Design of a Neural Prosthesis for Facial Reanimation and Assessment in a Rat Model

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For my family, my parents, and my patients

ABSTRACT

Facial palsy is a devastating condition potentially amenable to rehabilitation by functional electrical stimulation. In the introduction, this thesis presents a general approach to the management of this disease state, its impact on patient quality of life, and limitations of current treatment modalities. In the body, this thesis characterizes new methods for high-throughput quantitative assessment of facial nerve structure and function in a rodent model. A novel paradigm for unilateral facial reanimation via an implantable neuroprosthetic device is then presented. The paradigm comprises use of healthy-side electromyography activity as control inputs to a system whose outputs are neural stimuli to effect symmetric facial displacements. The vexing issue of suppressing undesirable activity resulting from aberrant neural regeneration (i.e., synkinesis) or nerve transfer procedures is addressed using proximal neural blockade. The feasibility of this approach is demonstrated in a live rodent model via implantation of epimysial and nerve cuff electrode arrays. Stimuli were delivered to evoke blinks and whisks of various durations and amplitudes. The dynamic relation between electromyography signals and facial displacements were modelled, and model predictions compared against measured displacements. Optimal parameters to achieve facial nerve blockade by means of high-frequency alternating current were determined, and safety of continuous delivery assessed over the medium term. Electrode implantation was well tolerated. Blinks and whisks of tunable amplitudes and durations were evoked by controlled variation of neural stimuli parameters. Facial displacements predicted from electromyography input modelling matched those observed with a variance accounted for exceeding 96%. Effective and reversible facial nerve blockade in awake behaving animals was achieved, without detrimental effects noted from continual use over several weeks. The use of proximal neural blockade coupled with distal functional electrical stimulation for the

rehabilitation of other peripheral motor nerve deficits is discussed, together with potential pathways for clinical translation.

RÉSUMÉ

La paralysie faciale est une condition dévastatrice potentiellement susceptible de rééducation par stimulation électrique fonctionnelle. En introduction, cette thèse présente une approche générale de la prise en charge de cet état pathologique, de son impact sur la qualité de vie des patients et des limites des modalités de traitement actuelles. Dans le corps, cette thèse caractérise de nouvelles méthodes d'évaluation à haut débit et quantitatives supérieures de la structure et de la fonction du nerf facial dans un modèle de rongeur. Un nouveau paradigme de réanimation faciale unilatérale via un dispositif neuroprothétique implantable est ensuite présenté. Le paradigme comprend l'utilisation de l'activité d'électromyographie du côté sain comme entrées de contrôle dans un système dont les sorties sont des stimuli neuronal pour effectuer des déplacements faciaux symétriques. Le problème épineux de la suppression de l'activité indésirable résultant de la régénération neurale aberrante (c'est à dire la syncinésie) ou des procédures de transfert de nerf est abordé en utilisant le blocage neural proximal. La faisabilité de cette approche est démontrée dans un modèle de rongeur vivant via l'implantation de réseaux d'électrodes épimysiales et nerveuses. Des stimuli ont été délivrés pour évoquer des clignements et des fouets de différentes durées et amplitudes. La relation dynamique entre les signaux d'électromyographie et les déplacements faciaux a été modélisée et les prédictions du modèle comparées aux

déplacements mesurés. Les paramètres optimaux pour obtenir un blocage du nerf facial au moyen d'un courant alternatif à haute fréquence ont été déterminés, et la sécurité de l'administration continue évaluée à moyen terme. L'implantation d'électrodes a été bien tolérée. Des clignements et des battements d'amplitudes et de durées réglables ont été évoqués par une variation contrôlée des paramètres des stimuli neuronaux. Les déplacements faciaux prédits à partir de la modélisation des entrées d'électromyographie correspondaient à ceux observés avec une variance expliquée dépassant 96%. Un blocage efficace et réversible du nerf facial chez des animaux éveillés a été obtenu, sans effet néfaste noté par une utilisation continue pendant plusieurs semaines. L'utilisation du blocage neural proximal couplé à une stimulation électrique fonctionnelle distale pour la réhabilitation d'autres déficits du nerf moteur périphérique est discutée, ainsi que des voies potentielles pour la traduction clinique.

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The work described herein is the result of tremendous support at the individual, industry, departmental, institutional, and national levels in Canada and the Unites States. I owe a deep debt of gratitude to my primary advisor Professor Robert Kearney, for his guidance, support, and time over the past six years. This research would not have been possible without his expertise and passion for biomedical system identification. His teaching has provided me with a powerful analytical skillset in biomedical signals and systems analysis that I hope to employ to advance patient care in the field of cranial nerve rehabilitation. His mentorship style is one I hope to emulate with my future students.

Another deep debt of gratitude is owed to Professor Tessa Hadlock, my co-supervisor on this dissertation. This research would not have been possible in the absence of her passion for advancing care of patients with facial palsy. Her systematic approach to management of this disease state, expertise with use of rodent model to study novel therapeutics, and writing talent were crucial to the realization of this work. Her teaching and research mentorship has provided me with crucial skills in the medical and surgical management of this disease state, use of animal research models, and grant writing. Her efficiency, drive, and resilience are inspirational.

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Minderler for his assistance with computer-aided design of our animal implants, ordering of components, animal care, sample processing, and manual segmentation of histopathologic images for training of artificial intelligence algorithms. I wish to thank Dr. Iván Coto Hernández for his assistance with image acquisition and expertise in image processing. I wish to thank Nat Adamian and Frank Ma for their time and expertise in coding algorithms for automated tracking of rat facial landmarks. I thank Dr. Danny McDonnall of Ripple Neuro for his expertise and assistance with epimysial electrode array design and manufacture, and for support of our National Institutes of Health R-01 grant. I thank Dr. Martin Bak of MicroProbes for Life Sciences for his expertise and assistance with nerve cuff electrode design and manufacture. I thank my patients with facial paralysis for providing a source of inspiration to advance therapeutic management of this disease state. I am grateful for the love and support of my wife Jacqueline over this long journey. I thank my mother and late father for valuing academics, excellence, and grit.

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THESIS FORMAT

This is a manuscript-based thesis comprised of six chapters, four of which include the text of research papers. Two papers have already been published in peer-reviewed journals over the course of my Ph.D. studies at McGill University, while the other two papers are planned for submission. Chapter 1 presents an introduction to the anatomy, pathophysiology, and sequelae of facial nerve injury, and the rationale for novel therapeutic approaches to the management of this disease state. Chapter 2 is a review article published in *Otolaryngologic Clinics of North America* in 2018. This article presents a conceptual framework for classification and management of facial palsy, together with a brief summary of contemporary medical and surgical therapeutic approaches to the disease. Chapter 3 is a review article to be submitted to *Current Opinion in Otolaryngology and Head & Neck Surgery*. This article provides a brief summary of functional electrical stimulation (FES) and neural blockade, and presents the case for exploitation of these methods for therapeutic management of facial palsy using a neuroprosthetic device (NPD). Chapter 4 is an original experimental manuscript published in *Plastic & Reconstructive Surgery* in 2019. This article demonstrates proof-of-principle of an NPD comprising FES paired with proximal electrical neural blockade for therapeutic management of unilateral facial paralysis in a rat model. Chapter 5 is an original methods manuscript to be submitted to the *Journal of Neuroscience Methods*. This article characterizes a novel comprehensive experimental platform for high-throughput functional and histomorphometric assessment of facial nerve function in a rat model. Chapter 6 presents a general discussion of the thesis findings and outlines a plan for future experiments and translation to clinical practice.

ORIGINAL CONTRIBUTIONS TO KNOWLEDGE

Facial Palsy Clinical Management

(1) I developed a graphical classification system for facial palsy based on the anatomic status of the facial nerve and duration of facial muscle weakness to guide clinical management of this challenging disease state (see Figure 2-2). Though multiple facial grading scales had been heretofore characterized, none had sought to classify patients into specific treatment domains.

Therapeutic Potential of Functional Electrical Stimulation (FES) in Facial Palsy

(2) I demonstrated that electromyography signals from implantable epimysial electrode arrays could be employed to predict facial displacements in mammals (Figure 4-8). This suggests the potential utility of epimysial EMG signals as control inputs for a FES system for rehabilitation of unilateral facial paralysis. Though prior work had demonstrated the utility of surface and needle EMG signals for prediction of facial displacements, neither approach is suitable for use with an implanted neuroprosthetic device (NPD).

Therapeutic Potential of Electrical Neural Blockade in Facial Palsy

(3) I demonstrated that proximal delivery of high-frequency alternating current (HFAC) via implanted nerve-cuff electrodes on the facial nerves of awake behaving mammals could inhibit physiologic neural activity without impacting distal neuromuscular excitability via targeted direct neural stimulation (Figure 4-9). This indicates a potential role for proximal neural blockade via HFAC combined with distal FES in the management of facial palsy.

Though prior work by others had established the efficacy and utility of HFAC use to suppress neural activity, use of proximal HFAC paired with distal FES for rehabilitation of unilateral facial palsy had heretofore not been described.

(4) I demonstrated feasibility, efficacy, and lack of neural dysfunction following prolonged delivery of HFAC to mammalian facial nerve branches over the medium term (Figure 4-10). This suggests that HFAC may be safely employed for long-term management of undesirable neural activity in the setting of postparalytic facial palsy and following nerve transfer procedures. Heretofore, no previously published work had assessed long-term motor neural structure and function following continual HFAC delivery.

Research Model for Study of Novel Therapeutics for Facial Palsy

(5) I characterized a research platform for high-throughput assessment of facial nerve structure and function in the rat, comprising a novel head fixation device and electrode array designed to permit long-term study of electrical stimulation (see Figure 5-3 and Figure 5-4). This platform has potential to accelerate knowledge discovery in the field of neurobionics. Though functional rat models for study of facial nerve regeneration had been described, the novel approach herein obviates the need for labour-intensive manual conditioning of animals to restraint, with potential to enable long-term implantation of neuromuscular electrodes in a cost-effective fashion.

(6) I demonstrated that a single cost-effective camera paired with a trained deep neural network could be employed for automated quantitative assessment of facial nerve function in head-restrained rodents (see Figure 5-6). This approach has potential to accelerate knowledge discovery in the field of facial nerve regeneration. Though

computer vision tracking of rodent facial displacements is not novel, this is the first approach employing deep neural networks for automated tracking of rat facial displacements from high-frame rate videography as a model for facial nerve regeneration.

Contributions to original knowledge in the field of neural rehabilitation from research completed over the course of my Ph.D. studies are evidenced by the awarding of a United States patent (for which I am co-inventor) titled "*Electrical neural blockade and functional stimulation of dysfunctional or transferred nerves*" (WO2017124019A1 / US US201922383A1 / US10850097B2). The date first conceived was August 2, 2014 and date of first disclosure was September 2014. The original filing date with the United States Postal Office was January 2016 and the patent was granted in December 2020. Further evidence that this work comprises a meaningful and original contribution to knowledge is the awarding of an R-01 grant (for which I serve as co-investigator) from the National Institute of Neurological Disorders and Stroke of the U.S. NIH (5R01NS071067) in 2016 based on the novel data presented herein.

CONTRIBUTIONS OF AUTHORS

Chapter 2

Jowett N. A General Approach to Facial Palsy. *Otolaryngol Clin North* Am. 2018 Dec; 51(6):1019-1031. PMID: 30119926.

I was the sole author on this clinical review paper. The approach outlined herein summarizes how I approach my own patients with this disease. The conception, writing, and figure preparation was my own work.

Chapter 3

Jowett N[*], Greene JG[*]. Neuroprosthetic Device Potential in Facial Palsy. To be submitted to *Current Opinion in Otolaryngology and Head & Neck Surgery*

[*]Authors contributed equally

I conceived and designed the format of this paper. I co-wrote the paper and assisted with figure preparation and referencing together with Dr. Jacqueline Greene, who has given her kind permission for it to be published as a chapter within this Ph.D. thesis. I critically revised and gave final approval to the manuscript.

Chapter 4

Jowett N, Kearney RE, Knox CJ, Hadlock TA. Toward the Bionic Face: A Novel Neuroprosthetic Device Paradigm for Facial Reanimation Consisting of Neural Blockade and Functional Electrical Stimulation. *Plast Reconstr Surg*. 2019 Jan; 143(1):62e-76e. PMID: 30589784.

I conceived and designed the format of this paper. Together with CJ Knox I acquired the data. Together with RE Kearney, I analyzed the data. I generated all figures for the manuscript. I drafted the manuscript and gave final approval. I served as corresponding author for this manuscript. In addition to expertise in biomedical system identification in this manuscript, RE Kearney critically revised the manuscript and provided guidance on the research. TA Hadlock supervised the research and provided critical revision of the manuscript.

Chapter 5

Jowett N, Ma F, Hadlock TA, Kearney RE. High-Throughput Platform for Study of Rat Facial Nerve Structure and Function. To be submitted to the *Journal of Neuroscience Methods*

I conceived and designed the format of this paper. I designed the custom head implant, screws, treadmill, recording setup, and surgical approach. I performed animal surgeries and acquired animal data. I created design parameters for a computer-vision algorithm to track facial displacements. I manually labelled acquired video and trained a deep neural network for automated facial landmark localization with an open-source toolbox. I analyzed data in MATLAB. I drafted the manuscript. Based on design parameters, F Ma wrote Python code to extract facial displacements from landmark positions outputted by the trained deep neural network. TA Hadlock provided guidance and supervision of the data capture aspects of the research. RE Kearney provided guidance and supervision on the data analysis aspects of the research and critical revision of the manuscript.

Additional Relevant Manuscripts Co-Authored During Ph.D. Studies Not Included Herein

Jowett N, Hadlock TA. An Evidence-Based Approach to Facial Reanimation. Facial Plast Surg Clin North Am. 2015 Aug; 23(3):313-34. PMID: 26208770.

In this paper, we provide a comprehensive evidence-based review of contemporary treatment strategies for facial palsy. I drafted the manuscript, prepared the figures, and served as corresponding author. TA Hadlock assisted with manuscript drafting, critical review, and final approval.

Wang W, Kang S, Coto Hernández I, **Jowett N**. A Rapid Protocol for Intraoperative Assessment of Peripheral Nerve Myelinated Axon Count and Its Application to Cross-Facial Nerve Grafting. *Plast Reconstr Surg.* 2019 03; 143(3):771-778. PMID: 30601328.

In this paper, we described a novel fluorescence-based neural tissue processing protocol that avoids challenges and costs of conventional histologic processing of nerve using osmium staining, resin embedding, and ultramicrotome sectioning. On this manuscript, I served as senior and corresponding author, wherein I contributed to the conception and design of the study, data acquisition, data analysis, data interpretation, statistical analysis, figure preparation, and manuscript drafting, critical revision, and final approval. W Wang contributed to the conception and design, data acquisition, and manuscript drafting. S Kang contributed to data acquisition. I Coto Hernández contributed to data acquisition and figure preparation.

Coto Hernández I, Yang W, Mohan S, **Jowett N**. Label-free histomorphometry of peripheral nerve by stimulated Raman spectroscopy. Muscle Nerve. 2020 07; 62(1):137-142. PMID: 32304246

In this paper, we described a label-free approach for rapid imaging of peripheral nerve frozen sections paired with a novel machine learning approach for automated quantitative neural histomorphometry. On this manuscript, I served as senior author, wherein I contributed to the conception and design of the study, data acquisition, data analysis, and manuscript drafting, critical revision, and final approval. I Coto Hernández contributed to the conception and design, data acquisition, data analysis, and manuscript drafting. W Yang contributed to the conception and design and data acquisition. S Mohan contributed to data acquisition and manuscript drafting.

Faris C, Tessler O, Heiser A, Hadlock T, **Jowett N**. Evaluation of Societal Health Utility of Facial Palsy and Facial Reanimation. JAMA Facial Plast Surg. 2018 Dec 01; 20(6):480-487. PMID: 30178066.

In this paper, we characterize community preferences for three facial palsy health states among healthy volunteers. I was responsible for study concept and design, survey creation, data analysis, figure preparation, and critical revision and final approval of the manuscript. C Faris contributed to survey creation, data acquisition, and manuscript drafting. O Tessler contributed to concept and design, survey design, and critical revision of the manuscript. A Heiser contributed to survey design, data acquisition. T Hadlock contributed to critical revision of the manuscript and survey design.

Dusseldorp J, Naunheim MR, Quetela O, Fortier E, Hadlock TA, **Jowett N**. Neurotization Preferences in Smile Reanimation: A Discrete Choice Experiment. Plas Reconstr Surg (in press, 2020)

In this paper, we employed choice models to characterize the value of restoration of spontaneous smile in facial palsy, discovering that a majority of survey respondents would accept a 40% risk of treatment failure. This highlights the importance of rehabilitation of spontaneous expression in management of facial palsy. I was responsible for study concept and design, survey creation, data analysis, figure preparation, drafting and critical revision and final approval of the manuscript. J Dusseldorp contributed to survey design and drafting of the manuscript. MR Naunheim contributed to survey design, data analysis, drafting and critical revision of the manuscript. O Quetela and E Fortier contributed to data acquisition. TA Hadlock contributed to survey design and critical revision of the manuscript.

LIST OF COMMON ABBREVIATIONS

ACE	angiotensin converting enzyme
ANA	anti-nuclear antibody
ANCA	anti-neutrophil cytoplasmic antibody
APLA	anti-phospholipid antibody
ASIC	application-specific integrated circuit
CAD	computer-aided design
CBC	complete blood count
CFNG	cross-facial nerve graft
CNC	computer numerical control
CPU	central processing unit
CRP	C -reactive protein
CUDA	compute unified device architecture
DAO	depressor anguli oris
DC	direct current
DLI	depressor labii inferioris
EEA	epimysial electrode array
EMG	electromyography
ENoG	electroneuronography
ERS	EMG response system
ESR	erythrocyte sedimentation rate
FDA	Food and Drug Administration
FES	functional electrical simulation
FFP	flaccid facial palsy
FPM	feet per minute
FGS	facial grading scale
FN	facial nerve
FP	facial palsy
GPU	graphics processing unit
HB	House Brackmann
HCl	hydrochloride
HFAC	high-frequency alternating current
HFD	head-fixation device
IRF	impulse response function
NCE	nerve cuff electrode
nFFT	nonequispaced fast Fourier transform
NLF	nasolabial fold
NPD	neuroprosthetic device
NTM	masseteric nerve
OSA	obstructive sleep apnea
PFP	postparalytic facial palsy
QOL	quality-of-life
RF	rheumatoid factor
RPM	revolutions per minute
SAQ	synkinesis assessment questionnaire

SRS	stimulus response system	VS	vestibular schwannoma
VAF	variance accounted for	VZV	varicella zoster virus

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CHAPTER 1: INTRODUCTION

1.1 Rationale and Objective

Facial expression is an evolutionary trait that facilitates social interactions (1-5). Mimetic movements are governed by a complex network of motor regions in the cortex and subcortex, which project to the facial motor nucleus in the brainstem. Lower motor neurons of the facial motor nucleus project via the seventh cranial nerve to neuromuscular junctions of specific facial muscles to effect voluntary and spontaneous expression. The seventh cranial nerve (known as the facial nerve) comprises a dominant trunk, which courses through a bony canal in the temporal bone of the cranium, and its terminal divisions within soft tissues of the face. Facial nerve dysfunction presents as facial palsy. Lower motor neuron facial palsy may be present from birth owing to a disorder in development, or arise secondary to neoplastic, traumatic, vascular, infectious, inflammatory, idiopathic, or iatrogenic insult. When facial palsy is acquired, the inciting insult to the facial nerve typically induces acute flaccid paralysis.

Seventh cranial nerve dysfunction results in functional loss beyond impairment in facial aesthetics and non-verbal communication. In the flaccid state, absence of frontalis muscle function results in ptosis of the brow that obscures vision. Loss of orbicularis oculi function results in the inability to blink, known as paralytic lagophthalmos. In the absence of treatment, paralytic lagophthalmos yields symptomatic eye irritation, which may progress to exposure keratitis, corneal scarring, and vision loss. Flaccidity of the nasalis muscle results in collapse of the external nasal valve and impaired nasal breathing. Loss of orbicularis oris muscle function yields oral incompetence and dysarthria. Smile is absent on the affected side.

Bell's palsy is the most common presentation of acute facial palsy (6). This idiopathic mononeuropathy occurs with a lifetime incidence of one in sixty and an annual incidence of 15-30 per 100,000 population (7, 8). Bell's palsy is thought to result from neurotropic viral reactivation (9-11) or autoimmune reactions (12, 13). Unlike neurons of the central nervous system, lower motor neurons retain capacity for regeneration of their axonal processes. Depending on the severity of facial nerve injury and therapeutic intervention, functional recovery may be complete, absent, or disordered resulting in a movement disorder known as postparalytic facial palsy (14, 15). Disordered or aberrant regeneration of facial motor neuron axons occurs following high grade facial nerve insult, where anatomic continuity of the nerve is preserved but internal architecture damaged. The inciting insult to the facial nerve results in flaccid paralysis that may last for several months, followed by gradual return of facial tone and movement over a period of one to two years.

Postparalytic facial palsy is a permanent end-state following recovery from high-grade facial nerve insult manifested by facial muscle hypertonicity and synkinesis. Facial synkinesis is the abnormal and involuntary activation of one or more facial muscles with voluntary or spontaneous expression. Postparalytic facial palsy presents with involuntary narrowing of the palpebral fissure with obstruction of visual fields, restriction of smile excursion, and facial muscle spasms yielding facial discomfort. Postparalytic facial palsy develops in up to 30% of patients following a single episode of Bell's palsy (16), corresponding to an annual incidence of up to 1700 cases in Canada alone. The incidence of postparalytic facial palsy following acute Lyme disease-associated facial palsy is in excess of 50% (17-19).

Health utility studies have demonstrated a majority of healthy individuals would favour monocular blindness over unilateral flaccid facial paralysis or severe postparalytic facial palsy

(20-22). Though manifold therapeutic options exist for management of flaccid facial paralysis and postparalytic facial palsy, clinical outcomes remain suboptimal (23). The objective of this thesis is to characterize and demonstrate the potential of a novel neuroprosthetic device approach to improve clinical outcomes in patients suffering from facial palsy.

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PREFACE TO CHAPTER 2

As indicated in the introduction, facial palsy comprises a broad spectrum of disease states. The etiology of facial palsy ranges from benign and fully reversible neural insults, such as mild cases of Bell's palsy, to those which are irreversible and life-threatening, such as malignancies of the parotid gland. Ultimate facial function following facial nerve insult may range from spontaneous and complete return of facial function to complete unilateral or bilateral paralysis. Accordingly, management of this disease is challenging. In this chapter, I present a conceptual framework for diagnostic and therapeutic management of facial palsy, together with a summary of current medical and surgical treatment options. Though not included herein, a comprehensive evidence-based review of contemporary facial palsy treatment strategies by Jowett and Hadlock is available in the published literature (23).

CHAPTER 2: A GENERAL APPROACH TO FACIAL PALSY

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Keywords: Facial palsy, facial reanimation, facial nerve, synkinesis, management

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2.1 Abstract

Management of facial palsy can be daunting. This article presents a conceptual framework for classification and therapeutic management of facial palsy.

2.2 Key points

1) Facial palsy is devastating condition encompassing a spectrum of movement disorders ranging from flaccid paralysis to postparalytic facial hyperactivity

2) Timing and selection of diagnostic and therapeutic interventions in facial palsy is critical

3) Therapeutic management may comprise medical therapy, surgical decompression, physical therapy, injectables, and surgical reanimation procedures

2.3 Introduction

Facial palsy (FP) is devastating condition with functional and esthetic sequelae resulting in profound quality-of-life (QOL) impairment (24, 25). When acquired, the inciting insult typically results in acute flaccid facial palsy (FFP). Depending on the degree of neural injury, ultimate outcomes range from persistent and complete FFP to full return of normal function. In between these extremes exist zonal permutations of hypo- and hyperactivity and synkinesis, often with symptomatic gustatory epiphora and facial discomfort, a condition known as postparalytic facial syndrome (14, 26) the result of aberrant regeneration of the facial nerve (27, 28). For clarity, a summary of pertinent definitions is provided in Table 2-1. This article provides a diagnostic and therapeutic management approach to facial palsy.

Table 2-1: Relevant definitions.

Term	**Definition**
Facial palsy (FP)	Term encompassing entire spectrum of facial movement disorders including flaccid facial palsy, facial paresis, and postparalytic facial palsy
Flaccid facial palsy (FFP)	Complete or near-complete absence of facial movement and tone, without synkinesis or hyperactivity
Postparalytic facial palsy (PFP)	Facial movement disorder occurring as a result of aberrant regeneration following facial nerve insult, comprising varying degrees of zonal synkinesis, hypo- and hyperactivity
Facial synkinesis	Involuntary and abnormal facial muscle activation accompanying volitional or spontaneous expression

2.4 History and Physical Examination

It is incumbent upon the treating clinician to establish a diagnosis for the underlying cause of the facial movement disorder. Common causes of acute facial palsy include Bell's,

Ramsay-Hunt syndrome (varicella zoster virus), Lyme disease, otic infections and cholesteatomas, post-surgical insult (eg. following vestibular schwannoma extirpation), benign tumors (eg. facial nerve schwannomas or venous vascular malformations of the facial nerve), or malignant tumors (eg. parotid or hematogenous primaries or metastases from primaries from skin), congenital malformations, and trauma (29). Though rare, pontine infarcts or hemorrhages may present with isolated facial palsy (30).

