-to-mouth were observed throughout the 20 trials. In contrast, stimulation with QHCl produced some mouth movements and paws-to-mouth primarily in the early trials and jumping behavior later on.

sucrose and then water. The most consistent pattern of labeling was in the external lateral subdivision following QHCl stimulation that was evident in nearly all the cases. In contrast, FLI in this subdivision following either sucrose or water stimulation was highly variable; present in 1 or 2 cases and nonexistent in others.

GAD1-EGFP and double-labeled neurons

The pattern of GAD1-EGFP in the rNST was very striking. As illustrated in Figure 5, the EGFP neurons labeled in the transgenic mice were densely distributed in the ventral half region of the rNST but were sparse dorsally. That only a subset of GABAergic neurons were labeled in the transgenic mice was evident from in situ hybridization for GAD1 that showed a more homogeneous distribution within the NST (Figure 6). It was clear from visual inspection that relatively few FLI neurons in the QHCl hotspot (dorsal-medial NST: Figure 5) were GAD1-EGFP positive. However, the potential for double labeling was much more likely in the ventral NST (Figure 5). Accordingly, double-labeled neurons were quantified in the ventral half of the NST for GAD-EGFP mice in each stimulus group. The mean proportion of double-labeled FLI neurons was 14.6 (SD = 5.8) and did not differ significantly across the 3 stimulus conditions.

Discussion

Behavioral distinction between sucrose, QHCl, and water

The results of the present study demonstrate that following intraoral gustatory stimulation, a behavioral pattern to "preferred" stimuli can be distinguished from responses to an "aversive" stimulus in the FVB/NJ mouse. When presented with sucrose or water, FVB/NJ mice put their paws to their mouth and engage in mouth movements and grooming significantly more than when presented with QHCl. QHCl stimulation produced jumping, paw flailing, and chin rubs, behaviors virtually absent following either sucrose or water. Although there are distinct similarities, these response profiles

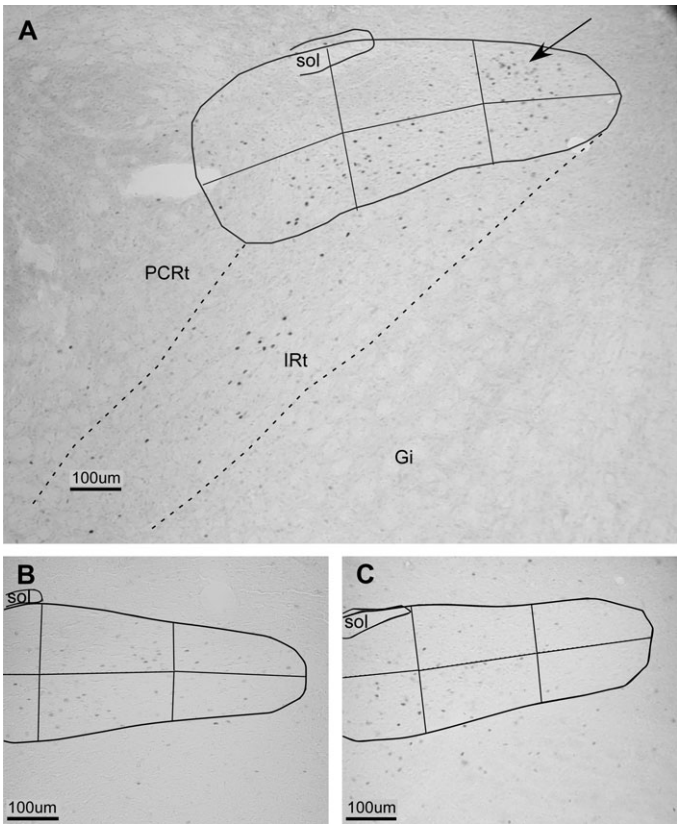

Figure 3 Stimulation with QHCl elicited more FLI in the dorsal-medial subfield of the rNST (A, arrow) compared with stimulation with either water (B) or sucrose (C). Although not quantified, the FLI following QHCl stimulation appeared much darker compared with FLI associated with other stimuli. Medial is to the right.

also suggest differences compared with other rodent species, that is, rats (Grill and Norgren 1978) and hamsters (Brining et al. 1991) as well as other mouse strains (Kiefer et al. 1998).

