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RAT WHISKER MOVEMENT AFTER FACIAL NERVE LESION: EVIDENCE FOR AUTONOMIC CONTRACTION OF SKELETAL MUSCLE

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Abstract—Vibrissal whisking is often employed to track facial nerve regeneration in rats; however, we have observed similar degrees of whisking recovery after facial nerve transection with or without repair. We hypothesized that the source of non-facial nerve-mediated whisker movement after chronic denervation was from autonomic, cholinergic axons traveling within the infraorbital branch of the trigeminal nerve (ION). Rats underwent unilateral facial nerve transection with repair ($N = 7$) or resection without repair ($N = 11$). Post-operative whisking amplitude was measured weekly across 10 weeks, and during intraoperative stimulation of the ION and facial nerves at $\geqslant$18 weeks. Whisking was also measured after subsequent ION transection ($N = 6$) or pharmacologic blocking of the autonomic ganglia using hexamethonium ($N = 3$), and after snout cooling intended to elicit a vasodilation reflex ($N = 3$). Whisking recovered more quickly and with greater amplitude in rats that underwent facial nerve repair compared to resection ($P < 0.05$), but individual rats overlapped in whisking amplitude across both groups. In the resected rats, non-facial-nerve-mediated whisking was elicited by electrical stimulation of the ION, temporarily diminished following hexamethonium injection, abolished by transection of the ION, and rapidly and significantly ($P < 0.05$) increased by snout cooling. Moreover, fibrillation-related whisker movements decreased in all rats during the initial recovery period (indicative of reinnervation), but re-appeared in the resected rats after undergoing ION transection (indicative of motor denervation). Cholinergic, parasympathetic axons traveling within the ION innervate whisker pad vasculature, and immunohistochemistry for vasoactive intestinal peptide revealed these axons branching extensively over whisker pad muscles and contacting neuromuscular junctions after facial nerve resection. This study provides the first behavioral and anatomical evidence of spontaneous autonomic innervation of skeletal muscle after motor nerve lesion, which not only has implications for interpreting facial nerve reinnervation results, but also calls into question whether autonomic-mediated innervation of striated muscle occurs naturally in other forms of neuropathy.

Key words: autonomic, infraorbital nerve, motor, paralysis, parasympathetic, reinnervation.

INTRODUCTION

Facial paralysis is experienced by approximately 40,000 individuals in the United States each year (Jackson and von Doersten, 1999), and can lead to permanent disfigurement and functional loss despite advances in static and dynamic surgical intervention (Guntinas-Lichius et al., 2006). Efforts to improve management of facial nerve injury have turned to animal models for studying nerve coaptation techniques, grafting approaches, and interventions intended to enhance axonal regeneration. These interventions must be compared to appropriate controls, including the chronically denervated state, representing long-term failure for reinnervation to occur.

Our research team studies facial nerve recovery in the rat model, tracking whisking function over time after nerve injury and repair. Whisking is a highly quantifiable, dynamic behavior controlled primarily by the buccal and marginal mandibular branches of the facial nerve (Dorfl, 1985; Semba and Egger, 1986; Henstrom et al., 2012). In the course of comparing facial nerve main trunk transection and suture repair to a chronically denervated state, we discovered that whisker movements would re-appear approximately 30 days after facial nerve resection, and grow stronger over the ensuing weeks. Although maximal whisking amplitude was generally greater in rats who had undergone facial nerve repair, there was nevertheless overlap in whisker movement amplitude for individual animals between repaired and

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Abbreviations: ACh, acetylcholine; ANOVA, analysis of variance; DNM, dilator naris muscles; GUI, graphic user interface; ION, infraorbital division of the trigeminal nerve; LLS, levator labii superioris; PBS, phosphate buffer; PFA, paraformaldehyde; RLN, recurrent laryngeal nerve.

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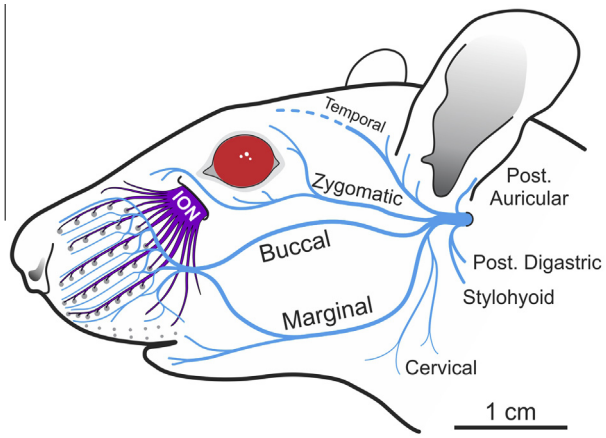

Fig. 1. Diagram of the rat facial nerve branches (in blue) beginning from the stylomastoid foramen, and infraorbital nerve (ION, in purple) beginning from the infraorbital fissure. Macro vibrissae follicles are represented by gray dots arranged in five major rows. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

resected groups. A deeper understanding of the source and mechanism of this non-facial nerve-mediated whisker pad motor innervation is imperative to the validity of vibrissal function as a metric for rat facial nerve recovery.

