Impact of Sensory and Motor Defects on Oral Function in an Animal Model

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Abstract

Objective. To evaluate the combined functional impact on swallowing of tongue sensory and motor loss using a rat model.

Study Design. Rats underwent selective neurectomies with transection of the motor (hypoglossal) nerve or motor and sensory (lingual) nerves. Postoperative functional parameters were followed for 2 weeks.

Setting. Translational research.

Subjects and Methods. Thirty-six adolescent male Wistar rats were divided into 4 groups: anesthetic (n = 6), sham surgery (n = 8), hypoglossal transection (n = 10), and hypoglossal and lingual transection (n = 12). Each morning on postoperative days 1 to 14, the water and food intake were quantified and the animal weighed. Two-way analyses of variance (SigmaPlot; SYSTAT, San Jose, California) were performed with factors of “group” and “postoperative day” (POD) to analyze whether a significant difference existed between water intake, pellet consumption, and weight change.

Results. The hypoglossal and lingual group consumed significantly less water during PODs 1 to 2 and significantly less food during PODs 1 to 3 than any other group. This established a significant difference in body weight between the hypoglossal and lingual group and all other groups for the duration of the study. Measured parameters in the hypoglossal group better approximated those of the control anesthetic and sham groups.

Conclusions. The addition of a sensory loss to a motor deficit involving the oral tongue results in a measurably significant difference in weight gain, a marker of function, compared with rats with only a motor deficit. Additional studies are needed to determine if there would be similar findings in a model of sensate vs asensate oral tongue reconstruction.

Keywords
glossectomy, rat, oral function, surgery, consumption
small animal model, which could more closely simulate the clinical scenario, we feel that the first step in modeling the impact of sensation on oral function would be in "sensory subtraction" from 1 of the 2 cohorts. Although it is extremely difficult to extrapolate to humans from this preliminary pilot experiment in a small animal model, it may shed light on the potential impact that sensation could have in human oral tongue reconstruction. Positive findings of this study could justify a subsequent larger study using an animal model with longer follow-up. Furthermore, without this solid preclinical evidence, it is difficult to justify a human trial.

Materials and Methods

This study was reviewed and approved by the Saint Louis University Institutional Animal Care and Use Committee. Thirty-six male Wistar rats ranging between 250 and 350 g were divided into 4 groups: anesthesia (A) (n = 6), sham surgery (S) (n = 8), hypoglossal transection (H) (n = 10), and a hypoglossal and lingual transection (H&L) (n = 12). The sample size for each surgical group was determined by power analysis to yield a significant difference of 10 g with α = 0.05 and confidence = 0.9. The A group evaluated weight gain in healthy animals. These animals received the anesthetic ketamine/xylazine (70 mg/mL and 9 mg/kg) at the same induction dose as the other groups and were allowed to recover from anesthesia without any surgical intervention. Their time from anesthetic injection to full recovery, approximately 2 hours, matched the other groups. The S group evaluated effects of surgical trauma on ability to gain weight. The surgical approach, which included anesthesia, incision, exploration, identification of the hypoglossal nerve, and wound closure, was identical to that of the hypoglossal group (H). However, in the H group, the hypoglossal nerve was transected. Finally, the hypoglossal and lingual transections in the fourth group (H&L) removed both sensory and motor innervation.

Surgical Procedure

Aseptic technique was followed. Rats were anesthetized with ketamine/xylazine. The right submandibular area was shaved and sterilized with 3 alternating swabs of alcohol and beta-hydroxybutyrate. A 2.5-cm incision was made through skin and platysma and sterilized with 3 alternating swabs of alcohol and beta-ketamine/xylazine. The right submandibular area was shaved. Rats were anesthetized with anesthesia, incision, exploration, identification of the hypoglossal nerve, and wound closure, was identical to that of the other groups. The S group evaluated effects of surgical trauma on ability to gain weight. The surgical approach, which included anesthesia, incision, exploration, identification of the hypoglossal nerve, and wound closure, was identical to that of the hypoglossal group (H). However, in the H group, the hypoglossal nerve was transected. Finally, the hypoglossal and lingual transections in the fourth group (H&L) removed both sensory and motor innervation.

