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Otolaryngology -- Head and Neck Surgery 2011 144: 915 originally published online 1 March 2011
DOI: 10.1177/0194599811399722

The online version of this article can be found at:
http://oto.sagepub.com/content/144/6/915
Effects of Sensory or Motor Nerve Deafferentation on Oromotor Function in Mice

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Sponsorships or competing interests that may be relevant to content are disclosed at the end of this article.

Abstract
Objective. To investigate the effect of sensory or motor nerve damage to the tongue using a mouse model.

Study Design. Animal study.

Setting. Research laboratory.

Subjects and Methods. Adult male and female mice from inbred strains B6 (n = 19) and D2 (n = 25). Following lick training, bilateral lingual–chorda tympani nerve cuts (LX) (n = 6 B6, n = 7 D2), unilateral hypoglossal nerve cuts (HX) (n = 7 B6, n = 9 D2), or sham surgery (n = 6 B6, n = 9 D2) was performed. Mice were lick tested postsurgically with both water and sucrose (4 days total). Following testing, post mortem dissections and microscopic analysis of tongue papillae were performed.

Results. In both strains, HX and LX mice demonstrated a significant reduction in volume per lick (VPL) in the surgical groups relative to shams. Neither motor nor sensory nerve transection affected local lick rate. In most LX mice in both strains, taste papillae were reduced compared with HX or sham mice.

Conclusion. Mice of either strain with either a sensory or a motor nerve injury have a significant loss of VPL during ingestion of either a neutral (water) or preferred (sucrose) stimulus. This reduction in VPL reflects a deficit in licking. Lick rate was not affected by deafferentation. A reduction in fungiform papillae following LX but not HX mice was noted.

Keywords
nerve transaction, oromotor, mice, tongue

Received September 16, 2010; revised January 13, 2011; accepted January 18, 2011.

Patients with head and neck cancer treated with radiotherapy commonly experience impairments of swallowing function, with reported incidence ranging from 50% to 100%.1 Documented findings include significantly reduced tongue strength, poor pharyngeal motility with subsequent pharyngeal residue, worse oropharyngeal swallow efficiency, epiglottic immobility, reduced laryngeal excursion, poor closure of the laryngeal vestibule, and altered duration of cricopharyngeal sphincter opening and aspiration (which is often silent).1 Patients who experience dysphagia often limit their oral intake as a result of these problems. Patients undergoing targeted chemoradiation therapy for advanced head and neck cancer lose about 10% of their pretreatment weight,2 which is consistent with patients who receive radiation therapy alone. This population also experiences a decline in the ability to eat, with an increase in reported swallowing difficulties and an increased need for percutaneous endoscopic gastrostomy tubes (26%).2 Patients who lose greater than 10% of their pretreatment body weight are considered to be at risk for malnutrition, and a weight loss above 20% may be associated with an increase in morbidity and mortality and poorer quality of life.3

Dysphagia in patients with head and neck cancer is multifactorial and has been evaluated in patients undergoing surgical ablation, radiation therapy alone, conventional chemoradiotherapy, and chemoradiotherapy protocols using intra-arterial chemotherapy.4 The contribution of oropharyngeal fibrosis and xerostomia in posttreatment dysphagia has been well established. In contrast, the contribution of decreased sensory and motor input to the tongue in post-treatment functional outcome has not been extensively studied. If the variables of xerostomia and pharyngeal dysphagia are eliminated, does disruption of sensory or motor innervation of the oral tongue result in significant weight loss or change in oral intake patterns?

Rodents, including laboratory mice, are a desirable model for examining the effects of sensory and motor...
deafferentation on oral behaviors. In both humans and rodents, the tongue receives motor innervation from the hypoglossal nerve and sensory innervation from branches of the trigeminal, facial, and glossopharyngeal nerves. On the anterior tongue, somatosensory information is carried centrally via the lingual branch of the trigeminal nerve, and taste information is transmitted via the chorda tympani (CT) branch of the facial nerve.