The time course of palsy onset, progression, resultant symptoms and their impact are documented. A thorough history is invaluable in establishing the diagnosis; the clinician may inquire as to the presence of otovestibular symptoms (hearing loss, hyperacusis, vertigo, imbalance, ottorhea, otalgia), other focal neurological deficits (eg. diplopia, facial anesthesia), constitutional symptoms (fever, chills, fatigue, malaise, sweats, weight loss), meningitic (headache, nuchal rigidity) and Lyme-specific symptoms (recent tick bite or exposure, erythema migrans rash, arthralgias, myalgias, low back pain), and inflammatory symptoms (eg. orofacial swelling or parotitis, uveitis) or known auto-immune conditions (eg. systemic lupus erythematosus, Sjogren's). In the setting of acute idiopathic facial palsy, red flags suggesting a diagnosis other than Bell's include bilateral paralysis, slow onset of facial weakness (weakness in Bell's palsy fully evolves over 24-72 hours), asymmetric weakness across facial zones at onset, constitutional symptoms (fever, lethargy, malaise, myalgias), headache (other than retro-auricular pain and otalgia, which occur frequently in Bell's palsy), presence of other focal neurologic deficits (diplopia, hearing loss, vertigo), and absence of recovery of facial tone within four months of palsy onset. Facial symptoms vary according to the timing of presentation and degree of recovery. Flaccid facial palsy (FFP) results in paralytic lagophthalmos and ocular irritation, loss of facial symmetry at rest, collapse of the external nasal valve, and oral

incompetence. Postparalytic facial palsy (PFP) presents with facial synkinesis, muscle hyperactivity, contracture, and epihora. Platysmal synkinesis results in neck discomfort and facial fatigue. Periocular synkinesis results in a narrowed palpebral fissure width. Lack of meaningful smile occurs in severe cases.

A thorough head and neck examination, including otoscopy and detailed cranial nerve examination is performed. Zonal assessment of facial function at rest and with movement is crucial (Figure 2-1). The brow position together with its effect on the periocular complex is noted. The degree of paralytic lagophthalmos, presence or absence of Bell's phenomenon, width of the palpebral fissure, and position of the lower lid is noted; laxity is assessed using distraction and snap-back tests. The depth and orientation of the nasolabial fold (NLF), position of the oral commissure, and presence and degree of brow, ocular, midfacial, depressor, mentalis, and platysmal synkinesis are evaluated. Attention is paid to the contralateral hemi-face with regard to whether weakening of a given paired muscle group – such as the hemi-brow or depressor labii inferioris – is likely to result in improved symmetry. Photo- and videography to document appearance at rest and with seven volitional facial movements (brow elevation, light and full-effort eye closure and smile, lip pucker, lower lip depression) on presentation and follow-up are essential. Spontaneous smile may be assessed by elicitation using humorous video clips (31).

Investigations

When the history and physical examination are consistent with Bell's palsy, further investigation is not required except in Lyme endemic areas, where serology is always prudent (17, 32, 33). Imaging studies (such as a fine cut CT of the temporal bone without contrast, and/or gadolinium enhanced MRI of the temporal bones and parotid gland) are indicated to rule out benign or malignant tumors affecting the facial nerve and should be ordered in the setting of

abnormal otoscopy or tuning fork findings, palpable parotid or neck mass, slow- or asymmetric-onset of facial palsy, slowly-progressive facial palsy, unilateral recurrent facial palsy, or recent facial palsy demonstrating absent recovery at four months. Blood work (CBC, ESR, CRP, RF, ANA, ANCA, APLA, ACE) is indicated in recurrent cases, or where autoimmune conditions are suspected. Electro-neuronography (ENoG) is indicated between three and 14 days of symptom onset in patients who present with delayed traumatic or idiopathic FFP who demonstrate complete absence of hemi-facial movement on examination.

2.5 Therapeutic Management

Given the breadth of therapeutic options, management of facial palsy can be daunting. It is useful to classify patients with facial palsy into one of five management domains, based on timing of presentation, and status of the facial nerve and facial musculature (Figure 2-2); acute FFP, FFP with potential for spontaneous recovery, FFP with viable facial musculature with low potential for spontaneous recovery, FFP without viable facial musculature, and postparalytic facial palsy. Therapeutic intervention may include pharmaceutical agents, corneal protective measures, physical therapy, chemo-denervation agents, fillers, and a myriad of surgical procedures. Organization of potential interventions by type and side of facial palsy, and by facial zone is valuable for developing a therapeutic plan (Table 2-2and Figure 2-3).

I - Acute Flaccid Facial Palsy (Intact Facial Nerve)

This domain encompasses the first 72 hours to two weeks following onset of acute facial nerve injury. The role of the clinician is to establish a diagnosis, initiate appropriate medical therapy (such as immunosuppressant, antiviral, or antibiotic), manage exposure keratopathy risk, and determine candidacy for acute surgical intervention. In the setting of Bell's palsy,

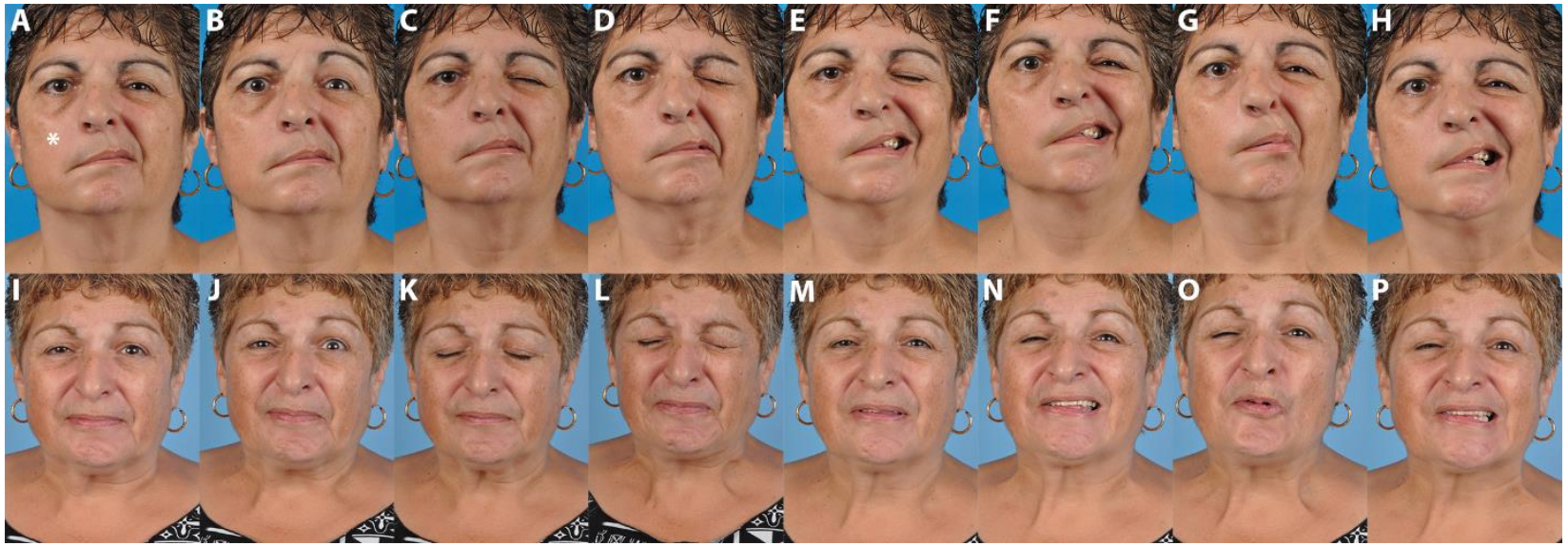

Figure 2-1: Acute flaccid facial palsy (top) and subsequent postparalytic facial palsy (bottom) in Ramsay-Hunt Syndrome (varicella-zoster viral facial palsy). Complete flaccid paralysis on the affected side (*) is demonstrated at rest (A), and with brow elevation (B), gentle eye closure (C), full-effort eye closure (D), gentle smile (E), full-effort smile (F), lip pucker (G), and lower lip depression (H). The patient lacks Bell's Phenomenon (C, D). At one year following symptom onset, the affected brow remains depressed, while hyperactivity has developed in the orbicularis oculi, mentalis and platysma muscles at rest (I). Volitional brow elevation remains impaired (J), while marked brow synkinesis is present with eye closure (K, L). As is usual in PFP, eye closure is adequate (K, L). Smile symmetry is improved with light effort (M); commissure restriction is noted with full-effort smile. Near normal return to function of the orbicularis oris muscle is noted (O). Lip depressor function remains weak on the affected side (P). Periocular, mentalis, and platysmal synkinesis is worsened by smile, pucker, and lip depression (N-P).

administration of high-dose corticosteroids within 72 hours of symptom onset shortens recovery time (34). Combined use of antivirals and corticosteroids in Bell's may be of additional clinical benefit, especially for those with severe to complete paralysis (35, 36), and good evidence supports combination therapy in VZV (37). Delayed onset or incomplete FP following trauma or iatrogenic insult warrants corticosteroids and observation. Iatrogenic injury resulting in immediate and complete paralysis of one or more FN branches warrants urgent surgical exploration. Patients with complete idiopathic or post-traumatic paralysis with an ENoG response demonstrating >90% degeneration and absent voluntary motor units on electromyography (EMG) are referred to neurotology for consideration of surgical decompression within 14 days of symptom onset (35, 38 55). Lyme disease-associated FP is treated with a prolonged course of oral doxycycline or intravenous ceftriaxone (39). Though commonly prescribed, the role of adjuvant corticosteroid therapy in Lyme is unclear (17, 19, 40). Otitis media-associated FP is treated with wide myringotomy with or without mastoidectomy, corticosteroids, topical and parenteral antibiotics (41). Eye lubrication with nighttime taping of the eye closed is typically indicated to prevent exposure keratopathy. Physical therapy (PT) for education and instruction on upper eyelid stretching to aid passive closure may be of benefit. Correction of paralytic lagophthalmos may be achieved by temporary tarsorrhaphy or upper eyelid weighting; indications include poor prognosis for rapid recovery, inability to work due to ocular symptoms, inadequate Bell's phenomenon, and absent recovery at 4 months (42).

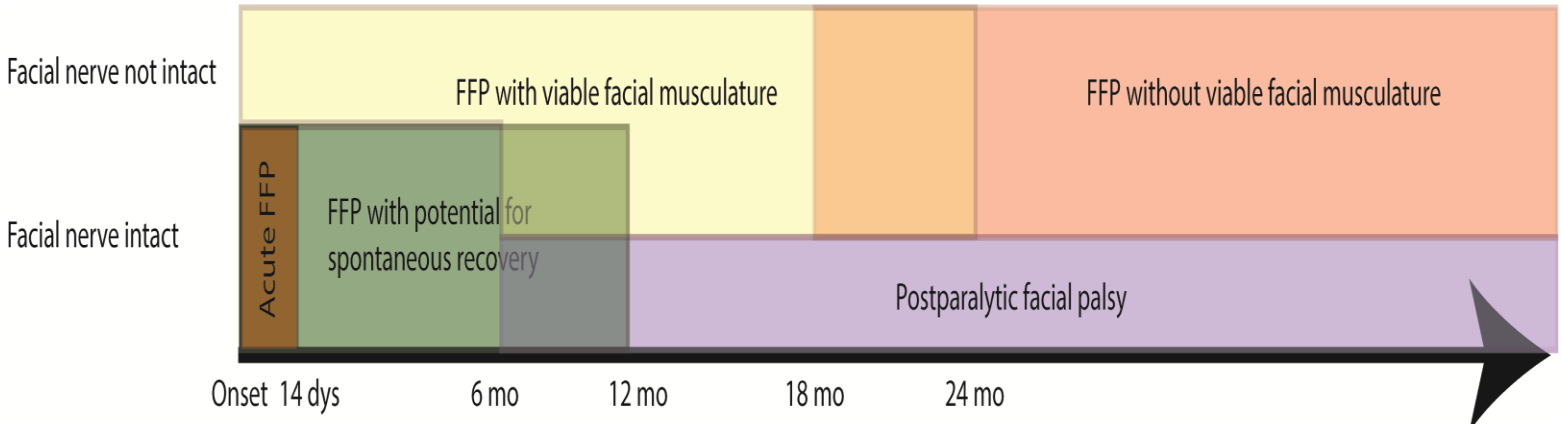

Figure 2-2: Facial palsy management domains. Patients may be categorized according to timing of presentation from palsy onset, and status of the facial nerve and facial musculature. This conceptual framework is helpful in selecting appropriate therapeutic interventions. Medical therapy is often indicated in acute flaccid facial palsy (FFP). Close observation is indicated for a period of several months in FFP where there exists potential for spontaneous recovery (for example, following extirpation of a vestibular schwannoma with facial nerve preservation). Where the facial nerve is discontinuous, nerve repair or transfers (such as hypoglossal-to-facial and/or nerve-to-masseter to branches controlling smile) are immediately indicated; such transfers are also indicated in the case of persistent FFP following 6-12 months of observation as native facial musculature remains viable (i.e., receptive to reinnervation) for a period of approximately two years. Where facial musculature is no longer viable (i.e., absent or unreceptive to reinnervation), muscle transfers are indicated for smile reanimation. Patients with postparalytic facial palsy (comprising synkinesis and varying degrees of zonal hypo- and hyperactivty) are typically managed with physical therapy and chemodenervation; surgical reanimation is appropriate in severe cases.

Table 2-2 Therapeutic options in flaccid facial palsy (FFP) and postparalytic facial palsy (PFP).

Setting	Acute FFP (intact facial nerve)	FFP with potential for recovery	FFP with viable musculature and low potential for recovery	FFP without viable facial musculature	PFP
Medical management	• Corticosteroids - idiopathic (Bell's), varicella zoster (VZV/Ramsay-Hunt), acute otitis associated, delayed traumatic, delayed iatrogenic • Antivirals - VZV, consider for idiopathic • Antibiotics (targeted) - indicated for Lyme disease or acute otitis • Eye protection is always indicated -Daytime lubricating eye drops • -Nighttime lubricating ointment, eyelid taping	• Corneal protection is always indicated -Daytime lubricating eye drops • -Nighttime lubricating ointment, eyelid taping	• Corneal protection is always indicated -Daytime lubricating eye drops • -Nighttime lubricating ointment, eyelid taping	• Corneal protection is always indicated -Daytime lubricating eye drops • -Nighttime lubricating ointment, eyelid taping	• Corneal protection if blink inadequate (rare) -Daytime lubricating eye drops • -Nighttime lubricating ointment, eyelid taping
Physical therapy	• Patient education • Eyelid stretching	• Patient education • Eyelid stretching	• Patient education • Eyelid stretching • Targeted physical therapy following dynamic reanimation	• Patient education • Eyelid stretching	• Patient education • Eyelid stretching • Biofeedback • Neuromuscular retraining • Targeted physical therapy following dynamic reanimation

Setting	• **Acute FFP (intact facial nerve)**	• **FFP with potential for recovery**	• **FFP with viable musculature and low potential for recovery**	• **FFP without viable facial musculature**	• **PFP**
Medical management	• Corticosteroids - idiopathic (Bell's), varicella zoster (VZV/Ramsay-Hunt), acute otitis associated, delayed traumatic, delayed iatrogenic • Antivirals - VZV, consider for idiopathic • Antibiotics (targeted) - indicated for Lyme disease or acute otitis • Eye protection is always indicated -Daytime lubricating eye drops -Nighttime lubricating ointment, eyelid taping	• Corneal protection is always indicated -Daytime lubricating eye drops -Nighttime lubricating ointment, eyelid taping	• Corneal protection is always indicated -Daytime lubricating eye drops -Nighttime lubricating ointment, eyelid taping	• Corneal protection is always indicated -Daytime lubricating eye drops -Nighttime lubricating ointment, eyelid taping	• Corneal protection if blink inadequate (rare) -Daytime lubricating eye drops -Nighttime lubricating ointment, eyelid taping
Injections	• None indicated	• Botulinum toxin - Contralateral brow • - Contralateral depressor labii inferioris (DLI)	• Botulinum toxin -Contralateral brow -Contralateral DLI • Volumizing fillers -Contralateral NLF • -Ipsilateral lips	• Botulinum toxin -Contralateral brow -Contralateral DLI • Volumizing fillers -Contralateral NLF • -Ipsilateral lips	• Botulinum toxin -Contra- or bilateral brow -Ipsilateral orbicularis oculi -Contralateral DLI -Ipsilateral DAO -Ipsilateral mentalis -Ipsilateral platysma • Volumizing fillers -Ipsi- or contralateral NLF • -Ipsilateral lips

Setting	Acute FFP (intact facial nerve)	FFP with potential for recovery	FFP with viable musculature and low potential for recovery	FFP without viable facial musculature	PFP
Surgical management	**Adjunctive** • Facial nerve decompression - indicated for idiopathic and post-traumatic complete FFP with ENoG response <90% and absent voluntary motor units on EMG between 3 and 14 days of symptom onset • Wide myringotomy +/- tube placement +/- mastoidectomy – indicated for acute otitis **Static reanimation** • Eyelid weight (reversible if recovery ensues)	**Static reanimation** • Eyelid weight • Consider lower lid tightening in elderly patients	**Static reanimation** • Brow ptosis correction • Eyelid weight • Lower lid tightening • External nasal valve correction • Nasolabial fold suspension • Oral commissure suspension **Dynamic reanimation** • Direct end-to-end repair or interposition grafting (for facial nerve transections/resections) • XII-VII for facial tone • Cross-facial nerve grafting or V-VII for targeted reanimation of expression (blink, smile)	**Static reanimation** • Brow ptosis correction • Eyelid weighting and lower lid tightening • Static facial sling: external nasal valve, NLF, oral commissure • Rhytidectomy • Contralateral DLI resection **Dynamic reanimation** • Smile reanimation • -Temporalis or free muscle transfer	**Static reanimation** **•Brow ptosis correction** **•Highly-selective neurectomy** **•Ipsilateral rhytidectomy** **•Contralateral DLI resection** **•Ipsilateral DAO resection** **•Platysmectomy** **Dynamic reanimation** **•Smile reanimation** **-Temporalis transfer or free muscle transfer**

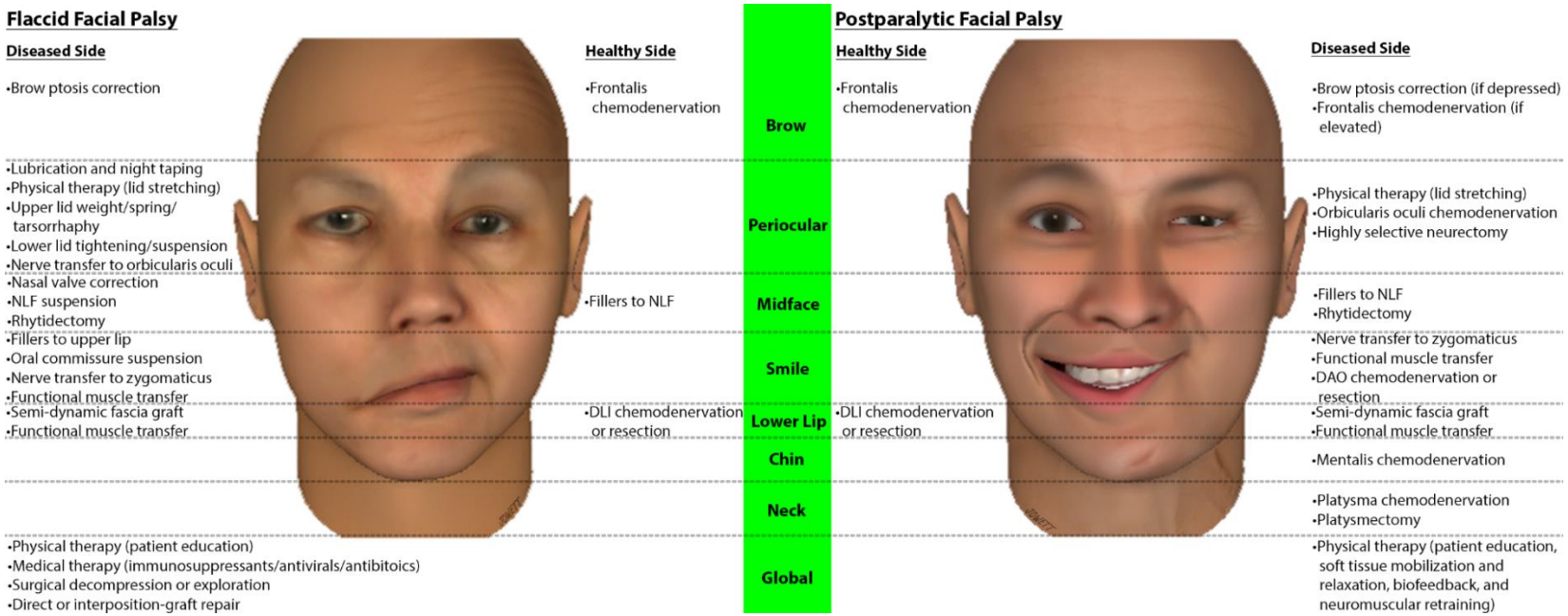

Figure 2-3: Therapeutic options in flaccid and postparalytic facial palsy, by facial zone and side. A plethora of targeted therapeutic interventions may be employed to restore balance and symmetry in hemi-facial palsy (NLF: nasolabial fold, DLI: depressor labii inferioris).

II - Flaccid Facial Palsy with Potential for Spontaneous Recovery

Where nerve continuity is believed intact in the setting of FFP – for example, following resection of a vestibular schwannoma (VS) where FN stimulation was noted prior to closure – a potential for spontaneous recovery exists, whereby return of facial tone and movement is expected within 6-12 months. Patients may benefit from physical therapy, corneal protective measures, static periocular reanimation, and temporary chemodenervation of the healthy-side depressor labii inferioris muscle to improve oral competence and articulation during this period. Close follow-up (every 3 months) is warranted to ensure recovery of function.

III - Flaccid Facial Palsy with Viable Facial Musculature and Low Potential for Spontaneous Recovery

Herein there exists discontinuity of the facial nerve or absent recovery of facial function noted within six to twelve months of facial palsy onset. Native facial musculature is intact and likely receptive to reinnervation. Common clinical scenarios involve patients presenting with dense FFP resulting from temporal bone tumors (such as facial nerve schwannomas, venous vascular malformations, or cholesteatomas), cerebellopontine angle tumor extirpations, or pontine hemorrhage. Though no definitive criteria exist, evidence from case-series suggests that facial musculature remains receptive to reinnervation for periods up to 24 months following denervation in adults (43-46), and for possibly longer periods in children. Within this timeframe, nerve repair and transfers are indicated. Interposition graft repair should be contemplated in the setting of neural discontinuity; split-hypoglossal nerve transfer to the main trunk of the facial nerve is an alternative option where interposition graft repair is unfeasible or where no recovery is noted within 12 months. The goal of main trunk repairs and transfers is to restore facial tone and some form of blink; meaningful reanimation of expression is rarely achieved. Volitional

expressions may be restored through targeted nerve transfers during this period, such as transfer of the masseteric nerve to lower zygomatic branches of the facial nerve for smile reanimation, or cross-face nerve grafting to upper zygomatic branches for blink restoration. Targeted nerve transfers should be considered in patients demonstrating minimal to no facial function (i.e, House-Brackmann Grade (47) V or VI) seven months following vestibular schwannoma resection, as the probability of ultimate recovery of meaningful expression in this scenario is less than ten percent (48). Static periocular reanimation (such as upper lid weighting and lateral tarsal strip procedure) is offered early in the course of palsy onset where recovery is likely to take several months.

IV - Flaccid Facial Palsy without Viable Facial Musculature

Where native facial musculature is absent (eg. following resection or congenital absence) or unlikely to be receptive to reinnervation (eg. long denervation period or marked distal perineural spread of a malignancy), nerve repair or transfers are no longer indicated. In addition to physical therapy and targeted chemodenervation of healthy side lip depression and brow elevation, surgical interventions include static facial suspensions, static periocular reanimation, and muscle transfers. Targeted suspensions of the brow, lower eyelid and mid-face, nasal valve, nasolabial fold, and oral commissure may be achieved using sutures, fascia lata, bioabsorbable or permanent implants. Tightening of the lower lid may be achieved by the lateral tarsal strip (LTS) procedure (49), with or without medical canthal tendon plication. Dynamic smile reanimation may be achieved through antidromic (50) or orthodromic (51) temporalis muscle transfer, or free muscle transfer (52, 53) with motor innervation provided through cranial nerve transfer. Options for dynamic reanimation of the lower lip include anterior digastric muscle transfer (54) or inlay of a T-shaped fascia graft (55).

V- Postparalytic Facial Palsy

Postparalytic facial palsy (PFP) develops six to eighteen months following severe facial nerve insult with spontaneous, yet aberrant, regeneration or following main trunk nerve grafting. Once present, it is permanent. Lagophthalmos is rare. PT is first-line treatment; a comprehensive program includes patient education, soft tissue mobilization, mirror and EMG biofeedback, and neuromuscular retraining (56). Blunting of hyperactivity through filler injection and weakening of hyperactive muscles through targeted chemodenervation, neurectomy, or resection in advanced disease is indicated in conjunction with PT. For many patients, targeted chemodenervation of diseased side orbicularis oculi, mentalis, and platysma offers significant improvements. Weakening of the diseased side depressor anguli oris muscle through chemodeneravation or resection can result in dramatic improvement in smile dynamics in select cases (57, 58). In cases with severe restriction of oral commissure excursion, regional (eg. temporalis) or free (eg. gracilis) muscle transfer may be considered for dynamic smile reanimation. Targeted nerve transfers – such as masseteric nerve transfer to diseased-side zygomatic branches for smile reanimation – are largely ineffective in the setting of PFP.