Postoperative Monitoring

The following parameters were evaluated: weight, food intake, water intake, mastication/drinking behavior, and tongue integrity. All animals were single housed for the entirety of the study. Members of the study who monitored animals were not blinded with regard to the group to which the animal belonged. Animals were evaluated daily between 6 AM and 8 AM to yield the maximum weight based on the rat’s nocturnal eating behavior. Monitoring continued until postoperative day (POD) 14. The rats’ eating behavior was observed. Animals displaying signs of oral trauma (bleeding, behavior change, loss of weight) were evaluated for a bitten tongue.

Water and Food Consumption

Water and food consumption was quantified. Each animal was provided with 100 mL of water for a 24-hour period. A graduated cylinder was used to measure the amount of water remaining in each bottle. Each animal was provided with 5-g food pellets. Initially, 5 pellets (25 g) were provided daily. One additional food pellet (5 g) was provided to each animal when any one animal was found to consume all provided pellets during a 24-hour period. To calculate the pellet number for a 24-hour period, remaining pellets and partial pellets were subtracted from the original number of pellets. Animals underwent two 23-hour fasts from food only on POD 5 and POD 10. Given the steady and continuous nature of rat feeding, introduction of the 23-hour fast allowed detection of differences in ability to optimize intake between cohorts when the rats were hungrier. The food pellets were removed after the daily morning weighing and were reintroduced postweighing the following morning.

Statistical Analysis

Baseline weights of the rats ranged from 288 to 351 g. To normalize the data, percent change in weight status of each rat’s “weight change” was referenced to baseline weight, calculated for each POD, and compared among the rat groups. Two-way analyses of variance (ANOVaras) (SigmaPlot; SYSTAT, San Jose, California) were performed with factors “group” and “POD” to identify significant differences in (1) weight change, (2) water intake, and (3) pellet consumption followed by the Holm-Sidak method of post hoc analysis. Significance was set at P < .05.

Results

Analysis of water and food consumption, body weight, and the appearance of the tongue was performed.

Water Consumption

Overall, the mean water consumption of each group ranged from 40 to 47 mL, consistent with the 10 to 12 mL/100 g body weight expected for water intake in adult rats. However, water consumption did vary among the groups as well as by postoperative day (2-way ANOVA: F(3, 446) = 15.56, P < .001; F(13, 446) = 28.5, P < .001, respectively). Moreover, there was a significant interaction between

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groups and postoperative day (2-way ANOVA: $F_{(39, 446)} = 3.354$, $P < .001$). Figure 1 shows that variance in water consumption was especially evident on POD 1, when the H&L group drank significantly less than all remaining groups. Although each group receiving surgery increased water consumption on POD 2, the H&L group continued to drink significantly less. From POD 3 onward, with the exception of the fasting periods during which average water consumption decreased by 50%, there was not a significant difference in water consumption between groups.

**Food Consumption**

Throughout this study, the mean pellet number consumed across groups was 5, approximating the daily intake of growing young rats (~25 g). However, the actual pellet number consumed differed among groups (Figure 2) and among the postoperative days (2-way ANOVA: $F_{(3, 380)} = 40.65$, $P < .001$; $F_{(11, 380)} = 81.5$, $P < .001$, respectively). Moreover, a significant interaction existed between groups and postoperative day ($F_{(33, 380)} = 1.88$, $P = .003$). As seen in Figure 2, overall, food intake was lowest in the H&L group, significantly so on PODs 1 through 3. During this same time period, there was no significant difference between the H and S groups. Following POD 3, pellet consumption in all surgical cohorts was equivalent.

