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Glossopharyngeal Nerve Transection Does Not Alter Taste Reactivity to Sucrose Conditioned to be Aversive

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Abstract

Glossopharyngeal nerve (GL) transection in rats is known to markedly reduce gaping, a stereotypical aversive oromotor behavior, in response to intraorally delivered quinine. In this experiment we tested whether GL transection would reduce gaping in response to an otherwise palatable stimulus (sucrose) but conditioned to be aversive. Sprague–Dawley rats were implanted with intraoral cannulae. Five received bilateral transection of the GL and five served as sham-operated controls. Water-deprived rats were presented with 0.3 M sucrose for 15 min immediately followed by an injection of 0.15 M LiCl on three occasions. Rats were then habituated to the taste reactivity chamber and intraoral fluid infusion for 3 days, and tested on day 4 with a 1 ml infusion (1 min) of 0.3 M sucrose. All rats drank negligible amounts of sucrose by the third conditioning session and there were no differences in sucrose intake between the groups. There were no significant differences in gapes, or any other measured oromotor response, to sucrose between GL-transected and sham-operated rats. These results show that the GL is not a necessary afferent limb for gaping in response to conditionally aversive taste compounds.

Introduction

The glossopharyngeal nerve (GL) innervates taste buds on the posterior tongue, which account for >60% of the total taste bud population in the oral cavity of the rat. Despite the pronounced innervation of taste buds by the GL, its role(s) in taste function in the rat remains unclear. Many taste-related behaviors associated with a variety of chemical compounds to which the GL responds, including quinine and sucrose (Pfaffmann *et al.*, 1967; Frank, 1991; Dahl *et al.*, 1997), are unaffected by transection of the nerve alone in the rat (St John and Spector, 1998).

One of the few well-established behavioral consequences of GL transection, however, is a marked reduction in the frequency of gapes elicited by intraoral quinine infusion (Travers *et al.*, 1987; Grill *et al.*, 1992; King *et al.*, 1999b). The gape is a member of a class of reflex-like oromotor and somatic responses collectively referred to as taste reactivity. Taste reactivity behaviors are stereotypically elicited by chemical compounds that come into contact with oral sensory receptors and can be divided into those associated with ingestion (ingestive responses) and those associated with rejection (aversive responses) of the taste stimulus (Grill and Norgren, 1978).

The effect of GL transection on quinine-elicited gaping has led to the hypothesis that the nerve may play a role in protective oromotor reflexes in general (Nowlis, 1977; Frank, 1991; Grill *et al.*, 1992; St John and Spector, 1998). At issue is whether the GL input is a critical component of the afferent limb of the gape response, regardless of whether the aversiveness of the taste stimulus is conditioned or unconditioned. In other words, it is possible that normal taste-induced gaping requires stimulation of the GL.

Accordingly, we tested whether GL transection would affect gapes elicited by sucrose after this normally palatable taste compound was used as a conditioned stimulus in a LiCl-induced taste aversion procedure.

Materials and methods

Subjects

Ten naive male Sprague–Dawley rats (Charles River) weighing 220–270 g were housed individually in wire mesh cages in a colony room where the temperature and lighting were controlled automatically. Subjects were fed laboratory chow (Purina 5001; PMI Nutrition International Inc., Brentwood, MO) and received distilled water *ad libitum*, except where noted otherwise.

Surgery

After a 5 day period of adjustment to the laboratory conditions, rats were randomly assigned to either the control or the experimental group. All rats were anesthetized (125 mg/kg body wt ketamine, 5 mg/kg body wt xylazine, i.m.) and two intraoral (IO) cannulae were surgically implanted anterolateral to the first maxillary molar on each

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Table 1 Taste aversion conditioning procedure