2.6 Clinical Outcomes

Systematic tracking of therapeutic outcomes is a prerequisite to clinical excellence. Outcomes tracking in facial palsy may entail patient-reported quality-of-life measures, clinician-assessed grading of facial function, and objective measurement of facial displacements. QOL impact may be assessed using generalized patient-graded scales such as the SF-36 (59). The Facial Disability Index (FDI),(60) the Facial Clinimetric Evaluation (FaCE) (24), and the Synkinesis Assessment Questionnaire (SAQ) (61) are patient-graded scales specifically designed and validated for use in facial palsy to concurrently assess symptom severity and impact on

QOL. Though global five- or six-point facial function scales exist including the House-Brackmann (47, 62), Fisch (63), and others (64, 65), such scales lack the resolution necessary to capture meaningful changes in zonal function over time. The Yanagihara scale (66) provides Likert-scale resolution of zonal appearance with movement but not at rest, and lacks separate grading of synkinesis. The Sunnybrook Facial Grading System (FGS) (67, 68) provides weighted scores of zonal symmetry at rest and with motion in addition to synkinesis. A recently validated electronic facial paralysis assessment tool (eFACE) provides even higher resolution zonal data through use of continuous visual analogue scales to assess five static, seven dynamic, and four synkinetic zonal parameters (69, 70). A computer vision-based facial landmark recognition algorithm has recently been employed within a novel freeware application (Emotrics, Massachusetts Eye and Ear) for objective measurement of various facial displacements (eg. smile excursion) from clinical photographs (71).

2.7 Summary

Management of facial palsy necessitates establishing a diagnosis and formulating a therapeutic plan according to the timing of presentation in flaccid cases, and the specific pattern of facial dysfunction in patients presenting with aberrant neural regeneration. Therapeutic interventions include physical therapy, injectables, and a plethora of surgical reanimation procedures to restore meaningful expression, facial balance and symmetry.

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PREFACE TO CHAPTER 3

In the previous chapter, a general approach to diagnostic and therapeutic management of flaccid and postparalytic facial palsy was presented. This primer may prove useful for clinicians unfamiliar with management of this complex and devastating disease state. Though manifold medical and surgical options are available for treatment of facial palsy as outlined in Chapter 2, existing strategies fail to adequately restore facial function. These disease states are characterized by poor or absent control of facial movement. Heretofore, the most significant barrier to more effective facial reanimation strategies has been a lack of effective control mechanisms for denervated or aberrantly re-innervated facial muscles. In Chapter 3, we summarize evidence suggesting facial palsy is amenable to rehabilitation by functional electrical stimulation (FES) in similar fashion to other neuromuscular disease states.

CHAPTER 3: NEUROPROSTHETIC DEVICE POTENTIAL IN FACIAL PALSY

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3.1 Abstract

Despite the success of neuroprosthetic devices for hearing loss, foot drop, and obstructive sleep apnea, there exists no FDA approved device for management of facial palsy. In addition to financial challenges associated with development of medical devices for rare diseases, prior technological and methodological limitations have impeded progress in the field. The goal of this review is to summarize recent advances in neuroprosthetic device development and their potential role in rehabilitation of facial palsy.

Recent advances in microfabrication techniques with flexible electronics and the evolution of nerve cuff electrodes have led to reduced local tissue inflammatory reactions and improved stability at the neural-electrode interface. Functional optogenetic stimulation carries potential as an alternative to electrical stimulation with potentially improved spatial selectivity and avoidance of long-term sequelae of chronic deposition of electrical charge. Neural blockade provides a means to control undesirable facial movements resulting from nerve transfer procedures or aberrant regeneration of the facial nerve. Miniaturization of powerful application-specific integrated circuits combined with advances in wireless charging technology render possible the development of a fully implantable neuroprosthetic device for facial palsy. Miniaturization of neural-electrode designs, application-specific integrated circuits, and wireless charging technology combined with novel approaches to management of undesirable facial activity herald the potential emergence of a neuroprosthetic device for facial palsy.

3.2 Introduction

High-grade insult to the facial nerve yields facial paralysis. Facial palsy is a devastating clinical condition resulting in functional, aesthetic, and communication deficits, including inability to raise the brow, blink, or smile. The condition may yield vision loss, speech and articulation difficulties, nasal obstruction, oral incompetence, drooling, and impaired nonverbal communication with profound impact on quality-of-life (20). Acute facial nerve transection may be treated with tensionless direct repair or interpositional nerve grafting. When repair of the main trunk is not feasible, nerve substitution techniques are indicated. Though cross face nerve grafting (CFNG) carries potential for reanimation of spontaneous expressions such as smile and blink, the procedure often fails to restore adequate movement. Masseteric nerve (NTM) transfer to select zygomatic branches of the facial nerve has emerged as a reliable technique for targeted smile reanimation, but lacks spontaneity and resting tone (72-76). Though hypoglossal nerve transfer to the main trunk of the facial nerve can restore adequate resting tone to the acutely paralyzed face, it does not restore symmetric expression (77, 78).

In long-standing facial palsy, facial musculature is unreceptive to motor neurotization. Functional muscle transfer is required for reanimation in this setting. Transferred muscle requires a donor nerve source of motor axons, for which trigeminal motor branches are often employed for smile vector reanimation (52). Disadvantages of trigeminally-driven smile include lack of spontaneity, lack of midfacial tone, and undesirable facial movements with prandial activity (79). Though normal facial expression and function necessitates activity of eleven paired muscles, dynamic facial reanimation is principally limited to the single vector of the zygomaticus major muscle to re-establish smile. No solution for multi-vector facial reanimation in long-standing facial palsy currently exists.

Where axons of the facial nerve are severely injured and the nerve sheath remains continuous, gradual recovery of facial tone and movement through axonal regeneration may occur. Postparalytic facial palsy occurs following aberrant recovery from high-grade facial nerve insult, and comprises varying degrees of zonal hypo- and hyper-activity and involuntary facial movements known as synkinesis (80, 81). The underlying pathophysiology is believed to comprise aberrant axonal sprouting and ephaptic conduction secondary to intraneural scarring and abnormal inter-neural connections (26, 82). In addition to physical therapy, postparalytic facial palsy may be managed via targeted weakening of specific hyperactive facial muscles via chemodenervation, neurectomy or myectomy to improve facial balance and symmetry. In severe cases, hypertonicity and co-contraction of modiolus levators and depressors can lead to restriction of oral commissure movements and lack of a meaningful smile. In these cases, functional muscle transfer for smile reanimation may be indicated.

The ultimate goal in the management of facial palsy is restoration of spontaneous, multi-vector spontaneous expression in flaccid and postparalytic states. Heretofore, the most significant barrier to more effective facial reanimation strategies in both flaccid and postparalytic facial palsy settings has been a lack of effective control mechanisms for denervated or aberrantly re-innervated facial muscles. Technological advances in signal processing techniques, implantable neural and muscular electrodes, and implantable microcontrollers, have led to remarkable breakthroughs in the design of neuroprosthetic devices (NPDs) for control of prosthetic limbs and ambulation in the setting of central nervous system disorders, for restoration of hearing and vision, and for treatment of obstructive sleep apnea and several neurological disorders (83-97). Herein we summarize recent advances in NPD development and the potential role of an NPD in the rehabilitation of facial palsy.

3.3 Functional Electrical Stimulation

The term functional electrical stimulation (FES) originated with Moe and Post in 1962 to describe electrical stimulation of denervated muscle to produce a functional muscular contraction in hemiplegic patients (98). Contemporary usage of the term FES may also comprise rehabilitation of sensory deficits. FES paradigms are implemented via NPDs. These devices deliver electrical current to excitable tissue to supplement or replace lost function in neurologically impaired individuals (Table 3-1) (99). Zoll's investigations of electrical stimulation to overcome cardiac asystole in two patients in 1952 led to the development of the modern cardiac pacemaker, the first implantable neuromodulatory device(100). In 1968, Brindley and Lewin reported that electrical stimulation delivered via electrodes implanted into the visual cortex of a blind patient elicited sensations of light flashes (101). The first neuroprosthetic device for FES of a peripheral motor deficit comprised rehabilitation of footdrop in hemiplegic patients as described by Liberson in 1961 (102). Other investigations into motor FES applications have focused on phrenic nerve stimulation for high level spinal cord injury patients (103) and for antagonist muscle activation for management of spasticity (104).

Table 3-1: Definitions of pertinent terms

Bioelectricity	Ability of biological tissues to propagate an electrical signal when stimulated
Functional electrical stimulation (FES)	Application of electrical current to excitable tissue to supplement or replace function that is lost in neurologically impaired individuals (sensory or motor)
Neuroprosthetic device (NPD)	A device to effect FES
Prosthetic	Artificial device that replaces a missing body part
Bionic	Replacement or enhancement of organs or body parts through electromechanical technology

The cochlear implant represents the prime example of an NPD in the field of otolaryngology. In 1950, Swedish neurosurgeon Lundberg stimulated a patient's auditory nerve with a sinusoidal current during a neurosurgical procedure and the patient perceived noise (105). French electrophysiologist Djourno and otolaryngologist Eyriès were the first to implant an electrode onto the auditory nerve during a facial nerve grafting procedure in a patient with a previous temporal bone resection for cholesteatoma (106, 107). Dr. William House, an American otologist, worked with electrical engineer Jack Urban and neurosurgeon Dr. John Doyle to develop and implant the first cochlear implant into the scala tympani partition of the human cochlea in 1961 (108). House and his colleagues faced skepticism from their contemporaries, as it was believed that simple electrical stimulation of the inner ear would be insufficient to restore speech comprehension and that implantation of such devices posed serious infection and wound healing risks (106, 107). The serendipitous development of speech comprehension following cochlear implantation was new evidence of the plasticity of the central nervous system to extract meaning from external electrical stimuli. Subsequent close collaboration between surgeons, engineers, and university-industry partnerships led to approval of the cochlear implant for adults by the FDA in 1984, launching the field of sensory neuroprosthetics.

Recent NPDs approved by the Food and Drug Administration (FDA) in the head and neck include the Inspire® hypoglossal nerve stimulator™ for severe obstructive sleep apnea (109, 110) (Figure 3-1), the Medtronic ® Interstim Therapy™ system, auditory brainstem implants (ABI) that stimulate second-order auditory neurons in the cochlear nucleus, deep brain stimulation for Parkinson's disease and epilepsy (111), and retinal neuroprostheses for vision restorationsuch as Second Sight Medical Products Inc (CA, USA) and Retina Implant AG

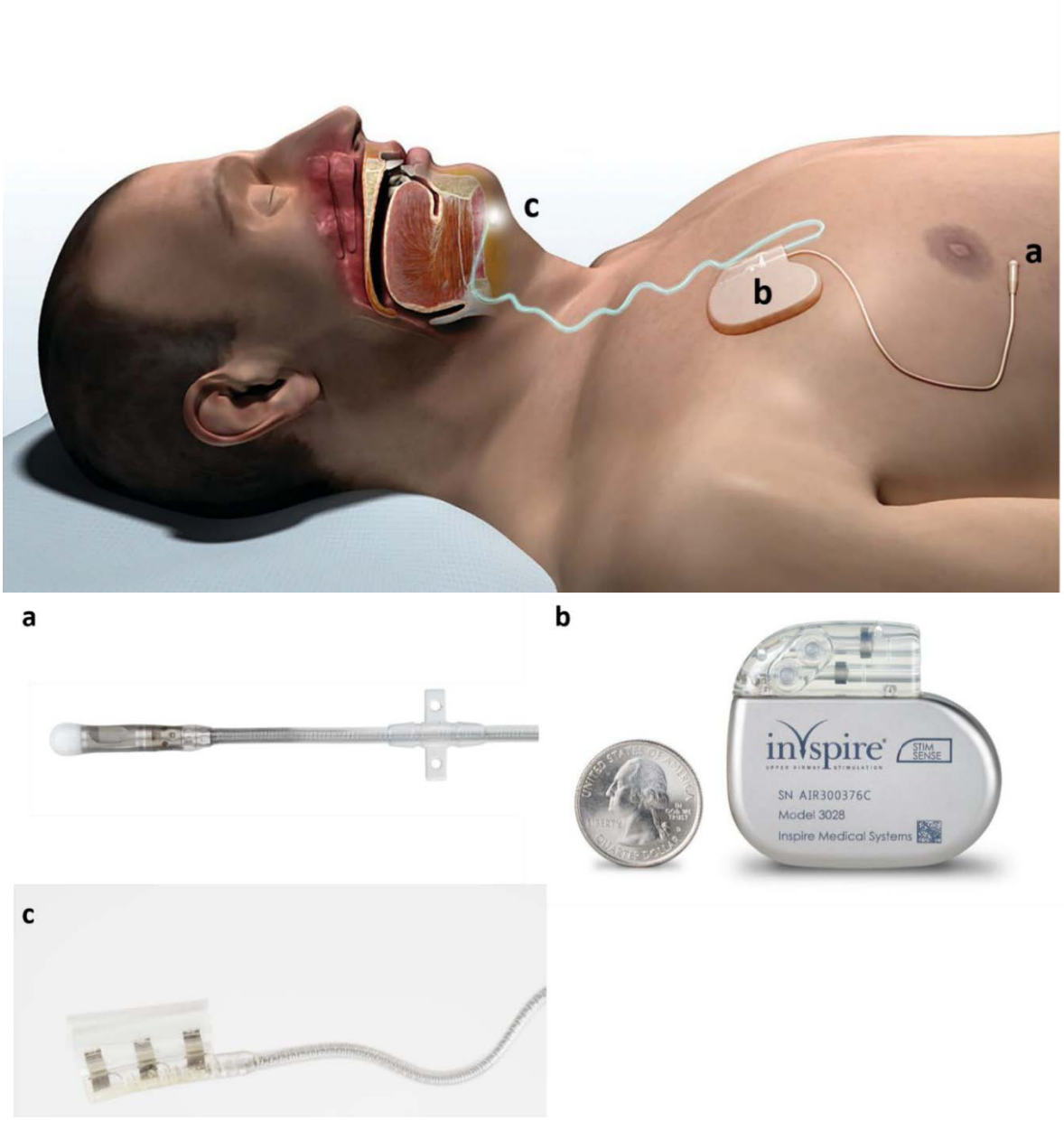

Figure 3-1: Hypoglossal nerve stimulator for the treatment of obstructive sleep apnea. The device comprises a functional electrical stimulation paradigm to mitigate oropharyngeal airway obstruction during sleep by eliciting tongue protrusion in response to inspiratory effort. The NPD comprises a (a) pressure sensor implanted into the intercostal space to capture respiratory cycles, (b) an implantable microcontroller to interpret the input signal from the pressure sensor and output electrical stimulation through (c) a nerve cuff electrode placed around the medial branch of the hypoglossal nerve to elicit tongue protrusion. (Published with permission from Inspire Medical Systems, Inc.)

(Reutlingen, Germany) (112). A fully implantable laryngeal pacemaker system has recently been tested in nine patients with bilateral vocal fold paralysis (113).

The general principle of FES comprises three components: a) an input (i.e. control) signal, b) a control algorithm, and c) an output signal to effect the desired response. An NPD comprises the hardware required to implement the FES paradigm, and consists of a sensor, processor, and effector that acts upon a targeted nerve or muscle to elicit a desired movement or sensation. Examples of various FES paradigms and their associated NPDs are shown in Figure 3-2.

3.4 Functional Electrical Stimulation for Facial Palsy

In 1977, Zealear and Dedo recognized the potential of FES in paired muscles systems that move symmetrically such as the laryngeal or facial muscles (114). Their work introduced the basic concept of employing contralateral healthy muscle activity as an input signal for FES of paralyzed cricothyroid muscles in dogs. Applications of FES to develop a NPD for facial palsy were carried on by multiple groups through the last few decades (115-124). A fully implantable NPD for restoration of eyeblink in patients with facial palsy has been reported by Ripple Inc., which utilizes a conductive polymer multi-electrode array for both EMG recording and healthy-side orbicularis oculi muscle activity and direct muscle stimulation on the paralyzed side (125, 126).

Heretofore, approaches to the development of an NPD for facial reanimation failed to achieve clinical translation due to financial, methodologic and technologic constraints. Surface electrodes, transcutaneous wires and bulky external hardware used in animal models are clinically unfeasible for long-term patient use. Although direct muscle stimulation of denervated muscle may prevent atrophy, it requires delivery of high and potentially injurious stimulus

DEVICE	Input Signal (sensor)	Control Algorithm	Output Signal (effector)	Desired Effect
Cochlear Implant	Sound (external microphone)	Converts input signal into electrical pulses that are sent transcutaneously to internal receiver via radio-frequency link	Electrical stimulation of the cochlear nerve (multichannel linear electrode array)	Restoration of hearing loss secondary to hair cell-dysfunction
Hypoglossal Nerve Stimulator	Thoracic pressure changes with respiration (implanted pressure sensor 4th intercostal space)	Converts input signal into electrical pulses to stimulate the hypoglossal nerve	Electrical stimulation of the hypoglossal nerve (Nerve cuff electrode)	Tongue Protrusion (relieving upper airway obstruction)
Proposed Facial Palsy NPD	Healthy facial muscle EMG (epimysial electrode array)	Converts input signal into electrical pulses to stimulate contralateral facial nerve	Electrical stimulation of affected-side distal facial nerve branches with proximal neural blockade signal (nerve cuff electrode array)	Reanimation of symmetric expression and suppression of undesirable facial activity in hemi-facial palsy

Figure 3-2: Functional Electrical Stimulation (FES) paradigms and the neuroprosthetic device (NPD) components. FES paradigms comprise an input signal, a control algorithm and an output signal to effect a desired outcome. NPDs are the devices to effect a particular FES paradigm and consist of a sensor, processor and effector that acts upon target tissue to evoke the desired effect.

amplitudes to evoke contractions (127-129). Such an approach is not feasible in the long-term due to excessive power requirements from an NPD, and regional nociceptive fiber activation that would result from the delivery of such high stimulus amplitudes.

The ideal device for reanimation of unilateral facial palsy would comprise a fully implantable solution with a robust input signal to predict contralateral healthy facial movements, a wirelessly chargeable miniature microcontroller, and an efficient means to effect synchronous and symmetric facial movements without potential activation of nociceptive fibers. Furthermore, an ideal device would also address the issue of undesirable facial activity that arises following aberrant regeneration and following nerve and muscle transfer procedures.

Input Signal and Sensors

As the majority of positive expressions are symmetric, the activity of contralateral healthy facial musculature may be used to drive an NPD. There are several means to capture healthy side facial activity, including facial landmark detection from video tracking, infrared sensors for blink detection, and EMG activity (123, 124, 130, 131). EMG signals are of sufficient amplitude for robust detection and precede muscle movements, rendering them ideal candidates for control of an FES paradigm for hemi-facial reanimation without conspicuous phase differences between normal and reanimated sides. Modeling the transformation of EMG activity to facial displacements has been demonstrated in rats (132) and rabbits (133). Recent work from our group has demonstrated greater than 96% variance-accounted-for (VAF) in a model using EMG input signals from epimysial EMG electrodes to predict whisking in a rat model of facial palsy (134).

EMG signals may be captured using surface electrodes, percutaneous needle electrodes, or implantable epimysial electrodes. Percutaneous needle and surface electrodes are an impractical solution for reasons including poor esthetics, discomfort, recurring costs, and variability in placement and subsequent signal acquisition. Ideal EMG electrode properties for a facial NPD include biocompatibility to avoid the need for external hardware, and robust signal capture over the long term through resistance to capsule formation, resistance to fracture, and biomechanical compatibility (135). Implantable epimysial electrode arrays (positioned on the surface of the muscle) are ideal for capture of facial EMG activity due to their relatively large surface area for signal capture that provides a high signal-to-noise ratio (SNR). A thin, flexible, and fully implantable conductive polymer epimysial multi-electrode array for EMG activity capture is shown in Figure 3-3 (125, 126).

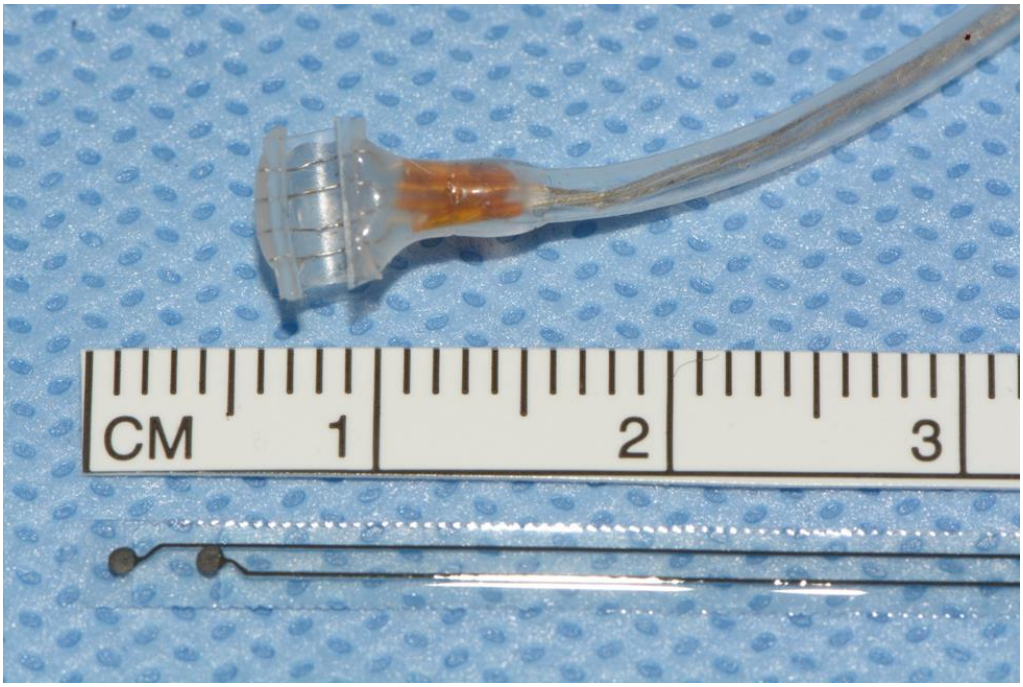

Figure 3-3: Experimental biocompatible electrodes. (Top) Implantable nerve cuff electrode comprising a silicone sheath with platinum-iridium electrodes (Microprobes for Life Science). (Bottom) Implantable epimysial conductive polymer electrode array for capture of EMG signals (Ripple LLC, proprietary materials).

Control Algorithm and Processors

Advances in signal processing techniques and miniaturization have led to the development of implantable application-specific integrated circuits (ASICs) for neuroprosthetic control of gait and grasp (136-140) and the treatment of obstructive sleep apnea. Modern implantable ASICs achieve delays of 5 ms or less on signal receiving and stimulus transmitting arms, with sub-millisecond processing times for most FES applications. Use of EMG activity to drive FES of hemifacial palsy requires real-time EMG signal conditioning, and digital signal processing of the conditioned signal for dynamic modulation of the output signal to achieve

desired facial movements. Output signal modulation may comprise variation in pulse amplitude, width, frequency, waveform and train rate of pulses. Existing technology would permit less than 20 ms delays between healthy-side detected EMG activity, signal processing, stimulus output, and subsequent paretic-side muscular contraction, well below the ~33 ms threshold above which humans are able to detect asymmetric movement (141, 142).

Output Signal and Effectors

Optimal FES of dysfunctional muscle requires three conditions: muscle must be neurotized, be capable of being stimulated in a purposeful and timely fashion, and be devoid of undesirable activity. Neurotization of muscle prevents denervation atrophy and permits FES through direct neural stimulation without the need for direct muscle stimulation. Such an approach is advantageous in that it requires far less energy, and is unlikely to result in inadvertent nociceptive fibre activation. An ideal means to drive FES for facial palsy would comprise implantable electrodes for direct neural stimulation.

Nerve cuff electrode designs have evolved significantly over time (143, 144). An implanted spiral nerve cuff electrode has been successfully employed in clinical use as the effector arm of the Inspire® Hypoglossal Nerve Stimulator, and thus makes an excellent candidate for use in an NPD for facial palsy. This design reduces potential nerve compression through a self-curling polymer sheath that 'self-sizes' to a given nerve, avoiding the need for securing of the cuff with sutures. Triolo described stable charge thresholds and functional muscle selectivity following implantation of spiral nerve cuff electrodes into 14 patients for periods of 2-11 years (145). More rigid readily customizable nerve cuff electrode designs are available for research purposes, permitting signal capture or stimulus delivery in mono- or multi-polar fashion (Figure 3-3) (146). Disadvantages of these rigid designs include variable contact between

electrodes and the nerve sheath about the circumference, and greater potential for nerve compression (144, 146).

