Published in final edited form as:

Ann Plast Surg. 2016 January ; 76(1): 94–98. doi:10.1097/SAP.0000000000000273.

The Dilator Naris Muscle as a Reporter of Facial Nerve Regeneration in a Rat Model

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Abstract

Objective—Many investigators study facial nerve regeneration using the rat whisker pad model, though widely standardized outcomes measures of facial nerve regeneration in the rodent have not yet been developed. The intrinsic whisker pad “sling” muscles producing whisker protraction, situated at the base of each individual whisker, are extremely small and difficult to study en bloc. Here, we compare the functional innervation of two potential reporter muscles for whisker pad innervation: the dilator naris and the levator labii superioris, to characterize facial nerve regeneration.

Methods—Motor supply of the dilator naris and levator labii superioris was elucidated by measuring contraction force and compound muscle action potentials during stimulation of individual facial nerve branches, and by measuring whisking amplitude before and after dilator naris distal tendon release.

Results—The pattern of dilator naris innervation matched that of the intrinsic whisker pad musculature (i.e. via the buccal and marginal mandibular branches of the facial nerve), whereas the levator labii superioris appeared to be innervated almost entirely by the zygomatic branch, whose primary target is the orbicularis oculi muscle.

Conclusion—While the levator labii superioris has been commonly used as a reporter muscle of whisker pad innervation, the present data show that its innervation pattern does not overlap substantially with the muscles producing whisker protraction. The dilator naris muscle may serve as a more appropriate reporter for whisker pad innervation because it is innervated by the same facial nerve branches as the intrinsic whisker pad musculature, making structure/function correlations more accurate, and more relevant to investigators studying facial nerve regeneration.

INTRODUCTION

Facial paralysis is a debilitating condition that generates both physical and social impairments^{1,2}. Amongst the symptoms, the most clinically relevant include oral incompetence, incomplete eye closure, and difficulty smiling³. Research to develop strategies and approaches to improve functional outcome following nerve injury or sacrifice has been executed by many investigators⁴⁻⁶, and further development of facial nerve-specific models would substantially benefit afflicted patients.

Numerous animal models have been employed to study nerve recovery following nerve crush or transection injury, with significant gains in our understanding of the neurobiology of regeneration over the past several decades⁷⁻¹¹. In rats, a highly quantifiable and commonly studied motor function of the facial nerve is movement of the vibrissal whiskers or “whisking”¹²⁻¹⁸. Rats whisk to explore their tactile environment through active protraction and retraction of approximately 25 whiskers embedded in each whisker pad¹⁸ (Figure 1). Whisker protraction is achieved primarily through contraction of sling muscles located at the base of each individual whisker follicle (intrinsic pad muscles), and whisker retraction stems from elastic tissue recoil and from contraction of muscles attached to the skull posterior (caudal) to each pad¹⁹. These extrinsic whisker retracting muscles include the levator labii superioris (LLS) and maxillolabialis (ML), which are enmeshed within the pad tissue between the rows of prominent whiskers. Although these extrinsic muscles play a relatively minor role in whisker movement, they are more frequently studied than the intrinsic sling muscles to represent whisker pad innervation due to the very small size and discontinuity of the individual sling muscles. For example, multiple studies have related immunohistochemical evidence of LLS reinnervation after facial nerve transection and repair rather than studying the whisker pad intrinsic muscles^{20,21}. However, our previous anatomical examination of whisker pad innervation suggests that the LLS is innervated primarily by the zygomatic branch of the facial nerve rather than the buccal and marginal mandibular branches supplying the sling muscles²². Therefore, the LLS might not provide an optimal representation of whisking recovery after facial nerve manipulation, but may better relate to eye blink. Alternatively, the dilator naris muscle (DNM) is immediately adjacent to the whisker pad and appears to receive its motor supply through the same facial nerve branches as the whisker pad intrinsic muscle, potentially making it a more appropriate reporter muscles for whisker pad function than the LLS.