The response in the FVB/NJ mouse following sucrose stimulation was similar to the rat and hamster in that there were rhythmic mouth movements. In response to sucrose stimulation, however, rats and hamsters also make more lateral tongue protrusions compared with water stimulation. Lateral tongue movements, however, were not observed in the mice. Regrettably, mice have the annoying habit of putting their paws to their mouths following either sucrose or water stimulation that precluded continuous observation. This may have contributed to the lack of notable differentiation between the response profiles to sucrose and water that are seen in the rat (Grill and Norgren 1978). Given that FVB/NJ mice are saccharin tasters with a strong preference compared with some other mouse strains (Reed et al. 2004), it is perhaps surprising that we could not differentiate sucrose from water profiles. A modified taste reactivity test in which the paws can be better controlled may reveal such differences.

Responses to gustatory stimulation in the FVB/NJ mouse also differed from those reported in the ICR outbred mouse (Kiefer et al. 1998). In the ICR strain, there were few differences in the behavioral responses across the 4 taste stimuli and water with the exception that overall sucrose produced fewer aversive responses. In fact, in this mouse strain, all "standard" taste stimuli (sucrose, NaCl, HCl, and QHCl) evoked gape responses. Interestingly, in contrast to the FVB/NJ mouse, the ICR mice did not make chin rubs in response to QHCl (or any of the other stimuli), and fluid

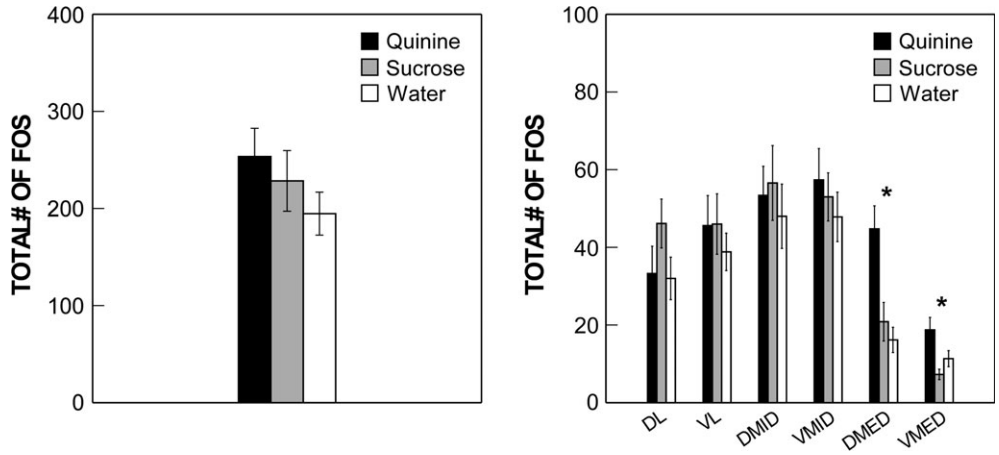

Figure 4 The total number of FLI cells were counted at one standard level of the rNST for rats stimulated with QHCl, sucrose, and water. Left: summed over the subfields, neither QHCl nor sucrose resulted in more FLI neurons than stimulation with water. Right: when the rNST was subdivided into 6 subfields (medial lateral thirds and then dorsal and ventral halves), quinine elicited significantly more FLI in the dorsal-medial ($F(2,18)$, $P < 0.001$) and ventral-medial subfields ($F(2,18)$, $P < 0.001$) than either sucrose or water. Note that counts were made of all FLI neurons regardless of staining intensity, across multiple focal planes. If staining intensity was taken into account (see Figure 3), it seems likely that the quinine pattern would appear more specific for the medial subfields and that this stimulus would be more effective than either water or sucrose. Abbreviations: DL, dorsal lateral; DMED, dorsal medial; DMID, dorsal middle; VL, ventrolateral; VMED, ventral medial; and VMID, ventral middle.