3.5 Control of Undesirable Neural Activity

In the setting of facial palsy, undesirable neural activity arises from aberrant regeneration of the facial nerve or normal physiologic action of transferred nerves, including where the masseteric nerve is employed for smile reanimation, or the hypoglossal nerve used to restore facial tone. Contemporary strategies to address facial muscle hyperactivity in the setting of postparalytic facial palsy include chemodenervation or selective neurectomy, which result in temporary or permanent flaccidity of the target region with loss of ability to effect movement through FES. Direct application of high-frequency alternating current (HFAC) to peripheral nerve sheaths has proven reversible and effective for localized blockade of propagating action potentials within mammalian axons, while maintaining excitability of distal neuromuscular activity necessary for extrinsic induction of muscle contraction (147-158). HFAC neural blockade was first described by Tanner in 1962 (159) and popularized by Kilgore and colleagues (160-164). We recently demonstrated the efficacy of HFAC to block undesirable facial activity while permitting distal FES via neural stimulation to effect purposeful movements in a live rodent model (134).

3.6 Emerging Technologies

Optogenetic Neural Stimulation and Inhibition

The emerging field of optogenetics combined with gene therapy approaches carries promise of providing an alternative means of electrical stimulation of nerves, with potentially less risk of neuropathy induction over the long term, improved spatial resolution, and cell-specific activation (165). This emerging field comprises transduction of neurons to express

transgenes encoding opsins, light-sensitive proteins that can modulate neural activity via light-induced depolarization or light-induced inhibition of depolarization (166-169). Proof of principle of 'functional optical stimulation' was demonstrated in a mouse model, wherein an optical peripheral nerve cuff was positioned about the sciatic nerve and selective optical stimulation delivered to effect leg movements in minimally restrained animals (165). In theory, facial nerves could be transduced with various opsins using viral vectors (eg. adeno-associated or similar viruses), after which functional stimulation of facial muscles could be achieved via optical stimulation delivered through optocuffs. Use of inhibitory and excitatory opsins could potentially enable inhibition of undesirable muscular activity with concurrent stimulation of desired contractions (167).

3.7 Wireless Power

An NPD for facial reanimation would require continuous power during waking hours. Implanted medical devices such as pacemakers and the hypoglossal nerve stimulator rely on non-rechargeable long-life lithium batteries that require surgical explant for replacement every 5-10 years (170). While such an approach could potentially be employed for an NPD for facial palsy, battery lifetime would be far shorter due to the higher processing demands of converting multiple EMG inputs into neural stimuli. Modern cochlear implants utilize radiofrequency transmission from a battery powered external ASIC to power signal transduction within the implanted receiver, and rely on periodical battery replacement for the external hardware (171). While external housing of the processor and sensor (i.e., microphone) is ideal for a cochlear implant, where the control signal (i.e., sound) is external to the body, the analog-to-digital converter for an NPD for facial reanimation based on healthy-side EMG control would ideally be implanted to reduce electrical noise during signal capture. The ideal NPD for facial palsy would be fully

implantable and comprise a wirelessly-chargeable battery with a long service life. Wireless charging capacity could reduce device bulk and lessen risk of infection and extrusion (172). Recent advances in wireless charging technologies carry great promise for the field of implantable medical devices. These approaches employ magnetic induction or magnetic resonance to charge or power an implantable device, with progress in the field largely fueled by consumer demand for wireless cell-phone charging capacity (173-177).

3.8 Future Directions

The ideal device for reanimation of unilateral facial palsy would comprise a fully implantable solution with a robust input signal to predict contralateral healthy facial movements, a wirelessly chargeable miniature microcontroller, and an efficient means to effect synchronous and symmetric facial movements without potential activation of nociceptive fibers. Furthermore, an ideal device would also address the issue of undesirable facial activity that arises following aberrant regeneration and following nerve and muscle transfer procedures.

In cases of long standing facial palsy, multiple free muscle transfers would be indicated to restore key vectors of movement, and innervation of these muscles performed with reliable neural sources. Temporalis muscle transfer would be avoided, as accessing the neural pedicle to place a nerve cuff would be extremely difficult. Each transferred muscle transfer could be independently controlled through EMG activity of its healthy-side correlate to achieve symmetric movements. Cross-facial nerve grafting surgery could be averted. Overall, the paradigm of facial reanimation would shift to earlier, more complete, reinnervation of the facial muscles using powerful regional nerves such as the ipsilateral masseteric nerve or hypoglossal nerve to neurotize the hemi-face. Spontaneous, symmetrical facial movement would be provided via the FES paradigm through the NPD, concurrent with elimination of undesirable physiologic

activation (e.g., via prandial activity with masseteric nerve transfer) of the transferred muscle through the use of proximal neural blockade via a separate nerve cuff electrode placed proximally.

Technologic advances in implantable neuromodulatory devices for management of peripheral nerve disorders carry hope for the future development of a neuroprosthetic device for facial palsy. Such a device would represent a paradigm shift in management of this devastating and vexing clinical condition.

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PREFACE TO CHAPTER 4

In the previous Chapter, we presented a brief summary of FES and its potential role in the management of unilateral facial palsy. We also introduced the concept of pairing proximal electrical neural blockade with distal targeted FES as means to control undesirable facial muscle hyperactivity. In Chapter 4, we demonstrate proof-of-principle of this approach in a live rat model.

CHAPTER 4: THE BIONIC FACE: A NOVEL NEUROPROSTHETIC DEVICE PARADIGM FOR FACIAL REANIMATION COMPRISING NEURAL BLOCKADE AND FUNCTIONAL ELECTRICAL STIMULATION

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4.1 Abstract

Background: Facial palsy is a devastating condition potentially amenable to rehabilitation by functional electrical stimulation. Herein, a novel paradigm for unilateral facial reanimation via an implantable neuroprosthetic device is proposed and its feasibility demonstrated in a live rodent model. The paradigm comprises use of healthy-side electromyography activity as control inputs to a system whose outputs are neural stimuli to effect symmetric facial displacements. The vexing issue of suppressing undesirable activity resulting from aberrant neural regeneration (i.e., synkinesis) or nerve transfer procedures is addressed using proximal neural blockade.

Methods: Epimysial and nerve cuff electrode arrays were implanted in the faces of Wistar rats. Stimuli were delivered to evoke blinks and whisks of various durations and amplitudes. The dynamic relation between electromyography signals and facial displacements were modelled, and model predictions compared against measured displacements. Optimal parameters to achieve facial nerve blockade by means of high-frequency alternating current were determined, and safety of continuous delivery assessed.

Results: Electrode implantation was well-tolerated. Blinks and whisks of tunable amplitudes and durations were evoked by controlled variation of neural stimuli parameters. Facial displacements predicted from electromyography input modelling matched those observed with a variance-accounted-for exceeding 96%. Effective and reversible facial nerve blockade in awake behaving animals was achieved, without detrimental effect noted from long-term continual use.

Conclusions: Proof-of-principal of rehabilitation of hemifacial palsy by means of a neuroprosthetic device has been demonstrated. The use of proximal neural blockade coupled with distal functional electrical stimulation may have relevance to rehabilitation of other peripheral motor nerve deficits.

4.2 Background

Facial palsy is a devastating clinical condition with functional, esthetic, and communication sequelae (61, 178-192), whose ultimate clinical course yields flaccid hemi-facial paralysis, postparalytic facial palsy, or combinations thereof (Figure 4-1). Dynamic reanimation in hemi-facial palsy is principally limited to smile restoration through nerve or functional muscle transfers. Commonly, smile is reanimated using non-emotional trigeminal motor tracts requiring a conscious bite effort that provides volitional but non-spontaneous smile, together with undesired prandial activation. Reanimation outcomes are further limited in that no approach to dynamic restoration of brow elevation, blink, lip pucker, or lower lip movement has achieved consistent success.

Heretofore, the most significant barrier to more effective facial reanimation strategies in both flaccid and postparalytic facial palsy settings has been a lack of effective control mechanisms for denervated or aberrantly re-innervated facial muscles. Recent technologic advances in signal processing techniques, implantable neural and muscular electrodes, and implantable application specific integrated circuits (ASICs) have led to remarkable breakthroughs in the design and control of prosthetic limbs, devices to restore hearing and other senses, devices to aid gait in the setting of central nervous system disorders, and devices to treat obstructive sleep apnea (109, 110, 136-140, 193).

This paper presents a novel implantable neuroprosthetic device (NPD) paradigm for functional electric stimulation (FES) reanimation of the hemi-paretic face (Figure 4-2). This paradigm addresses not only the challenge of evoking appropriate facial movements, but also the vexing issue of suppressing undesirable facial activity resulting from aberrant neural regeneration or nerve transfer procedures. The system uses healthy-side electromyography

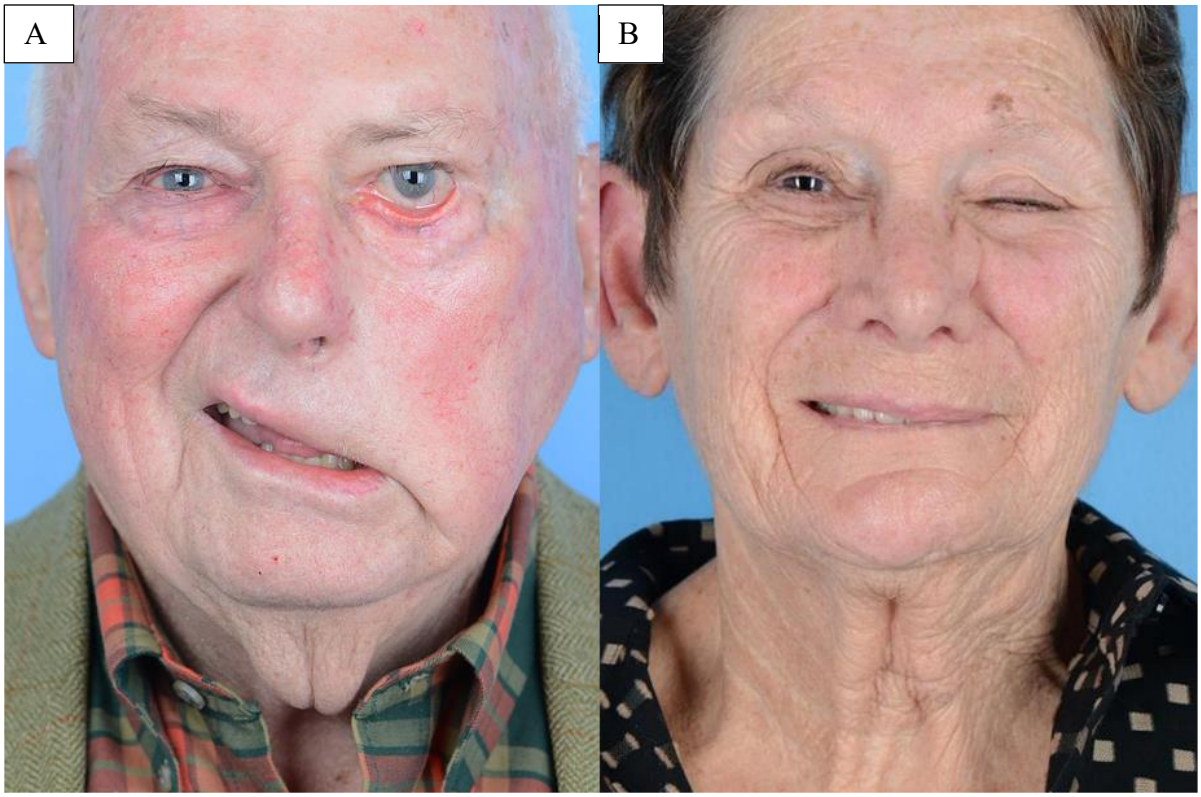

Figure 4-1: Hemifacial palsy. A – Left-sided flaccid facial paralysis, with severe paralytic lagophthalmos, lack of smile, and mid-facial ptosis. B – Left-sided post-paralytic facial nerve syndrome, with severe ocular and mid-facial synkinesis and smile restriction. This condition is the result of aberrant regeneration of the facial nerve following high-grade insults that include severe Bell's palsy, Lyme disease, Ramsay Hunt syndrome, and extirpation of cerebellopontine angle tumors

(EMG) signals as the control inputs to a NPD, whose outputs stimulate nerve branches on the paretic side to effect paired muscle contraction (Figure 4-3); concurrently applied high-frequency alternating current (HFAC) stimulation provides proximal neural blockade to prevent undesired physiologic muscle activation. Proof-of-principle of this paradigm is demonstrated in a rodent facial nerve model in a series of experiments, whereby implanted nerve cuff electrodes (NCEs) and epimysial electrode arrays (EEAs) are employed to deliver neural stimuli and capture facial muscle EMG activity.

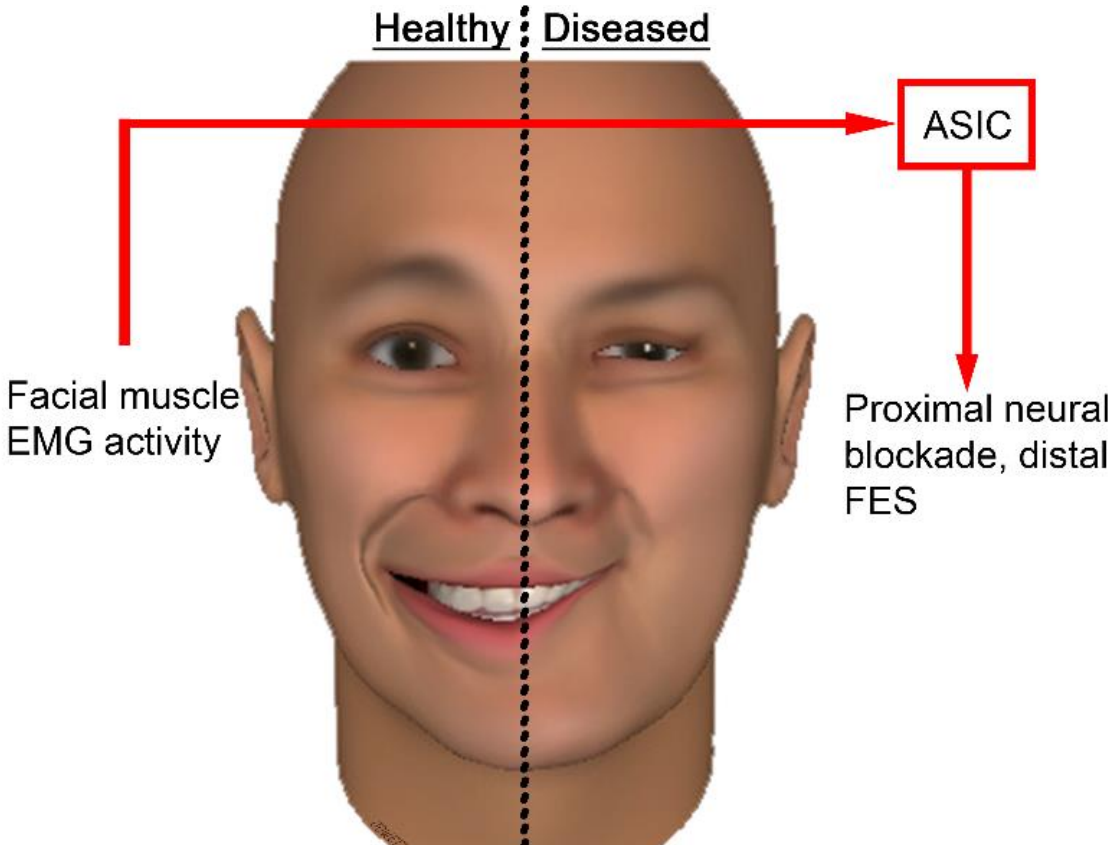

Figure 4-2: Neuroprosthetic device for hemi-facial reanimation. Implanted epimysial electrode arrays record EMG signals of healthy side facial musculature, which serve as inputs to an open-loop functional electrical stimulation control algorithm embedded into an application-specific integrated circuit (ASIC), that outputs concordant stimulatory signals to distal nerve branches via implanted nerve cuff electrodes on the diseased side. A constant high-frequency alternating current (HFAC) neural blockade signal is applied proximally on the affected side to prevent undesirable physiologic muscle activation.

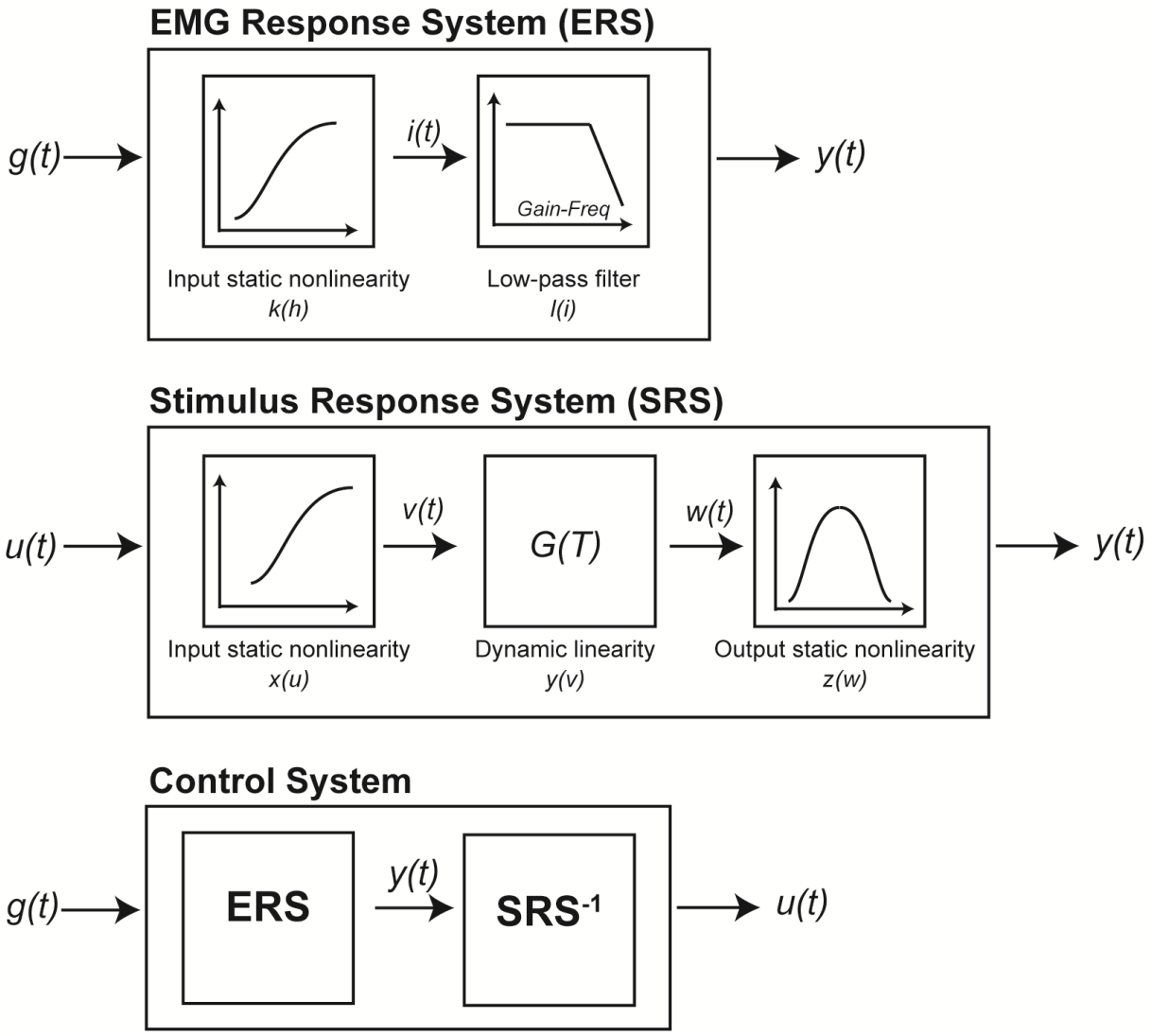

Figure 4-3: Proposed mathematical models for control of a neuroprosthetic device for hemifacial reanimation. Top – An input EMG signal g(t) is modeled to an output displacement y(t) on the healthy-side of the face using a Hammerstein system. Middle – An input electrical neural stimulus u(t) is modeled to the output displacement y(t) using an NLN structure). Bottom – An input EMG signal from one side of the face is modeled to an output neural stimulus that reproduces the displacement on the contralateral side by coupling the ERS with the inverse of the SRS

4.3 Methods

Overall approach: A three-part series of experiments was performed (Table 1) using a rodent facial nerve model (87, 88, 93, 194-202). Rat blink was used as a surrogate for human blink, while rat whisking (that occurs at different frequencies and amplitudes) was used as a surrogate for continuous proportional control of human facial muscle contractions responsible for movements such as smile or brow elevation. Implanted EEAs were used to record healthy-side facial muscle activity, while NCEs were used to deliver proximal neural blockade and distal stimulatory signals. Long-term tolerance of implanted electrodes and quality of EMG recordings were assessed, and the capacity to evoke facial displacements of varying amplitudes and durations evaluated. Dynamic relationships between EMG activity recorded from rat facial muscles and facial movements were modeled. Optimal neural blockade signal parameters to prevent synkinetic neuronal discharge while permitting distal branch FES were determined. The safety of continual delivery of HFAC to the facial nerve was assessed over the long-term.

Table 4-1: Live-rodent experiments

Part	**Experiment**	#
1 – Model establishment	Evoked facial displacements	5
	Evoked EMG	5
2 – Control system	Modelling of EMG to facial displacements	5
3 – Neural blockade	Efficacy	5
	Safety	5

Part 1: Establishment of a Rodent Model for Functional Electrical Stimulation of the Facial Nerve

Head fixation and conditioning: Ten female Wistar rats, 200-250 g, had titanium head fixation devices (HFDs) implanted and were conditioned to head fixation testing as previously described (87-89, 194-209). Briefly, rats were trained daily for two weeks to acclimate to handling and restraint. HFDs were then implanted under general anesthesia using ketamine HCl and dexmedetomidine HCl (60 mg/kg: 0.5 mg/kg) via a midline scalp incision. A subperiosteal plane was developed over the calvarium, the sterilized implant secured to the calvarium using titanium screws, and the incision closed in a single layer (Figure 4-4A). Daily head restraint training began two weeks later until animals tolerated ten-minute-long sessions. A customized resin top-hat enclosure to house the distal ends of electrode leads with their connectors was fabricated and secured to the HFD (Figure 4-4B). This enclosure provided protection and ease of access for subsequent recording and stimulation experiments.

Electrode implantation: Once conditioned, electrodes were implanted in the animals during a second procedure under general anesthesia. A 1 cm incision was made in the left cheek and flaps elevated immediately superior to the plane of the facial nerve. In five animals (group 1a), NCEs (custom bipolar design, MicroProbes for Life Sciences, Gaithersburg, MD, Figure 4-5, top) were implanted around intact and meticulously dissected zygomatic and buccal branches of the facial nerve. In five other animals (Group 1b), blunt dissection was carried deep to the center of the whisker pad musculature for EEA positioning (custom bipolar design, Ripple LLC, Salt Lake City, UT, Figure 4-5, bottom) concurrent with NCE placement around the buccal branch of the facial nerve. Electrodes were secured to the deep facial fascia overlying the masseter muscle using 5-0 polypropylene sutures, with leads tunneled in the subcutaneous plane

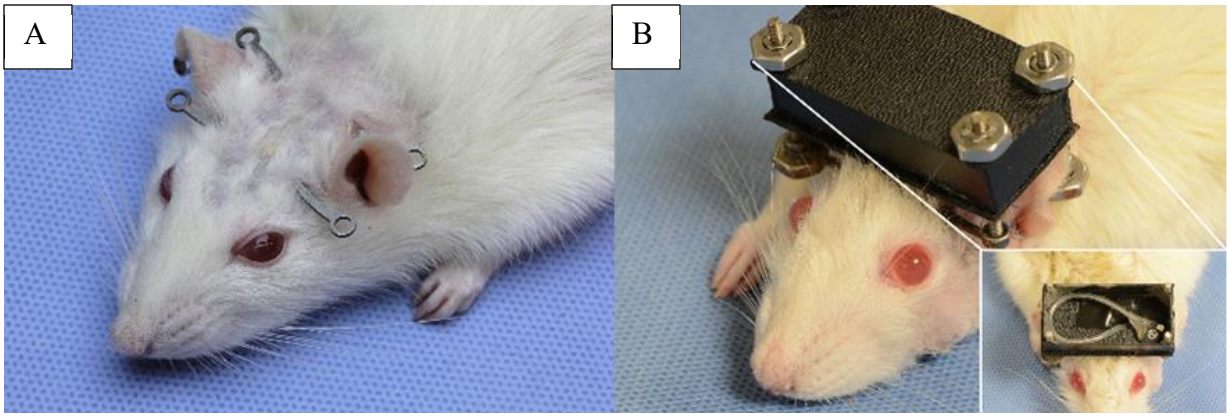

Figure 4-4: Rat head fixation device (HFD) and electrode lead enclosure. A – Typical healthy interface of the titanium HFD and scalp. B – Top-hat enclosure secured to the HFD with nuts (inset: open enclosure demonstrating electrode leads and pin connector).

to exit the skin over the occiput. Lead terminals were soldered to a pin connector (A11365-001, Omnetics Connector Corp, Minneapolis, MN) and housed in the customized top-hat enclosure (Figure 4-4B and Figure 4-6).

Quantitative whisker and eyelid displacement recording: The hardware and software used for monitoring whisking movements was adapted from Bermejo et al. (194-199, 210). A single whisker (C-1) on each side of the head was entubulated using a polyacrylamide tube to increase detectability. Whisker displacements were then independently tracked using commercial laser micrometer pairs (MetraLight, Santa Mateo CA). Blinks were detected using infrared sensors to measure light reflectivity from the cornea and eyelids as described by Thompson et al. (211). Computer-controlled air valves were used to deliver corneal air puffs and scented air flows to elicit blink and whisking behaviour, respectively.

