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Assessing White Matter Cortical Organization using Diffusion Tensor Imaging Post-Facial Reanimation Surgery

by

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Abstract

The purpose of this study was to determine whether diffusion tensor imaging (DTI) could detect cortical differences between people post-facial reanimation surgery compared to matched controls. Five primary white matter tracts were analyzed for fractional anisotropy (FA) and mean diffusivity (MD) and compared between 4 patients with facial reanimation surgery (ages 11 to 47 years) and their matched controls. Findings were in the predicted direction of lower FA and higher MD in regions of the brain containing corticobulbar tract (CBT) in the facial reanimation group compared to matched controls. This finding indicated that neural tracts associated with facial movements may have less white matter fibre tract integrity in this group of patients who have undergone reanimation surgery secondary to facial paralysis. The use of DTI tractography analysis may be useful in understanding underlying neural mechanisms of change following facial reanimation surgery and ultimately serve to inform surgical and rehabilitation processes.

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CHAPTER I: INTRODUCTION

Facial paralysis occurs following damage to the facial nerve (Gilchrist, 2009). This damage results in the inability to produce facial expressions which are an integral part of nonverbal communication, a visual component of facial aesthetics and the primary mode of expressing emotion (Gilchrist, 2009). Facial paralysis also can result in impaired speech and eating difficulties (Broussard & Borazjani, 2008). Therefore, any paralysis of the face can negatively impact day-to-day activities and overall quality of life.

Fortunately, several surgical procedures are available and provide some movement to native or transferred muscles of the face. Current reanimation surgical techniques typically involve a transfer of gracilis muscle from the leg to the face; this muscle is innervated with portions of salient facial or trigeminal nerve branches from another part of the body, known as a nerve graft (Zuker, Goldberg, & Manktelow, 2000; May & Schaitkin, 2000, Mehtha, 2009; Harrison, 2005). The goal of this surgery is to provide spontaneous movement of the facial muscles, which may increase oro-motor competence and ultimately lead to an increased ability to smile. Whereas neuromuscular changes after the surgery may be physically apparent, the detection of the reorganization of associated motor and sensory neural pathways is not straightforward. A vast amount of research has been presented on central nervous system reorganization following peripheral nerve injuries (Taylor, Anastakis, & Davis, 2009; Yogarajah et al., 2010; Navarro, Vivo & Velero-Cabre , 2007), but no research has been conducted on

cortical changes following facial reanimation surgery. However, advances in neuroimaging technology, specifically DTI, may provide an additional measurement technique, capable of detecting neural changes following reanimation surgery.

Diffusion tensor imaging is a Magnetic Resonance Imaging (MRI) technique that provides a sensitive measure of tissue microstructure and integrity of white matter tracts *in vivo* by measuring the diffusion of water in neural tissue (Sexton, Mackay, & Ebmeier, 2009). Diffusion tensor imaging has previously been used to describe white matter tract integrity in a variety of patient groups including those with amyotrophic lateral sclerosis (ALS), progressive bulbar paralysis, and those who have undergone neuronal damage due to cerebral artery infarcts, hemorrhages, or temporal lobe resections, among others (Sheikh, 2010; Wang & Melhem, 2005; Ulag, Moore, Bojko, & Zimmerman, 1999; Yoharajah, et al., 2010). To date, DTI has not been used to characterize neural structures in people who have undergone facial reanimation surgery.

The purpose of this study was to determine whether or not DTI could detect differences in neural integrity between people who have undergone reanimation surgery secondary to facial paralysis and matched healthy controls. This study used DTI to identify and measure the integrity of white matter fibre tracts thought to be involved in initial facial paralysis along with neural reorganization as a result of facial reanimation. The prediction was that DTI would able to detect white matter structural differences in selected

tracts of both the right and left cerebral hemispheres between patients with facial reanimation surgery and a group of matched controls. It was reasoned that if DTI could detect group differences in this small-sample study, then future studies could be designed to determine if DTI is sensitive to post-operative indicators of central nervous system neuroplasticity. To date, no studies have examined the cortical white matter changes following facial reanimation surgery. Understanding the relationship between central and peripheral neuroplasticity in this population may be beneficial in guiding surgical options and post-surgical rehabilitation.

The current study was conducted on archival data from a small sample of individuals who had facial reanimation surgery secondary to facial paralysis. There were no pre-surgical behavioural or neuroimaging measurements taken in the current study population. However, this study's causal comparative design aided in the understanding of the interaction effects of facial paralysis and facial reanimation surgery on white matter organization.

The current document presents literature related to the clinical populations representing the participants in the current study, as well as an overview of neuronal pathways thought to be involved in the clinical sequelae represented the current sample. An overview of facial reanimation surgeries and principles of neuroplasticity also are presented. Diffusion tensor imaging and measurement techniques are presented in the context of the methods for analyzing the current dataset. The rationale for using water

diffusion tractography to select and measure specific white matter tracts also are presented.

CHAPTER II: LITERATURE REVIEW

Background literature from several broad domains is examined in this chapter for the purpose of providing a framework and foundation related to the current study's research question. In order to demonstrate its relevance and importance to the field, it was necessary to consider literature in the following categories: (a) common types of facial paralysis, (b) facial nerve anatomy and corticobulbar contribution to facial movement, (c) treatment options, and (d) mechanisms of neuroplasticity following neural perturbation. The use of structural imaging (diffusion-tensor imaging) as a possible tool for measuring post-surgical neural changes in this population is also discussed.

Facial Paralysis

Bell's Palsy

The most common facial neuropathy is Bell's Palsy, which accounts for up to 70% of cases (Adour, Byl, Hilsinger, Kahn, & Sheldon, 1978). It has an annual incidence of 20 per 100,000 with one in 60 people being affected once in their lifetime (Holland & Weiner, 2004). Bell's Palsy is a lower motor neuron or peripheral palsy which affects unilateral facial muscles, resulting in drooping of the corner of the mouth and the inability to close the eyelid.

The diagnosis of Bell's Palsy can usually be drawn from the clear clinical symptoms evident in these patients. The acute onset and rapid evolvement of unilateral facial weakness followed by maximal asymmetry of facial movement, develops within two days (Holland & Weiner, 2004).

Because of its sudden onset, up to 75% of patients believe they have had a stroke or have a brain tumour (Holland & Weiner, 2004). Due its lower motor neuron phenomenon, Bell's Palsy also results in insufficient eyelid closure, during which the eye tries to compensate by producing the "Bell's phenomenon" of an upward gaze during an eye-closure attempt. This involvement of the eye muscles is clinically distinguishable from central nervous system palsies (Benatar & Edlow, 2004). Additional symptoms include hyperacusis due to stapedius muscle paralysis, altered taste on the anterior two-thirds of the tongue and hindered salivation and lacrimation (Gilchrist, 2009; Holland & Weiner, 2004). In some cases, gustatory tearing ("crocodile tears") may develop as a result of synkineses; a phenomenon whereby the intended activation of one muscle results in the contraction of other muscles (Gilchrist, 2009).