Studies in rodents have provided basic information about behavioral and anatomic changes following sensory and motor deafferentation of the tongue. However, few such studies have been conducted with mice, a species of critical importance to preclinical research given its advantages in terms of genetic and molecular techniques. Taste nerve transection has been used commonly in studies with rats to investigate the contribution of different taste bud populations to gustatory behavior. Concerning motor nerve injury, either bilateral or unilateral section of the lateral branches of the hypoglossal nerve leads to a reduction in water intake in young rats. In the current study, we examined the possible effects of sensory or motor denervation on fluid licking behavior to both neutral (water) and highly preferred (0.1 M sucrose) solutions in inbred mice. Fluid licking in mice can be easily quantified and is hence used as a surrogate for intake and eating behaviors in humans. Like mastication, suckling, or swallowing, licking is thought to be under the control of 1 or more central pattern generators (CPGs), intrinsic neural circuits responsible for generating rhythmic muscle movements. A tenet of CPG function is that the rhythmic muscular output is controlled independent of sensory inputs. In the current experiment, we performed combined lingual/chorda transections (deafferentation of the anterior tongue, thereby eliminating sensory feedback at the tongue–bottle spout contact) in 2 strains of inbred mice that differ in their native lick rate. C57BL/6J (B6) mice have a slower lick rate than do DBA/2J (D2) mice. Finding phenotypic differences between inbred strains indicates genetically based effects; therefore, examining potential effects in these strains will offer insights into the genetic basis of not only oromotor behavior but also susceptibility to neural damage. We also tested mice with a unilateral hypoglossal nerve transection, which should produce paralysis on 1 side of the tongue, also possibly affecting tongue–spout contact. We hypothesized that sensory and motor nerve damage impairs intake in mice and that there are genetically based effects (ie, strain differences) in degree of impairment.

Methods

Animals

Data were collected from adult male and female mice from inbred strains C57BL/6J (B6; n = 19) and DBA/2J (D2; n = 25). The median age of mice prior to testing was 103 days. Mice were generally age and weight matched between strains, weighing 19.5 to 38.9 g at the beginning of testing. The Animal Care and Use Committee at UTHSC approved this study, and all experiments were carried out in accordance with the National Institute of Health Guide for Care and Use of Laboratory Animals (NIH Publications No. 80-23), revised 1996.

Prior to testing, all mice were housed in plastic home cages with ad libitum food and water. Fresh bedding was provided, and water bottles were removed from the cages of singly housed mice, approximately 23 hours prior to testing in a lickometer. Thereafter, during the experiment, fluid was available during daily lickometer tests, with supplements available after testing on several days (see below).

Lick Testing

All licking behavior was measured using a computer-controlled lickometer (Davis MS-160, DiLog Instruments, Inc, Tallahassee, Florida). The apparatus and its operation have been described previously. Consumption during each session was measured by weighing bottles before and after on an analytic balance to the nearest hundredth of a milligram. Preoperatively, all mice were lick tested with deionized water for 2 consecutive days in the MS-160 (one 20-minute session per day). Following the preliminary test, water was returned to the home cage, and the mice rested 1 to 2 weeks prior to surgery. Five days following surgery, mice were again placed on water restriction and lick tested for 4 consecutive days, one 20-minute session per day. For the first 2 sessions, mice were tested with water; for the final 2 the mice were tested with 0.1 M sucrose. To limit weight loss due to water restriction, mice were given water supplements after testing on days 2 and 3 (testing was completed on day 4).

Surgery

Mice from either strain were placed into 1 of 3 groups: bilateral lingual nerve cuts (n = 6 B6, 7 D2), unilateral hypoglossal nerve cuts (n = 7 B6, 9 D2), or sham surgery (n = 6 B6, 9 D2). Mice were anesthetized (intraperitoneally) with ketamine/zylazine (86/13 mg/kg). The hypoglossal nerve was approached through a horizontal anterior neck incision in the skin in the space between the right and left angles of the mandible. Blunt dissection was used to visualize the strap muscles and the anterior belly of the digastric muscle. The sternohyoid muscle was retracted laterally, and the hypoglossal nerve was noted traveling in a superomedial direction just medial to the sternohyoid muscle and lateral to the thyrohyoid muscle. Microscissors were used to remove a 1- to 2-mm section of the hypoglossal nerve. The side on which to cut the nerve was chosen at random. The lingual nerve was approached through the lateral submental space. A 5- to 8-mm incision was made parallel to the body of the mandible. Blunt microdissection was used to divide the fibers of the mylohyoid muscle at its midpoint between the symphysis of the mandible and the angle of the mandible, and the nerve was found adjacent to the mandible where it coursed inferiorly and then anteriorly to travel toward the anterior tongue. A 1- to 2-mm section of
the lingual/CT nerve trunk was removed. Sham mice were prepared in the same way as the HX group, but only blunt dissection to loosen the skin from the underlying musculature was performed. Following each type of surgical procedure, the skin and subcutaneous layers were closed in 1 layer using 3-0 silk, antibiotic ointment was applied, and 0.3 mL of sterile NaCl was injected subcutaneously.