	Habituation days	Trial 1	2 Days	Trial 2	2 Days	Trial 3
a.m. session (15 min)	H_2O	CS → LiCl injection	H_2O	CS → LiCl injection	H_2O	CS → LiCl injection
p.m. session (60 min)	H_2O	H_2O	H_2O	H_2O	H_2O	H_2O

side so that taste stimuli could be infused directly into the mouth [for surgical details see Grill *et al.* and King *et al.* (Grill *et al.*, 1987; King *et al.*, 1999a)]. The five rats assigned to the experimental groups also had the GL transected (GLX) bilaterally at the time of cannulae implantation. An ~10 mm portion of the GL, starting at a point where the nerve travels along the external medial wall of the bulla and moving distally, was exposed, cut and removed. The other five rats, assigned to the control group, had their GL nerves exposed, as described above, but not transected (CON) [for more details on GL surgical procedure see St John and Spector (St John and Spector, 1998)]. After surgery, all rats were allowed to recover for at least 10 days. During this recovery time, both cannulae were cleaned daily with fine metal wire and the area around them was disinfected with Betadine and alcohol.

Taste aversion conditioning

The taste aversion conditioning phase took place over 10 days. All rats were deprived of water starting 24 h prior to the first session. During this phase of the experiment, all the rats were presented with one bottle of distilled water for 15 min in the morning and 1 h in the afternoon, 5 h after the morning session. Intake was measured only during the morning session. On days 4, 7 and 10 of conditioning the water was replaced with 0.3 M sucrose during the morning session. Intraperitoneal injection of 0.15 M LiCl (2.0 mequiv./kg body wt) immediately followed the sucrose intake test. The 1 h water session in the afternoon remained unchanged throughout this phase of the experiment (Table 1).

Taste reactivity

After a conditioned taste aversion to sucrose was established, taste reactivity was tested using a behavioral procedure based on that described by Grill *et al.* (Grill *et al.*, 1992). Briefly, rats were habituated for three sessions and then tested for their responses to sucrose in a cylindrical Plexiglas observation chamber. During habituation, the rats were placed in the chamber for 5 min prior to IO infusion of water for 60 s (1.0 ml over a 1 min period) via PE tubing from a syringe pump (Syringe Infusion Pump 22; Harvard Apparatus) connected to one of the cannulae. The left cannula was used unless clogged, in which case the right one was used instead. This procedure was repeated on the test day with a 1.0 ml infusion of 0.3 M sucrose in place of water. A mirror, positioned under the taste reactivity chamber, angled at 45° from the chamber floor and reflecting towards the camera, facilitated the observation of the face and mouth of the rat [for details see Grill *et al.* (Grill *et al.*, 1987)]. The oromotor responses of each rat, observed through the mirror, were videotaped (Panasonic 5100HS video camera) during this 1 min infusion and the behavior was subsequently analyzed in slow motion and frame by frame (JVC Video Recording System SR-S365U, JVC Editing Control Unit RM-G800U).

Table 2 Stereotyped taste reactivity behaviors

Ingestive behavior[a]	Aversive behavior[a]	Other
Tongue protrusions (TP)	gaping (G)	no data (ND)[b]
Lateral tongue protrusions (LTP)	chin rubbing (CR)	
Mouth movements (MM)	head shakes (HS)	
Paw licking (PL)	forelimb flails (FF)	

[a]Scored as discrete events and summed for each rat.

[b]Absence of the rat from the videorecord for at least 1 s (measured in s).

Eight stereotyped taste reactivity behaviors (Table 2) were counted and summed by an observer unaware of the rat's condition: mouth movements (MM), tongue protrusions (TP), lateral tongue protrusions (LTP), paw licking (PL), gapes (G), forelimb flails (FF), chin rubs (CR) and head shakes (HS). Total ingestive scores (TP + LTP + MM + PL) and total aversive scores (G + CR + HS + FF) were calculated for each rat (Grill and Berridge, 1985; Grill *et al.*, 1987; Spector *et al.*, 1988). At times, the view of the animal was obscured due to movement, position or focus. The duration of such episodes, lasting >1 s, was measured and rounded to the nearest second (no data, ND). In an additional analysis, the taste reactivity score was corrected for ND by dividing the summed counts of each behavior by the length of time in which the animal was clearly seen in the video record (60 – ND) and then multiplying by 60. This correction allowed for a fairer comparison between the stereotyped behaviors within the measured time (60 s) for the two groups, by using the rate of behavioral occurrence to adjust for seconds in which behavior might have occurred but could not be measured.

