The Necessity of the Glossopharyngeal Nerve in the Maintenance of Normal Intake and Ingestive Bout Size of Corn Oil by Rats

Yada Treesukosol, Ginger D. Blonde, Enshe Jiang, Dani Gonzalez, James C. Smith, Alan C. Spector

Department of Psychology & Program in Neuroscience, Florida State University, Tallahassee FL, USA

Running Head: Glossopharyngeal nerve transection and corn oil intake

Correspondence to be sent to:
Dr. Alan C. Spector
Department of Psychology
Florida State University
1107 West Call Street
P.O. Box 3064301
Tallahassee FL 32306-4301
Email: spector@psy.fsu.edu

Office: 850-645-7883
Fax: 850-644-7739
Abstract

Recent evidence in the literature suggest that signals carried by the glossopharyngeal nerve (GL), which supplies sensory and parasympathetic innervation of the posterior tongue, might be essential in the maintenance of normal gustatory responses to fat stimuli. Here, we report that GL transection (GLX) significantly decreased corn oil intake and preference in 23-h 2-bottle tests relative to sham-operated controls (SHAM). Drinking-pattern analysis of corn oil licking revealed that bout size, rather than the number of bouts initiated was lower in GLX compared with SHAM rats. We also tested a range of glucose concentrations and found that total licks over daily 23-h sessions significantly decreased in GLX rats compared with SHAM rats but this difference failed to reach significance when intake or any bout parameter was measured. These results show that the signals in the GL normally contribute to processes involved with corn oil bout termination as opposed to bout initiation. GL-derived signals could potentially provide input to “reward” circuits in the ventral forebrain that could serve to maintain ingestion during a meal or alternatively could act at the level of the brainstem to attenuate the inhibitory potency of vagal signals, thus delaying the onset of satiation, or perhaps contribute to a cephalic phase reflex modulation of the gut. In addition, parasympathetic efferents in the GL innervating the von Ebner glands which secrete lingual lipase thought to breakdown corn oil into detectable ligands could also be playing a role in driving corn oil intake. Whatever the mechanism, the presence of an intact GL is clearly necessary in maintaining normal intake of corn oil.

Keywords: fat taste, gustatory system, licking, meal patterns, drinking patterns
Introduction

The taste bud cells of the posterior tongue are innervated by the glossopharyngeal nerve (GL). This accounts for approximately 60% of the total taste bud population in the oral cavity of the rat, yet the role of the GL in taste function remains to be completely understood. Transection of the GL (GLX) appears to have little effect, if any, on a range of taste-related behaviors. Psychophysically assessed detection thresholds for quinine (52) and NaCl (3), salt discrimination (10), and concentration-dependent licking responses to quinine (50), sucrose, and maltose (48) measured presurgically in brief-access tests, were only marginally affected after GL transection, if at all. Although transection of the chorda tympani nerve, which innervates taste buds in the anterior tongue, alone or in combination with the greater superficial petrosal nerve, which innervates taste buds in the palate, substantially disrupts performance on several taste discrimination tasks tested in rats (20, 47, 51), transection of the GL does not (46-47, 51). It also appears that the GL is not necessary for maintaining normal sodium-specific, taste guided behavior (33). Collectively, these findings suggest that input from the remaining taste afferents is sufficient in maintaining a wide variety of taste-related behaviors in the absence of the GL despite that this nerve innervates the majority of taste buds in the rat oral cavity.

Although transection of the GL is without effect on performance in many taste-based tasks, this is not to say that removal of the nerve is benign from the standpoint of all taste-evoked responses. The GL does appear to be involved in unconditioned taste-elicited oromotor rejection reflexes to quinine – a stimulus that humans report as “bitter” and rats avoid. The frequency of quinine-stimulated gaping is reduced by GL transection, whereas transection of the chorda tympani nerve, which innervates taste buds of the anterior tongue, has little effect (24, 53). In gustatory relay areas of the brain stem,
characteristic patterns of quinine-stimulated neuronal Fos expression in the nucleus of the solitary tract (26) and the parabrachial nucleus (29) are eliminated by transection of the GL (31, 54), whereas transection of the CT has much less effect (31). Behaviorally, transection of the GL was shown to moderately attenuate intake of a novel quinine solution during a 45-min drinking test by decreasing lick volume and burst size in water-restricted rats (49). Furthermore, it has been shown that rats without presurgical experience with quinine display moderately shifted concentration-response functions after transection of the GL compared to controls (34). Quinine-evoked gaping is normal in rats when the CT cross-reinnervates the posterior tongue in the absence of the GL, but is severely suppressed when the GL cross-reinnervates the anterior tongue in the absence of the CT. This suggests that the effects of GL transection on certain quinine-evoked CNS and behavioral responses are due to the exceptional signal generated by the posterior tongue taste receptors, regardless of which nerve innervates them, upon application of this aversive stimulus (30). This conclusion is consistent with the dense expression of T2Rs in this oral taste receptor field (8). Nevertheless, as noted above, some taste-related behaviors involving quinine are not affected much by gustatory deafferentation of the posterior tongue.