**Postmortem Analysis**

Post mortem dissections were performed on all mice (1-20 days after behavioral testing) to verify that nerve cuts were successful. Following this, tongues were excised, rinsed, and stained with 0.5% methylene blue for approximately 90 seconds; rinsed again; and placed between 2 glass slides to flatten the tongue slightly for microscopic inspection. One of the authors blindly quantified fungiform taste papillae on the anterior tongues from the anterior margin of the median eminence to the tongue tip. Taste pores were counted as small blue dots in the center of fungiform papillae.

**Analysis**

Licking behavior data collected in each 20-minute test session was reported as total licks, total fluid intake (mL), and volume per lick (VPL; intake/total licks). To determine lick rate, we calculated the mean interlick interval (ILI) between 50 and 160 milliseconds. The ILI is the elapsed time between 2 lick contacts, and the majority of these in a given train of licks occur in this time window. Intervals longer than 160 milliseconds indicate longer pauses between bouts of licking. Behavior and weight data were analyzed using 3-way analysis of variance (ANOVA), with between-subjects factors for strain and group (surgery) and a within-subjects factor for test period. For VPL there were no significant effects of test period or strain, so this measure was compared. There were no significant differences in initial body weight (prior to onset of water restriction) between sham, HX, or LX. All mice progressively lost body weight across the 4-day test period, from about 89% of pretest weight on test day 1 to 86% on test day 4. However, no group (or strain) differences were found, indicating that all mice were equally able to consume food and water.

**Results**

**Pretest Findings**

Prior to surgery, licking behavior was measured in mice for 2 consecutive days (one 20-minute session per day) with water to establish baseline values. Lick counts, intake (mL), VPL, and lick rate in male and female B6 or D2 mice were extremely similar to values previously reported, collected in an identical manner. Essentially, lick counts averaged over 2 days differed significantly between strains, but not sexes, with B6 mice exhibiting higher mean counts (588.4 vs 406.3). A similar pattern of results was found for intake. Mice of both strains and sexes had a similar VPL, with a mean value for B6 mice of 1.16 μL and for D2 1.22 μL. There were highly significant differences in lick rate between strains, with B6 mice showing higher mean ILIs (116.1 milliseconds vs 92.9 milliseconds), but not between sexes.

**Posttest Findings**

Mice from each strain were grouped according to surgery (HX, LX, sham), and licking behavior across 2 consecutive days with water and 2 consecutive days with 0.1 M sucrose was compared. There were no significant differences in initial body weight (prior to onset of water restriction) between sham, HX, or LX. All mice progressively lost body weight across the 4-day test period, from about 89% of pretest weight on test day 1 to 86% on test day 4. However, no group (or strain) differences were found, indicating that all mice were equally able to consume food and water.

There was a general trend for HX and LX mice to lick more water or sucrose than sham mice in all sessions (Table 1). For intake, however, significant effects were not found between surgical groups (Table 1), indicating that all mice drank similar amounts of fluid during the test. If lick counts to water or sucrose are elevated in mice receiving hypoglossal or lingual nerve transections, yet consumption levels are more or less equivalent, then there must be differences in the amount of fluid animals obtain with each lick.

### Table 1. Mean Number of Licks and Mean Intake (mL) for Each Strain and Surgical Group in Each Test Period Post Surgery