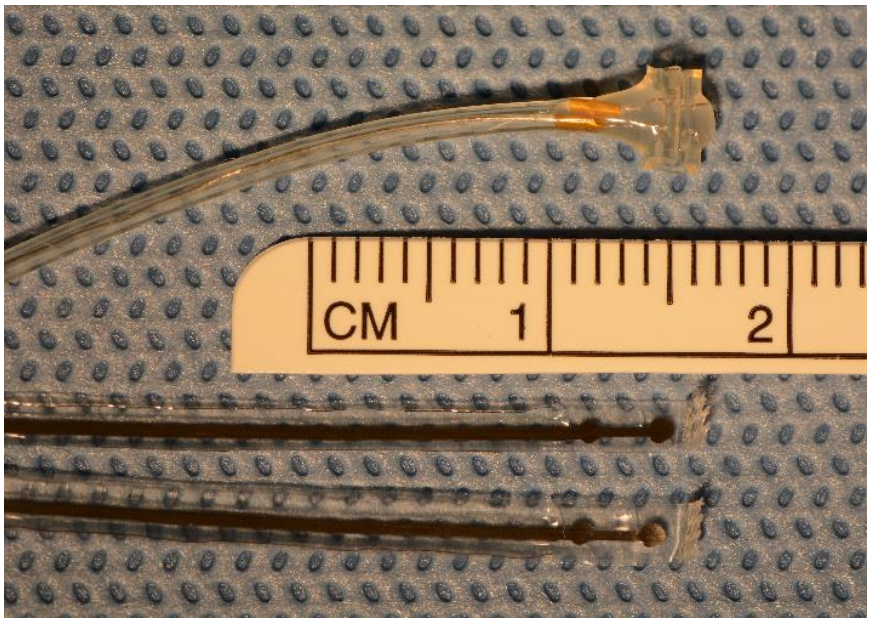

Figure 4-5: Electrodes. Silicone-sheathed nerve cuff with platinum-iridium electrodes (MicroProbes for Life Science, Gaithersburg, MD) (above) and a two-channel highly flexible conductive polymer electrode array (Ripple Neuro, Salt Lake City, UT) (below).:

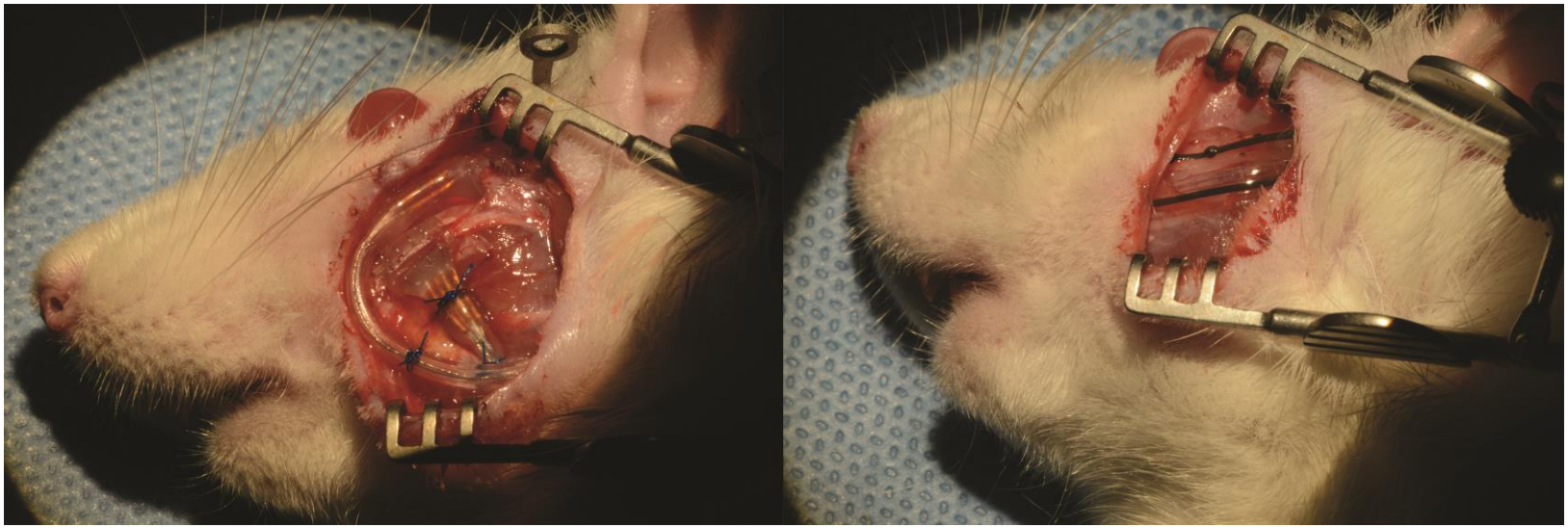

Figure 4-6: Electrode implantation. Right – NCEs are implanted on zygomatic and buccal branches of the FN. Left – EEAs are implanted underlying the orbicularis oculi and whisker pad musculature under general anesthesia.

Evoked stimuli: While under general anesthesia, animals in Group 1a received varying neural stimuli to zygomatic and buccal branch NCEs, with concurrent tracking of blink and whisker displacements. Animals in Group 1b received neural stimuli to the buccal branch NCE with concurrent capture of whisker pad EMG responses from implanted EEAs. A commercial electrophysiology system (CyberAmp 380 signal conditioner, Digidata 1322A digitizer, pCLAMP 10 software, Molecular Devices, Sunnyvale, CA) combined with analog stimulus isolator units (Analog Stimulus Isolator Model 2200, ADInstruments, Colorado Springs, CO) were used for stimulation and EMG signal acquisition. Neural stimuli comprised trains of current-controlled, charge-balanced square wave pulses (pulse width 0.4 ms, train durations 0.4 ms – 100 ms, repetition rates 1 - 2 Hz, peak-to-peak amplitudes 0.1 – 2 mA). EMG signals were measured with a differential amplifier with pre-filter gain of 10, high-pass filter at 10 Hz, low pass filter at 1000 Hz, notch filter at 60 Hz and post-filter gain of 100. EMG signals were then sampled at 10 kHz with 16-bit resolution, concurrent with whisker and blink displacement signals.

Part 2: Establishing Feasibility of Epimysial EMG as FES Control

Modeling of EMG activity to whisker displacement: Herein, the healthy-side EMG activity of the whisker pad musculature, as captured using implanted EEAs, were used as inputs and the resulting whisker displacements as the output. Animals from Group 1b above were placed under general anesthesia, and fixed stimuli delivered to the buccal branch NCE (constant current charge balanced square wave, peak-to-peak stimuli 0.5 mA, pulse width 0.4 ms, train width 1.2 ms, repetition rate 1 Hz). The resulting whisker pad EMG responses and C-1 whisker

displacements were measured. Methods for Hammerstein system[1] identification described by Jalaleddini and Kearney (212, 213) were used to identify models relating recorded EMG signals to measured whisker displacements in MATLAB (v2015b, The MathWorks Inc, Natick, MA). In this approach, muscle was treated as a nonlinear biological system where the relation between neural activation and force was modeled as the cascade of a static nonlinearity followed by a dynamic linear system (214, 215) (Figure 4-3, top).

Part 3: Establishing Neural Blockade Effectiveness and Safety

Determination of optimal HFAC parameters and efficacy: Three animals were implanted with three NCEs on the left buccal branch of the facial nerve, and the animal placed in a laser micrometer field to track evoked whisker displacements. Cathodic pulses from a pulse generator (S88, Grass Instruments, Astro-Med Inc., West Warwick, RI) coupled to stimulus isolators to achieve biphasic constant-current pulses (0.5 mA, 50 μs pulse width) were delivered to proximal (at 1 Hz) and distal (at 1.5 Hz) NCEs. High-frequency alternating current (constant-voltage sine wave) was delivered to the central NCE using a function generator (FG085 Kit, JYE Tech, Guilin, Guangxi, China). The peak-to-peak amplitudes (0 – 10 V) and frequency (2 kHz – 40 kHz) were varied to determine optimal blockade parameters. Two further animals, with HFDs implanted and conditioned to head fixation as described above, were implanted with NCEs to the buccal branch and zygomatic branches on the left side. Animals were placed in head fixation for quantitative tracking of awake behavioral whisking, with concurrent application of HFAC and distal FES neural stimuli to generate whisks and blinks.

[1] Hammerstein models comprise a static nonlinearity element in series with a linear dynamic subsystem. They are commonly employed in feedback-controlled FES strategies.

Establishing safety of continuous HFAC delivery: Once optimal HFAC parameters were identified, five animals underwent HFD device implantation and restraint training, followed by single bipolar NCE implantation to the buccal branch of the left facial nerve. The marginal-mandibular branch was resected bilaterally to eliminate its contribution to whisking. Beginning one week after NCE implantation, HFAC was delivered continuously for four hours, five days per week, for two weeks (total 40 hours) under general anesthesia. Three minute sessions of awake behavioral whisking assessment were recorded at baseline, after NCE implantation, and preceding each HFAC delivery session. The ratio of the whisking amplitudes between HFAC-blocked and non-blocked sides was tracked for each animal over time.

4.4 Results

Part 1: Establishment of a Rodent Model for Functional Electrical Stimulation of the Facial Nerve

Animals tolerated top-hat enclosures and electrode implantations for periods exceeding 40 days prior to scheduled euthanasia, without infection or severe foreign-body reaction despite the subcutaneous exit point of the electrode leads atop the head (Video 1). Robust evoked epimysial EMG responses were recorded from the implanted epimysial electrodes, with excellent signal-to-noise ratios (Figure 4-7, left) throughout this time. Electrical stimulation evoked blinks of varying durations and whisks of varying amplitudes in all NCE implanted animals (Video 2 and Figure 4-7, right).

Part 2: Establishing Feasibility of Epimysial EMG as FES Control

Hammerstein modelling of the relation between epimysial EMG signals (obtained from the undersurface of the whisker pad musculature) to evoked whisker movement demonstrated

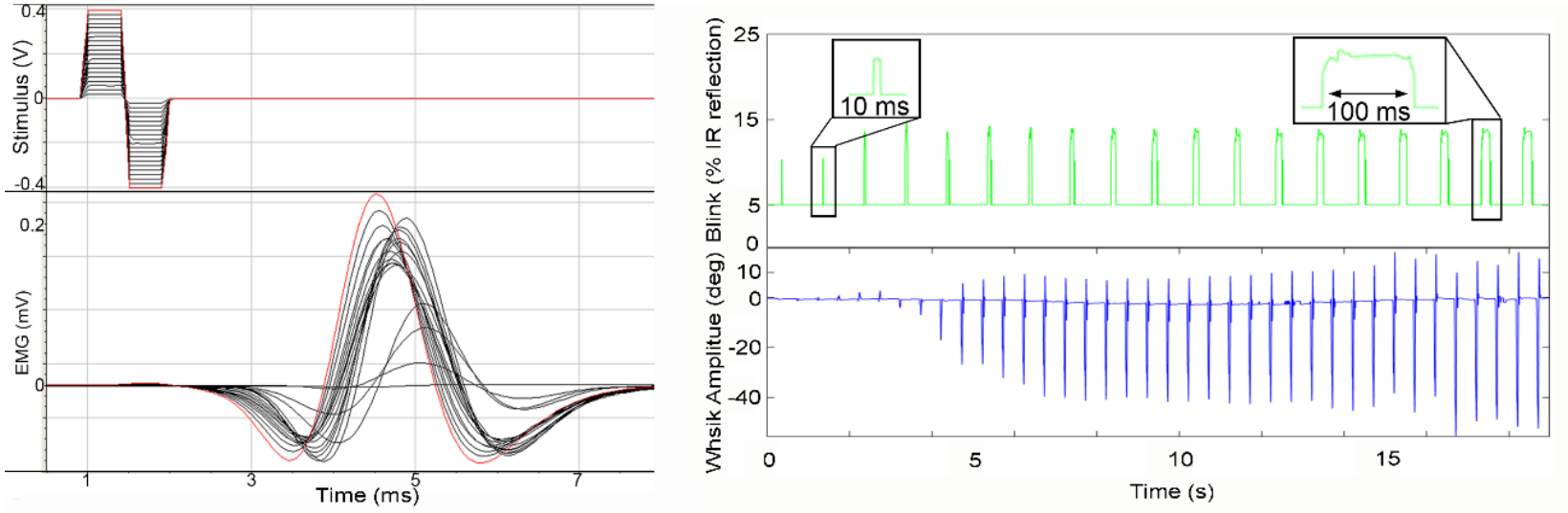

Figure 4-7: Evoked responses. Evoked EMG response captured using EEAs and elicited blink and whisker response from various neural stimuli delivered using NCEs of the types proposed herein on a live rat model. (Left) Noise-free differential compound muscle action potentials (CMAP, bottom) underlying the whisker pad (electrode spacing of 5 mm, initial gain of 10, sampling rate 10 kHz, AC input-coupled at 10Hz, low-pass filtered at 400 Hz, total gain 1000, stimuli shown above). (Right) Eyelid response (top) to biphasic train stimuli at 1 Hz of constant amplitude and increasing duration demonstrates ability to evoke faster or slower blinks, while whisker response (bottom) at 2 Hz stimulus of constant duration and increasing amplitude demonstrates ability to evoke whisks of lesser or greater amplitude

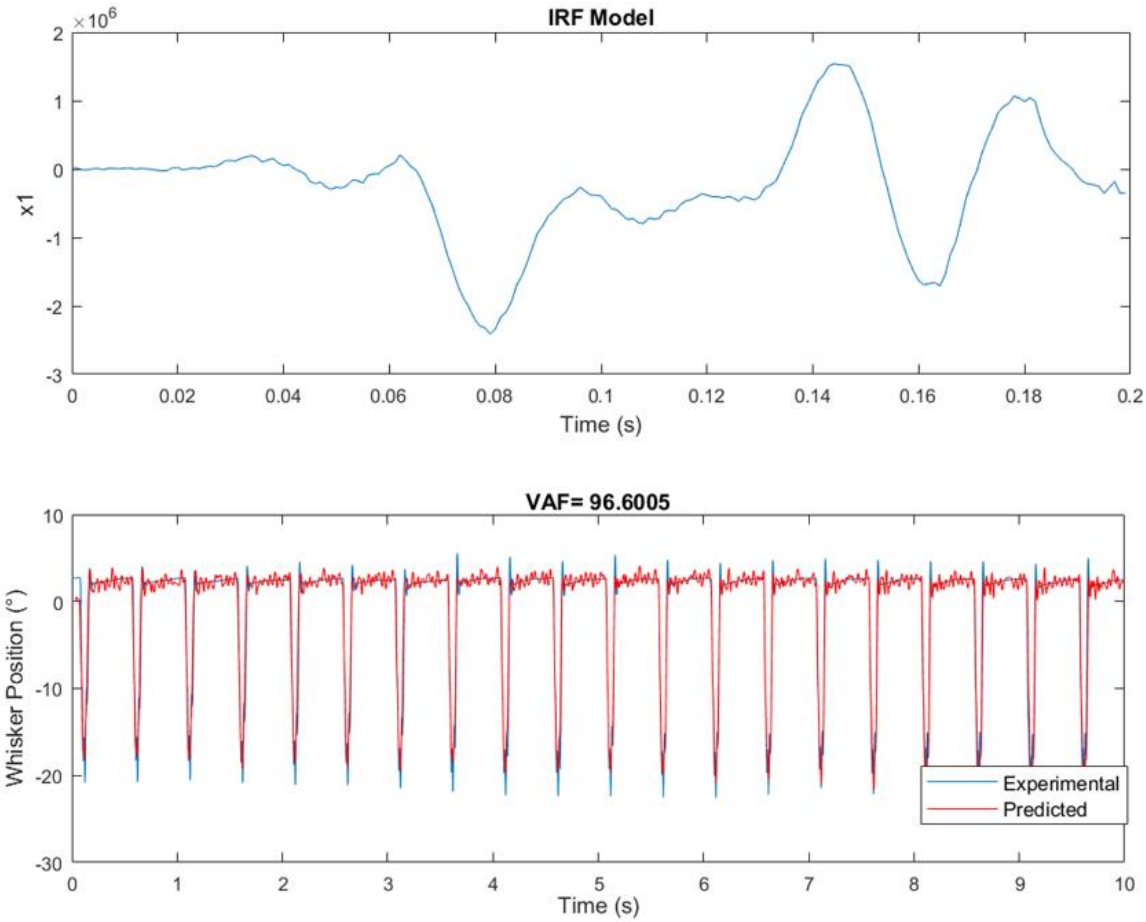

Figure 4-8: Modelling EMG activity to whisker displacement. Top – An impulse response function (IRF) indicates a clear dynamic relationship between an EEA-captured EMG input (implanted underlying the whisker pad) and recorded whisker position. Bottom – The model demonstrated a variance accounted for (VAF) in excess of 96% (below).

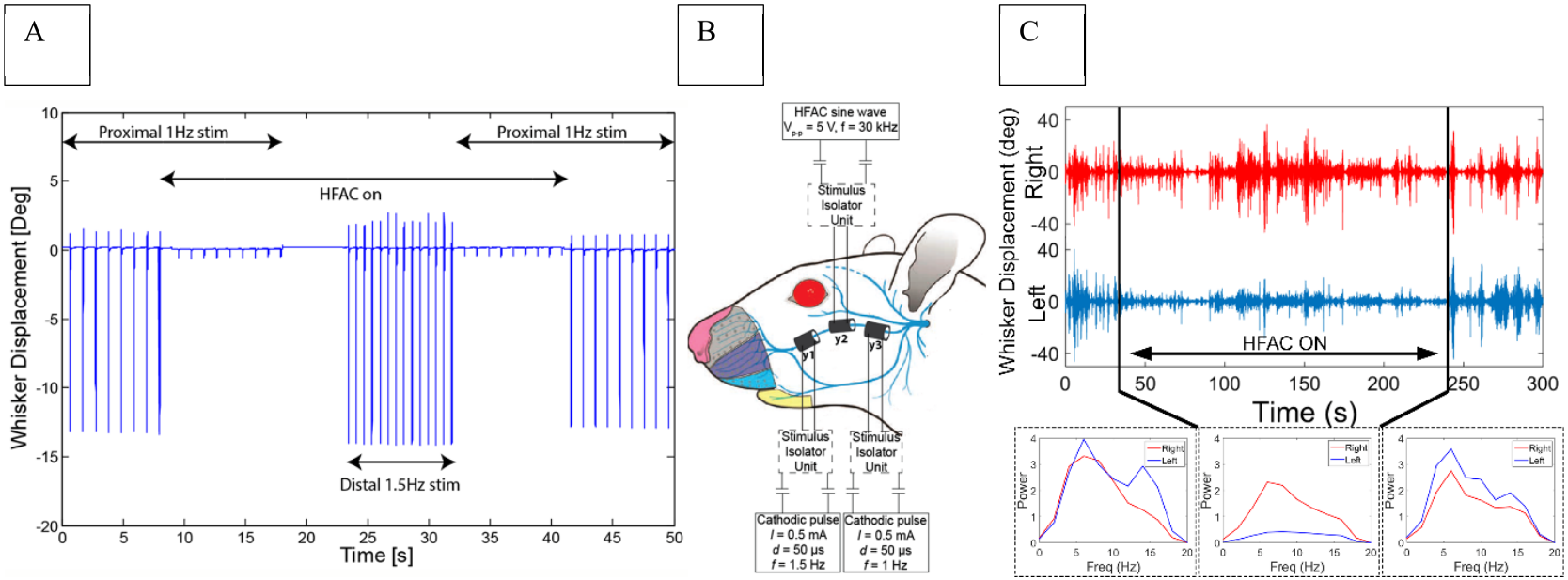

Figure 4-9: High-frequency alternating current (HFAC) neural blockade of whisker movement in an anaesthetized and awake rat. A – Three signals are delivered to the buccal branch of the FN (controls whisking): a proximal stimulation at 1 Hz, HFAC, and a distal stimulation at 1.5 Hz, with parameters as shown in (B). As is seen in (A), HFAC delivery results in a ~90% reduction in whisker amplitude while not affecting the ability to stimulate the nerve distally. B – Stimulation and blockade parameters and NCE positions are shown. C – Constant HFAC is delivered to the left buccal branch using an NCE from t=30s to t = 240s (above). Power spectra demonstrate near equal left and right-sided whisking power during the periods immediately before and after HFAC, with a dramatic drop on the left side seen with HFAC (below).

excellent predictive capacity (Figure 4-8), with the model accounting for more than 96% of the variance in whisker displacements.

Part 3: Establishing Neural Blockade Effectiveness and Safety

A 30-kHz sine wave with a peak-to-peak amplitude of 5V was found to be optimal for inducing blockade of evoked and spontaneous whisking activity in the rat using the customized NCE. In anesthetized animals, this achieved a ~90% reduction in evoked whisking amplitude, without restriction of distal neuromuscular excitability (Figure 4-9 A,B). In awake animals, a similar reduction in behavioral whisking power was observed between blocked and normal sides (Video 3 and Figure 4-9C) with HFAC application. No differences were observed in whisking amplitudes between sides with prolonged unilateral daily delivery of HFAC (Figure 4-10). Full results of this series of experiments will be reported in more detail in subsequent publications.

4.5 Discussion

While the basic concept of using signals from the contralateral face to drive FES of paralyzed facial muscles has been demonstrated (115-117, 121, 122, 124, 132, 216-219), prior work has not addressed critical issues relevant to the long-term implementation of this approach. Optimal FES of dysfunctional muscle requires three conditions: muscle must be neurotized, be capable of being stimulated in purposeful and timely fashion, and be devoid of undesirable activity. Neurotization of muscle prevents denervation atrophy and permits FES through neural stimulation without the need for direct muscle stimulation. Though direct muscle stimulation of denervated muscle may prevent atrophy, it requires delivery of high and potentially injurious stimulus amplitudes to evoke contractions (128, 220, 221). Such an approach is not feasible in the setting of facial palsy due to excessive power demands on an implanted NPD, coupled with

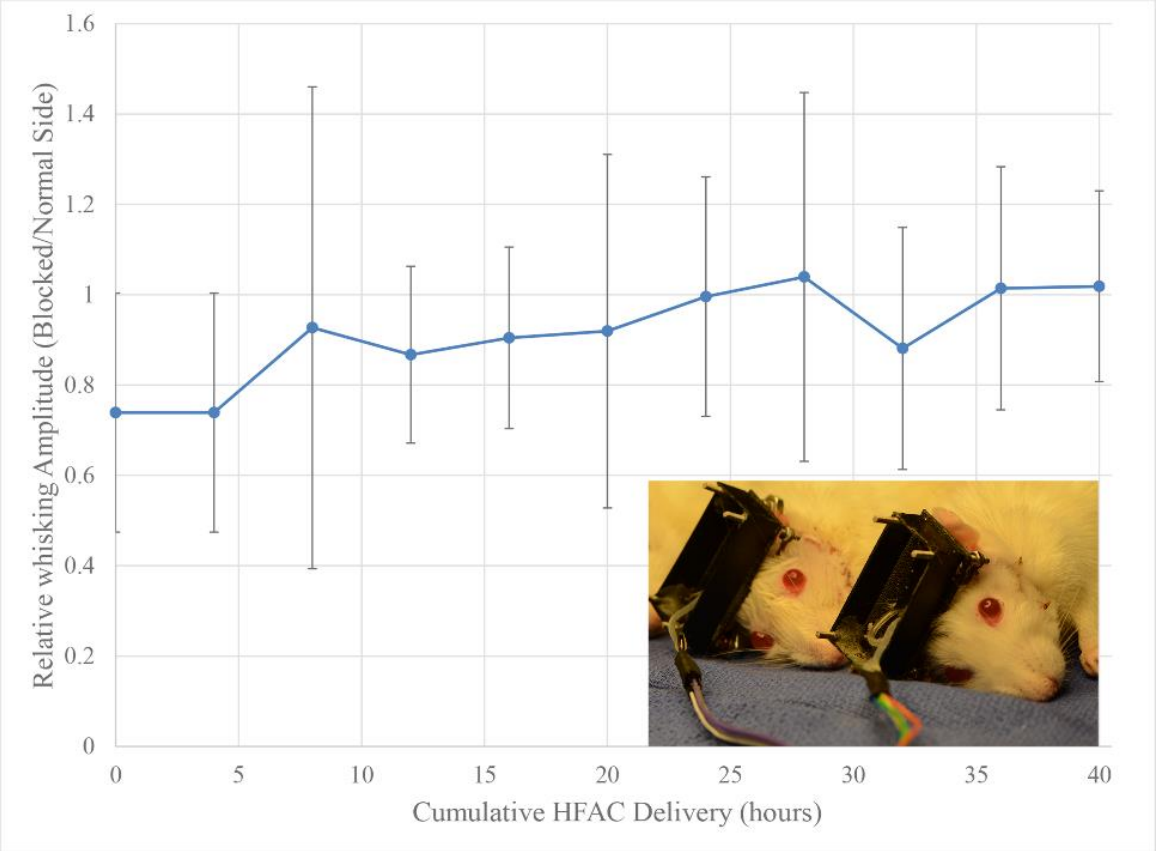

Figure 4-10: Prolonged high-frequency alternating current neural blockade delivery in the rat. Relative maximal whisk amplitudes between left-face (implanted with NCEs on nerve controlling whisking with 4 hours of daily continuous HFAC delivery - see inset) and right-face (normal-side) demonstrate normal or stronger whisker displacements on the side to which HFAC was delivered (green bar approximates normal range).

regional nociceptive fiber activation that would result from the delivery of high stimulus amplitudes. In the setting of facial palsy, neurotization of facial muscles may occur spontaneously following neural insult, through interposition graft repair, or through nerve transfer. In cases of long-standing or congenital facial palsy, dynamic facial reanimation may be achieved by functional muscle transfer. Here we propose that muscle contraction be evoked through stimulation of distal native facial nerve branches (or transferred nerves) using

electrically-shielded nerve cuff electrodes (NCEs). Such distal facial nerve branches to individual paired-muscles or functional muscle groups of the face are straightforward to access surgically. Likewise, in cases where nerve or muscle transfers are required for dynamic reanimation, nerve branches to the target muscle are readily accessible for cuff implantation at the time of reanimation surgery or during future re-exploration.