In most cases of Bell's Palsy, only one side of the face is affected; however, rare cases (1 - 2%) of bilateral paresis or paralysis have been noted (Kovo, Sagi, Lampl, & Golan, 2009; Adour et al., 1978). During reproductive years, there is a higher prevalence of Bell's Palsy in pregnant vs. non-pregnant females (Hilsinger, Adour, & Doty, 1975).

Although the causes of Bell's Palsy are still unclear, etiologies may include viral infections, trauma, diabetes, local infections, tumour, immunological disorders, or drug abuse. Viral infections are thought to be the primary cause of Bell's Palsy. More specifically, latent herpes virus Type 1 and herpes zoster virus account for over 84% of acute facial palsy (Liu, Li,

Yuan, & Lin, 2009; Holland & Weiner, 2004). Researchers suggest that the neuronal mechanism associated with facial neuropathy is the inflammation and demyelination of the facial nerve (Edstrom, Hanner, Karlsson, Andersen, Rosenhall, & Vahine, 1987).

The time course of recovery for patients with facial palsy is dependent on the degree and type of pathophysiologic lesion (Gilchrist, 2009). Generally, Bell's Palsy has very good prognoses, with 85% of cases reaching full recovery within nine months of the event (Sullivan et al. , 2007). The treatment strategy is to prevent worsening of facial palsy and to accelerate recovery via the use of steroids or acyclovir (Monnell & Zachariah, 2009). If the paralysis does not improve, surgical options for improving the movement and appearance of the face are considered. Surgical procedures are highly individualized and may include eyelid surgery, suspension techniques, facial nerve grafts, eyebrow lift, and face lift, among others.

Moebius Syndrome

Moebius syndrome is a congenital disorder that affects facial muscles bilaterally. This condition affects about 2000 people worldwide and may not be diagnosed until late childhood or early adulthood (Broussard & Borazjani, 2008). Moebius syndrome is characterized mainly by the inability to produce facial expressions or move the eyes laterally as a consequence of impairment to facial nerve (VII) as well as the abducens nerve (VI) (Broussard & Borazjani, 2008). The first sign of Moebius syndrome is paralysis of the orbicularis oculi muscle located around the eye, which can be observed soon

after birth (Terzis & Noah, 2002). As these children develop, the obicularis oris muscle paralysis becomes more noticeable, resulting in a clinically distinguishable "open-mouth" deformity coupled with difficulty smiling (Terzis & Noah, 2002). In many cases, other cranial nerves also may be involved and as a result, additional physical differences may be present including limb deformities, chest wall abnormalities, and missing teeth (Rizos, Negron & Serman, 1998; Broussard & Borazjani, 2008).

Children with Moebius syndrome may have multiple health problems, (i.e., dental, ophthalmologic) as well as difficulty with speech and mastication. Because newborns with facial paralysis of this type are unable to breast or bottle feed, food may be delivered via a gastrostomy tube (Broussard & Borazjani, 2008). This early lack of normal feeding can result in insufficient weight gain, swallowing difficulties, drooling. These sequelae may be associated with other feeding problems later on in life (Broussard & Borazjani, 2008). Ophthalmologic problems include irritation, corneal dryness and corneal erosion because of incomplete eyelid closure and difficulty blinking (Broussard & Borazjani, 2008). Like many patients with facial paralysis, those with Moebius syndrome also have speech difficulties. Incomplete lip closure results in lack of bilabial sound production and speech is further reduced due to poor tongue action (Broussard & Borazjani, 2008). Children with Moebius syndrome may experience many negative social consequences as a result of facial and eye paralysis, as they appear to be staring inappropriately and have little peripheral vision, which also causes

them to appear clumsy. Because people with Moebius syndrome have mask-like expressions, they are often viewed as being rude, bored or emotionless (Fitzgerald, 2006). This perception can lead to social isolation, depression, anger and other negative feelings.

There is no exact known cause of Moebius syndrome, however two major neuronal explanations have been proposed: an environmental cause and a primary genetic cause (Verzijl, van der Zwaag, Lammens, ten Donkelaar, & Padberg, 2005). Either embryologic or environmental toxic factors result in interruption in the blood supply of the brainstem during early embryologic development (Verzijl, Valk, de Vries, & Padberg, 2005). Drug abuse, including the consumption of a self-induced abortion pill, misoprostol, may lead to Moebius syndrome (Briegal, 2006). As a consequence, these children may be born with variety of sequelae including, jaw, ear, foot and hand anomalies, facial paralysis, mouth and tongue abnormalities (Miller, Stromland, Ventura, Johansson, Bandim, & Gillberg, 2004). Other drugs such as benzodiazepines, used to treat sleep disorders or anxiety, as well as cocaine use also may disrupt blood flow to the brain stem during pregnancy, which then leads to characteristic cranial nerve anomalies (Briegal, 2006).

Moebius syndrome also may have possible chromosomal causes, most notably with autosomal dominant transmission in the family (Terzis & Noah, 2002). Rizos, Negron and Serman (1998) also reported an increase in occurrence of this condition in marriages between blood relatives. Overall,

the pathology of Moebius syndrome is unclear because most cases are sporadic (Verzijl, van der Zwaag et al., 2005). Originally, P. J. Mobius stated that the syndrome may be the result of lesions of the pons, involving the abducens nucleus or its environment (1888). Research also has shown that there is marked decrease in the number of neurons in the facial motor nucleus with corresponding small facial nerve remnants (ranging from 280-1680 as compared to 5030-8700 for controls) (Verzijl, Valk et al., 2005). Brainstem hypoplasia in cranial nerve nuclei, as well as other abnormalities in the posterior fossa also have been observed (Verzijl, Valk et al., 2005; Towfighi, Marks, Palmer, & Vannucci, 1979). It appears that Moebius syndrome is part of a complex congenital anomaly involving hypoplasia of the brainstem and signs of neuronal degeneration (Verzijl, van der Zwaag et al., 2005).

Many options are available for the treatment of Moebius syndrome symptoms. To help with the eye irritation and eyelid closure, drops (artificial tears) and small gold weights can be stitched onto the eyelid (Broussard & Borazjani, 2008). To increase self-esteem and confidence, the Zuker procedure, commonly known as "smile surgery", is an effective way of producing movement in the face and some ability to smile (Broussard & Borazjani, 2008; Zuker et al., 2000). In order to fully understand the implications of this dynamic surgical reconstruction, which applies to various forms of facial paralysis, including Bell's Palsy and Moebius syndrome, it is necessary to first become familiar with the pathway of the facial nerve.

