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Terminal Segment Surgical Anatomy of the Rat Facial Nerve: Implications for Facial Reanimation Study

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Abstract

Introduction—Rodent whisking behavior is supported by the buccal and mandibular branches of the facial nerve, a description of how these branches converge and contribute to whisker movement is lacking.

Methods—Eight rats underwent isolated transection of either the buccal or mandibular branch and subsequent opposite branch transection. Whisking function was analyzed following both transections.

Anatomical measurements, and video recording of stimulation to individual branches, were taken from both facial nerves in 10 rats.

Results—Normal to near-normal whisking was demonstrated after isolated branch transection. Following transection of both branches whisking was eliminated.

The buccal and mandibular branches form a convergence just proximal to the whisker-pad, named the “distal pes.” Distal to this convergence, we identified consistent anatomy that demonstrated cross-innervation.

Conclusion—The overlap of efferent supply to the whisker pad must be considered when studying facial nerve regeneration in the rat facial nerve model.

Keywords

Facial Nerve; Anatomy; Rats; Movement; Vibrissae

Introduction

Many animal models have been employed for studying facial nerve repair, grafting, and regeneration, including rat[1–4], cat[5, 6], rabbit[7], and guinea pig[8]. Over recent years, the rat facial nerve has emerged as a particularly useful model, based upon investigators' ability to highly quantitatively measure whisking recovery. Amongst the existing descriptions of rat facial nerve anatomy, there exist contradictions in precise macro- and micro architecture [9–11]. We believed that these discrepancies required clarification through anatomical dissection and functional assays, because of the significant advantages of using the rat facial nerve over other models, which include ease of surgical manipulation, ability of animals to tolerate bilateral facial paralysis, and the ability to precisely measure return of whisking function [2, 3, 12].

The rat facial nerve exits the temporal bone at the stylomastoid foramen. The first branch is the posterior auricular branch. The main trunk continues distal to that branch, and travels several millimeters until it divides into multiple branches, as shown by Semba and Egger (Figure 1)[13]. While the buccal and marginal mandibular branches have been thought to extend to the upper and lower lip region respectively, the anatomic arrangement immediately proximal to the whisker pad has not been described. Mattox et al.[11] describe the position and length of the main trunk, the furcation and main branches of the nerve, and the relevant surrounding vasculature. They included axon counts from the main trunk, and marginal and buccal branches, as well as electrophysiological data reporting compound action potentials in the upper and lower lips when the proximal nerve was stimulated at various locations. They found cross-innervation to both the upper and lower lip regions, and concluded that fibers from both of these branches lead to both the mid- and lower face. However, a gross anatomical correlate to explain these findings was not elucidated.

In the current report, we performed distal rat facial nerve dissections and branch stimulation studies, in order to more thoroughly explain the above findings, and to provide additional proof of the multiplicity of innervation to the follicles of the whisker pad. Additionally, we assess the volitional whisking function of the whisker pad following transection of the buccal and marginal mandibular branches separately and in combination.

Materials and Methods

Eighteen female Wistar-Hannover rats (Charles River Laboratories, Wilmington, Massachusetts) weighing 200 to 250 grams were used in accordance with Massachusetts Eye and Ear Infirmary guidelines for animal care and use. For all surgical procedures, rats were anesthetized with an intramuscular injection of ketamine (50 mg/kg) (Fort Dodge Animal Health, Fort Dodge, Iowa) and medetomidine hydrochloride (0.5 mg/kg) (Orion Corporation, Espoo, Finland).

Isolated branch transections and whisker evaluation

Two groups of four rats each were randomly assigned. The first group had the buccal branch of their facial nerve transected approximately 5 mm distal to the main trunk. The second group had their marginal mandibular branch transected the same distance from the main

trunk. Subsequently, the opposite branch was transected seven days following the initial transection, as depicted in Figure 2. Whisker movement was assessed preoperatively, as well as three days after isolated branch transection and 3 days after completion transection of the additional branch. The relatively short latency from the first nerve transection to whisking assessment was relatively short (three days) with the intention of minimizing the amount of collateral sprouting of residual motor supply after partial whisker pad denervation, yet providing a few days for the rats to recovery from surgery. Post-surgical whisking amplitude was reduced for the intact (control) side of the face (see Results), suggesting that rats had not fully recovered from surgery. Nevertheless, all animals produced average whisks of at least 20 degrees, providing the basis for a meaningful comparison between intact versus manipulated sides of the face.

The hardware and software used for monitoring whisker movements [12] was adapted from that described by Bermejo and colleagues [14, 15]. Movement of a single whisker (C-1) on each side of the head, marked to increase its detectability by the monitoring system, was independently tracked on the horizontal plane using two sets of commercial laser micrometers. Computer controlled air valves were used to deliver sustained flows of scented air towards the snout to elicit whisking behavior. The timing of the stimulus delivery was on a random schedule within the 5 minute testing session for each animal on each testing day. Ten-second air flows were delivered at random time points for a total of 3 air flows per testing period.