In this study, we examined the electrophysiological and mechanical response of the LLS and DNM muscles while electrically stimulating individual facial nerve branches with the goal of identifying their source(s) of innervation. Additionally, we examined the effect of disinsertion of the DNM on whisking. Our hypothesis, based upon preliminary observations and dissections²², was that the DNM receives its motor supply from the same branches as those that innervate the whisker pad intrinsic, and thus may therefore represent a more appropriate reporter for studies of whisking recovery after facial nerve manipulation.

METHODS

Eight female Wistar Hannover Rats (Charles River Laboratories, Wilmington, MA), 75 to 90 days old weighing 200–250g, were employed for this study. Animals were treated following the Massachusetts Eye and Ear Infirmary guidelines for animal care and use; food and water were available ad libitum.

DNM and LLS Force Tension and Electromyography Experiments

Four animals underwent force tension and electromyography experiments. Animals were anesthetized by an intramuscular injection of ketamine (50 mg/kg; Fort Dodge Animal Health, Fort Dodge, Iowa) and dexmedetomidine hydrochloride (0.5 mg/kg; Orion Corporation, Espoo, Finland). The right pre-auricular area and nasal trunk were shaved and aseptically prepared. The facial nerve main trunk was exposed via a pre-auricular incision followed by parotid gland removal. The main trunk was identified at its emergence deep to the posterior belly of the digastric muscle, and its three distal motor branches, buccal, marginal mandibular, and zygomatic, were identified and dissected. A 2–3mm segment of each branch was freed from the surrounding fascia, 10–15mm from the main trunk trifurcation, and a blue powder-free nitrile background was placed under each segment for electrical stimulator placement. A background was likewise placed under the main trunk of the facial nerve proximal to the trifurcation (figure 2). Prior to nerve stimulation, mineral oil was placed on the nerve stimulator to electrically isolate the area. The exposed nerves and muscles were rinsed with saline throughout the experiments to prevent dehydration.

For LLS and DNM muscle exposure, a midline incision was made along the nasal trunk, and a skin flap was elevated to the second whisker row, exposing the superficial and superior surfaces of the LLS. The DNM distal tendon was identified immediately deep to the distal segment of the LLS along the nasal trunk, and dissected to its junction with the belly of the muscle. Dissection was then executed with meticulous attention to protect the neurovascular pedicle, and the distal tendon was transected and released (Figure 3).

Data were acquired using CyberAmp 380 (Molecular Devices, LLC, Sunnyvale, California) and a Digidata 1440a (Molecular Devices, LLC) acquisition system using pCLAMP 10 software (Molecular Devices, LLC). Data were analyzed using Clampfit 10.2 software (Molecular Devices, LLC)

Force Tension Experiment—For force tension experiments, a 6–0 braided undyed coated polyglactin 910 3/8 13mm suture (J492.P03 Ethicon, INC, Somerville, NJ) was secured between the DNM tendon and a micro force transducer. A second suture was secured superficially between the middle of the triangular muscular plane of the LLS and a micro force transducer. The sutures were aligned in positions that paralleled normal muscle movement. The protocol was as follows: 1st step: 0.1V, delta 0.1V, pulse width: 0.4ms, duration 500ms, train rate: 100hz, sweep number: 20. Three iterations were conducted at each stimulation site (main trunk, buccal branch, marginal mandibular branch, and zygomatic branch), and the voltage was calculated post each stimulation (Figure 4), which was then calibrated; contractile forces were average to compute cumulative average contractile force in in grams.

Electromyography Experiment—For electromyographic experiments, a 25mm, 30ga electrode needle; (TECA elite disposable concentric needle electrode; Carefusion, Middleton, WI) was placed superficially in both the DNM and LLS, in the vector of physiologic muscle contraction. Three iterations were conducted at each stimulation site exactly as conducted in the force tension experiments. The latency from onset of stimulus to first deviation in resting membrane potential was calculated post main trunk stimulation and Compound Muscle Action Potential (CMAP) amplitude data were analyzed using the greatest voltage delta from each action potential to compute average CMAP (Figure 5).