Histology

Following all behavioral testing, the rats were deeply anesthetized and perfused with isotonic saline followed

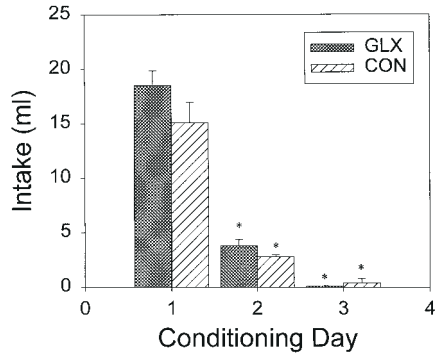

Figure 1 Sucrose one-bottle intake (in ml) on the three LiCl conditioning days (means ± SE) of GLX rats (cross-hatched bars) and CON rats (hatched bars). The asterisk indicates intake which is significantly different ($P < 0.05$) than intake on the first day of conditioning.

by buffered formalin (10%). The circumvallate papilla (CV) of each rat was removed, embedded in paraffin, sectioned (10 μm) and stained with hematoxylin and eosin. To confirm GL nerve transection, a person unaware of a rat's specific treatment counted taste pores under a light microscope.

Data analysis

Taste pores in the CV papillae from the CON and GLX groups were compared using a *t*-test in order to determine if GL transection was successful.

An analysis of variance (ANOVA, group × trial) was conducted on the one-bottle intake measures taken during conditioning trials. The data were examined for changes in intake over trials (within subjects) and for differences in intake between the two groups of rats (between subjects) in a 2-way ANOVA to reveal the effect of GL transection and conditioning on acquisition of the taste aversion.

The means of the counts for each oromotor behavior and the means for the total ingestive and aversive scores were compared between the two groups (CON and GLX) using a *t*-test. These oromotor behaviors were also compared by the same method after adjustment for ND. The conventional *P* value of 0.05 was used to determine significance in all statistical tests.

Results

Histology

Examination of tissue sections under a light microscope revealed that the CV papillae from CON ($\overline{x} = 403.5 \pm 26.74$) rats contained significantly more taste pores than those from GLX rats ($\overline{x} = 0.60 \pm 0.60$, one case was eliminated due to partial tissue loss; $t(7) = 17.15$, $P < 0.001$, *t*-test). No GLX rat had more than three taste pores in the CV papilla. Thus, successful GL transection was confirmed.

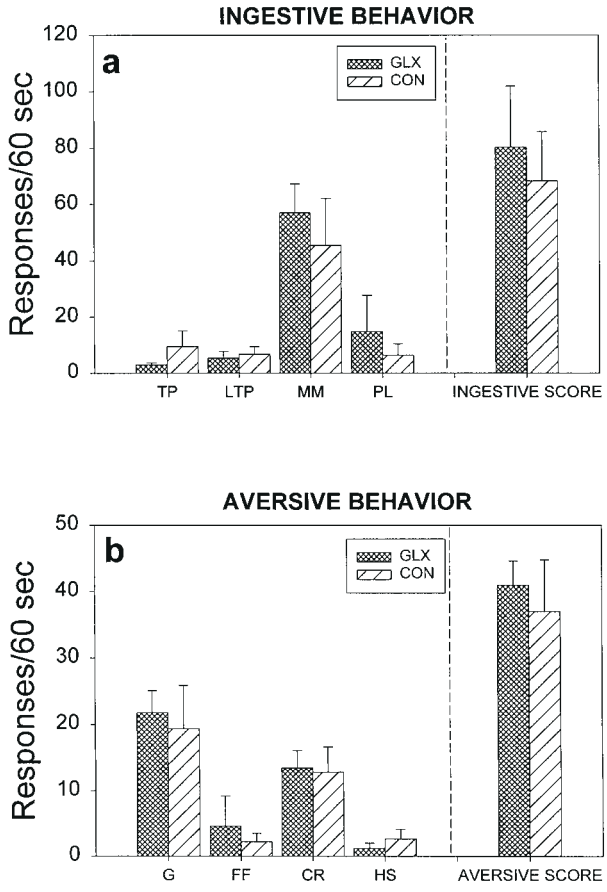

Fig. 2 Scored individual taste reactivity oromotor behaviors (means ± SE) for GLX rats (cross-hatched bars) and control rats (hatched bars) and a total score for **(a)** ingestive behaviors and **(b)** aversive behaviors. Ingestive behaviors scored were tongue protrusions (TP), lateral tongue protrusions (LTP), mouth movements (MM) and paw licks (PL). Aversive behaviors scored were gapes (G), forelimb flails (FF), chin rubs (CR) and head shakes (HS). The bars on the right of the dashed line represent the total scores for ingestive behaviors (TP + LTP + MM + PL) or aversive behaviors (G + CR + HS + FF). There were no statistically significant differences between the two groups.