A comprehensive understanding of the role of the GL in taste function is wanting. Recently, however, several lines of evidence suggest that signals carried by this nerve might be essential in the maintenance of normal gustatory responses to fat stimuli which brings us to the focus of the present report. First, the posterior tongue taste cells express both CD36, a lipid-binding transporter, which has been postulated to play a role in free-fatty-acid taste transduction (18-19, 32) and GPR120 (6, 35-36) and GPR40 (6), which are G-protein coupled fatty acid receptors thought to play a role in sensing dietary fat.
Second, the von Ebner’s glands, innervated by the GL and found at the base of the circumvallate and foliate papillae, secrete lingual lipase into the trenches (17, 25). Ingested fats are thought to be rapidly broken down by lingual lipase within 1-5 seconds (27). Thus, it is possible that transection of the GL would disrupt the oral hydrolysis of triacylglycerol. Third, transection of the GL in mice has been shown to decrease preference for 2% linoleic acid, a long chain polyunsaturated fatty acid known to block delayed rectifying K⁺ channels in a subset of taste bud cells (21-22), in a 48-h two-bottle test (19). Fourth, in this same study, mice lacking the GL displayed a decreased pancreato-biliary response to oral application of linoleic acid (19).

Accordingly, the purpose of this study was to test whether rats that had bilateral transections of the GL nerve would decrease their intake of corn oil, a triacylglycerol composed of predominantly long chain polyunsaturated fatty acids, across a range of concentrations presented in a series of 23-h two-bottle tests (vs. water). Expecting an effect of the neurotomy, which as will be shown was confirmed, we sought to determine whether the decrease was due to decreases in the number of ingestive bouts (i.e., meals), the size of those bouts, or both. Having such knowledge would allow us to determine whether the loss of GL input was affecting processes that terminate meals or processes that initiate them. As a source of comparison we also tested a range of concentrations of a preferred non-lipid caloric stimulus, glucose.
Materials and Methods

Subjects

Sixteen male Sprague-Dawley rats (Charles River Breeders, Wilmington, MA) with a mean weight of 324 ± 3.0 g at the time of surgery, were individually housed in polycarbonate cages with some modifications described below. Rats were housed in a room with automatically controlled temperature (21.9 – 22.6 °C), humidity (34 – 69%) and a 12 h light/ 12 h dark lighting cycle. Powdered laboratory chow (Purina Laboratory Chow 5001, St. Louis MO) and deionized reverse-osmosis water were provided ad libitum. All experimental protocols were approved by the Animal Care and Use Committee of Florida State University.

Surgery

Rats were anesthetized with a mixture of ketamine hydrochloride (125 mg/kg body weight) and xylazine hydrochloride (5 mg/kg body weight) delivered via intramuscular injection. Supplemental doses were administered as necessary. For rats receiving glossopharyngeal nerve transections (GLX), the sublingual and submaxillary salivary glands and the sternohyoid, omohyoid and posterior belly of the digastric muscles were retracted. The fascia below the hypoglossal nerve was dissected near the external medial wall of the bulla, exposing the GL. Approximately 10 mm of the GL was cut and removed. For rats receiving sham surgery (SHAM), the GL was exposed similarly to GLX surgeries, but the GL was not disturbed. In both surgical groups, procedures were conducted bilaterally and the incision was closed with sutures. Penicillin G Procaine suspension (30,000 units sc) and Ketorolac Tromethamine (2 mg/kg body mass sc) were administered
for 3 days following surgery. At least ten days were allowed for recovery before rats were tested with novel taste stimuli.