DNM Distal Tendon Release Experiment

Four animals were head-fixed, and normal whisking function amplitude was recorded using previously described methods¹⁴. Animals were then anesthetized, as previously described, and a 2cm lower midline incision was made along the nasal trunk. The DNM distal tendon was identified at its junction with the muscle belly and a 4mm segment was resected. The incision was closed with a 3-0 polyglactin suture, and postoperative whisking function was recorded one week later.

Data Analysis

For the force tension and electromyography experiments, each animal underwent three stimulations at each site, and data were analyzed to calculate an average excursion. These values from each animal were averaged to yield final measure, expressed as a percentage of the value achieved with main trunk stimulation, and are recorded as mean $\pm$ standard error. Data which were excessively noisy were omitted from analysis, and included a single set of CMAP data generated by the DNM upon marginal mandibular and zygomatic branch stimulation. Additionally, there was a single channel malfunction during one of the experiments; these data were eliminated from analysis, and included CMAP data for the LLS in one experiment. For the distal tendon release, data were analyzed using whisking software developed by Bermejo et al¹⁷, which calculates amplitude data for vibrissae excursion over a 5 minute period. Amplitudes of the top three most vigorous whisks from each animal were averaged. A two sample Student's t-test was used for statistical analysis. Significance was defined as $p < 0.05$.

RESULTS

Force Tension Analysis

There was no statistical difference in contractile force generated upon main trunk stimulation in the LLS and DNM (2-tail $p = 0.09$). The DNM generated a greater force than the LLS upon marginal mandibular and buccal branch stimulation (2-tail; $p < 0.05$). The LLS generated a significantly greater force than the DNM upon zygomatic branch stimulation (1-tail; $p < 0.04$).

There was no statistical difference in contractile force in the DNM between main trunk and marginal mandibular branch stimulation (2-tail; $p = 0.06$); however the DNM produced a significantly greater force upon main trunk stimulation than buccal and zygomatic branch stimulation ($p < 0.05$).

The contractile force generated by the LLS upon marginal mandibular and buccal branch stimulation was significantly less than the force generated upon main trunk stimulation (2-tail; $p<0.05$). There was no difference in contractile force generated by the LLS upon main trunk and zygomatic branch stimulation (2-tail; $p=0.39$) (Figure 6).

Electromyography CMAP Analysis

Average CMAP latency, measured from stimulation onset to CMAP, demonstrated that the LLS ($0.0030 \pm 0.00019\text{sec}$) responded significantly faster than the DNM to stimulation of the facial nerve main trunk ($0.0039 \pm 0.0002\text{sec}$) ($p<0.05$)

There was no significant difference in CMAP voltage change in the DNM compared to LLS upon main trunk stimulation (2-tail; $p=0.57$). There was no significant difference in CMAP peak-to-peak average voltage change in the DNM upon main trunk and the marginal mandibular (2-tail; $p=0.37$) stimulation; however, the average voltage delta was significantly less upon both buccal and zygomatic branch stimulation (2 tail; $p<0.05$) than main trunk stimulation in the DNM.

The CMAP delta voltage was significantly lower in the LLS upon marginal mandibular and buccal branch stimulation, compared to main trunk stimulation (2 tail; $p<0.05$). However, there was no significant difference in the CMAP action potential produced in the LLS upon main trunk and zygomatic stimulation (2 tail; $p=0.74$) (Figure 7).

Force Tension and CMAP Correlation

Force tension and CMAP peak-to-peak results at each stimulation site were correlated for both the DNM (Figure 8A) and LLS (Figure 8B) to account for variation among animals.

DNM Distal Tendon Release

Rat whisking amplitude measurements revealed that the DNM distal tendon release did not affect whisking amplitude (2 tail; $p=0.26$) (Figure 9).

Discussion

In this study, we investigated the role of different reporter muscles for studies of facial nerve regeneration, through clarifying the innervation patterns of the rodent LLS and DNM using force tension and electrophysiological analysis, two commonly used methods to examine target muscle innervation^{23,24}. Force tension recordings enabled maximum contractile force quantification and electrophysiological recordings quantified the latency and peak-to-peak times from each muscle's compound muscle action potential (CMAP) upon nerve stimulation. Together, the force tension and electrophysiological peak-to-peak recordings demonstrate consistency, and together provide a plausible description of the innervation patterns of the LLS and DNM facial muscles despite variation among animals. These results are important for investigators interested in studying facial nerve regeneration using the rat whisker pad model.