<table>
<thead>
<tr>
<th>Strain</th>
<th>Group</th>
<th>Licks$^a$</th>
<th>Intake$^b$</th>
<th>Licks$^a$</th>
<th>Intake$^b$</th>
<th>Licks$^a$</th>
<th>Intake$^b$</th>
<th>Licks$^a$</th>
<th>Intake$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>B6</td>
<td>Sham</td>
<td>412.17</td>
<td>0.54</td>
<td>685.83</td>
<td>0.90</td>
<td>1285.5</td>
<td>1.63</td>
<td>1157.5</td>
<td>1.56</td>
</tr>
<tr>
<td></td>
<td>HX</td>
<td>893.57</td>
<td>0.55</td>
<td>1294.86</td>
<td>0.83</td>
<td>2173.43</td>
<td>1.29</td>
<td>2027.57</td>
<td>1.41</td>
</tr>
<tr>
<td></td>
<td>LX</td>
<td>766.83</td>
<td>0.41</td>
<td>1782.33</td>
<td>0.94</td>
<td>2452.67</td>
<td>1.62</td>
<td>2013.67</td>
<td>1.38</td>
</tr>
<tr>
<td>D2</td>
<td>Sham</td>
<td>386.33</td>
<td>0.44</td>
<td>641.56</td>
<td>0.78</td>
<td>1023.78</td>
<td>1.26</td>
<td>1102.78</td>
<td>1.56</td>
</tr>
<tr>
<td></td>
<td>HX</td>
<td>455.00</td>
<td>0.37</td>
<td>785.78</td>
<td>0.63</td>
<td>1196.78</td>
<td>0.93</td>
<td>1605.56</td>
<td>1.22</td>
</tr>
</tbody>
</table>

The first water test (day 1) occurred 6 days after surgery for all mice.

$^a$For licks, significant main effects for strain, surgical group, and test period (Fs $> 6.2$; Ps $< .02$) and significant strain × test period interaction ($F_{3,114} = 8.0$; $P < .0001$).

$^b$For intake, significant main effects for strain and test period (Fs $> 5.3$; Ps $< .03$) and significant strain × test period interaction ($F_{3,108} = 3.49$; $P < .02$).
Indeed, VPL was significantly decreased in the surgical groups relative to shams (Figure 1). The strains did not differ significantly from each other in direct comparisons within each surgery group. These results suggest that the major consequence of either sensory or motor denervation of the tongue is an attenuation of not overall intake but rather intake efficiency—in other words, compromised mice had to lick more times to ingest the same amount of fluid.

Lick rates, that is, mean ILIs (milliseconds), were also compared among each strain and surgery group (Figure 2). Motor or sensory nerve transection did not significantly affect lick rate. Mice of all surgery groups demonstrated mean lick rate values very close to strain presurgical values, and B6 mice had a slower lick rate than D2 mice, regardless of surgery.

**Distribution of Taste Papillae**

Counts of fungiform papillae and taste pores on the anterior tongue were determined following postmortem verification of nerve transections (Figures 3 and 4). Notably, in most of the LX mice, taste papillae were reduced compared with HX or sham mice. A limitation of the count results is that the postmortem measurements were not made at a standard time point following surgery and behavioral testing; rather, they were made during an interval ranging from 11 to 31 days, with the mean time in each group/strain ranging from 15.4 to 19.8 days post surgery. Nonetheless, in both strains, there was a significant decrease in number of fungiform papillae in the LX group. B6 LX mice possessed significantly fewer fungiform taste papillae than sham but not HX mice (Figure 4). In the D2 strain, mice undergoing lingual nerve transection showed a significantly greater reduction in taste papillae relative to both HX and sham mice. In neither strain did HX mice differ from sham mice, and there were no differences in number of taste papillae relative to the side of the nerve injury. When B6 LX mice were compared directly to D2 LX mice, the difference (less papillae in D2 mice) reached significance (t test; \( P < .05 \)). Generally, only a small percentage of fungiform papillae counted in any of the groups did not have stained taste pores (2%-6%), with the exception of D2 LX mice: in this group, only 60% of the remaining taste papillae had taste pores, confirming that taste bud loss after lingual nerve transection was much more dramatic in this strain.

**Discussion**

Coordination of oromotor movements, such as tongue protrusion and retraction, jaw opening and closing, and swallowing, is under the control of central pattern generators, which are motor “programs” among premotor neuron networks that send rhythmic inputs to motor neuron pools in cranial nerve nuclei V, VII, and XII.\(^{12,15}\) We found that lick rate in B6 and D2 mice did not vary as a consequence of either bilateral lingual nerve transection or unilateral hypoglossal nerve transection. This finding is consistent with a model of CPG function where the CPG is unaffected by peripheral sensory feedback.