**Behavioral procedure**

Rats received GLX (n=8) or SHAM (n=8) surgery and were placed in specially designed cages described elsewhere (see 43) with some modifications. These cages allow continuous monitoring of chow and fluid ingestion in 6-s time bins over 23-h daily test sessions. Powdered laboratory chow (Purina Laboratory Chow 5001, St. Louis MO) was provided *ad libitum* in a food jar placed in the feeding compartment of the cage. As the rat enters the feeding area, its head breaks an infrared beam and these beam-break signals were recorded by a computer. On the other side of each cage is a stainless steel rack that holds two fluid bottles. Licks on the drinking spouts activated an electronic contact circuit which was registered by a computer.

Baseline measures for food and water intake from two bottles were taken for at least four days before surgery. At least ten days were allowed for recovery from surgery before the rats were tested with novel taste stimuli presented at room temperature. For testing, one bottle was filled with the taste solution, prepared fresh with deionized reverse osmosis water daily, and the other with deionized reverse-osmosis water. The bottles were rinsed daily and then refilled with fresh solutions. Test compounds consisted of 0.06, 0.12, 0.25, 0.5 and 1.0 M glucose (BDH Chemicals, West Chester, PA) and 1, 2, 4, 8 and 16% (v/v) corn oil (J.M. Smucker Company, Orrville, OH). The corn oil solutions were prepared by blending corn oil with 5 ml of the emulsifier Tween 80 (Sigma Aldrich, St. Louis, MO) in 1000 ml mixtures for at least 2-min before presentation. Although with the use of the emulsifier the corn oil went into solution, over time there was some partial
separation of the oil and water. Nevertheless, separation of corn oil mixed in this fashion appears to reach an asymptote at about 1.5 h (45) and most importantly all of the animals were treated identically.

In the two bottle test between glucose and water, the glucose was presented across the days in ascending order of concentration. At the end of the glucose testing, the rats were presented two days of water alone in both of the bottles. This was followed by the two bottle test between corn oil and water. Again, the concentration of the corn oil was presented in ascending order across days. Each concentration of glucose and corn oil was tested for 2 consecutive days. Intake was measured and the position of the bottles for each daily test was determined by an ABBA order across 4 day blocks.

Data analysis

A feeding bout was operationally defined as starting with a 3 s beam-break of the food jar hopper and lasting at least 30 s. A drinking bout was operationally defined as requiring a minimum of 3 licks for initiation and at least 30 licks thereafter. An interval of 5 min or more of no beam-breaks (for feeding bouts) or licking (for drinking bouts) defined the termination of an ingestive episode. Feeding and drinking bouts often overlapped in time. These bout criteria have been successfully used in previous studies (eg. 1, 7, 9, 16, 44) and accounted for approximately 97% of the feeding data and approximately 99% of the licking data for chow and fluid respectively on average in the present study.

In addition to intake and preference, the total number of licks, the number of bouts, bout licks (mean number of licks per bout) and bout rate (number of bout licks divided by bout length) were compared using a two-way ANOVA (group x concentration). The mean data for each of the two sessions for a given concentration were averaged and used as
scores in the statistical analysis ($\alpha=.05$). When a significant interaction was observed, pair-wise comparisons at each concentration were conducted using the false discovery rate procedure (2).

**Histology**

At the conclusion of the experiment, rats were deeply anesthetized with sodium pentobarbital and transcardially perfused with saline followed by 10% buffered formalin. The tongue was removed from each rat and placed in 10% buffered formalin. The circumvallate papilla was embedded in paraffin and sectioned on a rotary microtome. These 10-μm sections were mounted on slides and stained with hematoxylin and eosin. The circumvallate papillae sections for each tongue were analyzed under a light microscope by an observer unaware of the surgical condition of the rat to determine the presence or absence of intact taste buds. It is quite clear when the GL supply to the tongue has been interrupted because virtually the entire epithelium of the circumvallate papilla is devoid of taste buds; normally there are hundreds of taste buds. If very few intact taste buds were present, the number was quantified. If a GLX rat had more than 30 taste buds in the circumvallate papilla, it was discarded from the behavioral analysis (this did not occur). When numerous taste buds were observed in a given circumvallate papilla, they were not counted. The primary purpose of the histology was to simply confirm that GL transections were complete and regeneration had not occurred.
Results

Histology

Rats in the GLX group had very few taste buds in the circumvallate papilla when compared with SHAM rats. The mean number of taste buds in GLX rats was 1.8 ± 0.8 and the range was 0 - 11. In contrast, the circumvallate papillae of SHAM rats were replete with many taste buds. These results indicate that the nerve transections were successful and that no significant regeneration occurred.