Taste aversion conditioning

Transection of the GL did not affect the acquisition of conditioned taste aversion by the GLX group. Sucrose intake did not significantly differ between water-deprived GLX and CON groups [$F(1,8) = 2.67$, $P = 0.141$, ANOVA] (Figure 1), but it did significantly decrease across sessions [$F(2,16) = 165.06$, $P < 0.001$, ANOVA]; in both groups, intake of sucrose was significantly higher on the first trial day than in the second and third trials [all $t(4) > 6.89$, all P values ≤ 0.002] (Figure 1).

Taste reactivity

In both groups of rats, ingestive as well as aversive oromotor behaviors could be seen during IO sucrose infusion. The mean number of individual ingestive oromotor behaviors counted in both groups of rats can be seen in Figure

Table 3 Literature survey of taste reactivity procedures used to measure quinine-induced gaping

	No. of test sessions	Habituation	Highest conc. tested	Time before infusion	Infusion length	Infusion rate or amount	No. of gapes in shams	No. of gapes in GLX
Present study	1	3 days	0.3 M sucrose (conditioned)	5 min	1 min	1 ml/min	19	22
(Travers *et al.*, 1987)	3–18	–	3 mM quinine	–	1 min	1 ml/min or 50 μl	~12	~5
(Grill *et al.*, 1992)	12 (only 1 for 3 mM quinine)	3–5 days	3 mM quinine	5–10 min	1 min	1 ml/min	~16	~5
(King *et al.*, 1999b)	1	3 days	3 mM quinine	1 h	30 min	0.233 ml/min	32	8

2a and the aversive behaviors can be seen in Figure 2b. No significant differences were found between the two groups for total ingestive (Figure 2a) or aversive scores (Figure 2b) or for individual oromotor responses [all $t(8) < 0.81$, all P values > 0.25, t-test], including gapes. Of the 60 s measured, the mean time in which the rat was out of view or its response could not be measured (ND) was 10.2 ± 2.3 s. The correction for ND did not affect the statistical outcomes [all $t(8) < 0.6$, all P values > 0.35, t-test].

Discussion

The significant decrease in sucrose intake over the three conditioning trials displayed by rats from both the CON and GLX groups indicates that a conditioned taste aversion to this stimulus was acquired in both groups. This is, to our knowledge, the first demonstration of a sucrose taste aversion in GL-transected rats. It is interesting to note, therefore, that there was no significant difference in the rate of acquisition between rats that had the input from the GL (CON) and those that did not (GLX). This can be explained by the contribution of input from the chorda tympani (CT) and greater superficial petrosal (GSP) branches of the seventh nerve (and perhaps the superior laryngeal nerve), which remained intact. The GSP in particular, which innervates palatal taste buds, is highly responsive to sugars in the rat (Nejad, 1986). Although the GL innervates ~64% of the total taste buds in the oral cavity (Miller, 1977) and 20% of single taste fibers sampled in the GL respond well to sugars (Frank, 1991), the input provided by this nerve is apparently unnecessary for the successful acquisition of a conditioned taste aversion to 0.3 M sucrose as assessed by a single-bottle intake test.