The major consequence of both sensory and motor nerve deafferentation was an increase in number of licks relative to sham mice that occurred without a concomitant increase in consumption. We interpreted the increase in licks to be a decrease in the average VPL in mice in both surgical groups. Results of previous studies support this interpretation. Dotson and Spector\(^ {16}\) showed that either intact (nonataxic) B6 or D2 mice took nearly twice as many licks in a 30-minute session when presented with a 1.5-mm sipper tube orifice than with a 2.7-mm orifice, although neither the total amount consumed nor the lick rate was changed. The authors concluded that the mice were able to proportionally adjust their licking as a function of VPL. Bryant et al.\(^ {17}\) found a significant decrease in lick volume in ataxic cerebellectomized mice relative to controls. Chronic treatment...
of rats with haloperidol also produced a decrease in lick volume, as did the development of a conditioned taste aversion. In our experiment, despite an unaltered primary rhythm, the deafferented mice were much less efficient in their licking behavior. In general, surgical effects on oromotor behavior were similar in magnitude between the 2 inbred strains (eg, Figure 1).

The effects of combined lingual–CT nerve transection, or CT transection alone, on fungiform taste bud maintenance and regeneration have been relatively well studied in rat but not mouse models. Transection of the CT nerve causes degeneration of many, but not all, fungiform taste buds on the anterior tongue within several days, along with morphological alterations to the papillae. Combined transection generally results in more severe taste bud and papillae degeneration. This degeneration and the eventual regeneration of both nerves and taste buds depend on the species, location, and extent of nerve injury. In a recent lingual-CT transection study with B6 mice, taste bud loss was evident 5 days following surgery, and numbers of fungiform taste buds decreased to their minimum at 20 days. In particular, taste bud loss on the 1-mm “taste bud–rich” tongue tip was 50% of controls in unilaterally denervated mice. In our study we found a reduction in fungiform taste papillae in both B6 and D2 mice following lingual cuts, with a greater effect in D2 LX mice. Moreover, the ratio of taste pores to papillae was greatly reduced in this group of D2 mice relative to all other groups. These findings point to intriguing phenotypic variation in taste bud degeneration/maintenance between these strains, and further study into the genetic basis of neurotrophic interactions in the taste system is warranted.

The results of this study indicate that both sensory and unilateral motor denervation primarily affect licking efficiency in inbred mice. However, the underlying rhythmicity of licking was not affected by this discoordinated movement. Hypoglossal and lingual nerve injuries in humans can occur as a result of disease, trauma, or injury during treatment. It is clinically observed that patients lose interest in oral intake secondary to the lost of taste and to oral phase discoordination. However, confounding factors (eg, radiation therapy, discrepancy in medical care availability, poor dentition) limit interpretation when evaluating human subjects who have experienced an isolated injury to the motor or sensory supply of the tongue. Although peripheral

Figure 3. Dorsal surface of the anterior tongue of mice representative of (A) sham, (B) HX, and (C) LX mice. Taste pores are small dots in the center of fungiform papillae and reflect the presence of taste buds (inset: arrow points to pore). Scale bar = 0.5 mm.

Figure 4. Mean counts (± standard error of the mean) of fungiform papillae in both strains, in each surgical group. Asterisks are used to show significant differences among groups (P < .05; post hoc tests), which are denoted by horizontal lines. For mice of both strains, significant main effects of surgery were found (F2,16-22 > 4.89; P values < .03).
motor or sensory nerve injury in humans would be expected to cause similar decreases in oral-phase ingestive efficiency. Our results suggest that the overall coordination of rhythmic oral movements such as mastication, swallowing, and protective reflexes may not be adversely affected, as this coordination is presumably the domain of CPG circuits in the brainstem.  

Author Contributions

Courtney B. Shires, acquisition of data, interpretation of data, creation of early draft, final approval of submitted manuscript; Jennifer M. Saputra, acquisition of data, critical revision of early draft, final approval of submitted manuscript; Rose Mary S. Stocks, interpretation of data, critical revision of early draft, final approval of submitted manuscript; Merry E. Sebelik, interpretation of data, critical revision of early draft, final approval of submitted manuscript; John D. Boughter, concept and design, acquisition of data, analysis of data, critical revision of early draft for important intellectual content, creation and final approval of submitted manuscript.

Disclosures

Competing interests: None.

Funding source: This research was supported in part by NIH grant NS052366 to J.D.B, and by NIH grant NS060887 to Detlef H. Heck, UTHSC, Department of Anatomy & Neurobiology.

References