Average contractile force and CMAP amplitude data indicate that the LLS is innervated primarily by the zygomatic branch of the facial nerve, with minor contributions from the

marginal mandibular and buccal branches. We further observed that the DNM is innervated primarily by the marginal mandibular and buccal branches, with little input from the zygomatic branch. Additionally, our CMAP and force tension cumulative averages at each stimulation site demonstrate a strong correlation in both the DNM and LLS, which accounts for physiological variation observed among animals.

The rhythmic pattern controlled by the intrinsic and extrinsic muscles of the rat whisker pad influence whisking and nostril movement through the facial nerve. Intrinsic muscles form a sling around each whisker follicle in the whisker pad, and control vibrissae protraction through the buccal and marginal mandibular branches of the facial nerve^{19,22,25,26}. Our results show that the DNM is innervated in large part by the same branches that control intrinsic whisking. The extrinsic muscles (LLS, m. maxillolabialis, m. transversus nasi, m. nasalis), have been associated with vibrissae retraction^{19,25,26} and our present results demonstrate that LLS retraction is primarily supplied by the zygomatic branch, unlike the intrinsic pad muscles. It is likely that previous investigators examined the LLS due to its convergence with the intrinsic whisker pad muscles, yet the two groups appear to receive their neural input through different pathways. Moreover, we observed that the LLS responded faster to stimulation of the main trunk compared to the DNM, which further suggests an alternative pathway.

Whisking function and whisker pad immunohistochemistry are common reporters of facial nerve regeneration^{12–18} following surgical manipulation. However, the orientation of the individual sling muscles makes them notoriously difficult to assess histologically since the muscle in its entirety cannot be sectioned based upon its oblique position around each follicle. Thus, it would be useful to identify a surrogate reporter facial muscle that displays a common innervation pattern, which would allow analysis of histological muscle changes along with functional whisking behavior. The DNM is primarily involved in nostril dilation, in addition to nose deflection and reflection during sniffing²⁷. Although we demonstrated that the DNM distal tendon release did not change active whisking function, others have suggested that the DNM is involved in vibrissae protraction during the first stage of the sniff cycle²⁷. Our work herein demonstrates that the DNM is mostly supplied by the buccal and marginal mandibular branches; thus, the muscle is likely to experience similar histological changes post- facial nerve manipulation as the whisker pad intrinsic musculature.

In addition to its motor supply, the DNM possesses several advantages over the LLS as a reporter muscle. The DNM can be harvested en bloc due to its ease of dissection from the surrounding soft tissues, and its long single nasal tendon. In contrast, the LLS fans out distally into multiple radians, converging with the whisker pad. The DNM is composed of parallel muscle fibers (optimal for cross-sectional cryostat cuts), surrounded by a thin fascia layer. Additionally, using the LLS may introduce inaccuracies in experiments in which only the buccal and marginal mandibular branches are manipulated. We propose that the DNM is the most suitable reporter for whisker pad innervation in rat model of facial nerve regeneration based upon its more closely matched innervation patterns to the whisker pad musculature, and have now integrated its histologic analysis into our rodent facial recovery paradigms post-facial nerve manipulation.

In summary, facial nerve regeneration remains suboptimal following crush, transection and repair, and following cable grafting. Laboratory models to investigate manipulations of the neuromuscular environment are of critical importance in identifying the utility of novel regeneration approaches. Tools such as DNM re-innervation patterns will serve to further characterize any regenerative benefit derived by different experimental maneuvers.