Anatomy and Function of the Facial Nerve

The most commonly diagnosed cranial neuropathy is facial nerve (CN VII) neuropathy (Gilchrist, 2009). The facial nerve has many branches consisting of both sensory and motor pathways, that collectively control muscles of facial expression, contribute to aurical sensation, taste sensation on the anterior two thirds of the tongue, and promote secretions of the lacrimal, submandibular and sublingual glands (Gilchrist, 2009; Kosins, Hurvitz, Evans, & Wirth, 2007).

These functions begin with activation of neurons in the cerebral cortex, specifically efferent projections from the pre-central and post-central motor cortex, which are carried through fascicles of the corticobulbar tract (CBT). The upper face receives corticobulbar contribution from both ipsilateral and contralateral motor cortices, resulting in less detrimental effects from a lesion including spared forehead movement. In contrast, the lower face receives contribution from only the contralateral motor cortex. Lesions involving pathways to the lower facial muscles are therefore more severely affected, because their motor neurons depend on significant cortical innervation (Kosins et al., 2007). The location of the damage affects the type of accompanying paralysis. For example, an upper motor neuron lesion (above the level of decussation) results in weakness of the contralateral lower face with preservation of spontaneous movement and tone of the upper facial musculature (Kosins et al., 2007). If the lesion occurs below the

level of pyramidal decussation in the caudal medulla, then clinical signs will present ipsilateral to the site of damage (Bhatnagar, 2008).

Regardless of the type of lesion, this CBT follows a pathway that courses to the posterior limb of the internal capsule near the genu, then through the upper midbrain to the lower brain stem where it synapses to the facial nerve nucleus in the pons (Nelson, 1974; Bhatagnar, 2008). At the lower pons, anterior to the fourth ventricle, these fibres reach the contralateral motor nucleus. The corticobulbar projections to these cranial nerves cross the midline at multiple points, rather than at a single point, before synapsing on the cranial lower motor neurons (LMNs) of the facial nerve nucleus (Bhatagnar, 2008).

The course of the facial nerve and its central connections can be segmented into the meatal segment, labyrinthine segment, tympanic segment, mastoid segment, and extratemporal segment (Nelson, 1974). The facial nerve (CN VII) emerges from the brainstem at the level of the pons. It then enters the internal auditory meatus of the temporal bone, where the facial nerve divides into the labyrinthine, tympanic and mastoid segments (Kosins et al., 2007). At the end of meatus is the geniculate ganglion, where the facial nerve fibers separate from the vestibulocochlear nerve (CN VIII). The facial nerve then continues above the oval window and stapes as it approaches the mastoid segment (Bhathagnar, 2008; Kosins et al., 2007). The next three branches of the facial nerve to arise from the mastoid segment include the nerve to the stapedius muscle, the sensory auricular nerve and

the chorda tympani nerve. Finally, as a purely motor nerve, the facial nerve exits the skull base through the stylomastoid foramen and courses through the parotid gland to provide movement to the face (Kosins et al., 2007). Collectively, a rich network of nerve fibres is formed from these five branches, that target the muscles of the face. As facial paralysis arises from damage to the facial nerve, the most direct treatment option would be to repair the facial nerve, through surgical techniques such as facial reanimation surgery. Based on the significant contribution of the CBT in skilled and fine movements of facial muscles, it was hypothesized that differences (from matched controls) in this tract may be present before and following facial reanimation surgery.

Treatment of Facial Paralysis

Many treatment options are available for the paralysis of the face, including botulinum toxin injection, nerve decompression, neurorrhaphy, and nerve transfer procedures involving nerve grafts and neuromuscular transfers (Mehta, 2009; Burgess & Goode, 1994).

Static treatment options can be used to add support to the face, with focus on paralyzed forehead and eye muscles. In unilateral facial palsies, for example, an endoscopic eyebrow lift as well as botulinum toxin injection in the contralateral functional side of the face can add support and symmetry to the face (Harrison, 2005). Gold weights placed on upper eyelid may assist in eyelid closure and decrease chronic dryness of the eye (Harrison, 2005). Static suspension procedures also can be performed to lateralize the corner

of the mouth and lip-cheek crease (Snow, Wackym, & Ballenger 2009). These static procedures may be used to add support and symmetry to the face; however, they will not provide movement of the facial muscles (Harrison, 2005).

In cases where more dynamic facial movement results are desired, the paralyzed face can be rehabilitated using one of three surgical techniques: (1) direct repair of the facial nerve, (2) the use of nerve transfers and/or cross-facial nerve grafts, or (3) a nerve graft in combination with a neuromuscular transplant (Klebuc & Shenaq, 2004). For acute paralysis of the face that lasts less than three weeks in duration, the restoration of impulses from the facial nerve is the desired outcome, through techniques such as facial nerve decompression and neurorrhaphy (direct facial nerve repair) (Mehta, 2009; Burgess & Goode, 1994).

Over time, intact facial nerves may not fully recover due to original injury or to surgical trauma. In cases where the paralysis has lasted from three weeks to two years, nerve transfers and cross-facial nerve grafting using nerves from another part of the body are used. Many factors must be considered before planning and selecting a nerve donor site, including: (a) the functional deficit produced by sacrificing the donor nerve (i.e., speech or swallowing problems), (b) the need for nerve grafts, (c) nerve topography (diameter, axon count, fibre count), and (d) the donor nerve's ability to provide adequate power without inducing mass movement (Klebuc & Shenaq, 2004). Other variables such as the disease state, possible

development of additional cranial nerve deficits due to the selection and the presence of coexistent cranial nerve lesions, also must be considered (Klebuc & Shenaq, 2004).