Dynamic reanimation of muscle requires an adequate control signal to effect desired movements at appropriate times. Use of myoelectric activity to reliably drive neuroprostheses has already been demonstrated (222, 223). Herein, we have demonstrated that electromyography (EMG) signals from contralateral, healthy facial muscles may be utilized as control signals to drive FES of the paretic hemi-face. Herein, EMG activity was captured through the use of implanted epimysial electrode arrays (EEAs), obviating the need for external sensors, and a clear mathematical relationship capable of predicting facial displacements from EMG inputs was demonstrated. Use of the contralateral healthy hemi-face for dynamic reanimation of unilateral facial paralysis is a natural choice, as the majority of expressions – especially positive expressions – are symmetric (224). The NPD proposed herein could evoke paired-muscle contraction without a conspicuous phase delay in movement onset between sides. Modern implantable ASICs achieve delays of 5 ms or better on signal receiving and stimulus transmitting arms, with sub-millisecond processing times for most FES applications. When coupled with typical delays on the order of 10 ms for physiologic transduction of the neural stimulus to effect myocyte depolarization, the maximum expected delay between healthy-side detected EMG activity – and subsequent muscular contraction – and paretic-side neural stimulation is on the order of 20 ms, below the ~33 ms threshold above which humans are able to detect asymmetric movement (225, 226).

While stimulation of desirable muscle activity is necessary for functional reanimation, concurrent inhibition of undesirable neural activity is often just as important. No prior work has addressed the vexing issue of how to prevent undesirable muscle activation from aberrantly regenerated axons (i.e. in the case of aberrantly regenerated native facial nerve) or from the normal functioning of transferred cranial nerves (e.g. prandial activation of muscle innervated by transfer of the masseteric branch of the trigeminal nerve). Herein, we describe for the first time the use of concurrent proximal application of HFAC over prolonged periods to prevent such undesired facial contractions. Delivery of HFAC to peripheral nerve trunks results in reversible induction of localized blockade of propagating action potentials, without impeding distal neuromuscular excitability (147-158); proximal application of HFAC to a motor nerve squelches physiologic activity while maintaining the capacity for distal FES. While there exist brief tetanic onset and offset responses with HFAC application (147, 150, 227), such repeated responses would be avoided herein through continual proximal delivery of HFAC during waking hours concurrent with distal FES to drive expression. The device could also be implemented in 'FES-only' mode to allow physiologic action potentials to pass concurrent with evoked potentials, or 'neural blockade-only' mode to induce targeted flaccidity.

In contrast to a recent publication (132), the novelty of the work herein lies in (a) use of clinically relevant biocompatible epimysial electrodes to capture EMG signals as opposed to transdermal wires, (b) use of clinically relevant implantable neural cuffs to evoke facial displacements through neural as opposed to direct muscle stimulation via transdermal wires, (c) characterization of a mathematical model capable of predicting whisker displacements from EMG signals as opposed to correlation of signal envelopes, (d) recognition of the clinically vexing issue of undesirable physiologic neural activity that occurs with aberrantly innervated

muscle, and (e) proposing and demonstrating the long-term efficacy of proximal HFAC application concurrent with distal FES as a solution.

Though the ultimate goal of reanimation is to restore dynamic function of the entire facial musculature, restoration of three symmetric facial movements alone – brow elevation, blink, and smile – would dramatically improve clinical outcomes. Such an approach would require implantation of only three miniature EEAs and NCEs coupled to an implanted ASIC, and would represent a paradigm shift in management. The proposed NPD could also be readily utilized in patients undergoing, or who have already undergone, trigeminal nerve-driven smile reanimation to re-establish spontaneity. In this realization, smile activation – resulting from the contraction of transferred free functional muscle or native facial musculature driven by the masseteric nerve – could be controlled through EMG signals from healthy side zygomaticus major activity through the FES paradigm proposed herein, with elimination of highly undesirable prandial activation through proximal HFAC neural blockade (Figure 4-11). Similar to cochlear implant programming, stimulation parameters could be tuned wirelessly as innervation and tissue response to implanted electrodes reach a steady state.

This work has demonstrated the feasibility of employing epimysial EMG signals from healthy-side facial musculature captured using biocompatible and fully-implantable miniature electrodes as a means for control of a FES paradigm to drive reanimation of symmetric expression in hemi-facial palsy. The capacity to effect independent facial movements of varied duration and amplitudes by means of FES of distal facial nerve branches was established. Importantly, the efficacy and safety of proximal neural blockade by means of continuous HFAC delivery as a means to extinguish undesirable facial muscle activity arising from the intrinsic activity of damaged or transferred nerves has been proposed and demonstrated.

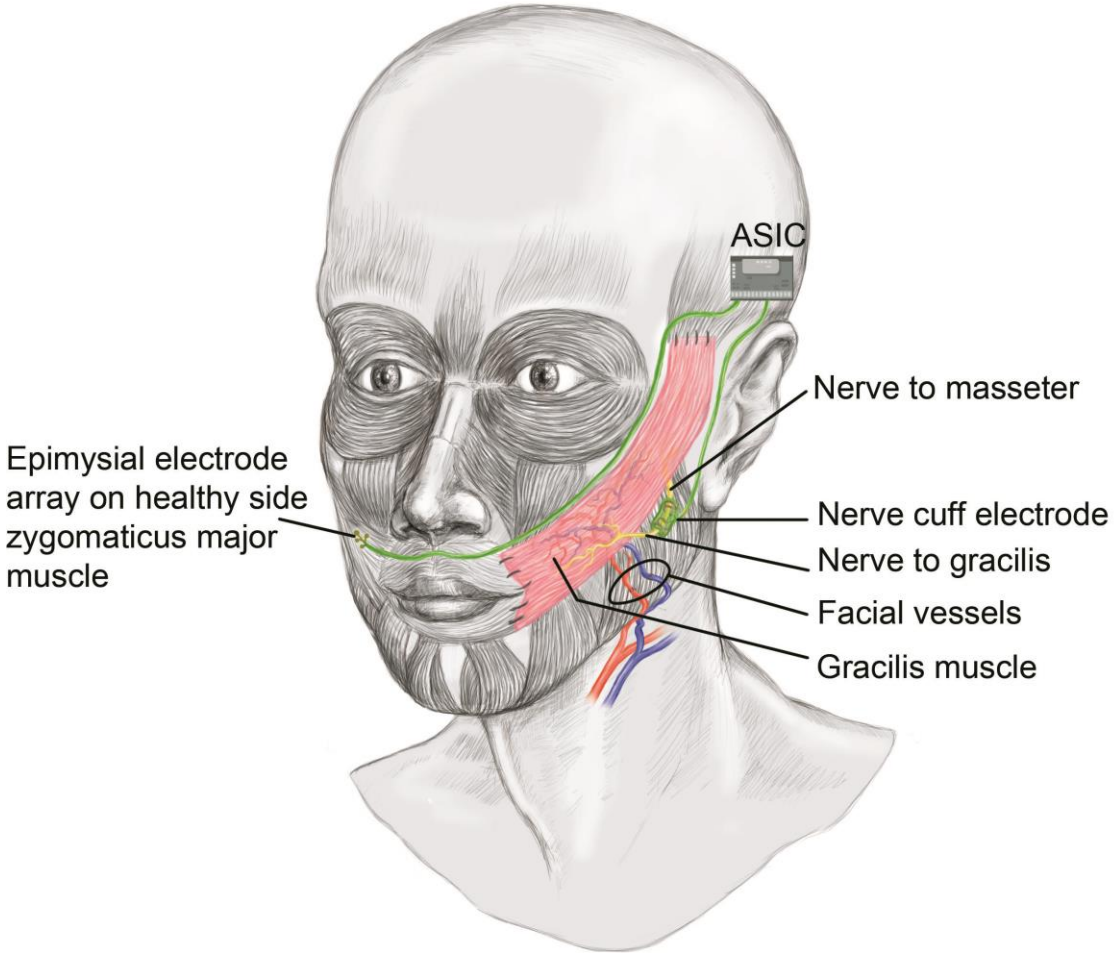

Figure 4-11: Proposed paradigm for reanimation of spontaneity of trigeminal nerve-driven smile. An implanted epimysial electrode array captures contralateral healthy zygomaticus major EMG activity with leads tunneled subcutaneously to an implanted application-specific integrated circuit (ASIC). Output leads from the ASIC connect to a nerve cuff electrode on the nerve driving smile (e.g. the masseteric nerve, a branch of the trigeminal nerve). A proximal neural blockade signal is delivered to eliminate undesired prandial activation of the muscle, concurrent with distal functional electrical stimulation signals from the ASIC to reanimate symmetric spontaneous and volitional smile.

4.6 Conclusions

The combination of proximal HFAC with distal FES to achieve total extrinsic control over a motor nerve has clinical implications elsewhere where undesirable activity of a peripheral nerve resulting from disease, injury, or nerve transfer exists. Future work will seek to study this paradigm over the long term using a fully implantable miniaturized ASIC currently under development. Beyond FES applications, application of HFAC to peripheral nerves might ultimately prove efficacious for management of spastic disorders and painful neuropathies, as focal blockade of action potential propagation occurs for efferent and afferent pathways (228).

4.7 References

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PREFACE TO CHAPTER 5

In the previous chapter, we employed a live rat model to illustrate the potential for a novel neuroprosthetic device approach to management of unilateral facial palsy. We demonstrated how electrical signals from implanted epimysial electrode arrays could be employed to predict facial displacements, how stimuli delivered through nerve cuff electrodes could evoke specific facial expressions, and how delivery of HFAC to the proximal facial nerve could suppress physiologic neural activity while allowing for distal targeted FES of specific facial expressions. We demonstrated in a small series of otherwise uninjured animals that continual delivery of HFAC to the facial nerve via a nerve cuff electrode appeared safe over a period of several weeks. Prior to investment in clinical translation, further work is required in animal models to further validate the safety and efficacy of this approach over the long-term. Such work will require a high-throughput animal model that permits unbiased, quantitative, and high-resolution assessment of facial nerve structure and function. Owing to space limitations of the peer-reviewed manuscript format, we did not discuss in detail the manifold challenges and limitations of the research model used in Chapter 4. Major issues included animals tearing out and chewing through expensive electrode arrays over the course of the experiments, limited resolution of blink quantification, and need for extensive manual conditioning of animals to head and body restraint over a period of several weeks prior to testing. The following chapter presents our subsequent soon to-be-published work on the development of a comprehensive research platform for high-throughput assessment of facial nerve function in the rat.

CHAPTER 5: HIGH-THROUGHPUT PLATFORM FOR STUDY OF RAT FACIAL NERVE STRUCTURE AND FUNCTION

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5.1 Abstract

Background: Facial palsy is a devastating clinical condition yielding functional, aesthetic, and communication impairments. Advances in neuroprosthetic device design, bioengineered nerve conduits, and genetic therapies carry potential to advance management of this orphan disease state. Murine models offer opportunity to investigate these emerging therapeutic approaches in cost-effective fashion.

New method: We describe the design, assembly, and validation of a novel research platform enabling high-throughput assessment of facial nerve function and histomorphometry in the rat. The platform comprises a biocompatible cannulated metallic skull implant enabling head restraint and connection to implanted electrodes, a miniature conveyor belt to mitigate need for body restraint and labor-intensive conditioning, cost-effective high-frame rate videography for capture of dynamic facial movements, a deep-learning based computer vision algorithm for quantitative tracking of facial displacements, and a rapid non-toxic frozen section approach for nerve imaging paired with a machine learning-based segmentation algorithm for automated quantitative histomorphometry.

Results: Metallic skull devices were implanted in four Lewis rats with two rats undergoing unilateral facial nerve buccal branch crush injury at the time of implantation. Rats were readily conditioned to handling and head restraint on the moving treadmill-like platform. Implant stability was noted over the four-week study period. Quantitative assessment of whisking function and nerve histomorphometry at four weeks post-operative revealed differences between animals with uninjured and crushed facial nerves.

Comparison with existing methods: This approach obviates labor-intensive behavioural conditioning and tissue processing steps inherent to exiting rodent models of facial nerve function. Conclusions: Described is a comprehensive research platform for high-throughput study of facial nerve structure and function in the the rat.

5.2 Background

Congenital absence or injury to the facial nerve yields devastating consequences. Loss of facial nerve function (Figure 5-1) results in impaired blink impacting sight, nasal valve collapse impacting respiration, lip flaccidity impacting articulation and oral competence, and impaired facial expression, with profound negative impact on emotional well-being and social development (1, 3, 20).

State-of-the-art management of facial paralysis may entail microsurgical nerve repair or nerve-transfer procedures, wherein axons from a healthy donor nerve are re-routed towards a distal target to restore function (23, 42). For example, the masseteric nerve may be transferred to select zygomatic branches of the facial nerve for targeted smile reanimation (74, 75, 229), while a portion of the hypoglossal nerve may be transferred to the main trunk of the facial nerve to restore resting facial tone (77, 78). Though targeted physical therapy to exploit cortical plasticity improves patient outcomes, restoration of spontaneous expression following facial reanimation by nerve transfer is not commonly achieved (230, 231). Further, nerve transfer procedures result in undesirable activation of facial musculature arising from normal activities, such as smile activation with prandial activity with masseteric nerve transfer (232).

The tendency of positive facial expressions to demonstrate symmetry with the contralateral side offers unique potential for management of unilateral facial paralysis (224). Recently we proposed a neuroprosthetic device (NPD) paradigm for reanimation of hemi-facial paralysis (233). This functional electric stimulation (FES) paradigm was designed to address the challenge of evoking appropriate facial movements while suppressing undesirable facial activity resulting from nerve transfer procedures. The approach proposes use of healthy-side electromyography activity as control inputs to a system whose outputs are neural stimuli to effect symmetric facial

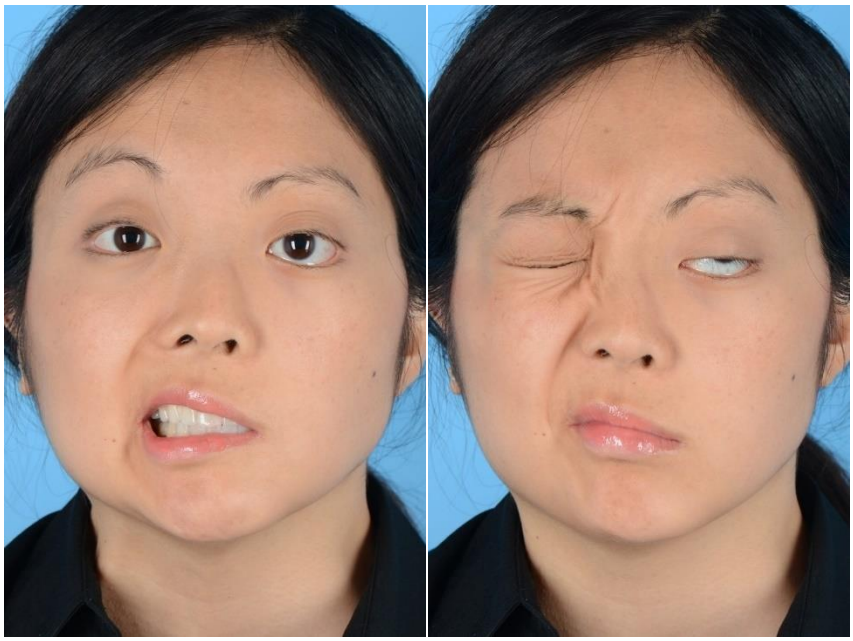

Figure 5-1: Left facial palsy following skull base tumor resection. (A) On the affected side, smile is absent. (B) Blink is incomplete placing the ocular surface at risk of injury with subsequent vision loss.

displacements, concurrent with use of proximal neural blockade using high-frequency alternating current. Though proof-of-principle was demonstrated in a small series of animals, this approach requires further study to assess long-term safety and efficacy.

Biological alternatives to a neuroprosthetic device to optimize facial reanimation outcomes exist. In unilateral facial palsy, redundant healthy-side facial nerve branches supplying distinct facial muscle action groups may be re-routed to paired contralateral facial muscles to restore spontaneous symmetric expressions in a procedure known as cross-facial nerve grafting (CFNG) (234). The basis for CFNG is the capacity for axons of peripheral nerves to regenerate over long distances. The current standard for CFNG involves harvest of a less-critical sensory nerve from the extremities, typically the sural nerve, to appropriately guide, protect, and support

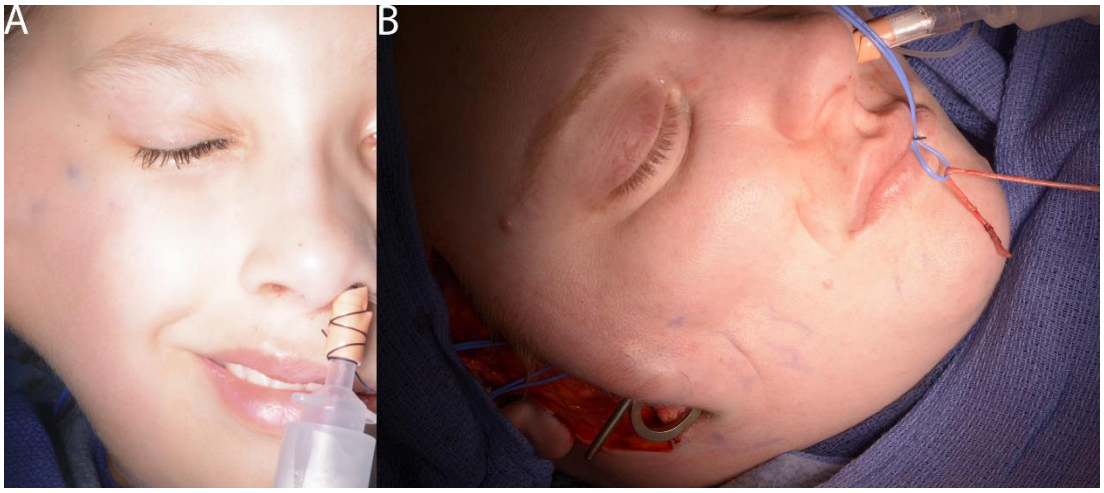

Figure 5-2: Cross-facial nerve grafting. (A) A facelift incision and tetanic nerve stimulation is employed to identify ideal donor nerve branches on the healthy side of the face. (B) An interposition nerve graft is then tunneled across the face using a fascia needle; in this case the graft is passed across the upper lip in a two-pass technique via a small mucosal incision.

regenerating axons along their journey (Figure 5-2). Despite meticulous surgical technique, CFNG carries a 20-30% failure rate (73, 235), thought to result from inadequate growth of donor axons across the long distance spanned by the CFNG (73, 235-242). Evidence suggests that increased transcription and neurotrophic factor expression may result in improved axonal regeneration over long distances (243-245). Our long-term goal seeks to harness progress in genetic therapies to enhance axonal regeneration and subsequent functional outcomes across long nerve gaps.

Rigorous assessment of novel therapeutics for facial reanimation requires thorough evaluation in animal models prior to clinical translation. Neural regeneration may be assessed using functional, electrophysiologic, and histologic outcome measures (246). Despite capacity for neural regeneration far superior to humans (247, 248), rodents remain commonly utilized for

study of neural regeneration due to their low cost, small size, broad strain diversity including transgenic models, and well-characterized genomes (248-255). Rats are preferred over mice among surgeons for their ease of handling, ease of conditioning to functional assays, and larger size that constitutes a higher-fidelity model of microsurgical neural repair and grafting (256, 257). Originally developed for behavioral analysis (194, 195), a model for tracking rat facial displacements was popularized for study of facial nerve regeneration (83-94). This model comprised surgical placement of a head fixation device and subsequent restraint of the animal for tracking of whisker movements using laser micrometers and blink activity using infrared diode and detector pairs. Key limitations of this model included complex hardware, need for whisker tagging, several weeks of labor-intensive conditioning to body restraint within a cloth sac, and low resolution tracking of blink movements.

Histologic assessment is critical to understanding the relationship between peripheral nerve structure and function following injury and regeneration. Conventional preparation of nerve tissue for histomorphometry is time and resource intensive, necessitating glutaraldehyde fixation, serial dehydration, resin embedding, ultramicrotome sectioning, mounting and staining, and acquisition of multiple high powered fields using conventional light-microscopy with oil immersion. Automated and expert morphometric analysis of conventional toluidine blue stained ultrathin sections of regenerating nerves is frought with challenge (258). Accordingly, some investigators opt to forego histologic assessment (86, 91, 205, 259-262).

Herein, we characterize a novel research platform to address current limitations of functional and histologic assessment of murine facial nerve function and regeneration. The platform comprises a custom-designed biocompatible cannulated metallic skull implant enabling head restraint and connection to implanted electrodes, a miniature conveyor belt to mitigate the

need for animal body restraint and labor-intensive manual conditioning, cost-effective high-frame-rate videography for capture of dynamic facial movements, a deep-learning based computer vision algorithm for quantitative tracking of facial displacements, and a rapid non-toxic frozen section approach for nerve imaging paired with a machine learning-based segmentation algorithm for automated quantitative histomorphometry. We demonstrate the utility of this approach in a small series of Lewis rats.

5.3 Materials and Methods

Head fixation device and implantable electrodes

Figure 5-3 demonstrates the custom-designed skull implant and mutlichannel implantable electrode array. A cannulated head fixation device comprising a flat base with six arms and metric threaded cylinder was designed in commercial computer-aided design (CAD) software (Fusion 360, Autodesk Inc., San Rafael, CA) (Figure 5-3A). The arms of the flat base contain screw holes positioned specifically for securing the device to the rat skull; holes were positioned to align with the frontal, temporal and occipital bones of the skull on each side of an adult rat. The cylinder was designed to house a miniature eleven pin circular female connector (A79103-001, Omentics Connector Corp, Minneapolis MN) while permitting head-restraint of the animal. Devices were manufactured using pure austenitic Society of Automotive Engineers (SAE) grade 316 stainless steel (selected for its favorable biocompatibility properties) by a commercial computer numerical control (CNC) mill (eMachineShop, Mahwah, NJ). Miniature self-drilling bone screws were designed using CAD, and manufactured using grade II titanium (selected for its favorable osseo-integration properties) by a commercial CNC mill (US Micro Screw, Seattle, WA) (Figure 5-3B). A custom implantable electrode array comprising two bipolar nerve cuff electrodes and seven bare wire leads connected to an eleven pin circular connector (Omnetics

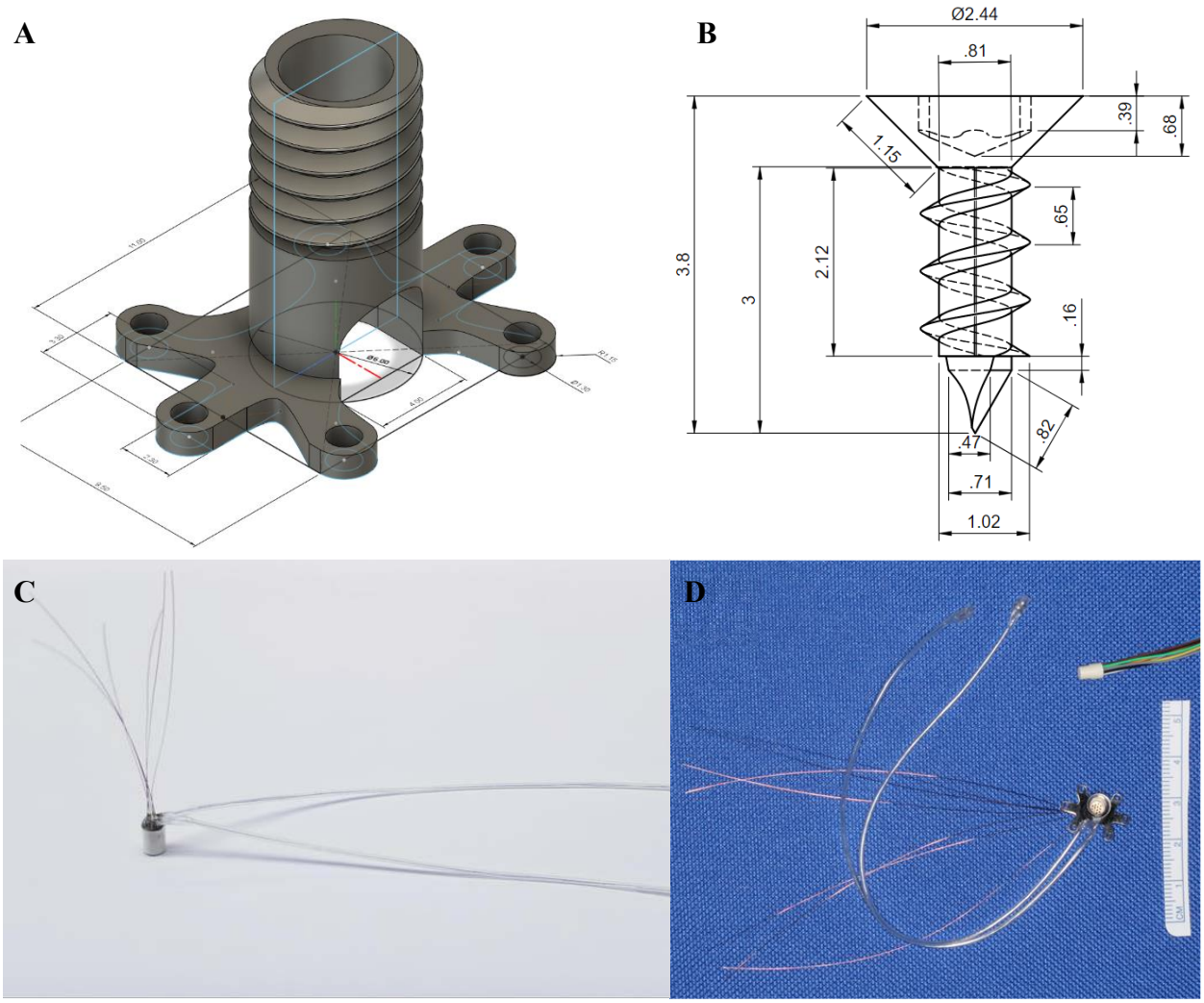

Figure 5-3: Custom-designed cannulated murine head-restraint system. (A) Computed-aided design (CAD) rendering of cannulated skull implant. (B) CAD rendering of self-drilling miniature bone screws to secure implant to rat skull. (C) Photograph of custom-designed commercial muti-channel implantable electrode array comprising two biopolar nerve cuff electrodes suitable for use on rat facial nerve branches, and seven insulated bare wire leads for optional fiber electromyography recording. Electrode leads are micro-soldered to a commercial miniature circular male pin connector. (D) Photograph of miniature male pin connector within canulated port of skull implant with attached electrodes existing flush at the base of the implant, together with corresponding female pin connector leads (upper right of panel) for connection to EMG and neural stimulation hardware.