The rodent whisker pad receives a dense motor and sensory nerve supply from the facial and trigeminal nerves, respectively (Fig. 1) (Dorfl, 1985; Semba and Egger, 1986; Henstrom et al., 2012). Although the infraorbital division of the trigeminal nerve (ION) provides dense sensory innervation to the whisker pad (Dorfl, 1985), it is also known to contain postganglionic, parasympathetic, cholinergic axons (Wilke et al., 1992) that innervate blood vessels of the whisker pad (Fundin et al., 1997). We hypothesized that these parasympathetic, cholinergic fibers were the source of motor innervation causing whisker movement in rats with chronic facial nerve discontinuity. We tested this hypothesis in rats at 10 or more weeks after unilateral facial nerve resection by (1) electrically stimulating the ION intraoperatively and observing possible whisker movements, (2) transecting the ION and observing possible cessation of whisker movements, (3) administering the autonomic blocking drug hexamethonium to see if ION-based whisker movements were diminished, (4) cooling the snout to see if the vasodilation reflex would increase ION-based whisker movement, and (5) immunohistochemical identification of somatic and autonomic innervation of whisker pad intrinsic and extrinsic muscles.

EXPERIMENTAL PROCEDURES

Animals and procedures

Eighteen female Wistar–Hannover rats (Charles River Laboratories, Wilmington, Massachusetts) weighing 250–300 g were obtained in accordance with Massachusetts Eye and Ear Infirmary guidelines for animal care under an approved protocol. For all surgical procedures, rats were anesthetized with an intramuscular injection of ketamine hydrochloride (50 mg/kg) (Fort Dodge Animal Health, Fort Dodge, Iowa) and medetomidine hydrochloride (0.5 mg/kg) (Orion Corp., Espoo, Finland). For assessment of fibrillation-related whisker movements, rats were sedated with intramuscular injection of medetomidine hydrochloride (0.5 mg/kg), which was subsequently reversed with atipamezole hydrochloride (0.5 mg/kg) (Pfizer Inc., New York, NY). At the conclusion of the study, animals were euthanized according to NIH guidelines.

Head restraint device implantation and restraint conditioning

Whisking assessment was performed under head and body restraint, requiring surgical implantation of a titanium head fixation device 5–6 weeks before testing (see Hadlock et al., 2007 for details). Prior to restraint device implantation, rats were handled daily for 2–3 days in order to acclimate them to handling. After device implantation, rats were handled daily for two weeks, and then gradually conditioned to restraint over an additional two-week period (see Heaton et al., 2008 for a complete description of restraint training).

Facial nerve transection and repair or resection

All rats underwent left facial nerve (VII) transection at the main trunk distal to the stylomastoid foramen. The main trunk was exposed by removal of the parotid gland and divided with microsurgical scissors under an operating microscope (Leica Wild M65m, Wetzlar, Germany). In 7 rats, the transected facial nerve was repaired with two or three interrupted 10-0 nylon epineurial sutures (VII Repaired group). In 11 rats, the incision exposing the facial nerve was extended to the lateral border of the whisker pad, permitting extirpation of the buccal and marginal mandibular branches from the pes anserinus proximally to their distal convergence at the whisker pad (VII Resected group; see Fig. 2). The proximal and distal nerve stumps were then sutured into silicone tubes sealed with cyanoacrylate to prevent axons from exiting or entering the cut nerve ends, respectively. Whisking assessment began one week after nerve manipulation and continued weekly for 10 weeks. The capped main trunk in rats undergoing nerve resection (VII Resected group; $N = 11$) was exposed through a small incision once per month during the recovery period, and a Montgomery bipolar nerve stimulator (Boston Medical Products, Inc., Westborough, MA) was used to stimulate around the capped nerve in order to confirm that axons had not escaped encapsulation and re-innervated the whisker pad.

Rats from the VII Resected group underwent ION exposure at $\geqslant$16 weeks after their initial surgery ($N = 11$). The ION was transected just anterior to its exit point from the infraorbital fissure, and the distal fascicles were electrically stimulated while whisker movements were observed ($N = 11$). Five rats from the VII Resected group were euthanized at the end of the ION transection surgery, and the remaining 6 were