The following donor nerves have been used for transfer to cranial nerve seven: (a) hypoglossal nerve (CN XII), (b) masseteric branch of the trigeminal nerve (CN V) (c) spinal accessory nerve (CN XI), and (d) facial nerve (CN VII) with cross-facial nerve graft (Klebuc & Shenaq, 2004; Mehta, 2009). The hypoglossal to facial crossover is a commonly used procedure. However, this method is no longer considered the "gold standard" due to its significant donor site morbidity, postoperative difficulties with eating and development of impaired speech in over twenty-five percent of patients (Klebuc & Shenaq, 2004; May & Schaitkin, 2000). Difficulties can be reduced by utilizing a jump graft technique, where symmetry is enhanced by strengthening the paretic side and weakening the overactive normal side (Klebuc & Shenaq, 2004). The motor branch of the trigeminal nerve (masseteric nerve) to the masseter muscle has commonly been used for reinnervating paralyzed mimetic muscles in the mid-face and for powering free-muscle flaps (Klebuc & Shenaq, 2004). Although the most commonly used procedure has been the hypoglossal-facial transfer (Mehta, 2009), the trigeminal cranial nerve transfer may be advantageous for several reasons. It has limited donor site morbidity due in part, to the nerve's oblique branching pattern including multiple smaller proximal branches. In addition, the duplication of activity provided by the masseter and temporalis muscles

working together in mastication; significantly reduce the donor deficit produced by harvesting the motor nerve branch of the masseter muscle (Klebuc and Shenaq, 2004). Another donor nerve is the spinal accessory nerve (XI). Whereas the spinal accessory nerve is not considered a first-line donor site for facial reanimation, it is best suited for situations when the motor branch to masseter and hypoglossal nerves are absent and/or pre-existing speech and swallowing problems are present (Klebuc & Shenaq, 2004). The phrenic nerve also may be used when more acceptable donor nerves are unavailable (Klebuc & Shenaq, 2004). A significant drawback of nerve transfers is the possible development of unwanted mass motion (i.e., facial spasms, shoulder movements) (Klebuc & Shenaq, 2004).

Procedures of facial reanimation using nerve grafts are now commonly used. If the contralateral facial nerve is intact and functioning, then a cross-facial nerve graft procedure can be used (Mehta, 2009). This two-stage procedure begins with a pre-auricular face-lift, with an incision on the functional side of the face, after which the branches of the facial nerve are identified using a nerve stimulator (Mehta, 2009). Nerve branches are carefully selected for sacrifice and a crossed facial nerve graft is used to extend the facial nerve branch across the upper lip with its distal end fixed to the tragus of the ear on the paralyzed side. The proximal end of the nerve graft is then coapted to the donor facial nerve branches (Mehta, 2009). The second stage of operation occurs 6-12 months later (Harrison, 2005; Mehta,

2009), when secondary nerve repairs are performed between selected facial nerve branches and the cross facial nerve grafts.

When facial paralysis has lasted for over two years, the native facial musculature may have atrophied and will require the use of alternative muscles for facial reanimation (Mehta, 2009). Muscle transfer techniques include regional muscle transfers and free muscle transfers. Regional muscle transfers, involving a temporalis muscle, are commonly utilized for patients who have normal trigeminal nerve use and muscle that has not atrophied. The correction of unilateral facial palsy in the lower face may be accomplished using a sling procedure using the transfer of the temporal muscle. The temporalis muscle, however, is powered by the fifth cranial nerve and therefore does not respond to motor commands for expressing emotion; however conversion from mastication to emotion has occurred when the patient is under the age of seven years and had long periods of retraining. In general, a good smile can be achieved on command but not to uninhibited emotion (Harrison, 2005). Other regional muscle transfers include the masseter muscle for smile reanimation and the digastric muscle transfer for marginal mandibular nerve injuries. The masseteric muscle transfer is considered inferior to the temporalis muscle because of its more lateral vector of pull (Mehta, 2009). A free muscle transfer can be used if the native facial musculature has been rejected (Mehta, 2009). Muscles used in this procedure include the gracilis, pectoralis minor, serratus anterior, latissimus dorsi and others. The gracilis muscle is the most commonly used,

as it is easily harvested and provides excellent neurovascular support (Mehta, 2009).

It is uncommon to see any movement in the muscle graft for six months because it takes this time for the axons to traverse the nerve repair and enter the motor end plates of the muscle itself (Harrison, 2005). Children animate within three to four months because of the more rapid migration of axons. (Harrison, 2005). Prior to the surgery, the careful selection of the donor muscle must be decided upon by the surgeon and is dependent on the desired functional outcomes for the patient. Muscle selection is as integral to the success of the surgery as is the selection of donor nerve.

Many advances in the facial reanimation of patients with unilateral and bilateral facial palsies have been made in the past 30 years. The advances in the ability to transfer axons over significant distances via nerve grafts, together with the successful vascularisation of transferred muscles via microvascular surgery, have significantly improved outcomes of facial reanimation surgery (Harrison, 2005). Rehabilitative therapy and motor re-education for an extensive period of time in combination with surgical intervention has been shown to result in impressive motor outcomes (Klebuc & Shenaq, 2004; Hadlock, Greenfield, Wernick-Robinson & Cheney, 2006). These changes in movement may be explained by possible mechanisms of neuroplasticity following facial reanimation surgery.

Mechanisms of Neuroplasticity

Mechanisms of neural plasticity (i.e., production of new neural connections) can occur when the peripheral nervous system is disturbed. Adaptations can occur in response to new learning in the intact brain or relearning in the damaged brain (Kleim & Jones, 2008). The following sections will review the intrinsic mechanisms of neuroplasticity, as well as external factors that contribute to neural changes including activity-dependent neuroplasticity.

Intrinsic mechanisms of neuroplasticity

Intrinsic mechanisms contribute to neural changes, including (a) neurochemical factors, (b) alterations of excitatory and inhibitory synaptic connections, (c) sprouting of new connections, and (d) reorganization of sensory and motor central maps (Navarro, 2009). Following a nerve injury, for example, the reinnervation of denervated targets occur through the regenerating of injured axons, the collateral branching of undamaged axons, and the remodeling of nervous system circuitry related to the lost functions (Navarro, 2009). Changes in plasticity that develop after peripheral nerve injuries occur at the molecular, cellular, and circuit levels and progress through changes at the spinal cord, brainstem nuclei, thalamus, and brain cortex as well as neurons that interconnect them (Navarro, 2009).

Following a facial nerve injury such as Bell's Palsy, the neuroplastic potential of the brain facilitates development of new neurocranial pathways among the first and seventh cranial nerves, partly due to the proximity of

cranial nuclei within the pons, such as the facial and trigeminal nuclei (Klebuc & Shenaq, 2004). Studies using electroneurography , which measure conduction velocities associated with the electrical stimulation of peripheral nerves have provided evidence suggesting that the damaged facial nerve undergoes ipsilateral neurotization, or nerve regeneration, via the trigeminal nerve. Spontaneous recovery after facial nerve damage may be due to the recruitment of aberrant pathways between the fifth and seventh nerves (Klebuc & Shenaq, 2004).

Other factors such as the severity of the injury, the distance of the lesion to the cell body, the type of neuron and the patient's age may affect how the damaged brain relearns behaviours and how the intact brain learns novel behaviours and encodes experiences (Navarro, 2009; Plowman & Kleim, 2009). Animal studies have shown age differences in the motor representations and cortical organization following facial nerve severing in newborn rats as compared to adult rats (Franchi & Veronesi, 2004).