A79103-001) was designed; manufacture was by a commercial vendor (MicroProbes for Life Sciences, Science, Gaithersburg, MD) (Figure 5-3C). The nerve cuff electrodes were silicone-based, housing 0.5 mm diameter circular platinum iridium contacts, and stainless steel leads encased in thin silicone tubing; cuffs were designed with electrode spacing of 1 mm, inner diameter of 1.5 mm, and total length of 10 mm. Bare wire stainless steel leads with polyimide insulating coating were connected to remaining terminals of the micro-connector for optional capture of electromyography signals via direct intramuscular positioning. Figure 5-3D demonstrates the circular connector with attached leads positioned within the skull implant, together with corresponding male pin connector (A79102-001, Omentics Connector Corp, Minneapolis MN) used to establish a connection with the implanted electrodes during experiments.

Rat treadmill and head-restraint hardware

Figure 5-4 demonstrates the custom-designed experimental setup for high-frame-rate capture of head-restrained ambulating rats. A custom-designed miniature conveyor belt to serve as a rodent treadmill was designed in commercial web-based software (Conveyor Configurator, QC Industries LLC, Batavia, OH) and manufactured by a commercial vendor (QC Industries LLC). The treadmill comprises a 6″ wide by 18″ long track with stainless steel frame, right-hand pulling top-mounted brushless direct current variable speed 200 W (1/4 HP) 3000 RPM motor with 10:1 parallel shaft gearhead. A motor driver allows for manual control of the belt between speeds of 0 and 500 FPM or 9.1 km/h, according to observed animal comfort. The treadmill was secured with ¼″ screws using custom-designed L-brackets to a 24″ by 24″ optical breadboard (B2424FE, Thorlabs, Inc., Newton, NJ). Adjacent to the conveyor belt is mounted a rail system designed to enable rapid mounting and dismounting of animals from the treadmill surface

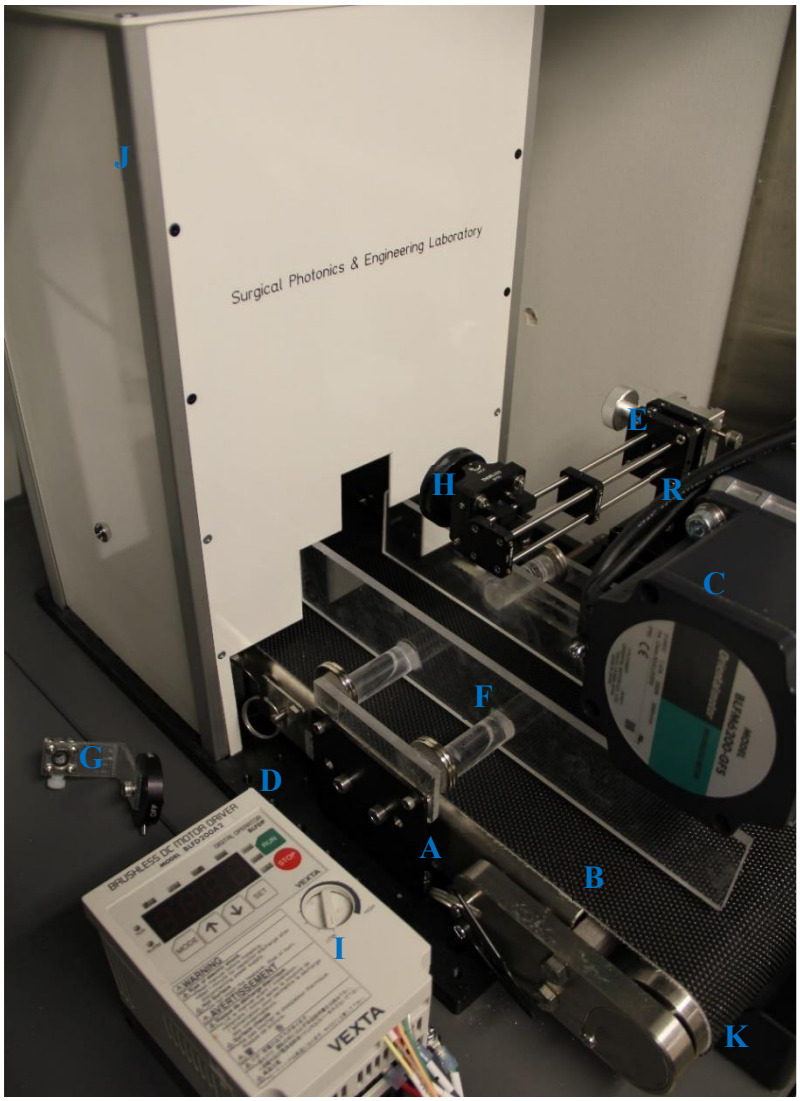

Figure 5-4: Experimental platform for high-frame rate capture of facial displacements in the head-fixed ambulating rodent. A custom-designed commercial stainless-steel frame (A) conveyor belt (B), powered by a top-mounted DC brushless motor (C) is mounted to an optical breadboard (D). Adjacent to the conveyor, a z-height adjustable optical rail system (E) is mounted securely to a linear-glide rail with locking thumbscrew (not visible). Magnetically-mounted clear side rails (F) maintain linear orientation of rats while ambulating on the track. A cannulated thumbscrew hub to receive the skull-implant is secured to an L-bracket (G) with attached circular magnetic base for rapid coupling of animals to the optical rail system (H) atop the track. Treadmill speed is readily adjustable via a turn-dial on the brushless DC motor driver (I). Once mounted on the track, the optical rail is slid forward to position the head of the animal inside the recording enclosure (J), wherein red-light illumination is employed for high-frame rate capture of facial displacements (see next Figure). Excrement is captured by a trap positioned below the edge of the track (K).

and rapid adjustment of head position within the recording field. The system comprises an 18″ long by 4″ wide single-axis linear motion rail with movable platform with thumbscrew for rapid and secure positioning (UGA040C-1P1G10 and UGA040R-0300-000, PBC Linear, Roscoe, IL). A rack and pinion z-axis stage (55-024, Edmund Optics Inc., Barrington, NJ) was mounted to the linear rail platform to enable optimal z-axis positioning of animal heads atop the treadmill surface. A 30 mm optical cage system (ER6 cage assembly rods and CP3 cage plates, Thorlabs Inc) was mounted to the z-axis stage; at the end was affixed the bottom plate of a magnetic circular retention base (SB1, Thorlabs, Inc). The corresponding top plate of the magnetic circular retention base was mounted to a custom 1.5″ x 1.5″ steel L-bracket; a 6 mm bore hub with thumbscrew of 1″ by 1″ outer diameter (Actobotics 545576, ServoCity Inc., Winfield, KS) was mounted to the opposite end of the L-bracket. The bore hub was selected to enable rapid coupling of the L-bracket to the skull implant, while enabling wire connection to an enclosed pin connector. The magnetic retention base was designed to facilitate rapid mounting and dismounting of animals from the testing track. Neodynium disc magnets were employed to enable rapid placement and removal of acrylic plexiglass side-rails (measuring 12″ long by 3″ high x 1/8″ thick), used for maintaining animal bodies in the forward facing direction during testing.

Surgery and conditioning

Four adult Lewis rats (male and female) weighing 200-300g were employed. General anesthesia was induced in an induction chamber using 1.0 L/min O_2 with 3% isoflurane gas. Buprenorphine (0.05 mg/kg subcutaneously) and meloxicam (1.0 mg/kg subcutaneously) were administered for analgesia. Animals were placed in clean surgical field and transitioned to nose cone isoflurane anesthesia at a maintenance rate of 1-3%. The top of the head was shaved and

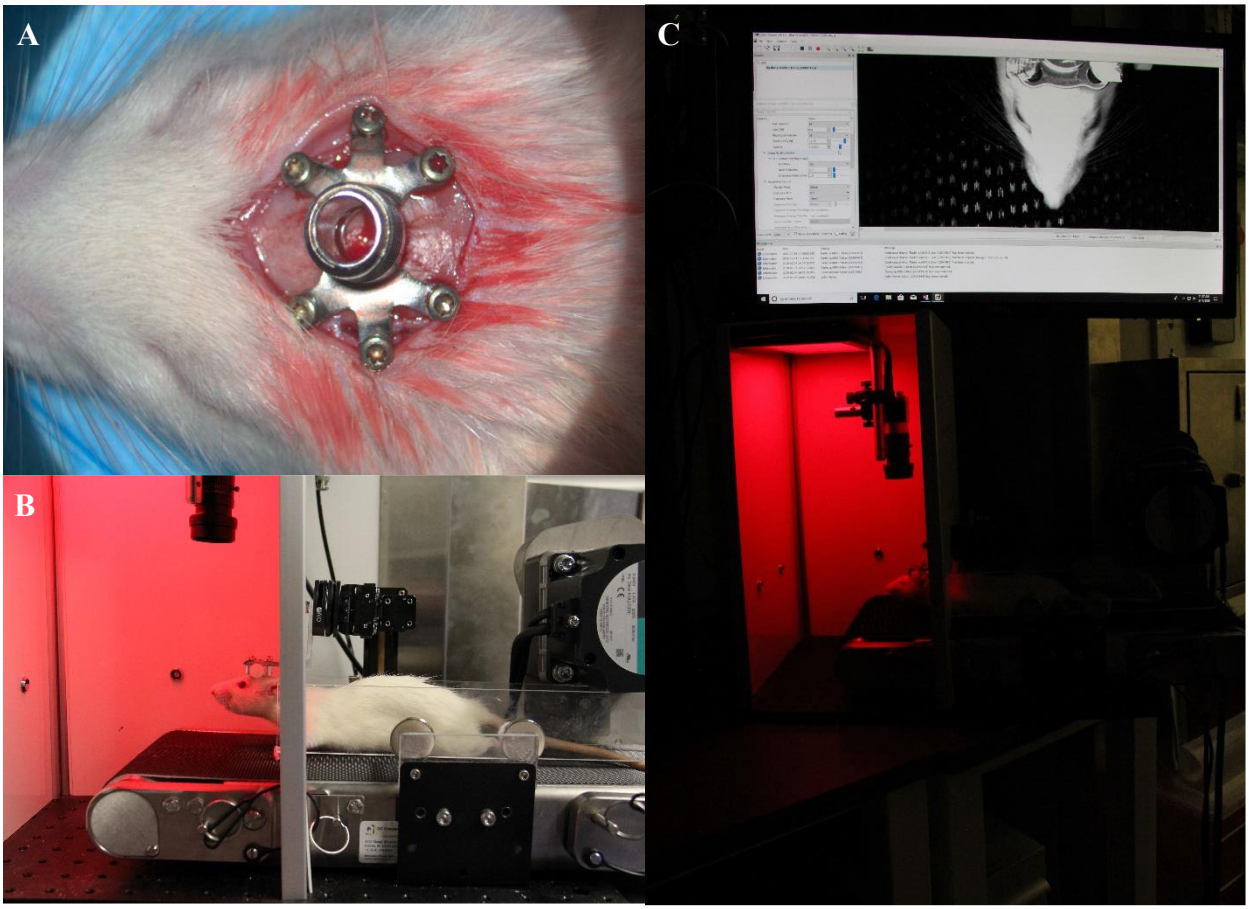

Figure 5-5: High-speed videography of head-restrained amubulating rat. (A) Intraoperative photograph of custom-designed cannulated stainless-steel implant secured to the skull with six custom-designed miniature titanium bone screws. (B) Live rat shown on treadmill in head-restraint with head positioned inside recording enclosure (side panel removed for visualization). A commercial high-frame rate camera is visible (cam) inside the enclosure. (C) A live-feed is displayed on a wall-mounted monitor for monitoring of animal during recordings.

prepped with alcohol. A #15 blade surgical scalpel was used to create a 1.5 cm incision in the mid-sagittal plane atop the cranium. Periosteum overlying the skull was carefully teased away to expose raw bone. The arms of the head fixation devices were gently bent to fit the skull curvature, and devices secured by placement of six miniature bone screws (Figure 5-5A). In two

animals, the left facial nerve extratemporal main trunk was circumferentially dissected and crushed for 30 seconds using a needle driver to induce a high-grade neural injury-in-continuity. In the other two animals, the facial nerves were left undisturbed. The skin was closed around the device using veterinary skin adhesive (VetBond Tissue Adhesive, 3M, Minneapolis, MN), antibiotic ointment was placed, and the animals recovered from general anesthesia. Animals were co-housed following surgery, two per cage, for social interaction and well-being. An additional dose of meloxicam (1.0 mg/kg subcutaneously) was administered for post-operative analgesia on the first post-operative day. Beginning two-weeks after surgery, animals were conditioned to mobilizing on the treadmill by twice weekly sessions, increasing from 30 seconds to five minutes of head restraint.

Video Recording

Figure 5-4 and Figure 5-5 illustrate the video recording hardware employed. A custom enclosure (measuring 9″ wide by 9″ long by 16″ high) was designed using commercial software (Front Panel Designer v 6.2.1, Shaeffer AG, Berlin, Germany) and manufactured using reflective white-coated aluminum (selected to maximize light signal for recordings) through a commercial vendor (Front Panel Express, Kent, WA). The enclosure was designed for precise positioning atop the treadmill surface, with a receiver port for the treadmill side rails and animal head (Figure 5-4). To maintain animal comfort and encourage ambulation during head-restraint within the enclosure, red light illumination was chosen for video recordings. Rats lack red-light sensitive retinal cone cells and are functionally blind at wavelengths over 600 nm (263). A 3.5″ by 6″ red light-emitting diode backlight (85-257, Edmund Optics) was mounted within the enclosure to the top panel. Adjacent to the light source was mounted an optical post (TR6, Thorlabs Inc.), from which was suspended a commercial high-frame-rate miniature universal

serial bus (USB) camera (acA800-510um Basler ace, Ahrensburg, Germany). The camera was equipped with a 6 mm fixed-focal length lens (33-301, Edmund Optics) and longpass filter at 610 nm (FGL610H, Edmund Optics). The camera was connected to a performance workstation (Intel i9-10920X 12 core 3.5 GHz CPU, 128 GB DDR4 3200 MHz RAM, 1 TB NVMe M.2. SSD, NVIDIA Titan RTX GPU).

Videos of facial displacements of rats in head-restraint using the aforementioned platform were captured beginning four weeks following surgery. This time period was chosen to ensure adequate osseointegration of the devices, while allowing time for some regeneration of crushed axons among injured animals. Figure 5-5B and C illustrate video capture of facial displacements during experiments. Long-segment high-frame-rate video at 500 frames-per-second (fps) at VGA resolution (640 pixels by 480 pixels) was captured to system memory using commercial software (Streampix v8, Norpix Inc, Montréal, QC), and compressed using a CUDA-accelerated H.264 video codec (Norpix Inc.) prior to saving to the system hard drive for subsequent analysis.

Rat facial landmark and displacement tracking

An open-source Python-based computer vision toolbox (DeepLabCut) was employed. This toolbox facilitates training of deep neural networks to track user-defined features from digital media with limited training data (264) and accuracy that rivals human labeling for markerless tracking of anatomic features in laboratory animals and human subjects (265, 266). DeepLabCut employs the feature detector architecture from a powerful pose estimation algorithm (DeeperCut (267, 268)), based on extremely deep neural networks pre-trained using ImageNet,(269) a huge generalized dataset for object recognition. Specifically, DeepLabCut employs pre-trained deep, residual networks comprising 50 or 101 layers (known as ResNets(269)) and deconvolutional layers developed in DeeperCut,(267, 268) whose weights are

adapted through further training to predict user-desired features within novel datasets. The DeepLabCut toolbox comprises a graphical user interface for rapid neural network training via labelling of user-defined points from sub-samples of frames within videos of interest. Trained algorithms yield confidence scores for each automatically localized landmark, representing the likelihood that each distinct anatomic landmark has been labelled correctly. Processing of unseen test frames within the DeepLabCut GPU enables rapid identification of frames yielding low confidence scores, which may then be manually labelled for additional training rounds to improve algorithm performance. For well-trained algorithms, low confidence scores occur primarily where an anatomic landmark is not present or visible within a given frame.

The Python-based toolbox was installed per published guidelines by Nath on the same performance workstation employed for video acquisition (264). For rat whisker and blink tracking, we defined 26 distinct landmarks (Figure 5-6A); five points were placed equidistant along the C-1 whisker on the right and left sides, single points placed at the lateral and medial canthi and two points along the upper and lower eyelids on the right and left sides, three points along the sagittal midline of the nose, and one point at the center of the nasal tip. Two long-segment videos (one uninjured and one crush-injured animal) were employed for training. The selected input parameters for DeepLabCut training employed herein included video resolution (640 x 480 pixels), dot size (3), ResNet layers (50), inference batch size (N=8),and maximum number of iterations (1030000). Training required only a single step, and progressed through all iterations in approximately 24 hours.

Next, secondary code was written within a Python programming environment for automated framewise estimation of whisking and blink activity. Figure 5-6B graphically depicts

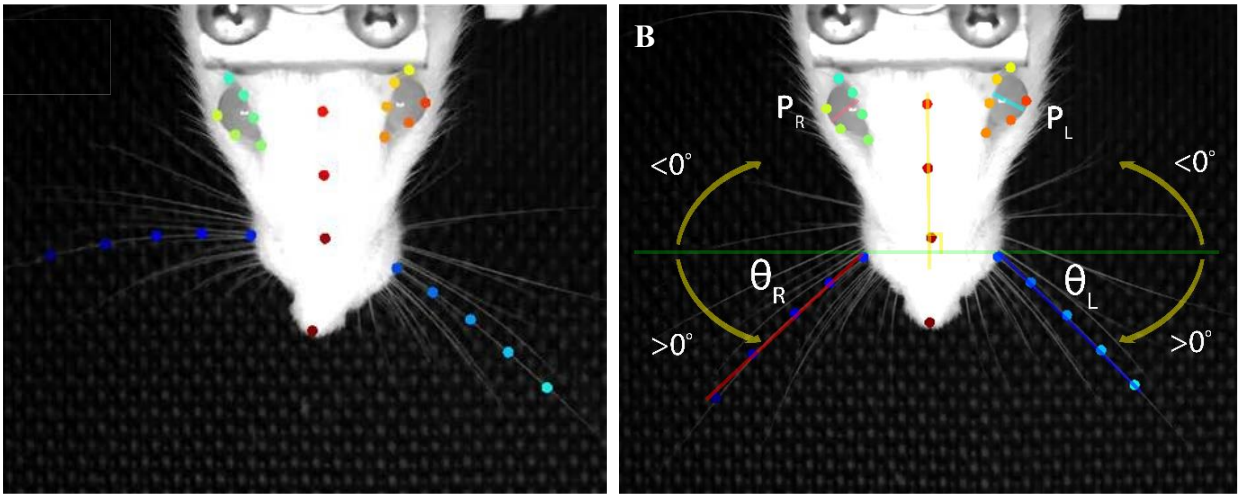

Figure 5-6: Automated pose estimation and calculation of whisker and blink displacements from high-frame rate videography of the head-fixed rat. (A) A deep neural network was trained to localize 26 distinct points on the rat face using an open-source computer vision toolbox (DeepLabCut). (B) Point locations were employed for automated plotting of regression lines to the sagittal midline and C-1 whiskers (right, red line; left, blue line). Right (θ_R) and left (θ_L) whisking angles were defined relative to the coronal plane (green line), with protraction considered positive and retraction negative (yellow curved arrows). Right (P_R) and left (P_L) palpebral fissure width was calculated in pixel distance for lines spanning the average distance between upper and lower lid points (right - pink link, left – cyan line).

whisking and blink measures obtained from DeepLabCut landmarks for each frame. The sagittal midline was defined as a linear regression line through the three center points (excluding the nasal tip point). Right and left C-1 whiskers were defined by linear regression lines through their five landmark points. Whisker displacements were defined as the angle formed by the right and left C-1 whisker with the coronal plane of the face, defined as an axis orthogonal to the sagittal midline. Blink displacements where defined as the mean pixel difference between two lines connecting opposing point on upper and lower eyelids on right and left sides. To minimize errors, regression lines were plotted (and values calculated) only for frames where all points

along each specific landmark were localized with high confidence by the trained deep neural network.

Quantitative Analysis of Whisking Function

An open-source toolkit for the identification of biomedical systems in MATLAB (R2018a, The MathWorks Inc, Natick, MA) was employed for whisker signal processing (270)[2]. Linear interpolation was employed for missing data points. First derivatives were taken and signals detrended. A nonequispaced fast fourier transform of 5000 bin length was employed for generation of power spectra for comparison of whisking activity between right and left sides and between injured and uninjured animals.

Nerve histomorphometry

Four weeks following surgery, one control and one crush-injured animal were humanely euthanized via CO_2 inhalation in accordance with institutional animal care protocols. The corpses were immediately transcardially perfused with 4% phosphate-buffered paraformaldehyde (PFA). Left facial nerve buccal branches were harvested and post-fixed by immersion in 4% PFA for 48h, followed by cryoprotection in 30% sucrose for 24h. Axial cryosections at 1 μm were obtained, mounted on silane-coated glass slides, stained with a myelin-specific dye (FluoroMyelin® Green, 1:300 diluition, Molecular Probes, Eugene, OR), and cover-slipped according to previously described methods (271, 272). Fluorescent images of nerve cross-sections were collected by confocal microscopy (Leica Microsystems SP8, Wetzlar, Germany). The microscope was equipped with a resonant scanner and high-magnification oil-immersion objective lens (HC PL

[2] Tools employed within the nonlinear system identification toolbox (‘nlid’) included the data object ‘nldat’, ‘ddt’ and ‘detrend’ methods, and power spectrum object ‘spect’

APO 63x/1.40 Oil CS2, Leica Microsystems). Samples were excited using a 488 nm argon laser, and signal collected using a 550/50 nm band-pass filter using hybrid detectors and images acquired using commercial software (Leica HyD and Leica Application Suite X) Commercial deconvolution software (Lightning, Leica Microsystems) was employed for image enhancement, and image brightness and contrast adjusted for enhanced visualization using ImageJ software (Fiji Distribution, Version 1.52e) (273, 274).

Myelinated axon counts were quantified from digitized images using commercial machine learning software (Aivia v8.5, DRVision Technologies LLC, Bellevue, WA) (275). Briefly, a random forest pixel classifier was trained by manual painting of a few myelin rings and adjacent background within a digitized image. A shallow decision tree was employed for rapid preview, and iterative repainting of suboptimally labelled regions was performed until a suitable result was obtained. A deep decision tree model comprising forty ensembles was then trained, each reaching a maximum depth of 16. The model output is an 8-bit confidence image, wherein each pixel of an input image is assigned a value between 0 and 255, representing 0-100% confidence that the pixel belongs to the trained class. Thresholds are then selected to separate myelin from background and to define the lowest allowed cross-sectional area for myelin ring segmentation.