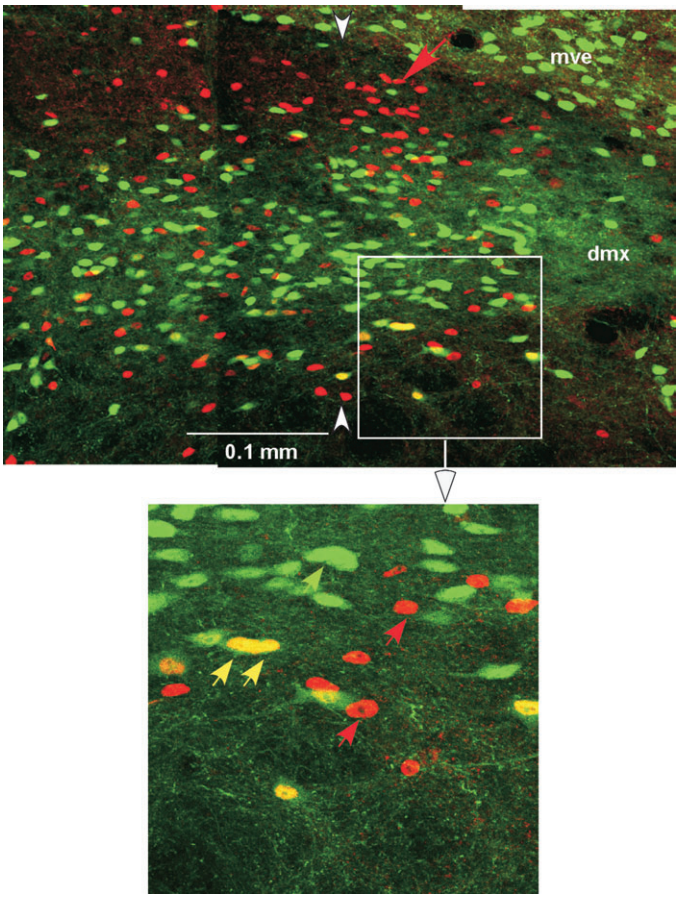

Figure 5 Photomicrographs of Fos and GAD1-EGFP in the rNST. Upper panel: low magnification photomicrograph. Borders of the nucleus are demarcated with arrowheads. The medial border of the nucleus is just beyond the right hand edge of the photomicrograph. Large red arrow points to a cluster of Cy3-labeled Fos nuclei. The boxed region includes several double-labeled neurons and is shown at a higher magnification in the lower panel. Lower panel: significant numbers of double-labeled neurons (yellow arrows) are evident, along with single-labeled Fos (red arrows) and GAD1 (green arrow) neurons in the ventral half region. Abbreviations: dmx, dorsal motor nucleus of the vagus and mve, medial vestibular nucleus.

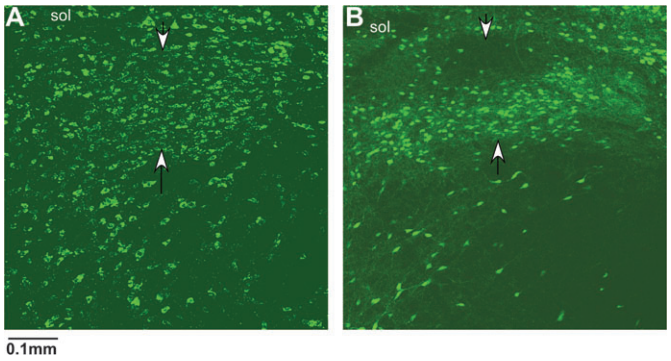

Figure 6 **(A)** Photomicrograph of rNST and subjacent RF showing uniform distribution of in situ hybridization for GAD1 in the FVB/NJ mouse in comparison to the ventral distribution of EGFP in the transgenic mouse. **(B)** Arrows demarcate the borders of the nucleus of the solitary tract. Abbreviation: sol, solitary tract.

expulsion was not observed, despite the other "aversive" behaviors. Methodological differences may explain the discrepancy between our results and those of the ICR mouse. We used a significantly higher concentration of QHCl (0.003 vs. 0.0005 M) and delivered it at half the rate (100 vs. 200 µl/min). We have observed in rat (Travers JB, unpublished observations) that the initial mouth movements following the rapid infusion of a fluid stimulus can induce a mouth opening (and EMG profile) that is intermediate between a full-fledged gape response and a "normal" mouth opening. Thus, the observation of gapes in response to all types of fluid stimulation in the ICR mouse in the absence of either chin rubs or fluid expulsion may indicate that the gape response observed reflected the intense mechanical attribute of the stimulus delivery and not the gustatory quality of the QHCl. Alternatively, these behavioral differences in taste reactivity could reflect strain differences in gustatory sensitivity that are clearly evident in mice (Glendinning 1993; Whitney and Harder 1994; Bachmanov et al. 2002; Boughter et al. 2005).