Nowlis (Nowlis, 1977) proposed two separate mammalian oral reflex systems, one mediating ingestive behavior with the CT nerve as its 'afferent limb' and the other mediating aversive behavior with the GL nerve as its 'afferent limb'. Extending the earlier work of Travers *et al.* (Travers *et al.*, 1987), Grill *et al.* (Grill *et al.*, 1992) showed that the GL and the CT are indeed associated with the two classes of taste-elicited oromotor responses in a manner resembling Nowlis' hypothesis, because transection of the GL nerve significantly reduced aversive responses to taste stimuli without a change in ingestive responses, while transection of the CT led to the converse. Nowlis' hypothesis was tempered, however, by the fact that transection of either nerve did not eliminate any one oromotor behavior completely; both aversive and ingestive oromotor behavior could be seen in either GL-transected or CT-transected rats. Thus, Grill *et al.* (Grill *et al.*, 1992) suggested that neither nerve exclusively controls either class of oromotor responses. Nevertheless, the fact remains that GL transection does lead to striking reductions in the frequency of quinine-elicited gapes (Travers *et al.*, 1987; Grill *et al.*, 1992; King *et al.*, 1999b).

The fact that CON and GLX rats still displayed some ingestive responses to sucrose after three taste aversion conditioning trials is not unusual. For example, Breslin *et al.* (Breslin *et al.*, 1992) showed that sucrose-elicited ingestive responses progressively decreased while aversive responses concomitantly increased over the course of three taste aversion conditioning trials. Importantly, TPs were the most sensitive behavior dropping to minimal numbers after a single sucrose–LiCl pairing. Indeed, our rats displayed relatively few TPs in response to this normally 'palatable' solution. Nevertheless, in both the latter study (Breslin *et al.*, 1992) and ours, ingestive behavior was not entirely eliminated. Moreover, ingestive scores are sometimes even higher than aversive scores elicited by either conditionally or unconditionally aversive stimuli (depending on stimulus concentration and conditioning parameters) because the duration of the behavioral components comprising the ingestive class are shorter and tend to be generated in longer bursts (especially TP and MM). It is difficult to say more on this issue regarding the present data set because a NaCl-injected control group was not included in the design. Rather, our focus was on the effectiveness of GL transection to attenuate gape occurrence to a conditioned stimulus. Nevertheless, the fact that intake of sucrose dropped to negligible levels in both groups coupled with the relatively high frequency of gapes as well as other aversive responses,

which are rarely elicited by this sugar unconditionally, leave little doubt that a robust taste aversion was evident in both groups of rats.

A distinct reduction in the occurrence of quinine-induced gaping as a consequence of GL transection is well documented in the literature, despite differences in procedures (Table 3) (Travers *et al.* 1987; Grill *et al.*, 1992; King *et al.*, 1999b). Recent work in our laboratory (King *et al.*, 1999b) suggests that recovery of normal levels of quinine-elicited gaping in GL-transected rats is entirely dependent on regeneration of the nerve. Furthermore, a relationship appeared to exist between the number of quinine-elicited gapes and the number of CV taste buds ($r = 0.81$, $n = 12$), a finding that supports the role of the GL in oromotor rejection responses to unconditionally aversive taste stimuli. In contrast, our results show that a 'palatable' stimulus (sucrose) conditioned to be aversive produces clear rejection behavior, including gaping, that is not significantly altered by GL transection. Thus, the input of the GL is not necessary for normal taste-elicited aversive behavior to be expressed and the well-established decrease in quinine-elicited gaping in GL-transected rats must depend on the interaction between the specific stimulus properties of the alkaloid and the neurotomy.

The failure of GL transection to affect the acquisition and expression of a conditioned taste aversion to sucrose in rats adds to the growing list of taste-related behavioral tasks in which performance is unaltered by the neurotomy. Based on their results and on a survey of findings in the literature, St John and Spector (St John and Spector, 1998) speculated, as have others (Atema, 1971; Nowlis, 1977; Finger and Morita, 1985; Travers *et al.*, 1987; Frank, 1991; Grill *et al.*, 1992; Caprio *et al.*, 1993; Spector *et al.*, 1996), that the gustatory nerves are functionally specialized. They suggested that the GL might play a greater role in protective oromotor rejection responses, whereas the input of the gustatory branches of the seventh facial nerve may be more involved in qualitative recognition and discrimination of taste stimuli. It is possible that the GL does indeed significantly contribute to reflex-like rejection responses for a variety of taste compounds that are unconditionally aversive, but the nerve does not seem to play a critical role in the rejection responses elicited by taste compounds that are conditionally aversive.

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