**Body Weight**

The course of weight change differed among the groups (Figure 3). The H&L group lost 18.8 g on POD 1. In comparison, the H group gained 0.8 g, the S group gained 7 g, and the A group lost 5 g on POD 1. Thus, during PODs 1 through 3, every group except the H&L group was able to exceed or maintain (within 5 g) their baseline weight. In fact, the weight of the H&L group was significantly lower than the other 3 groups at all postsurgical time points (2-way ANOVA: $F_{(3, 447)} = 81.086$, $P < .001$). While differences in overall weight change from PODs 1 to 14 (Figure 3) existed during the study (2-way ANOVA: $F_{(13, 447)} = 25.436$, $P < .001$) for the H, S, and A groups, the H&L group barely exceeded baseline weight at POD 14. Furthermore, the percentage of weight change from PODs 0 to 14 was significantly lower in the H&L group than all other groups (Table 1).

**Rate of weight change.** The rate of weight change differed among groups for only POD 1 (2-way ANOVA: $F_{(3, 128)} = 9.91$, $P < .001$). Post hoc analysis showed that all groups significantly differed from one another in the amount of weight lost between weight at time of surgery and 24 hours later (POD 1). The rate of weight loss was greatest in the H&L group, while both the H and S groups showed a slight positive change. After POD 1, the rate of weight gain (slope of each line) was equivalent across groups.

**Physical Exam of the Tongue**

As expected, following resection of the hypoglossal nerve, a deviation to the right was noted in both the H and H&L groups during tongue protrusion. Interestingly, a substantial subset of animals within the H&L group bit their tongues during PODs 1 to 5. At POD 5, 67% (8/12) showed evidence of a bitten tongue on physical examination: hole in the tissue, bleeding from tissue, or evidence of wound healing. By the conclusion of the study (POD 14), 12 of 12 had healed. In contrast, the H group showed no evidence of these injuries.
Discussion

Surgical management of oral cancer frequently results in a significant resection of tongue tissue with the possible result of dysfunction in the oral phase of swallowing. Surgeons reconstructing glossectomy defects have the option to reliably restore sensation to the reconstructed tongue. However, no high-level evidence exists that demonstrates that a sensate reconstruction confers a functional benefit over an asensate reconstruction. Studies claiming a functional benefit rely on subjective measures or objective outcomes in populations with heterogeneous surgical resections. Given the degree of heterogeneity of clinical surgical defects, including such factors as tumor stage, defect size, flap size, type of flap reconstruction, recipient nerve selected for sensate reconstruction, quality of neurorrhaphy, degree of sensory restoration, and the impact of adjuvant therapy, the specific impact of sensation alone on human tongue reconstruction is difficult to measure. The goal of the present study was to objectively measure the impact of the presence or absence of sensation on a hemitongue that lacked motor innervation in an animal model. The ability to objectively measure the isolated impact of sensation may shed some light on this issue in clinical oral cavity reconstruction. Careful interpretation of the findings of this study, with consideration of the limitations of extrapolating animal studies to human clinical conditions, may shed some light on the potential impact of sensory restoration in oral cavity reconstruction following major extirpative surgery.

The dramatic loss of weight during PODs 0 to 2 in the H&L group demonstrates the morbidity associated with sensory loss. During this interval, the animals had not yet developed compensatory functional strategies. This period demonstrates the significant impact of uncompensated combined sensory and motor loss and demonstrates the functional disadvantage that this combination of deficits has over motor loss alone. This weight loss was severe and far greater than the weight change observed in the H and S groups, which both gained weight during POD 1. In fact, the H and S groups closely mimicked each other with regard to weight gain for the duration of the entire study. Thus, weight of the H&L group was chronically less than that of the other groups during our study. We postulate that loss of compensatory weight gain was detrimental to the H&L group, highlighting the morbidity associated with loss of sensation. They were unable to increase their daily consumption so as to “catch up” and achieve a weight equal to the S group. This inability can be seen in the shallower slope of the H&L group at PODs 12 to 14. Although not statistically different, this gradual change may have resulted in a greater degree of difference between all other cohorts, if followed for a longer period of time. Another possibility is that they would eventually achieve the expected weight for their age but take a much longer time to attain normal body habitus. Either scenario indicates functional improvement of the H group over the H&L group or a functional disadvantage of the H&L group compared with the H group.