The average amplitude and velocity of the C1 whisks from onset to peak protraction was calculated for the largest three whisks recorded on each day from each side of the face. Whisking amplitude was compared within each rat before surgery, after single nerve branch transection (marginal or buccal), and after transection of the remaining nerve branch supplying the whisker pad (see Figure 2) using multiple two-tailed paired t-tests. Differences were considered statistically significantly if the p value was < 0.05 .

Facial nerve dissections

Ten rats were used for evaluation of the macroanatomy of the facial nerve. Following anesthesia induction, both right and left infra and pre-auricular areas were shaved. All microsurgical dissections were performed at 40x magnification. On each side, an incision was made and the hemifacial skin was removed up to, but not including, the whisker pad. The parotid gland was removed and the main trunk of the facial nerve was identified as it emerged from the stylomastoid foramen into the soft tissues of the neck. The facial nerve was dissected circumferentially off the soft tissues, all the way from the main trunk out to the distal entrances of nerve bundles into the muscles of the whisker pad. The length and diameter of each segment of the facial nerves were measured using digital calipers (Ultratech, General Tools, New York, NY), and photographed (see Table 1).

Facial nerve stimulation and videographic recording of whisker movement

Following the recording of all length and diameter measurements, electrical stimulation and simultaneous video recording of whisk movements were performed on the 10 right-sided nerves. Each nerve was stimulated with a bipolar nerve stimulator (Montgomery Nerve

Stimulator; Boston Medical Products, Westford, MA), set on 1 milliamp, at each of the following locations as shown in figure 3: (A) main trunk, (B) buccal branch, (C) marginal mandibular branch, (D) “distal pes”, and individual branches after the distal pes {(E) superior, (F) middle, (G) inferior}. The movement of the whisker pad was videotaped during each stimulation, using a high definition video camera (Canon HF M300). A black background was placed between the whiskers and the stimulating probe, both to improve visualization of the whisker movement, as well as to blind the observers to the location of the stimulating probe. Recordings for each stimulation point were stored as individual files for subsequent blinded rating of elicited movement.

Videographic analysis of whisking movements

Video clips were independently analyzed by three blinded observers. Custom software written in MATLAB (MathWorks, Natick, MA) presented video segments of each nerve stimulation in random order, and observers indicated on a rat anatomy illustration what face zones had elicited movement (scored as present or absent in each zone for each video clip; see Figure 4). Zones used to describe movement on videographic analysis included: upper (including C1 whisker) and lower whisker pad, nose, and the upper and lower lips. The pooled rating among observers for presence (score of 1) or absence (score of 0) of movement for each zone was calculated at each nerve stimulation location (see Table 2).

Results

Isolated Branch Transections and Whisker Evaluation

Average whisking amplitude was significantly reduced (by approximately 30%) for both the left and right sides of the face after surgery compared to average pre-surgical values ($p = 0.012$, $p = .034$, respectively), but did not differ between the left (unmanipulated) and right (lesioned) sides of the face for rats after having received either right buccal ($n=4$) or right marginal mandibular ($n=4$) nerve cuts ($p = .093$, $p = .4$, respectively). This suggests that rats were still affected by surgery at the time of whisking assessment, but that C1 whisking amplitude was not reduced by partial denervation of the right whisker pads compared to the intact left side after cutting either the buccal or marginal mandibular nerve branch (see Figure 5).

After transection of both the buccal and the marginal mandibular branches, average whisking was essentially eliminated according to our standard of requiring measured movements to achieve at least 3 degrees in order to be considered valid whisks. The group undergoing initial buccal branch transection had an average final whisking amplitude of 1.56 degrees (SD 3.13). The group undergoing initial marginal mandibular branch transection had an average final whisking amplitude of 2.46 degrees (SD 2.86) (see Figure 5).

Facial Nerve Dissections

All animals possessed consistent proximal branching and distal fusing architecture, with three terminal branches emerging from the distal pes area (Figure 6). The width and length of designated positions along the extratemporal facial nerve are presented in Table 1. The average straight-line distance from the main trunk trifurcation to the distal pes was $25.0 \pm$

1.2 mm. The average length of the distal pes was 3.0 ± 0.8 mm, and the average width of the pes was 2.3 ± 0.4 mm. The branches of the distal pes were highly consistent in width across all animals (see Table 1).