Glucose

Accompanying results of ANOVAs for glucose concentration-response functions for intake, licking and the various meal patterns can be found in Table 1. Mean values for glucose intake were lower in GLX rats compared to SHAM rats, but did not reach significance (upper graph of Figure 1; Table 1). The total number of licks to glucose over daily 23-h sessions, however, was significantly lower in the GLX group compared with SHAM rats (lower graph of Figure 1; Table 1). A two-way ANOVA revealed a main group effect for preference but based on the significant group x concentration interaction this appears to be due to slightly lower preferences observed for the GLX group relative to the SHAM group only at the lower concentrations (top left graph of Figure 2; Table 1). Nevertheless, even at the lower concentrations, pair-wise comparisons did not reveal significant group differences. From the nature of the curves, the lower total number of licks to glucose observed for the GLX compared with the SHAM rats is more likely attributed by a decrease in the number of licks during a bout (bout licks) rather than by a decrease in the number of bouts across the daily sessions (bottom left and top right graphs of Figure 2). A two-way ANOVA for mean bout licks, however, failed to reveal any
significant differences between the two groups. There was also no group difference for
mean bout lick rate which was calculated by dividing the number of licks in a bout by the
bout length (bottom right graph of Figure 2). Thus, across the concentration range,
although there was evidence that preference for low concentrations was slightly attenuated
and total number of licks for glucose was significantly lowered by the absence of GL input,
this did not translate into significant differences in intake, or in any drinking pattern
parameter measured.

Water intake during glucose sessions decreased as glucose concentration
increased. A two-way ANOVA (Group x Concentration) revealed a main effect of
concentration but not a main effect of group nor a significant interaction effect (middle
graph of Figure 1; Table 2). Particularly at the higher concentrations of glucose, licking to
water was negligible and in most cases did not meet the criteria for bout analysis.

Chow intake did not significantly differ between the two groups during glucose
testing (Table 2), but did significantly decrease for both groups as glucose concentration
was raised (upper graph of Figure 3). Accordingly, the animals in both groups appeared to
attempt to regulate their calories, but SHAM rats showed significantly higher intake of total
calories from chow and glucose (middle graph of Figure 3; Table 2).

Although the body weight of rats in the GLX group did not significantly differ from
those in the SHAM group on the day of surgery, these animals were significantly lighter on
average compared with their controls during the glucose testing phase (lower graph of
Figure 3, Table 2). This is likely due to the initial drop in body weight in the GLX rats in the
days soon after surgery and although GLX rats recovered their body weight they never
caught up with SHAM rats. Nevertheless, because the body weight was different between
the two groups during glucose testing, we conducted the above analyses with the scores
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divided by body weight. In general, this did not change the basic effects, but the main
effect for an increase for total caloric intake failed to reach significance when scores were
divided by body weight.

Corn Oil

In contrast to what was observed for glucose, the effects of GL transection on
intake (Figure 4), preference, and licking pattern parameters (Figure 5) for corn oil were
much more striking (Table 3). Transection of the GL significantly decreased intake (top
graph of Figure 4), total licks (bottom graph of Figure 4), and preference (top left graph of
Figure 5) across the range of corn oil concentrations presented. As corn oil concentration
increased, the number of bouts decreased for both surgical groups and there was no
significant group difference (top right graph of Figure 5; Table 3). The lower corn oil intake
in GLX rats compared with their SHAM controls appears to be due to a significantly lower
bout size (number of licks per bout) rather than a group difference in the number of bouts.
Because there was an effect of concentration and a significant group x concentration
interaction with respect to bout size, we ran separate one-way ANOVAs across
concentrations for each group separately. This analysis revealed that the SHAM animals
significantly increased their corn oil bout size across concentration (F(4,28)=7.478,
p<0.001), whereas there was no effect of concentration in the GLX group (F(4,28)=0.340,
p=0.848), a finding consistent with the flat shape of the concentration-response function.
Pair-wise comparisons revealed a significant difference between the groups for bout size
at the highest corn oil concentration presented.

Interestingly, there was also no significant difference between the groups in the
mean bout lick rate suggesting that there was no substantial difference in how vigorously
the animals consumed the corn oil during bouts (bottom right graph of Figure 5, Table 3). In fact, although there was a tendency for corn-oil concentration to increase bout lick rate especially in the SHAM rats, there was no significant main effect of concentration nor was the interaction between group and concentration significant. Accordingly, corn oil did not seem to vary its potency to maintain uninterrupted licking by rats during bouts as a function of concentration in this testing context.