The cutting or severing of an axon results in a neuronal shift from a transmitter to a regenerative phenotype, which in turn, activates molecular pathways and promotes axonal regeneration and neuronal survival (Navarro, 2009). It is important to note, however, that reinnervation of target organs does not always lead to adequate recovery of motor and sensory functions, as it depends on the selectivity of axon-target reconnection. Misdirection of regenerated axons may occur, which leads to inappropriate outcomes such as synkinesis, or the involuntary movement of part of the face during voluntary

movement of another part of the face (Choi, Raisman & Phil, 2002). Animal studies by Lu, Wang, Qui, Liu and Wu (2002) also found that misdirected regeneration occurs among motoneurons innervating different branches of the facial nerve following facial nerve axotomy in rats. Current research is focused on developing therapeutic strategies that enhance axonal regeneration, promote selective target reinnervation, and consequently amplify positive changes and improve recovery related to lost movement (Navarro, 2009). The next section will discuss the extrinsic features associated with neuroplasticity. Specifically, activity-dependent neuroplasticity and its role in dynamic outcomes will be described.

Extrinsic mechanisms of neuroplasticity

Many extrinsic mechanisms are believed to be responsible for central nervous system changes in activity-dependent neuroplasticity, including electrical stimulation, motor learning and relearning, and rehabilitation (Plowman & Kleim, 2009). One of the key neural strategies that have been found to improve functional movement is retraining (Plowman & Kleim, 2009).

Kleim and Jones (2008) discussed the mechanisms by which the damaged brain relearns lost behaviour in response rehabilitation. The brain has the capability to restore and compensate for functions that have already been compromised or lost (as in the case of facial paralysis) (Kleim & Jones, 2008). This can be described by studies examining the effects of constraint-induced therapy. When forced use of affected limbs and constraint of

unaffected limbs are applied, changes in brain organization occur (Taub & Uwasatte, 2005). Norton and Gorassini (2006) also have found that increasing the use of the spared CST through intensive, repetitive treadmill training contributes to locomotor recovery, i.e., increases in corticospinal drive to muscles of the leg during walking, in patients with incomplete spinal cord injury.

Many neural connections may be driven to reorganize by the production of compensatory behaviour changes, even in the absence of rehabilitation (Kleim & Jones, 2008). When the brain loses some of its neural connections due to lack of use, as may be the case in chronic facial paralysis, it undergoes vast changes of degeneration, commonly referred to as the "Use It or Lose It" principle (Kleim & Jones, 2008). As a result, new neural connections which produce compensatory behaviours arise (Kleim & Jones, 2008). This has important implications when considering the varying degree of muscular movement seen following facial reanimation surgery, as pre-operative compensatory behaviours may have developed. Studies have shown that motor re-learning results in more functional movement than compared to no motor re-learning (Plowman & Kleim, 2009; Klebuc & Shenaq, 2004). After dynamic methods of facial reanimation surgery are performed, motor re-learning must take place in order for the free muscle transfer to function as facial musculature. Animal studies with adult rats have been used to demonstrate long-lasting changes at a higher motor cortical level following the repair of the facial nerve (Franchi, 200). It was

suggested that central supranuclear mechanisms may be involved in the disorder of facial movements observed after reinnervation of the facial nerve; however, the researcher noted that facial motor control is affected more by facial neuron modifications than by limits in supranuclear motor out system plasticity (Franchi, 2000).

By studying activity-dependent plasticity, new insight can be gained into the neural mechanisms underlying improvements in functional movement after injury or repair. In this study, the presence of any changes detected by DTI may have been a combination of the original infarct as well as a combination of intrinsic and extrinsic mechanisms of neuroplasticity.

Diffusion Tensor Imaging

Whereas muscular changes after the surgery may be physically apparent, the detection of neural reorganization is not as straightforward. Based on previous studies that have successfully used DTI to describe neural integrity, it is possible that this technique may allow further descriptions of structural features of white matter tracts in the current study population. Diffusion tensor imaging is a non-invasive method for indirectly examining white matter fibre tract integrity by measuring the diffusion of water in neural tissue and allows the three-dimensional study of white matter fibre bundles at the macroscopic level (Sexton et al., 2009; Mori, Wakana, Nagae-Poetscher, & Van Zijl, 2005). Diffusion refers to the molecular movement of bulk water (Wang & Melhem, 2005). These water molecules are usually in random motion, called isotropic diffusion. Within white matter, a number of

structures could restrict molecular motion and give it a particular direction, including axonal membranes, and to a lesser extent, myelination and neurofibrils. This movement is known as anisotropic diffusion (Beaulieu, 2002).

Diffusivity can be described mathematically by a tensor, characterized by its three eigenvectors and the corresponding eigenvalues (λ). If diffusion is isotropic (λ1=λ2=λ3), diffusion in all three main axes is equal and the shape is a sphere. If diffusion is anisotropic (λ1>λ2>λ3), the largest eigenvalue reflects the direction of maximum diffusivity, which reflects the orientation of fibre tracts, and the shape is an ellipsoid (Sexton et al., 2009; Wang & Melhem, 2005).

Research in DTI makes use of some equations to provide measurable data on the integrity of the brain tissue and the degree of alignment of cellular structures. The apparent diffusion coefficient, also known as MD, gives a measure of the directionally averaged magnitude of diffusion. This is calculated as:

$$MD = \frac{(\lambda_1 + \lambda_2 + \lambda_3)}{3}$$

This value gives a measure of the overall displacement of molecules in the white matter tracts. Another important value derived from DTI is FA, the degree of diffusion anisotropy or the extent of alignment between these cell structures (Wang & Melhem, 2005). Oishi, Faria, van Zijl and Mori (2011) show this calculation as:

$$FA = \sqrt{(½)}\ \frac{\sqrt{[(\lambda_1 - \lambda_2)^2 + (\lambda_2 - \lambda_3)^2 + (\lambda_3 - \lambda_1)^2]}}{\sqrt{(\lambda_1^2 + \lambda_2^2 + \lambda_3^2)}}$$

FA and MD values are normally inversely related (Wozniak, Mueller, Chang, Muetzel, Caros & Lim, 2006). A DTI-based colour map allows mapping of the white matter tracts in the brain. Orientation is coded using red, green and blue (RGB) colour channels, whereas the degree of anisotropy is coded by the brightness of the assigned colour (Wang & Melhem, 2005).