Acknowledgments

Financial Support: RO1NS071067

References

1. Coulson SE, O'Dwyer NJ, Adams RD, Croxson GR. Expression of emotion and quality of life after facial nerve paralysis. *Otol Neurotol*. 2004; 25(6):1014–1019. [PubMed: 15547436]
2. Sinno H, Thibaudeau S, Izadpanah A, et al. Utility outcome scores for unilateral facial paralysis. *Ann Plast Surg*. 2012; 69(4):435–438. [PubMed: 22964665]
3. Labbe D, Bussu F, Iodice A. A comprehensive approach to long-standing facial paralysis based on lengthening temporalis myoplasty. *Acta Otorhinolaryngol Ital*. 2012; 32(3):145–153. [PubMed: 22767978]
4. Glaus SW, Johnson PJ, Mackinnon SE. Clinical strategies to enhance nerve regeneration in composite tissue allotransplantation. *Hand Clin*. 2011; 27(4):495–509. ix. [PubMed: 22051390]
5. Willand MP, Holmes M, Bain JR, Fahnestock M, De Bruin H. Electrical muscle stimulation after immediate nerve repair reduces muscle atrophy without affecting reinnervation. *Muscle Nerve*. 2013; 48(2):219–225. [PubMed: 23637030]
6. Bain JR, Hason Y, Veltri K, Fahnestock M, Quartly C. Clinical application of sensory protection of denervated muscle. *J Neurosurg*. 2008; 109(5):955–961. [PubMed: 18976091]
7. Magill CK, Moore AM, Borschel GH, Mackinnon SE. A new model for facial nerve research: the novel transgenic Thy1-GFP rat. *Arch Facial Plast Surg*. 2010; 12(5):315–320. [PubMed: 20855773]
8. Bain JR, Mackinnon SE, Hudson AR, Falk RE, Falk JA, Hunter DA. The peripheral nerve allograft: an assessment of regeneration across nerve allografts in rats immunosuppressed with cyclosporin A. *Plast Reconstr Surg*. 1988; 82(6):1052–1066. [PubMed: 3264409]
9. Ladak A, Olson J, Tredget EE, Gordon T. Differentiation of mesenchymal stem cells to support peripheral nerve regeneration in a rat model. *Exp Neurol*. 2011; 228(2):242–252. [PubMed: 21281630]
10. Konofaos P, Burns J, Terzis JK. Effect of low-dose FK506 after contralateral C7 transfer to the musculocutaneous nerve: a study in rats. *J Reconstr Microsurg*. 2010; 26(4):225–233. [PubMed: 20169524]
11. Guntinas-Lichius O, Glowka TR, Angelov DN, Irintchev A, Neiss WF. Improved functional recovery after facial nerve reconstruction by temporary denervation of the contralateral mimic musculature with botulinum toxin in rats. *Neurorehabil Neural Repair*. 2011; 25(1):15–23. [PubMed: 20930211]
12. Hadlock T, Kowaleski J, Lo D, et al. Functional assessments of the rodent facial nerve: a synkinesis model. *Laryngoscope*. 2008; 118(10):1744–1749. [PubMed: 18641527]
13. Heaton JT, Kowaleski JM, Bermejo R, Zeigler HP, Ahlgren DJ, Hadlock TA. A system for studying facial nerve function in rats through simultaneous bilateral monitoring of eyelid and whisker movements. *J Neurosci Methods*. 2008; 171(2):197–206. [PubMed: 18442856]
14. Hadlock T, Kowaleski J, Mackinnon S, Heaton JT. A novel method of head fixation for the study of rodent facial function. *Exp Neurol*. 2007; 205(1):279–282. [PubMed: 17397835]
15. Harvey MA, Bermejo R, Zeigler HP. Discriminative whisking in the head-fixed rat: optoelectronic monitoring during tactile detection and discrimination tasks. *Somatosens Mot Res*. 2001; 18(3):211–222. [PubMed: 11562084]
16. Bermejo R, Houben D, Zeigler HP. Optoelectronic monitoring of individual whisker movements in rats. *J Neurosci Methods*. 1998; 83(2):89–96. [PubMed: 9765121]