A two-way ANOVA (Group x Concentration) for water intake during corn oil sessions did not reveal a main effect of group, concentration nor a significant interaction effect (middle graph of Figure 4; Table 4).

Chow intake did not significantly differ between the two groups (Table 4) during corn oil testing (top graph of Figure 6) and remained relatively unchanged across varying corn oil concentrations. Thus, unlike what was seen during glucose testing, there was no evidence that SHAM rats attempted to regulate their caloric intake. The outcomes of the two-way ANOVA on total caloric intake indicates that the concentration-dependent increase in the SHAM group was greater than in the GLX group based on the significant main effects and interaction. Pair-wise comparisons conducted at each concentration revealed group differences in total caloric intake at 4% and 16% corn oil. Nevertheless, separate repeated-measures one-way ANOVAs conducted demonstrated that both groups increased their total caloric intake as corn oil concentration was raised (SHAM (F(4,28)=12.759, p<0.001); GLX (F(4,28)=5.153, p=0.003)).

Similar to body weights during glucose testing, GLX rats were significantly lighter on average compared with their controls during the corn oil testing phase (lower graph of Figure 6, Table 4). As was done for the analysis from the glucose testing data, we
conducted the above analyses with the scores divided by body weight. This did not change the basic significance of the outcomes of statistical tests.
Discussion

Recent evidence in the literature suggests that signals carried by the glossopharyngeal nerve might be essential in the maintenance of normal gustatory responses to fat stimuli. Here, GL transection resulted in a decrease in both intake and preference for corn oil across a range of concentrations in a long-term two-bottle test. These findings are consistent with a study in which mice with GL transection showed lower intake and preference for linoleic acid, a fatty acid component of corn oil, compared with controls (19). In the present study analysis of patterns of ingestion revealed that bout licks, but not number of bouts or mean bout lick rate (calculated by dividing the number of licks in a bout by the bout length), to corn oil were lower in GLX rats compared with their sham-operated counterparts. These findings indicate that it is not the number of bouts initiated but rather how long the bout is maintained and when it is terminated that contributes to the decreased corn-oil intake in the nerve-transected animals. Moreover, because bout number dropped in the SHAM animals across concentration as well, the stability of corn oil intake in that group during those tests was attributable to the counteracting increase in bout licks.

As a comparison, we also tested a range of concentrations of a preferred non-lipid caloric stimulus, glucose. The total number of licks to glucose over daily 23-h sessions was significantly decreased in GLX rats compared with their SHAM controls, but the concomitant decrease in total intake failed to reach statistical significance. The transection-induced drop in licking did not appear to be due to a decrease in the number of bouts initiated or bout lick rate but rather a decrease in the number of licks in a bout. However, unlike the striking effect of GL transection on corn oil, these various ingestive pattern parameters for glucose were not significantly different between the two groups.
Thus, overall, the effect of GL transection on glucose licking was quite marginal relative to the robust effect the neurotomy had on corn oil ingestion.

For corn oil, the results clearly point to bout size as being the central feature of the ingestive pattern that was sensitive to our manipulations of concentration and peripheral gustatory input. It is generally well accepted that at least three factors can influence meal size. First is the physiological state of the animal at the start of a meal. Food deprived animals will eat a larger meal when food first becomes available. During ongoing ingestion with unlimited food access, however, the physiological status of the animal from meal to meal becomes more complex because a given meal can modulate internal state. Second is the strength and temporal trajectory of inhibitory signals from the gut as a result of the accumulating postingestive load during the bout. Third, are the excitatory signals arising from the oral cavity and other head receptor systems such as olfaction (see 11, 12-14).

Within this framework, by definition, the transection of the GL virtually eliminates signals from the posterior tongue. Whether the critical signals are of a taste or somatosensory origin remains undetermined because the GL is a mixed nerve carrying afferent input that contributes to both gustatory and trigeminal pathways (see below for more discussion). Also, the central fate of these GL-derived signals from the standpoint of processing related to the control of corn oil intake remains unclear. One possibility would be that they simply contribute excitatory input to “reward” circuits in the ventral forebrain known to be influenced by preferred taste compounds (see 38). Another interesting possibility, however, is that the signals act at the level of the brainstem by exerting an inhibitory influence on inhibitory signals (i.e., double negative) arising from vagal input associated with the growing postingestive load during a bout. If the latter were true, then one would expect GL transection to have little effect on corn oil intake in a sham drinking
paradigm – a testable prediction based on the outcomes of our experiment. Finally, although speculative, it is also possible that GL transection changes postingestive digestive processing of corn oil by removing the afferent limb of cephalic phase reflex modulation of the gut; this could potentially promote earlier satiation or perhaps even mild aversion.