Previous literature has shown many successful examples of the use of DTI as a sensitive tool for the detection of group differences in a variety of populations. For example, the examination of corticospinal degeneration in patients with ALS and Primary Lateral Sclerosis (PLS) was done using DTI as an upper-neuron motor marker (Wang & Melhem, 2005). The segmentation of the corticospinal tract (CST) at every cross-sectional slice was performed, with focus on these values along the CST: posterior limb of internal capsule, cerebral peduncle, corona radiata, and the pons and pyramids. Analysis revealed that diffusion anisotropy was high in the internal capsule, as it contained very coherent and tightly packed CST fibres. In the pons and medulla, CST fibres are less coherent due to the presence of transverse pontine fibres and nuclei of the cranial nerves. This study concluded that the posterior limb of the internal capsule was where CST degeneration occurred in ALS patients. This example demonstrates the potential use of DTI to assist in diagnosis and the extent to which disease progression can affect fibre damage and neuronal degeneration (Wang & Melhem, 2005). The DTI

method also has been used to examine the extent of Wallerian degeneration in patients with neuronal damage (cerebral artery infarcts, haemorrhages), as well as case studies on patients with progressive bulbar paralysis (Ulag et al., 1999). These researchers concluded that DTI is useful for detecting differences between diffusion of water molecules in normal and pathologic tissue, and may serve as a feasible technique to quantify neural integrity when there is known or suspected damage to white matter tracts (Ulag et al., 1999).

Another study examined cortical changes using DTI, following peripheral nerve damage (Taylor et al., 2009). Specifically this work used DTI to characterize white matter tract integrity of patients who had undergone upper limb peripheral nerve transection and surgical repair. In this study, a region-of-interest-based approach revealed reduced FA in white matter adjacent to the right anterior and posterior insula in these patients. Some regions of interest, such as the left thalamus and left insula did not show group differences (Taylor et al., 2009). Taken together, these studies indicate the potential for DTI to detect white matter tract differences between various clinical groups and healthy controls.

Current research has linked cognitive abilities to white matter integrity, with the use of DTI in a variety of populations (Lebel, Rasmussen, Wyper, Andrew & Beaulieu, 2010; Lebel & Beaulieu, 2009). For example, in children with fetal alcohol spectrum disorder, it was found that correlations existed in mathematic scores and FA in white matter of the left parietal lobe,

left cerebellum, and bilateral brainstem (Lebel et al., 2010). Being able to correlate brain wiring connectivity with function is an important step forward in research. This knowledge may help determine what factors contribute to the variety of dynamic outcomes following nerve injuries.

Based on current studies that have compared the diffusion anisotropy with known normal values or with unaffected, normal portions of the same brain, it appears possible to assess the degree to which tissues have maintained or lost their normal degree of organization. Whether DTI is sensitive enough to detect differences in white matter of facial reanimation and the normal population is unknown, thus this research question was used to guide the current study.

Primary Research Question

The primary research question asked in the current study was: Is DTI sensitive to differences in white matter structural organization (FA, MD) in selected tracts of the right and left hemispheres between patients with facial reanimation surgery and their matched controls?

CHAPTER III: METHODOLOGY

Participants

The current study was conducted on archival data from a sample of eight participants. The four treatment participants were selected from a convenience sample of patients who previously had received facial reanimation surgery at the University of Alberta Hospital, and were matched by gender and age with healthy control participants. Table 1 shows the salient demographic information for the patient group.

Table 1. *Participant Information*

Participant	Sex	DOB	Paralysis	Type of Surgery	Date Surgeries Were Performed	Date Images Were Taken	Age at Time of Study
S1	F	Aug/10/1959	Right-sided Bell's Palsy	Cross-facial nerve graft with gracilis muscle transfer	1st surgery: Dec 2/05 2nd surgery: May 5/06	Jan/24/2007	47
S5	M	Mar/23.1996	Right-sided paralysis due to trauma	Cross-facial nerve graft with gracilis muscle transfer	1st surgery: Mar 23/05 2nd surgery: Oct 19/05	Mar/21/2007	11
S4	M	Apr/29/1987	Moebius syndrome	Gracilis muscle transfer with unilteral masseteric nerve innervation	1st surgery: May 15/07 2nd surgery: Hadn't occurred at time of study	Mar/3/2006	19
S7	M	Sept/17/1992	Moebius syndrome	Gracilis muscle transfer with bilateral masseteric nerve innervation	1st surgery: July 9/03 2nd surgery: Apr 8/04	Jul/18/2007	14

Participants were between the ages of 11 years and 47 years old at the time of the initial study and presented with facial palsy due to Bell's Palsy, Moebius syndrome, or trauma to the facial nerve. They received facial reanimation using specific muscle transfers and nerve grafts based on their distinctive paralysis. Measurements were taken from the structural organization of white matter tracts in this population, as well as a group of control participants (matched for age and sex; ages 11 to 47 years) who had no history of facial paralysis or other abnormalities to head and or neck structures. All study procedures were approved by the University of Alberta Health Research Ethics Board.

Data Analysis

Diffusion tensor imaging was used to compare the orientation and integrity of each patient's white matter tracts with that of the matched control. Analysis was performed on archival data of scans that were performed on a 1.5-T MRI scanner (Siemens Sonata, Erlangen, Germany) in the University of Alberta Hospital's NMR Research Centre. For each participant, 40, 3-mm thick contiguous axial slices were captured, with a diffusion tensor acquired for each slice with six sets and the diffusion weighting sample (b-value) set to 1000.

Images were processed on a personal computing system in the Edmonton Oilers Community Foundation Children's Speech Research Laboratory at the University of Alberta, equipped with DTI-Studio Version 2.4 software (Baltimore, MD). This program generates FA, MD, vector maps,

and color-coded maps. Analysis began with the "DTI Mapping" function, which processed all slices using a gradient system. The gradient table was set for 6 directions; 6-orientation DTI with 6 diffusion weighted images (DWI) and an individual set without diffusion weighting (b0). Each DWI has a resolution of 256 x 256 x 40. In order to minimize the detection of fibres that are not part of the tracts of interest, threshold parameters were set within the DTI Studio software. Background noise threshold was set to 30, with an automatic outlier rejection at 2.3. The "fiber tracking" operation was used to analyze the white matter tracts for each region of interest (ROI). The FA threshold was set at .25, and tracking at 70 degrees in order to exclude unwanted fibres from the analysis.

Selection of White Matter Tracts

Each ROI was selected based upon predicted changes in white matter integrity in brain regions associated with facial movements. The primary ROI in the current study was the CBT, because of its role in driving muscles of the face for non-speech and speech-related facial movements. Unfortunately, the proposed method of study using DTI prohibited analysis of this tract in isolation, due to a number of crossing fibres from other smaller or larger tracts. When a smaller white matter tract crosses over a larger white matter tract, the software will inherently measure the larger tract which may not be associated with the intended ROI. (Holodny, Watts, Korneinko, Pronin, Zhukovskiy, Gor, et al., 2005). Fortunately, previous studies have successfully selected several tracts containing corticobulbar fibres and

reliably assessed them using DTI (Holodny et al., 2005, Wang & Melhem, 2005; Ulag et al., 1999; Johansen-Berg & Behrens, 2009), thus it was hypothesized that changes following facial reanimation may be apparent in these particular sections of tracts due the predominant representation of CBTs in those segments.