In addition to displaying the same aversive responses reported in rats and hamsters, the FVB/NJ mouse made vertical "popcorn" jumps in response to QHCl. These responses were never observed in the early trials but, rather, developed over time. Vertical jumps in mice, variously termed "popcorn" or "popping" behavior, can be elicited following a variety of behavioral or pharmacological treatments, including escape from localized pain (Suaudeau et al. 2005), environmental heat stress (Harikai et al. 2004), withdrawal from morphine addiction (Zarrindast et al. 2002), PCP administration as a model of psychotic behavior (Tizabi et al. 1998), and finally as a model of hyperflexia following systemic cannabinoid treatment (Patel and Hillard 2001). With the possible exception of the last example, vertical jumping appears as a response to aversive stimuli, to which we now add inescapable stimulation with a bitter-tasting (to humans) stimulus.

Distribution of Fos and double labeling with GAD1-EGFP

QHCl stimulation induced a pattern of FLI in the NST that was very similar to that observed in the rat (Harrer and Travers 1996). In particular, there was a dense concentration of FLI in the medial region of the rNST, a pattern not evident following stimulation with either sucrose or water. When the oral rejection (gape) response in rat is attenuated by cutting the ninth nerve (King et al. 1999), this pattern of FLI is eliminated, suggesting that these FLI-positive neurons may be part of the circuit for processing bitter stimuli that lead to this aversive response. Although the pattern of gustatory-induced FLI expression in the mouse appeared quite similar to that in the rat, there were some differences. In the rat, sucrose stimulation typically produces more FLI in the rNST compared with stimulation with water (Harrer and Travers 1996), but this was not the case in the mouse. In addition,

in the rat, QHCl stimulation induces more FLI in the medial subfields than in the other subfields. In the present study, FLI expression was roughly equivalent across all 6 subfields following QHCl stimulation when based on counts of all stained nuclei, regardless of staining intensity. Inspection of Figure 3, however, makes it evident that many of the FLI neurons in the mid- and lateral subfields were quite lightly stained following QHCl stimulation. Thus, if intensity were taken into account in the quantitative analysis, it seems likely that the quinine pattern would appear more specific for the medial subfields, so that the within-animal FLI pattern would more closely resemble the rat (see Figure 3). Nevertheless, the FLI plotting was carried out in the same manner as our previous studies in rat, and thus other factors most likely contribute. One possible explanation is that the FLI expression is more prevalent in the middle and lateral subfields because the mouse generally exhibits higher levels of overall motor activity. Unlike the rat, the mouse is almost in continuous motion in between stimulations, engaging in locomotion, rearing and in particular grooming. The oral tactile stimulation of grooming, in particular, could raise background levels of activity in and around the NST. Because neurons in this nucleus are responsive to tactile as well as taste stimulation (Travers and Norgren 1995), this same explanation may also account for why there is apparently no difference between FLI expression in the NST following sucrose and water stimulation in the mouse.

In the forebrain, EGFP-expressing neurons in GIN transgenic mice are associated with a subpopulation of somatostatin (SOM) neurons (Oliva et al. 2000). In the hippocampus, 99% of the EGFP neurons coexpressed GAD1 and 95% of the EGFP neurons also stained positive for SOM. However, less than 30% of SOM staining neurons were EGFP positive, indicating that only a subset of SOM neurons were GABAergic. The pattern of EGFP in the present study showed EGFP-positive neurons disproportionately represented in the ventral half of the rostral solitary nucleus. Given that the EGFP expression is under the control of the GADI promotor, it is likely that these neurons are GABAergic; however, it is unlikely they contain SOM. In the rat, SOM-positive neurons are primarily located in the medullary ventrolateral RF, and these neurons are glutamatergic rather than GABAergic (Stornetta et al. 2003). Only the occasional SOM-positive neuron was found in the NST. Thus, it is unclear which particular subpopulation of GABAergic neurons are disproportionately labeled in the ventral part of the rNST in this mouse strain. We have previously demonstrated that neurons in the ventral subdivision of the rat rNST are a major source of projections to the subjacent RF (Halsell et al. 1996); however, there are no reports of neurotransmitter phenotypes specific to this subdivision.

Relatively, few of the GABAergic neurons in the ventral region were FLI positive, and there was no difference in the number of double-labeled neurons as a function of stimulus. Thus, the present study provides no evidence for our hypothesis that QHCl-induced gapes derive from disinhibition of preoromotor neurons. A role for GABA in mediating rejection responses to aversive stimuli, however, cannot be entirely ruled out. Infusions of a diazepam binding inhibitor into the fourth ventricle increased the aversive response to intraoral infusions of 0.9% NaCl in the ddY mouse (Manabe et al. 2001). Because the EGFP label in the present study was only on one (unidentified) subset of GABAergic neurons, we may not have detected a differential increase/decrease in FLI in the relevant set of GABAergic neurons following QHCl stimulation.