As noted above, the effects of GL transection may be related to the elimination of somatosensory or taste input from the posterior tongue, but it also might be related to the removal of parasympathetic efferents innervating the von Ebner’s glands. In rats, these glands secrete lingual lipase, thought to play a role in breaking down ingested fats, into the trenches of the circumvallate and foliate papillae (17) (27). Parasympathetic fibers in the GL supply the von Ebner’s glands (28), thus it is possible that GL transection disrupts lingual lipase production or secretion. This would, in turn, disrupt the breakdown of corn oil into free fatty acids for detection by taste receptor cells innervated by uncut nerves thus contributing to the lowered intake and preference of corn oil observed in GLX rats in the present study. In a short-term two bottle preference test, rats show a preference for dietary triacylglycerol, thought to be broken down into free fatty acids by lingual lipase, over xanthan gum solution and mineral oil (27). Lingual lipase inhibition by orlistat, an inhibitor of digestive lipases, reduced triacylglycerol preference. Rats also showed a preference for free fatty acids over the other oils, but lingual lipase inhibition had little effect on the preference for free fatty acids (27). These reported findings are consistent with the hypothesis that in the present study, GLX resulted in decreased corn oil preference, in part, due to a decrease in lingual lipase secretion.

Transection of the GL results in the loss of taste buds in the posterior tongue. Indeed, in the present study, this was confirmed using histology. Thus, even if lingual
lipase production was not disrupted, there would not be taste receptor cells in the posterior tongue for the free fatty acids to stimulate. These cells have been shown to express CD36 (18-19, 32), GPR120 (6, 35-36) and GPR40 (6), all of which are thought to play a role in sensing dietary fat. Accordingly, GL transection results in the removal of receptor cells that appear to play a role in fatty acid reception. This may lead to a severe attenuation of the signals sent to the nucleus of the solitary tract and its downstream pathways, ultimately resulting in a decrease in intake as observed in the GLX group of the present study.

Finally, the GL not only carries gustatory signals from the posterior tongue but also provides somatosensory input to the brain (4-5). The texture of corn oil is a salient feature of the stimulus (eg. 15). Transection of the GL would therefore also result in the reduction of somatosensory signals from the posterior tongue and provide another explanation for the decreased response to corn oil observed in GLX rats. These possible explanations of the effect of GL transection on corn oil intake discussed above are not necessarily mutually exclusive. In fact, it is likely a combination of various factors that lead to the effect observed in GLX rats.

In the current study, at least for glucose, preference in the two-bottle test was lower for GLX rats only at the lower concentrations, and thus it does not appear that GL transection substantially alters the hedonic component (related to ingestive motivation) of the stimulus. On the other hand, preference ratios for glucose in both groups were high and thus it is possible that in the absence of a ceiling effect, group differences for preferences at the higher concentrations could have been observed. Nevertheless, it is fundamentally clear that the effect of the neurotomy was far more robust on the ingestion of corn oil than glucose. Evidence in the literature indicates that interlick intervals or licks to water do not significantly differ between rats that received GLX surgery or SHAM
surgery (48, 50) suggesting the decrease in intake observed in GLX rats in the present study is not attributable to any substantial motor impairment. Rather, there appears to be some degree of chemospecificity associated with the effect of GL transection on the ingestion of caloric fluids. The scope of this specificity remains to be investigated.
Perspectives and Significance

The findings discussed here interestingly connect two vexing questions that have confronted gustatory researchers. First, what is the role of the GL in taste function, and, second, is there a fat taste quality? Although GL transection does produce some deficits in some taste-related behaviors, such as a reduction in quinine-stimulated gaping, for the most part, the removal of this nerve, which innervates the majority of taste buds in the rat oral cavity, is without functional consequence. As detailed above, the case for the existence of fat taste has been growing but a clear understanding of its perceptual characteristics has been elusive. The results presented here show that the GL is critical in maintaining normal ingestive responses to corn oil. Whether this effect is attributable to a loss of taste or somatosensory input, changes to postingestive digestive processing of corn oil, or is due to removal of parasympathetic innervation of von Ebner’s glands remains to be determined, but, nonetheless, a link between the function of the glossopharyngeal nerve and oil ingestion is clear, at least in the rat. It would be instructive for investigators to establish the boundaries of this linkage by testing the consequences of GL transection on the ingestion of different types of fat stimuli. The characterization of the multi- and single-fiber response properties of the glossopharyngeal nerve to a panel of various oils and fatty acids applied to the posterior tongue could provide insight into the nature of potential peripheral signals generated by such stimuli (see 6). Finally, more detailed psychophysical approaches might help define the sensory properties of fat stimuli in both intact and gustatory nerve-transected animal models (see 23, 37, 39-42).
Acknowledgements