Due to the limited number of participants available for this research, only a minimum number of tracts were assessed. To limit the number of tracts measured, only five primary white matter tracts were targeted. The following white matter tracts were analyzed based on their containment of corticobulbar fibres, robustness, and reliability of selection in DTI images: (1) the posterior limb of the internal capsule, (2) the corona radiata, (3) the cerebral peduncle, (4) the sagittal stratum and (5) the cingulum (Fan, Yu, Quan, Sun, & Guo, 2005; Nagae et al., 2007; Mori et al., 2005). The posterior limb of the internal capsule (specifically the dorsal third quarter) has been shown to contain many corticobulbar fibres and has been reliably assessed in a number of studies (Wang & Melhem, 2005; Ulag et al., 1999; Johansen-Berg & Behrens, 2009). The corona radiata and sagittal stratum are not related to a single specific tract, however they both contain parts of the CBT and have been reliability assessed in previous studies (Mori et al., 2005; Peterson et al., 2011). The cerebral peduncle was chosen as a tract of interest, because it also contains CBT fibers and some researchers have noted that the entire cerebral peduncle is a reliable ROI for tracing the pyramidal tract (Mori, et al., 2005; Johansen-Berg & Behrens, 2009). Also, supranuclear lesions, such as in

the cerebral peduncle, may cause dissociation of emotional and voluntary facial movements and some degree of paralysis of the arm and leg (Victor & Ropper, 2001). The cingulum was selected, because it is a major association tract that is part of the limbic system and subsequently thought to be involved in the expression of emotions (Tate & Tollefson, 2006). These primary tracts of interest were selected based on expected differences in white matter integrity either due to clinical aetiology, following facial reanimation surgery, or both.

Another set of four tracts were selected in cortical association areas, not directly related to the facial reanimation. These four secondary tracts also were examined for exploratory purposes only. These tracts included: (1) the uncinate fasciculus, (2) the corpus callosum, (3) the medial lemniscal system and (4) the pontine region. The uncinate fasciculus connects parts of the limbic system in the temporal lobe to regions of the frontal lobe (Taylor, MacFall, Gerig, & Krishnan, 2007). Due to its involvement of the limbic system, the uncinate fasciculus may play a role in facial expression. The corpus callosum connects the right and left hemisphere, thus it may be a cortical region that shows group differences in cortical connectivity (Mori et al., 2005). The medial lemniscal system is easily identified on multidirectional DTI and is involved in touch, vibration and proprioception. The trigeminal lemniscus conveys tactile, pain and temperature impulses from the skin of the face as well as proprioceptive information from the facial and masticatory muscles (Johansen-Berg & Behrens, 2009). Finally, pontine

fibres were also of interest because they contain nuclei that may be involved with eye movement, facial expression and facial sensation (Saladin, 2007).

Predictions

The following predictions were used to drive the hypotheses of the current study:

1. The facial reanimation group will have lower FA and higher MD when compared to the control group.
2. There will be lower FA and higher MD corresponding to the areas of suspected damage (containing corticobulbar fibres: posterior limb of the internal capsule, cerebral peduncle, corona radiata) in the facial reanimation group when compared to the control group.
3. There will be hemisphere asymmetry in the control group, and hemisphere symmetry in the facial reanimation group, in terms of FA and MD.

Measurement Protocol

The selection of both primary and secondary tracts using DTI Studio were acquired using a ROI method that applies existing anatomic knowledge of tract location and manually defined ROIs (Oishi, Faria, van Zijl, & Mori, 2011). Each tract was examined through different orientations: axial, coronal or sagittal. The orientation that gave the best representation of the tract was chosen to produce the tract isolation. In order to ensure consistency of tractography in all images between the participants, a protocol was developed and followed for each individual tract. An ROI was first selected using the "cut" option. Five to ten slices of the tract were cut, depending on

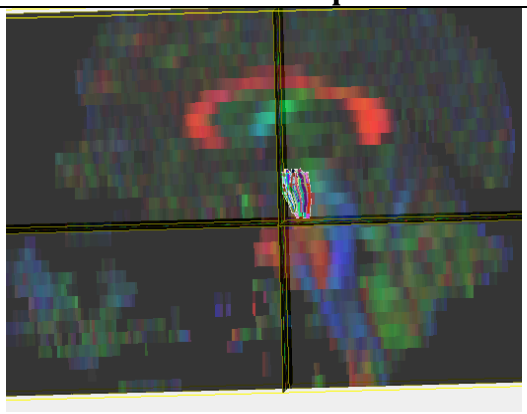
the orientation and robustness of the tract. The "cut" option was again selected to remove reconstruction results outside the two ROIs where only the trajectories between the ROIs were recorded and outside regions were erased. This created a five to ten slice isolated piece of the tract in a particular orientation. When possible, this piece of the tract was further modified using the ROI option "not". If the piece was over 10 slices in another orientation, the "not" option was then used to further remove a group of fibres outside the desired isolated piece of the tract. Once the centre of the tract had been located, the plane view button was selected to move the plane five slices in one direction. From here the "not" option was selected. Once again, the line was moved over the centre, then over five slices to the other side and "not" was selected again. This gave a 5x10 slice isolated piece of the tract, so that relatively consistent portions of the tracts could be examined for each participant and reliable measures could be attained and compared. This type of protocol was successfully used on four brains and matched controls in a previous study (Nickerson, Lebel, Beaulieu & Boliek; in preparation). Table 2 shows the specific sample size for each tract, and the percent of the tract measured. Table 3 presents the protocol used for the selection of each tract and a visual exemplar for each portion of the primary tracts selected.