Observed Stimulation

Stimulation studies revealed considerable overlap of vibrissal movement when either the marginal mandibular, buccal, distal pes, or individual distal pes branches were stimulated (Table 2). Marginal mandibular branch stimulation resulted in observable movement 100% of the time in both the upper and lower pad areas. Additionally there was a higher rate of observable movement with the upper lip (89%) versus the lower lip (67%). Similar to the mandibular branch, the buccal branch stimulation showed high rates of observable movement in both the upper and lower pad (97% and 93%). The rates of movement in the nose, upper lip and lower lip were observed less frequently with buccal stimulation versus marginal stimulation (Table 2).

Individual distal pes branch stimulation revealed that superior branch stimulation nearly always resulted in movement of the entire whisker pad and the nose, while the middle branch had less control over the superior half of the whisker pad and nasal movement, and the inferior branch had very little upper whisker pad control and caused no nasal movement. Individual stimulation of all three distal pes branches caused movement in the lower half of the whisker pad nearly all of the time showing a greater degree of cross-innervation between distal pes branches to the lower half of the pad than to the upper half of the whisker pad.

Discussion

The rat facial nerve has many similarities to other mammals. This includes the location, number and general pattern of peripheral branching. In addition, the nerve is easily exposed surgically, and the main trunk and peripheral branches are readily identifiable for manipulation. This model has become successfully employed in experiments designed to understand regeneration of the facial nerve following manipulation. Previously, investigators have described the extrinsic and intrinsic musculature, the proximal anatomy, and the presence of dual innervation from individual facial nerve branches into the whisker pad [13, 16]. In the current report, we sought to better understand the distal macroanatomy and functional anatomy of the rat facial nerve, and to better and more thoroughly elucidate the phenomenon of dual innervation at the whisker pad. Prior investigators clearly demonstrated mass upper face and snout movement with stimulation of either the buccal or marginal mandibular branches of the facial nerve [13]. We quantitatively demonstrate the overlap of both the buccal and marginal mandibular branches of the facial nerve in the whisking function of rodents as measured by C1 whisker movement. The amplitude of volitional C1 whisker movement is indistinguishable from that of the contralateral (healthy) C1 movement if either the buccal or marginal branches are cut unilaterally, indicating a large degree of functional overlap in whisking control for these two neural pathways to the whisker pad. In addition, transection of both nerve branches is required for cessation of whisking function. Previously published schematics [11] from anatomic dissection of the rodent facial nerve do not illustrate a distal pes immediately proximal to the whisker pad, as we describe herein.

The current report both expands on their anatomic findings, provides quantitative length and width data in the various segments of the rat facial nerve, and provides functional information through review of video recordings of vibrissal movement during selective branch stimulation.

For decades, the prevailing notion was that all vibrissal piloerector muscles are innervated by the buccal branch of the rat facial nerve[16]. The consistently observable movement of different regions of the whisker pad during stimulation of specific areas of the facial nerve, and preservation of whisking function after buccal or marginal mandibular branches lesions reported herein, unequivocally demonstrates that the whisker pad receives innervation from both the buccal and marginal branches. Specifically, we have found that both the upper and lower halves of the whisker pad move 93–100% of the time with isolated stimulation of either the buccal or marginal mandibular nerve branch based on human visual assessment, and that quantified whisking amplitude is equally unaffected by cutting either branch in isolation. Taken together, these findings are important to consider when studying facial nerve manipulation and recovery, particularly when utilizing whisking as the recovery parameter. As put forth by other investigators[13, 17], we have added further data to support the fact that both buccal and marginal mandibular branches of the facial nerve, or the main trunk, must be divided in order to denervate the region.

Conclusion

We have confirmed with microsurgical dissection, individual branch transection, and observed stimulation of the whisker pad, that fibers from both the buccal and marginal mandibular branches of the rat facial nerve provide axons to both the upper and lower halves of the whisker pad and can independently support whisking. These proximal nerve branches fuse to form a distinct “distal pes” which then gives rise to 3 distinct branches that enter the whisker pad and face. These observations confirm multiple neural inputs to the rat whisker pad. This improved understanding must be considered, and perhaps exploited, in further rat facial nerve regeneration studies.

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Figure 1.

Diagram of the extratemporal rat facial nerve anatomy as presented in Figure 1 of Semba and Egger (1986) showing the major nerve branches, but lacking detail regarding how the buccal and upper division of the marginal mandibular branches fuse prior to entering the whisker pad. (Reproduced from: Semba, K. and M.D. Egger, *The facial “motor” nerve of the rat: control of vibrissal movement and examination of motor and sensory components*. *J Comp Neurol*, 1986. **247**(2): p. 144–58 with permission.)

Figure 2.

Diagram showing the location of buccal and marginal mandibular branch transections for the two experimental groups tested for whisking function after partial and then subsequent complete whisker pad denervation.

Figure 3.