We would like to thank Lee Carella, Melissa Isaacs, Steven Janasik and Anthony Muller for their technical help in this experiment. Portions of this work were presented at the Annual Meeting of the Society for the Study of Ingestive Behavior, Paris, France July, 2008. Supported by NIH R01-DC01628 (A.C.S.)
References


Figure captions

Fig 1: Mean (±SE) intake (top) to glucose as a function of concentration (top graph) and water (middle graph) for SHAM (closed symbols) and GLX (open symbols) groups. Mean (±SE) total licks (bottom) to glucose as a function of concentration for SHAM (closed circles) and GLX (open circles) groups.

Fig 2: Mean (±SE) preference (top left), number of bouts (top right), bout licks (bottom left) and mean bout lick rate (bottom right) to glucose as a function of concentration for SHAM (closed circles) and GLX (open circles) groups.

Fig 3: Mean (±SE) chow intake (top), total calories from chow and glucose (middle) and body weight (bottom) as a function of available glucose concentration for SHAM (closed circles) and GLX (open circles) groups.

Fig 4: Mean (±SE) intake to corn oil as a function of concentration (top graph) and water (middle graph) for SHAM (closed symbols) and GLX (open symbols) groups. Mean (±SE) total licks (bottom) to corn oil as a function of concentration for SHAM (closed circles) and GLX (open circles) groups.

Fig 5: Mean (±SE) preference (top left), number of bouts (top right), bout licks (bottom left) and mean bout lick rate (bottom right) to corn oil as a function of concentration for SHAM (closed circles) and GLX (open circles) groups. Because there was a significant interaction for bout licks, pairwise comparisons were conducted using the False Discovery
Rate procedure, * denotes significant difference between the groups at the signified concentration.

Fig 6: Mean (±SE) chow intake (top), total calories from chow and corn oil (middle) and body weight (bottom) as a function of available corn oil concentration for SHAM (closed circles) and GLX (open circles) groups. Because there was a significant interaction for total calories, pairwise comparisons were conducted using the False Discovery Rate procedure, * denotes significant difference between the groups at the signified concentration.
Table 1: Two-way ANOVA values for bout pattern analysis measures to glucose

<table>
<thead>
<tr>
<th>Measure</th>
<th>Group</th>
<th>Concentration</th>
<th>Group x Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intake</td>
<td>$F(1, 14) = 4.450$, $p = 0.053$</td>
<td>$F(4, 56) = 32.678$, $p &lt; 0.001$</td>
<td>$F(4, 56) = 0.765$, $p = 0.553$</td>
</tr>
<tr>
<td>Total Licks</td>
<td>$F(1, 14) = 5.541$, $p = 0.034$</td>
<td>$F(4, 56) = 31.142$, $p &lt; 0.001$</td>
<td>$F(4, 56) = 0.696$, $p = 0.598$</td>
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<tr>
<td>Preference</td>
<td>$F(1, 14) = 6.495$, $p = 0.023$</td>
<td>$F(4, 56) = 41.475$, $p &lt; 0.001$</td>
<td>$F(4, 56) = 3.511$, $p = 0.013$</td>
</tr>
<tr>
<td>Total Number of Bouts</td>
<td>$F(1, 14) = 0.100$, $p = 0.757$</td>
<td>$F(4, 56) = 12.886$, $p &lt; 0.001$</td>
<td>$F(4, 56) = 0.278$, $p = 0.891$</td>
</tr>
<tr>
<td>Bout licks</td>
<td>$F(1, 14) = 2.725$, $p = 0.121$</td>
<td>$F(4, 56) = 15.285$, $p &lt; 0.001$</td>
<td>$F(4, 56) = 0.731$, $p = 0.574$</td>
</tr>
<tr>
<td>Bout rate</td>
<td>$F(1, 14) = 0.012$, $p = 0.914$</td>
<td>$F(4, 56) = 5.134$, $p &lt; 0.001$</td>
<td>$F(4, 56) = 0.552$, $p = 0.698$</td>
</tr>
</tbody>
</table>
Table 2: Two-way ANOVA values for measures during glucose sessions