Acknowledgements

This work was supported by DC00416 and DC00417 to S.P.T. and J.B.T., respectively. We wish to thank Colleen Kershaw for assistance in the behavioral analysis.

References

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

Boughter JD Jr, Raghow S, Nelson TM, Munger SD. 2005. Inbred mouse strains C57BL/6J and DBA/2J vary in sensitivity to a subset of bitter stimuli. BMC Genet. 6:36.

Bradley RM, Grabauskas G. 1998. Neural circuits for taste. Excitation, inhibition, and synaptic plasticity in the rostral gustatory zone of the nucleus of the solitary tract. Ann N Y Acad Sci. 855:467–474.

Brining SK, Belecky TL, Smith DV. 1991. Taste reactivity in the hamster. Physiol Behav. 49:1265–1272.

Chan CY, Yoo JE, Travers SP. 2004. Diverse bitter stimuli elicit highly similar patterns of Fos-like immunoreactivity in the nucleus of the solitary tract. Chem Senses. 29:573–581.

Chen Z, Travers JB. 2003. Inactivation of amino acid receptors in medullary reticular formation modulates and suppresses ingestion and rejection responses in the awake rat. Am J Physiol Regul Integr Comp Physiol. 285:R68–R83.

Dewey WL. 1986. Cannabinoid pharmacology. Pharmacol Rev. 38:151–178.

DiNardo LA, Travers JB. 1997. Distribution of fos-like immunoreactivity in the medullary reticular formation of the rat after gustatory elicited ingestion and rejection behaviors. J Neurosci. 17:3826–3839.

Ganchrow D, Oppenheimer M, Steiner JE. 1979. Behavioural displays to gustatory stimuli in newborn rabbit pups. Chem Senses Flavour. 4: 49–61.

Ganchrow JR, Steiner JE, Bartana A. 1990. Behavioral reactions to gustatory stimuli in young chicks (Gallus gallus domesticus). Dev Psychobiol. 23:103–117.

Glendinning JI. 1993. Preference and aversion for deterrent chemicals in two species of Peromyscus mouse. Physiol Behav. 54:141–150.

Grabauskas G, Bradley RM. 1996. Synaptic interactions due to convergent input from gustatory afferent fibers in the rostral nucleus of the solitary tract. J Neurophysiol. 76:2919–2927.

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

Halsell CB, Travers SP, Travers JB. 1996. Ascending and descending projections from the rostral nucleus of the solitary tract originate from separate neuronal populations. Neuroscience. 72:185–197.

Harikai N, Sugawara T, Tomogane K, Mizuno K, Tashiro S. 2004. Acute heat stress induces jumping escape behavior in mice. Physiol Behav. 83:373–376.

Harrer MI, Travers SP. 1996. Topographic organization of Fos-like immunoreactivity in the rostral nucleus of the solitary tract evoked by gustatory stimulation with sucrose and quinine. Brain Res. 711:125–137.

Hoffman GE, Smith MS, Fitzsimmons MD. 1992. Detecting steroidal effects on immediate early gene expression in the hypothalamus. Neuro Protocols. 1:52–66.

Hu HH, Travers JB, Travers S. 1997. Subnuclear location of orosensory neurons in the nucleus of the solitary tract. Soc Neurosci Abstr. 23:1036.

Kiefer SW, Hill KG, Kaczmarek HJ. 1998. Taste reactivity to alcohol and basic tastes in outbred mice. Alcohol Clin Exp Res. 22:1146–1151.

King CT, Garcea M, Spector AC. 2000. Glossopharyngeal nerve regeneration is essential for the complete recovery of quinine-stimulated oromotor rejection behaviors and central patterns of neuronal activity in the nucleus of the solitary tract in the rat. J Neurosci. 20:8426–8434.

King CT, Travers SP, Rowland NE, Garcea M, Spector AC. 1999. Glossopharyngeal nerve transection eliminates quinine-stimulated fos-like immunoreactivity in the nucleus of the solitary tract: implications for a functional topography of gustatory nerve input in rats. J Neurosci. 19:31073–21.

Lamb CF, Finger TE. 1995. Gustatory control of feeding behavior in goldfish. Physiol Behav. 57:483–488.

Liu H, Behbehani MM, Smith DV. 1993. The influence of GABA on cells in the gustatory region of the hamster solitary nucleus. Chem Senses. 18: 285–305.