17. Bermejo R, Szwed M, Friedman W, Ahissar E, Zeigler HP. One whisker whisking: unit recording during conditioned whisking in rats. *Somatosens Mot Res.* 2004; 21(3–4):183–187. [PubMed: 15763903]
18. Bermejo R, Vyas A, Zeigler HP. Topography of rodent whisking—I. Two-dimensional monitoring of whisker movements. *Somatosens Mot Res.* 2002; 19(4):341–346. [PubMed: 12590835]
19. Hill DN, Bermejo R, Zeigler HP, Kleinfeld D. Biomechanics of the vibrissa motor plant in rat: rhythmic whisking consists of triphasic neuromuscular activity. *J Neurosci.* 2008; 28(13):3438–3455. [PubMed: 18367610]
20. Angelov DN, Ceynowa M, Guntinas-Lichius O, et al. Mechanical stimulation of paralyzed vibrissal muscles following facial nerve injury in adult rat promotes full recovery of whisking. *Neurobiol Dis.* 2007; 26(1):229–242. [PubMed: 17296303]
21. Guntinas-Lichius O, Irintchev A, Streppel M, et al. Factors limiting motor recovery after facial nerve transection in the rat: combined structural and functional analyses. *Eur J Neurosci.* 2005; 21(2):391–402. [PubMed: 15673438]
22. Henstrom D, Hadlock T, Lindsay R, et al. The convergence of facial nerve branches providing whisker pad motor supply in rats: implications for facial reanimation study. *Muscle Nerve.* 2012; 45(5):692–697. [PubMed: 22499096]
23. Sobotka S, Mu L. Force characteristics of the rat sternomastoid muscle reinnervated with end-to-end nerve repair. *J Biomed Biotechnol.* 2011; 2011:173471. [PubMed: 22203781]
24. Navarro X, Udina E. Chapter 6: Methods and protocols in peripheral nerve regeneration experimental research: part III-electrophysiological evaluation. *Int Rev Neurobiol.* 2009; 87:105–126. [PubMed: 19682635]
25. Dorfl J. The musculature of the mystacial vibrissae of the white mouse. *J Anat.* 1982; 135(Pt 1): 147–154. [PubMed: 7130049]
26. Berg RW, Kleinfeld D. Rhythmic whisking by rat: retraction as well as protraction of the vibrissae is under active muscular control. *J Neurophysiol.* 2003; 89(1):104–117. [PubMed: 12522163]
27. Haidarliu S, Golomb D, Kleinfeld D, Ahissar E. Dorsorostral snout muscles in the rat subserve coordinated movement for whisking and sniffing. *Anat Rec (Hoboken).* 2012; 295(7):1181–1191. [PubMed: 22641389]

Figure 1.

A, Rat facial nerve anatomy, showing the arrays of whiskers, organized in 5 distinct rows (gray dots) (Ref 22). B, In vivo CN VII exposure revealing the main trunk and zygomatic, buccal, and marginal mandibular branches. C, Rat whisker pad extrinsic muscles, exposing the DN muscle: (1) LLS, (2) DN, (3) zygomatic arch, (4) mandibular branch of VII, (5) buccal branch of VII, (6) masseter, (7) zygomatic branch of VII, and (8) temporal branch of VII. Drawing is from Muscle Nerve. 2001;4:1202–1212 with permission. Adaptations are themselves works protected by copyright. So in order to publish this adaptation, authorization must be obtained both from the owner of the copyright in the original work and from the owner of copyright in the translation or adaptation. Photograph insert shows DN under slight tension.

Figure 2.

A, Example of force tension in DN during facial nerve main trunk stimulation with a 100-Hz biphasic pulsetrain. B, Example of CMAP in LLS during zygomatic branch stimulation of 0.40 milliseconds biphasic pulses. Both techniques used increasing stimulation amplitudes until responses plateaued.

Figure 3. DN (in blue) and LLS (in red) contraction force as a percentage of the force and CMAP generated during facial nerve main trunk stimulation ($n = 4$). Bars are $\pm$ SE.

Figure 4. DN (in blue) and LLS (in red) CMAP peak-to-peak amplitude (B) as a percentage of the force and CMAP generated during facial nerve main trunk stimulation ($n = 4$). Bars are $\pm$ SE.

Figure 5. DN (A) and LLS (B) average contractile force and CMAP amplitude correlation at each stimulation site with Pearson correlation coefficient R^2 .

Figure 6.
Whisking amplitude pre- and post-DN distal tendon release.