<table>
<thead>
<tr>
<th></th>
<th>Group</th>
<th>Concentration</th>
<th>Group x Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chow intake</strong></td>
<td>$F(1, 14) = 2.826, F(4, 56) = 76.016, F(4, 56) = 0.728,$</td>
<td>$p = 0.115$</td>
<td>$p &lt; 0.001$</td>
</tr>
<tr>
<td></td>
<td>$F(1, 14) = 2.791, F(4, 56) = 31.164, F(4, 56) = 2.247,$</td>
<td>$p = 0.117$</td>
<td>$p &lt; 0.001$</td>
</tr>
<tr>
<td><strong>Water intake</strong></td>
<td>$F(1, 14) = 8.392, F(4, 56) = 115.508, F(4, 56) = 0.826,$</td>
<td>$p = 0.012$</td>
<td>$p &lt; 0.001$</td>
</tr>
<tr>
<td></td>
<td>$F(1, 14) = 6.062, F(4, 56) = 263.783, F(4, 56) = 1.402,$</td>
<td>$p = 0.027$</td>
<td>$p &lt; 0.001$</td>
</tr>
</tbody>
</table>
Table 3: Two-way ANOVA values for bout pattern analysis measures to corn oil

<table>
<thead>
<tr>
<th></th>
<th>Group</th>
<th>Concentration</th>
<th>Group x Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intake</td>
<td>F(1, 14) = 6.512, p = 0.023</td>
<td>F(4, 56) = 0.217, p = 0.928</td>
<td>F(4, 56) = 1.186, p = 0.327</td>
</tr>
<tr>
<td>Total Licks</td>
<td>F(1, 14) = 5.622, p = 0.033</td>
<td>F(4, 56) = 0.998, p = 0.417</td>
<td>F(4, 56) = 1.463, p = 0.226</td>
</tr>
<tr>
<td>Preference</td>
<td>F(1, 14) = 6.061, p = 0.027</td>
<td>F(4, 56) = 0.373, p = 0.827</td>
<td>F(4, 56) = 1.987, p = 0.109</td>
</tr>
<tr>
<td>Total Number of Bouts</td>
<td>F(1, 14) = 0.013, p = 0.909</td>
<td>F(4, 56) = 2.574, p = 0.047</td>
<td>F(4, 56) = 0.826, p = 0.515</td>
</tr>
<tr>
<td>Bout licks</td>
<td>F(1, 14) = 5.072, p = 0.041</td>
<td>F(4, 56) = 3.259, p = 0.018</td>
<td>F(4, 56) = 5.063, p = 0.001</td>
</tr>
<tr>
<td>Bout rate</td>
<td>F(1, 14) = 0.30, p = 0.846</td>
<td>F(4, 56) = 2.344, p = 0.066</td>
<td>F(4, 56) = 1.274, p = 0.291</td>
</tr>
<tr>
<td></td>
<td>Group</td>
<td>Concentration</td>
<td>Group x Concentration</td>
</tr>
<tr>
<td>------------------</td>
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<td>---------------</td>
<td>-----------------------</td>
</tr>
<tr>
<td>Chow intake</td>
<td>( F(1, 14) = 1.288, ) ( p = 0.258 )</td>
<td>( F(4, 56) = 1.832, ) ( p = 0.136 )</td>
<td>( F(4, 56) = 1.017, ) ( p = 0.406 )</td>
</tr>
<tr>
<td>Water intake</td>
<td>( F(1, 14) = 3.722, ) ( p = 0.074 )</td>
<td>( F(4, 56) = 0.295, ) ( p = 0.880 )</td>
<td>( F(4, 56) = 2.055, ) ( p = 0.099 )</td>
</tr>
<tr>
<td>Total calories</td>
<td>( F(1, 14) = 12.732, ) ( p = 0.003 )</td>
<td>( F(4, 56) = 17.414, ) ( p &lt; 0.001 )</td>
<td>( F(4, 56) = 5.208, ) ( p = 0.001 )</td>
</tr>
<tr>
<td>Body weight</td>
<td>( F(1, 14) = 4.795, ) ( p = 0.046 )</td>
<td>( F(4, 56) = 148.558, ) ( p &lt; 0.001 )</td>
<td>( F(4, 56) = 0.880, ) ( p = 0.482 )</td>
</tr>
</tbody>
</table>