Manabe Y, Toyoda T, Kuroda K, Imaizumi M, Yamamoto T, Fushiki T. 2001. Effect of diazepam binding inhibitor (DBI) on the fluid intake, preference and the taste reactivity in mice. Behav Brain Res. 126:197–204.

Oliva AA Jr, Jiang M, Lam T, Smith KL, Swann JW. 2000. Novel hippocampal interneuronal subtypes identified using transgenic mice that express green fluorescent protein in GABAergic interneurons. J Neurosci. 20:3354–3368.

Patel S, Hillard CJ. 2001. Cannabinoid CB(1) receptor agonists produce cerebellar dysfunction in mice. J Pharmacol Exp Ther. 297:629–637.

Reed DR, Li S, Li X, Huang L, Tordoff MG, Starling-Roney R, Taniguchi K, West DB, Ohmen JD, Beauchamp GK, Bachmanov AA. 2004. Polymorphisms in the taste receptor gene (Tas1r3) region are associated with saccharin preference in 30 mouse strains. J Neurosci. 24:938–946.

Smith DV, Li C. 2000. GABA-mediated corticofugal inhibition of taste-responsive neurons in the nucleus of the solitary tract. Brain Res. 858:408–415.

St John SJ, Spector AC. 1998. Behavioral discrimination between quinine and KCl is dependent on input from the seventh cranial nerve: implications for the functional roles of the gustatory nerves in rats. J Neurosci. 18: 4353–4362.

Steiner JE. 1979. Human facial expressions in response to taste and smell stimulation. Adv Child Dev Behav. 13:257–295.

Steiner JE, Glaser D. 1984. Differential behavioral responses to taste stimuli in hon-human primates. J Hum Evol. 13:709–723.

Stornetta RL, Rosin DL, Wang H, Sevigny CP, Weston MC, Guyenet PG. 2003. A group of glutamatergic interneurons expressing high levels of both neurokinin-1 receptors and somatostatin identifies the region of the pre-Botzinger complex. J Comp Neurol. 455:499–512.

Suaudeau C, do-Rego JC, Costentin J. 2005. Modifications in avoidance reactions of mice, on a second exposure to the hot plate, resist to various amnesia-inducing treatments. Brain Res Cogn Brain Res. 25:339–347.

Takeuchi HA, Masuda T, Nagai T. 1994. Electrophysiological and behavioral studies of taste discrimination in the axolotl (Ambystoma mexicanum). Physiol Behav. 56:121–127.

Tizabi Y, Mastropaolo J, Park CH, Riggs RL, Powell D, Rosse RB, Deutsch SI. 1998. Both nicotine and mecamylamine block dizocilpine-induced explosive jumping behavior in mice: psychiatric implications. Psychopharmacology (Berlin). 140:202–205.

Travers JB, Grill HJ, Norgren R. 1987. The effects of glossopharyngeal and chorda tympani nerve cuts on the ingestion and rejection of sapid stimuli: an electromyographic analysis in the rat. Behav Brain Res. 25:233–246.

Travers JB, Urbanek K, Grill HJ. 1999. Fos-like immunoreactivity in the brain stem following oral quinine stimulation in decerebrate rats. Am J Physiol. 277:R384–R394.

Travers JB, Yoo JE, Chandran R, Herman K, Travers SP. 2005. Neurotransmitter phenotypes of intermediate zone reticular formation projections to the motor trigeminal and hypoglossal nuclei in the rat. J Comp Neurol. 488:28–47.

Travers SP. 2002. Quinine and citric acid elicit distinctive Fos-like immunoreactivity in the rat nucleus of the solitary tract. Am J Physiol Regul Integr Comp Physiol. 282:R1798–R1810.

Travers SP, Hu H. 2000. Extranuclear projections of rNST neurons expressing gustatory-elicited Fos. J Comp Neurol. 427:124–138.

Travers SP, Norgren R. 1995. Organization of orosensory responses in the nucleus of the solitary tract of rat. J Neurophysiol. 73:2144–2162.

Travers SP, Travers JB. 2005. Reflex topography in the nucleus of the solitary tract. Chem Senses. 30 (Suppl 1):i180–i181.

Whitney G, Harder DB. 1994. Genetics of bitter perception in mice. Physiol Behav. 56:1141–1147.

Zarrindast MR, Habibi M, Borzabadi S, Fazli-Tabaei S, Hossein Yahyavi S, Rostamin P. 2002. The effects of dopamine receptor agents on naloxone-induced jumping behaviour in morphine-dependent mice. Eur J Pharmacol. 451:287–293.

Accepted October 5, 2006