A possible etiology for this loss of compensatory weight gain is loss of masticatory efficiency. Kapur and colleagues studied masticatory performance in healthy subjects who underwent maxillary anesthesia or unilateral anesthesia (lingual, inferior alveolar, and maxillary) and a control group. They found that the most detrimental sensory

<table>
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<th>Group</th>
<th>% Weight Change</th>
<th>Significance</th>
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<tr>
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<td>b</td>
</tr>
<tr>
<td>S</td>
<td>8.896</td>
<td>b</td>
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<tr>
<td>A</td>
<td>16.601</td>
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Abbreviations: A, anesthesia group; H, hypoglossal transection group; H&L, hypoglossal and lingual transection group; POD, postoperative day; S, sham surgery group.

*At the conclusion of the study, the H&L group had significantly lower weight change compared with remaining groups.

Same letter denotes no significant difference.
loss occurred with total unilateral anesthesia intraorally. Our study differs in that a lesion was specifically made to the lingual nerve. We postulate that unilateral anesthesia impaired localization of food in the mouth and affected the creation of a bolus necessary for successful swallowing. The H&L animals displayed the ability to accommodate this deficit and return to a rate of weight gain that was similar for the H and S groups. However, we do not know if their swallowing returned to normal. This observation warrants further evaluation with a videofluoroscopic swallow study (VFSS) as described by Lever and colleagues.11

Further evidence of accommodation is the learned ability to avoid self-inflicted tongue trauma. Hara et al12 investigated the impact of a transected hypoglossal nerve on jaw movement. They found only a slight impairment of mastication following bilateral severing of the hypoglossal lateral branches. In the current study, while unilateral hypoglossal transection caused protrusion of the tongue ipsilaterally to the lesion in both H and H&L groups, only the H&L rats bit their tongues. This indicates a protective functional advantage when sensation was preserved in the H group. However, after 5 days, the H&L rats appeared to accommodate this defect since no new lesions were observed. This adaptation most likely represents behavior modification.

Several limitations of this study direct future research on the topic of sensate reconstructions: (1) The authors did not blind themselves to the group designation during collection of objective measures: pellet consumption, water intake, and weight. (2) Growing adolescent rats may have confounded the use of weight change as a functional measure. (3) Rats have a known ability to continuously eat, consuming 80% of food during the night hours.13,14 It is possible that even with a large sensorimotor deficit, their behavior is only slightly affected. (4) The present results are based on small cohorts of adolescent rats followed for 2 weeks. A longer study is warranted to completely evaluate the long-term functional effect of these neural transections in the rat since the slower weight gain of the H&L group from PODs 12 to 14 leads us to believe that had we monitored these animals for a longer period of time, more of a difference possibly would have been observed. (5) Finally, implementation of objective measures of deglutition such as VFSS would improve our ability to study physiologic changes in swallowing that occur with sensory and/or motor denervation.

In conclusion, this pilot study suggests that the combined surgically inflicted loss of motor and sensory innervation to half of the oral tongue appears to have an impact on the ability to gain and maintain weight compared with those rats with only a motor deficit. The average weight of the H&L group was significantly lower than the H group for the duration of the study, and at no point were they able to demonstrate the compensatory ability to increase consumption to allow them to catch up to what should have been their normal weight. This difference was established by the dramatic loss of weight due to lower intake of water and food in the H&L group during POD 1. In contrast, the ability of the H group to eat, drink, and maintain body weight demonstrates the importance of sensation in this animal model. This pilot study suggests that the combination of both a motor and sensory deficit results in a functional disadvantage compared with the presence of only a motor deficit. Loss of motor function unilaterally causes the expected ipsilateral deviation of the tongue during protrusion but is not detrimental to weight gain. Additional preclinical studies with longer follow-up are needed to determine if these findings have applicability to human oral cavity reconstruction.

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Author Contributions
Edward J. Doyle III, design, data collection, analysis, authorship of manuscript; Michael Anne Gratton, design, data collection, analysis, authorship of manuscript; Mark A. Varvares, design, data collection, analysis, authorship of manuscript.

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