Diagram of the rat extratemporal facial nerve anatomy consistent with our rat facial dissections. Note the presence of a distal pes just proximal to the whisker pad. Labeled areas represent points of bipolar stimulation for observation of movements: A) Main trunk, B) Buccal Branch, C) Marginal Branch, D) Distal Pes, E) Superior Branch of distal pes, F) Middle Branch of distal pes, and G) Inferior Branch of distal pes.

Figure 4.

Zones used to describe movement on videographic analysis. Whisker pad was divided into upper (including C1 whisker) and lower halves, other areas included the nose and the upper and lower lips.

Figure 5.

Average amplitude of the three strongest whisks are plotted for individual rats comparing their left (control) versus right (experimental) C1 whisking amplitude. Data on the left half of the plot reflect whisking amplitude after sustaining right buccal branch transection (red group) or right marginal mandibular branch transection (black group) versus the left (control) side in each rat (connected by lines). Data on the right half of the plot are after rats sustained subsequent transection of their remaining nerve branch on the right side, causing complete whisker pad motor denervation and loss of meaningful whisks.

Figure 6.

Facial nerve motor supply of the whisker pad, (A) showing convergence of the buccal and marginal mandibular branches (dotted lines) into the distal pes, (B) which then diverge as superior (S), middle (M) and inferior (I) branches before entering the whisker pad. Note the infraorbital nerve (ION) deep to the facial nerve as it exits the infraorbital foramen.

Table 1

Rat facial nerve length and width measurements.

			Nerve Segment Lengths (mm)				Nerve Location Widths (mm)				
Animal	Weight	Face Side	M. T. to Z. B.	M. T. to D.P.	D. P.	M.T.	D.P.	D.P. Superior	D.P. Middle	D.P. Inferior	
1	306	R	*	23.9	3.1	1.7	2.6	1.3	1.2	1.4	
		L	9.4	25.9	2.1	1.5	2.3	1.6	1.3	1.4	
2	276	R	7.9	24.0	2.3	1.4	2.2	1.0	1.4	1.0	
		L	8.0	25.4	2.0	1.4	2.5	1.2	1.3	1.3	
3	276	R	7.7	25.7	2.6	1.7	2.0	1.2	1.3	1.1	
		L	9.7	25.3	3.0	1.0	2.2	1.5	1.5	1.1	
4	298	R	10.7	25.3	2.5	1.5	2.8	1.4	1.6	1.6	
		L	10.0	25.4	2.9	1.1	2.1	1.5	1.3	1.8	
5	280	R	7.0	23.8	4.1	1.3	2.7	2.0	1.8	1.8	
		L	*	24.0	4.9	1.5	2.9	2.0	2.0	1.9	
6	268	R	*	24.1	2.9	1.4	2.3	1.4	1.5	1.5	
		L	10.7	23.5	4.1	1.6	2.4	1.4	1.4	1.5	
7	256	R	10.6	24.7	2.3	1.1	1.8	1.1	0.9	1.1	
		L	*	25.6	2.4	1.1	1.9	1.2	1.4	1.8	
8	307	R	6.1	28.7	3.8	1.3	2.6	2.0	1.9	2.0	
		L	7.0	26.7	3.8	1.6	2.5	1.8	2.4	1.7	
9	276	R	11.5	25.1	3.9	1.1	2.3	1.1	1.0	1.3	
		L	11.9	25.3	2.1	1.2	1.6	1.1	0.9	1.1	
10	270	R	11.1	24.6	2.7	1.1	2.0	1.3	1.1	1.3	
		L	10.1	23.9	2.8	1.1	1.4	1.1	1.4	1.5	
		Mean	9.3	25.0	3.0	1.3	2.3	1.4	1.4	1.5	
		St Dev	1.8	1.2	0.8	0.2	0.4	0.3	0.4	0.3	

M.T.=Main Trunk, Z.B.=Zygomatic Branch, D.P.=Distal Pes

* unable to get measurement because zygomatic branch diverged directly off of the main trunk.

Table 2

Percentages represent the pooled observational rate among observers for presence (score of 1) or absence (score of 0) of movement of each facial zone following stimulation of 10 facial nerves. C1 whisker was included in the upper half of the pad for reporting.

Stimulation Branch	Zones Of Movement				
	Lower Lip	Upper Lip	Lower Half Pad	Upper Half Pad	Nose
Main Trunk	54%	60%	87%	90%	63%
Mariginal Branch	67%	89%	100%	100%	97%
Buccal Branch	25%	67%	93%	97%	67%
Distal Pes (D.P.)	40%	67%	97%	97%	87%
D.P. Superior Branch	0%	57%	100%	97%	100%
D.P. Middle Branch	38%	57%	100%	33%	20%
D.P. Inferior Branch	5%	93%	87%	7%	0%