5.4 Results

There were no perioperative complications. Head fixation devices demonstrated good osseointegration enabling head-fixation for a minimum up four weeks in all animals; the two longer surviving animals herein were noted to have good head-fixation after eight weeks, prior to scheduled euthanasia. Animals were noted to readily take to the treadmill apparatus, with immediate and excellent front limb gait noted at speeds around 3 km/h. Rats were noted to

intermittently drag their hindlimbs on the treadmill track in early conditioning trials, which improved with over time and with adjustment of head position.

Qualitative visual analysis of the trained deep neural network for facial landmark localization after a single round of training demonstrated excellent results. Subsequent analysis of captured videos returned data points for over 99.8% of frames, indicating the algorithm was localizing landmarks with high consistency.

Figure 5-7 demonstrates the time series of whisking and blink activity on right and left sides in an uninjured and a facial nerve crush-injured animal over a representative 60 second recording four weeks following surgery. In the uninjured animal, right and left side whisker tracings appear similar. In contrast, C-1 whisker tracings demonstrate obvious differences in amplitude between left and right sides in the crush-injured rat, with lower amplitude whisks observed on the affected side. Differences in whisking power between right and left sides in the uninjured and injured states are better visualized with power spectra as shown in **Figure 5-8**. During this sequence, animals demonstrated a bimodal whisking frequency with peaks around 5Hz and 11 Hz, corresponding to exploration of novel environments (276). In the uninjured animal, power was uniformly distributed across left and right sides, while a stark difference in power up to frequencies of 25 Hz was noted in the injured state. Blink tracings showed scant blinking activity for all animals tested at all time periods.

Figure 5-9 demonstrates images obtained of the buccal branch of the facial nerve in an uninjured animal and in a crush-injured animal four weeks following insult. High-throughput rapid frozen section was sufficient for resolving myelinated axons by conventional fluorescence microscopy. Training of the machine learning based pixel classifier for myelinated axon segmentation was readily performed in under five minutes. Subsequent automated segmentation

of axons across the entire cross-sections demonstrated good performance as shown in Figure 5-9B,D. Compared to the uninjured state, axon diameters were smaller and myelin thickness decreased as demonstrated by the increased spacing between axons seen for the injured nerve.

5.5 Discussion

Challenges inherent to the study of peripheral nerve regeneration leave many clinical questions without answers supported by evidence from rigorously controlled animal studies. For example, there exists no controlled animal study that has characterized the impact of surgical decompression timing on long-term outcomes following acute entrapment of the facial nerve. Such entrapment is thought to result from viral reactivation-induced inflammation of the nerve in Bell's palsy (10, 11, 277), with subsequent pressure-induced axonal degeneration and ischemic neural fibrosis (11, 63, 278-281). In 15-30% of such cases, post-paralysis facial palsy (PFP) subsequently develops as axons regenerate in random fashion to the distal facial musculature (28, 282, 283). The result is an irreversible state of facial hypertonicity, with multiple manifestations of aberrantly regenerated axons, amongst them involuntary eye closure during smiling, speaking, or eating, and massive neck and mid-facial contraction during voluntary eye closure (Fig. 6). Though facial nerve decompression surgery in acute cases of Bell's palsy has been shown to impressively lessen the risk of PFP development among high-risk patients in prospective clinical studies (38, 63), its use remains sporadic. Failure of the procedure to gain widespread acceptance is based on the absence of evidence of effectiveness from randomized controlled animal and human studies.

Herein we sought to demonstrate the proof-of-principle of a high-throughput platform for assessment of facial nerve structure and function in the rat to address unanswered questions and assess novel therapeutics in the field of facial reanimation and facial nerve regeneration. This

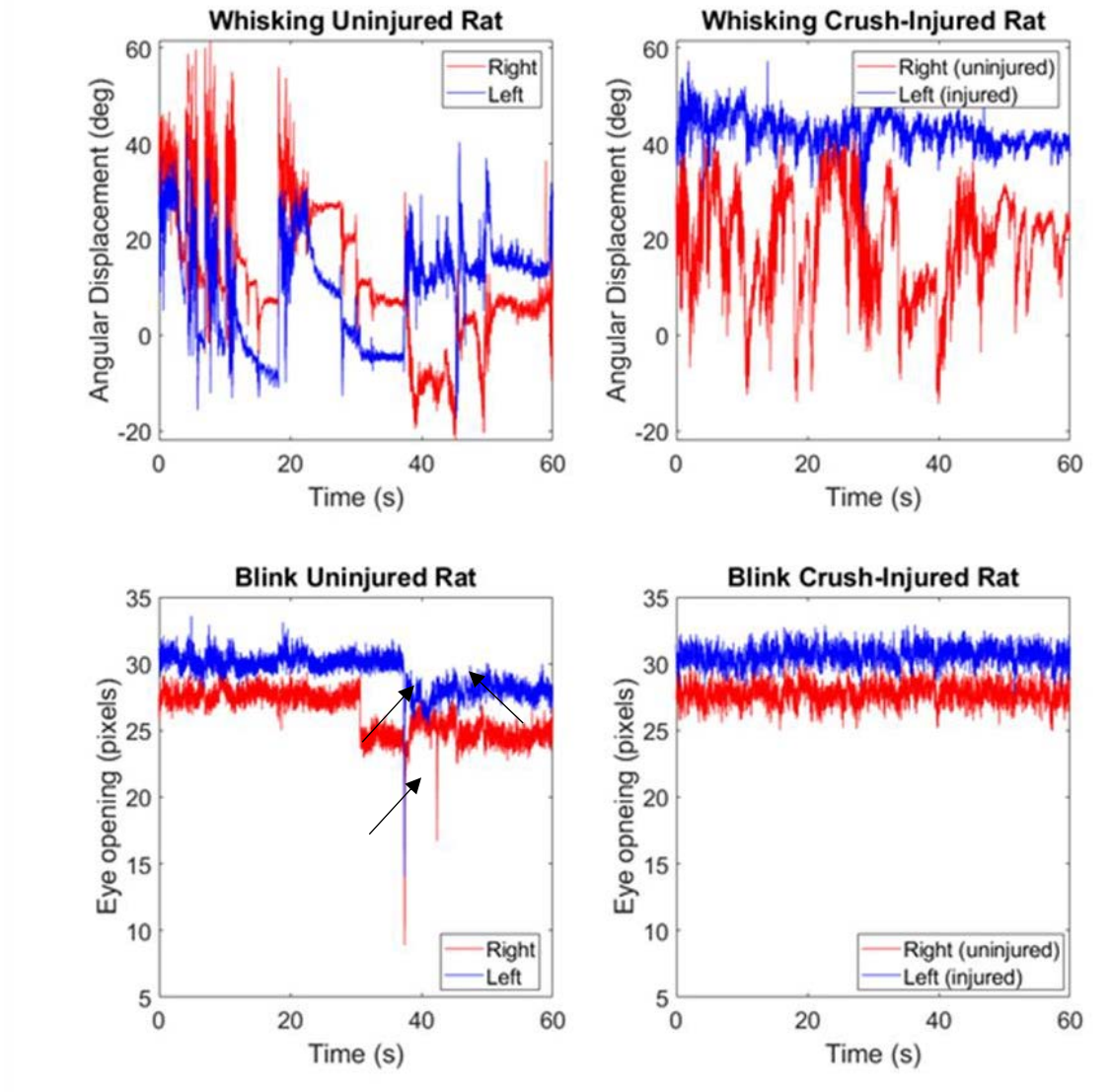

Figure 5-7: Right- and left-sided whisker and blink tracings in the uninjured and unilateral facial nerve-crush injured rat. Tracings were recorded four weeks after head-implant surgery and facial nerve crush. Tracings represent unfiltered raw-data obtained using the automated computer-vision algorithm developed herein over a single 60 second, 500 frame-per-second recording length in each animal. Note sparse blink activity; only thee blinks are noted in the uninjured rat (arrows) during the recording session, while no blinks are noted in the injured rat.

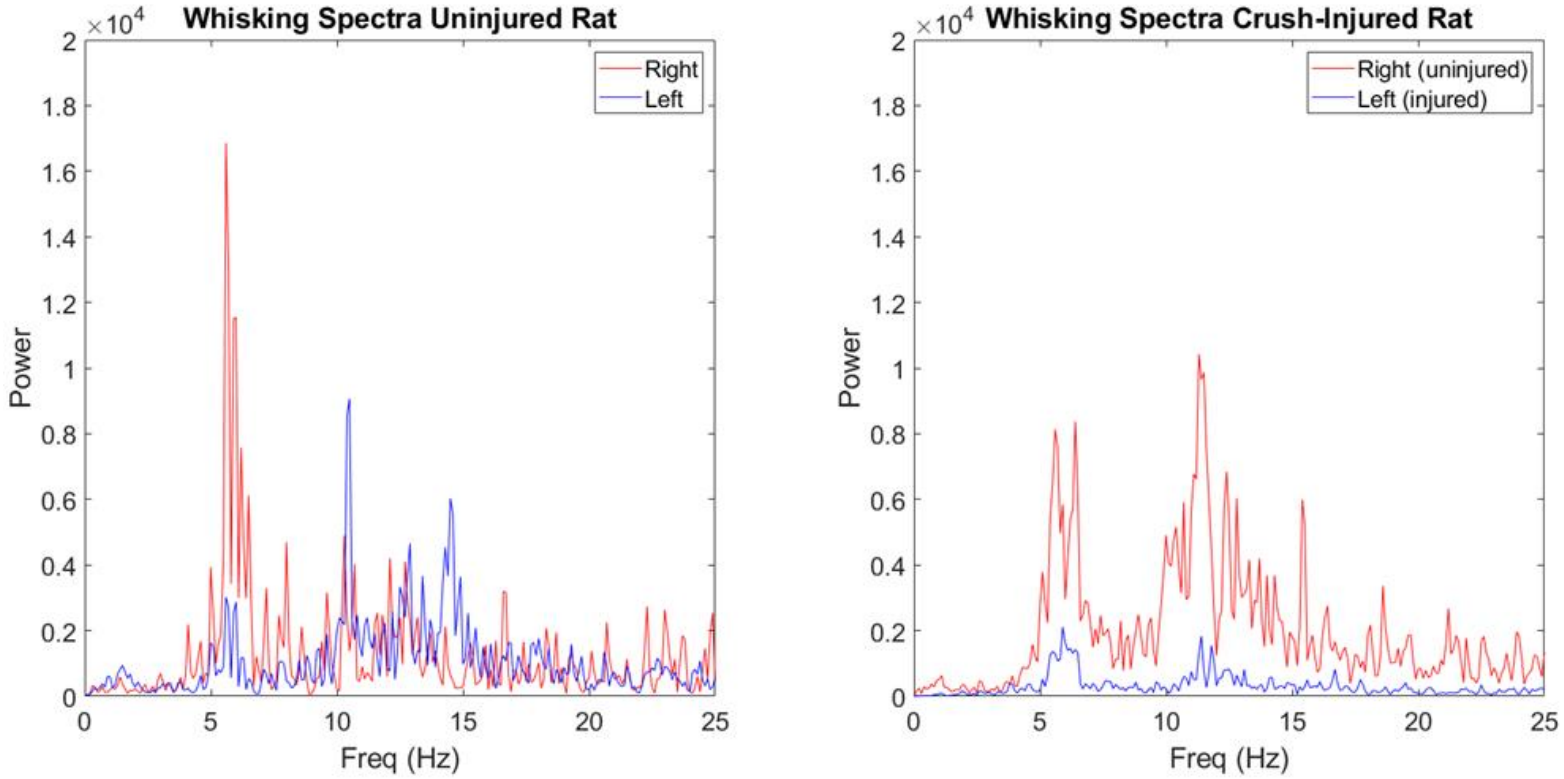

Figure 5-8: Power spectra of whisking activity compared between sides in the uninjured and unilateral facial nerve-crush injured rat. The spectra correspond to the whisking displacement time series in the previous figure. In the uninjured rat, whisking power is similar between right and left sides, while a stark decrease in whisking power is noted on the damaged side of the facial-nerve crush injury rat.

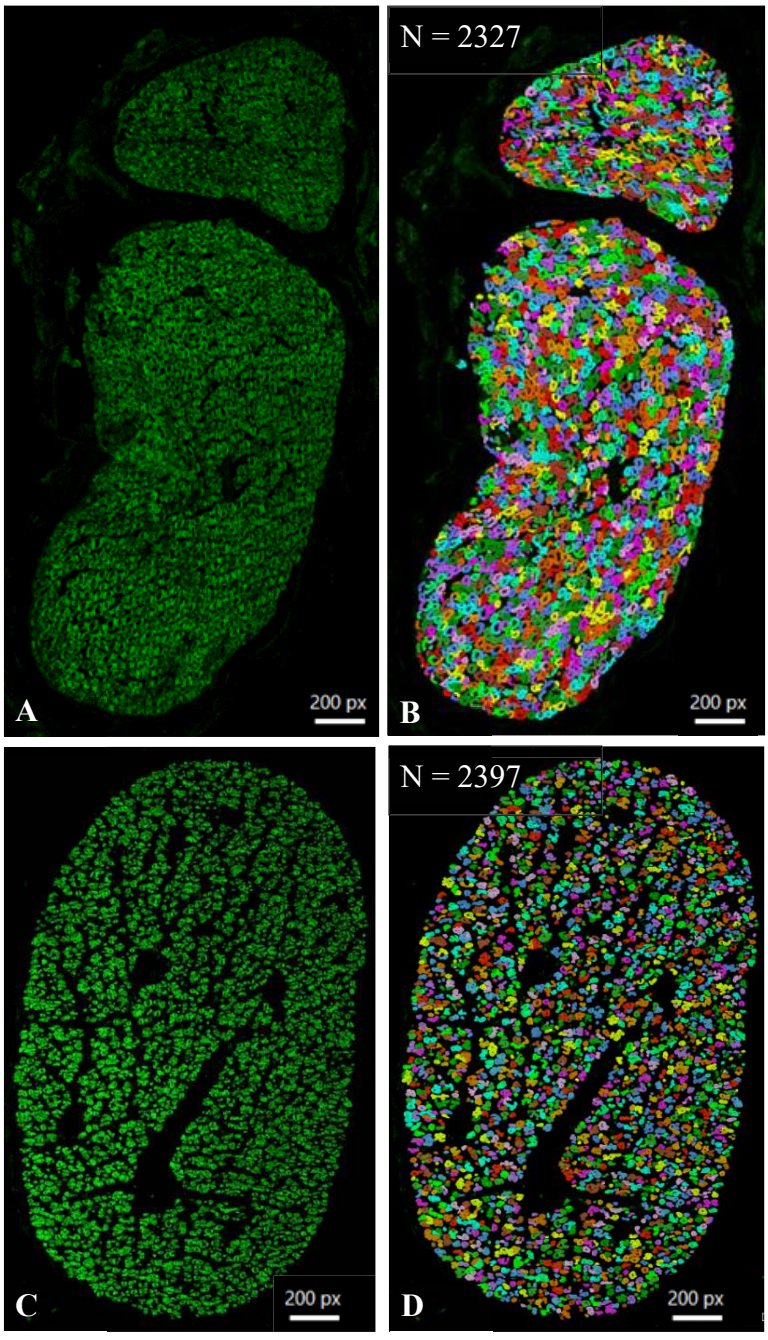

Figure 5-9: Rapid histomorphometry of rat facial nerve. (A, C) The buccal branch of the facial nerve in an uninjured (A) and facial nerve crush injured (B) mouse stained with a myelin-specific dye and visualized using fluorescent microscopy. (B, D) Automated segmentation of myelin rings with quantification of myelinated axons.

platform enables quantitative assessment of facial nerve function, while avoiding the need for labour-intensive conditioning to full-body restraint. In contrast to prior hardware setups for functional assessment of rat whisking and blink (194, 195), use of high-frame-rate video for data capture paired with automated quantification of facial displacements using trained deep neural networks requires minimal hardware. Setup is straightforward, requiring only an adequate high-frame-rate camera and personal computer. Future work will seek to further optimize the functional component of this model by combining camera control, video compression, landmark localization, metric quantification, and data analysis within a single user-friendly graphical user interface.

This study demonstrated clear functional differences between whisking power of right and left C-1 whiskers at four weeks in a facial nerve crush-injured rat; such differences were not seen in an uninjured control. This suggests this model may prove useful for quantification of differences in facial nerve function in various disease states. Future work will seek to validate the efficacy of specific parameters including cross-correlation coefficients and root-mean-power in detecting differences between healthy and injury states in larger numbers of animals across multiple time points to establish means and standard deviations on which power studies for other work may be based. Herein, scant blink activity was noted, which is typical of the rat (194, 195). Future work will seek to add high-throughput blink conditioning capacity to the platform comprising air puffs delivered with sound stimuli (284).

Histologic assessment is critical to understanding the relationship between peripheral nerve structure and function following injury. The most commonly assessed parameters of periphearal neural regenation in cross-section include myelinated axon count, density, and diameter, in addition to myelin thickness, and g-ratio: the ratio of the inner axonal diameter to

the total diameter including myelin sheath (285, 286). Recent work by our group has demonstrated that frozen section and rapid non-toxic fluorescent dye staining paired with machine learning-based pixel classifiers can be employed for rapid neural histomorphometry of human peripheral nerve (271, 275). Herein, we demosntrated use of this high- throughput approach for quantification of rat facial nerve structure in health and injured states. Though only axon counts were quantified herein, future work will seek to quantify all commonly assessed measures of nerve regeneration in cross-sections.

This work has other limitations. Though a custom electrode array and head-fixation device to enable long-term implantation of nerve cuff and EMG wires in the rat was developed herein, only the head-fixation device was tested. Future work will investigate whether this approach enables long-term electrode implantation in the rat, and whether neural stimuli may be delivered and EMG signals obtained using this approach Though adequate whisking activity was achieved herein to make observations between left and right side function, as animals become habituated to the experimental setup it is likely that their exploratory whisking activity will decrease. Without sufficient whisking effort, functional differences between sides would be obscured. Future work will seek to add stimulation to the recording environment to encourage whisking activity over repeated testing periods. Though gross visualization of landmark localization and myelinated axon segmentation by the artificial intelligence algorithms trained here suggested high accuracy, future work will require comparison against manual human markings to validate this approach.

5.6 Conclusion

A high-throughput platform for quantitative assessment of facial nerve structure and function in the rat has been described. This model may prove useful for study of facial nerve regeneration and novel therapeutic approaches to management of facial palsy.

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CHAPTER 6: GENERAL DISCUSSION

6.1 Discussion

The importance of facial expression is demonstrated by its conservation and increasing complexity among higher-order animals through natural selection (1). For example, smile facilitates social interactions by conveying happiness, intelligence, and social status (1-5). Facial palsy yields functional, esthetic, and communication impairments with devastating consequence (20, 61, 178-187, 189-192, 287). In this thesis, we presented a general approach to management of facial palsy, summarized contemporary treatments while noting their limitations, and proposed an original approach to therapeutic management of this challenging disease. We demonstrated in a live rat model the potential for therapeutic management of unilateral facial palsy with a NPD. We discussed how this novel approach pairing proximal neural blockade by HFAC with distal targeted FES addresses the challenges associated with rehabilitation of aberrantly innervated musculature. We then characterized a comprehensive research platform to facilitate future work to assess the safety and efficacy of this FES paradigm over the long term.

Realization of an NPD for facial palsy would inform the acute and long-term management of the disease. Modern treatment algorithms in the setting of new-onset facial palsy involve performing interventions in response to lack of clinical improvements over time. The innovation described herein would permit active intervention to mitigate or avoid the functionally crippling long-term sequelae of facial palsy. For example, the degree of spontaneous recovery following manipulation of the FN in the cerebellopontine angle or skull base is notoriously difficult to forecast as there are no reliable electrophysiologic tests capable of predicting functional outcomes (288, 289). Here, a watchful waiting approach is undertaken, whereby the expectation for spontaneous recovery is abandoned after one year of observation.

By that time, the receptivity of muscle to alternative neural inputs has diminished (43-46, 290, 291), and the prospect for adequate restoration of dynamic movement to the native facial musculature is low. Establishing that facial muscles with any kind of motor axonal input—whether from the native proximal FN stump or an alternative motor neural source (such as the hypoglossal nerve or a motor branch of the trigeminal nerve)—may be reanimated by means of a NPD would promote earlier intervention to preserve native facial muscle viability. Beyond improving functional and communication outcomes and restoring currently neglected facial movements, such a device has the potential to substantially reduce the number of surgical, medical, and physical therapy interventions necessary to treat the persistently flaccid state, whose cost over time may be staggering.

The ultimate success of the proposed NPD approach for facial reanimation is dependent on the following conditions being maintained over long periods of time: (a) the capacity for EMG signals from the contralateral healthy-side musculature to be used as control signals to deliver optimal FES parameters, (b) the capacity of the EEAs to capture EMG signals in robust fashion without detrimental effect on the adjacent muscle, (c) the capacity of the NCEs to remain in place and robustly deliver FES and HFAC signals without inducing an inflammatory, compressive, or other form of neuropathy, (d) the absence of patient discomfort resulting from the implanted electrodes, leads, and ASIC, and from the outputted FES and HFAC signals, and (e) the capacity for the ASIC to function reliably under the rigors of clinical use.

Evidence from the literature, our results presented herein, and clinical outcomes of similar FDA-approved NPDs suggest that the aforementioned conditions will be met. First, it has been demonstrated that rectified and low-pass filtered EMG signals may be used as an input to a non-linear system to predict muscle force activation in limbs (292) and that relationships exist

between facial muscle EMG signals and force (120). Similarly, previous studies have established methods for modeling the dynamics of electrically stimulated muscle (293, 294). Furthermore, our preliminary data has demonstrated that a clear impulse response function exists between differential EMG signals recorded from whisker pad musculature using EEAs and whisker displacements in our rodent model. Though use of healthy-side EMG activity to extract command signals might prove less adjustable than traditional FES control signals (e.g. pressure, tilt, and accelerometer signals used in footdrop neuroprotheses), use of myoelectric activity to reliably drive neuroprostheses has been previously demonstrated (222, 223). Further, use of EMG activity as control inputs does not preclude future development of configuration software enabling clinicians and patients to individually tailor the NPD parameters (such as stimulus timing, amplitude, pulse frequency, and pulse width) to optimize clinical outcomes and compensate for inter-patient variability. Second, numerous studies have demonstrated long-term success with EMG signal recording from implantable electrodes (137, 295-297). The biocompatible EEAs used herein demonstrated the ability to accurately capture and amplify differential EMG signals with very low noise while implanted in a canine limb model (298). Third, numerous studies have demonstrated the feasibility of long-term implantation of NCEs (299-302). As discussed in Chapter 2, use of implanted NCEs for long-term stimulation of a cranial nerve has precedent; a recently FDA-approved NPD utilizes an NCE around the hypoglossal nerve to evoke contraction of oropharyngeal airway dilator muscles triggered by changes in thoracic pressure (109, 303-307). Our findings herein demonstrated no significant functional or histologic insult to the neuromuscular unit in rodents four weeks after NCE implantation on facial nerve branches. Further, over the period examined herein, no neuropathy or myopathy was noted following prolonged daily application of HFAC to the facial nerve.

Fourth, the small size of the EEAs and NCEs makes it unlikely that patients would take notice when implanted in the sub-superficial aponeurotic system plane of the face. The small stimulus amplitudes required to depolarize nerve (as opposed to direct muscle stimulation) are unlikely to trigger regional nociception fibre activation, especially when delivered in a bipolar fashion within an electrically shielded NCE. Considering that an FDA-approved NPD for sleep apnea is well tolerated by sleeping patients, significant patient discomfort resulting from a similar device to stimulate facial motor nerve branches is unlikely. Although motor nerves contain sensory fibers that convey information from the target muscle to the central nervous system, an additional benefit of the proximal HFAC delivery proposed here is that the transmission of these retrograde sensory action potentials would also be blocked, as HFAC has been demonstrated to result in localized blockade of sensory and motor axons (148, 308). Finally, as no spontaneous clinical improvements occur in the setting of long-term FFP and PFP, long-term FES would be required to maintain the clinical benefits offered by this device. Fortunately, as discussed in Chapter 2, there is a great deal of experience and published evidence within the field of otolaryngology on long-term use of implanted NPDs for neural stimulation, with cochlear implants having proven safe, effective, and cost-effective in children and adults (309) with high rates of use and benefit at follow-up extending over a decade (310, 311). Tremendous patient benefit and long-term effectiveness has also been demonstrated from motor neuroprostheses used in other clinical scenarios, an example being FES stimulation of the peroneal nerve during gait for footdrop (138, 193).

6.2 Future Directions

Future work will seek to employ our novel high-throughput rat model to implement a paradigm of stimulated hemi-facial movements, whereby EMG signals from one side of the rat

face will be used to generate symmetric facial movements via distal nerve branch stimulation, with and without concurrent proximal neural blockade of native conduction on the stimulated side. We aim to achieve this using implanted microelectrode arrays with connection leads secured within the head-fixation devices as proposed in Chapter 5. Next, we will seek to determine the long-term feasibility of facial reanimation using the FES paradigm described herein, via gradually increasing conditioning to head restraint on our treadmill to enable continual experiments of long duration in otherwise uninjured rats. We will then study the approach in rats who have undergone unilateral hypoglossal to facial nerve transfers, to characterize whether symmetric facial expressions may be restored using this paradigm. If these sets of experiments confirm efficacy and safety of the approach, we will then seek to partner with industry to develop a customized implantable ASIC paired with appropriate electrode arrays that meets the requirements of our FES paradigm. Presently, miniaturization of hardware necessary for HFAC signal generation poses a considerable challenge.

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