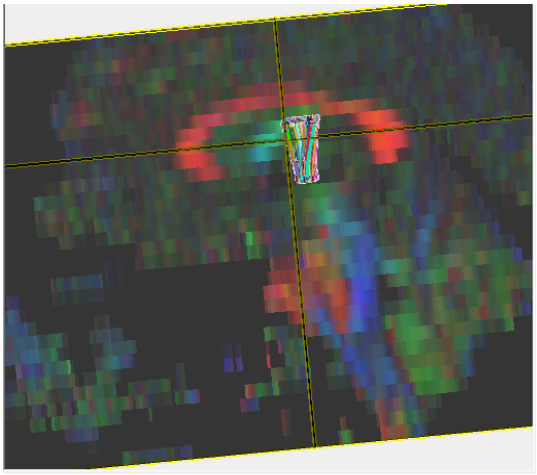
Table 2. *Sample size for primary tracts*

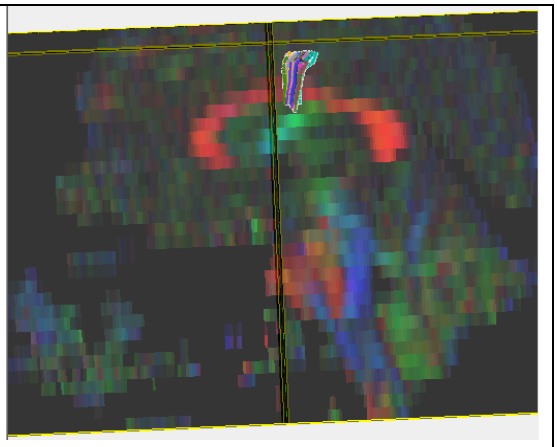
Tract	Sample Size	% of Tract
Cingulum (hippocampal) (CG)	5 slices sagittally	21% of hippocampal region of CG; 8% of

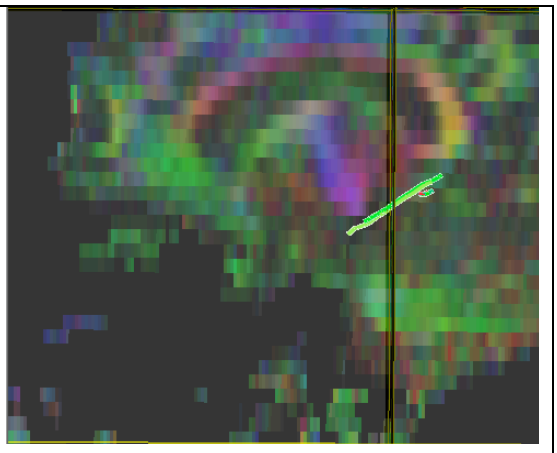
		whole CG
Cerebral Peduncle (CP)	5 x 10 axial x coronal	65% of entire CP
Posterior Limb of Internal Capsule (PLIC)	5 x 10 axial x coronal	50% of entire PLIC
Corona Radiata (CR)	5 x 10 axial x coronal	10% of entire CR
Sagittal Stratum (SS)	5 slices sagittally	22% of the entire SS

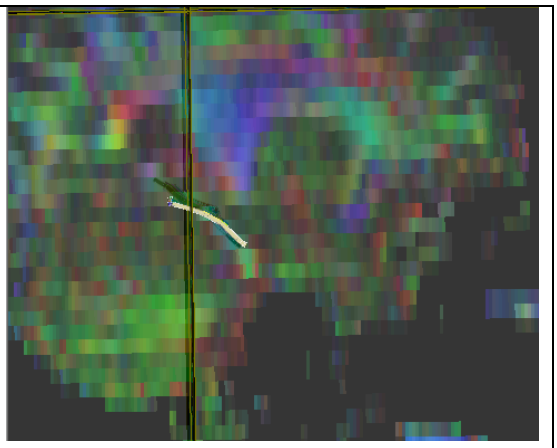
Table 3. *Primary tract selection and visual exemplar of the tracts identified*

Tract	Tract Selection	Visual Exemplar
Cerebral Peduncle (CP) *p.57-69 (Oishi et al., 2011)*	• Under *Image tab* move axial plane until image looks similar to p.57 (around axial 11-18) • Under *Fiber tab* use POLY shape with CUT operation to select purple and blue area in axial plane • Move plane 5 slices up using *Image tab*, you should see an image similar to p.69 • Under *Fiber tab* press CUT again and select appropriate blue-purple area • Use NOT operation with RECTANGLE shape to remove extra tracts outside of isolated piece (move coronal slice over area you want to remove) and lower red part on sagittal	 To create a 5x10 isolated piece: Move plane in the middle of current piece. Move 5 slices one way – select area in coronal plane and press NOT to remove, move 5 slices in the other direction if needed and press NOT to remove again. Count to ensure that it is 10 slices coronally by 5 slices axially.

	plane End result: 5 x 10 isolated piece of CP (axial x coronal)	
Posterior Limb of Internal Capsule (PLIC) *p. 71-87 (Oishi et al., 2011)*	• Under *Image tab* move axial plane until image looks similar to p.71 (should be following last cut of CP slice in axial) • Under *Fiber tab* use POLY shape with CUT operation to select purple and blue area in axial plane • Move plane 5 slices up using *Image tab*, you should see an image similar to p.87 • Under *Fiber tab* press CUT again and select appropriate blue-purple area • Use NOT operation with RECTANGLE shape to remove extra tracts outside of isolated piece (lateral projections on sagittal plane) End result: 5 x 10 isolated piece of CP (axial x coronal)	 To create 5x10 isolated piece: select dorsal third quarter of fan if possible (i.e. if you divided it up into 4 parts go from the middle to dorsal side)
(Superior) Corona Radiata (CR) *p.89-105 (Oishi et*	• Under *Image tab* move axial plane until image looks similar to p.89 (should be following last cut	

al., 2011)	of PLIC slice in axial) • Under *Fiber tab* use POLY shape with CUT operation to select large blue area starting from the top beside SFO green area down to bottom of blue (avoiding PLIC) in axial plane • Move plane 5 slices up using *Image tab*, you should see an image similar to p.105 • Under *Fiber tab* press CUT again and select appropriate blue and purple/red area starting again beside green area (CG) • Use NOT operation with RECTANGLE shape to remove extra tracts outside of isolated piece (corona radiata can be very wide); Ex: green area on sagittal slice End result: 5 x 10 isolated piece of CR (axial x coronal)	 To create a 5x10 isolated piece: Move plane in the middle of current piece. Move 5 slices one way – select area in coronal plane and press NOT to remove, move 5 slices in the other direction if needed and press NOT to remove again. Count to ensure that it is 10 slices coronally by 5 slices axially
Cingulum (CG) *p.227-237 (Oishi et al., 2011)*	• Under *Image tab* move sagittal plane until image looks similar to 'cgh' on p.235 (as	

	soon as you see a diagonal blue/green line coming out of the CC area, stop); usually around sagittal slice 150 for RIGHT and 108 for LEFT • Under *Fiber tab* use poly shape with CUT operation to select diagonal green/blue area • Move plane 5 slices up for RIGHT/down for LEFT using *Image tab*, while following the blue/green area and you should see an image similar to p.227 • Under *Fiber tab* press CUT again and select poly shape to select appropriate diagonal green area End result: 5 sagittal slice cingulum in hippocampal area	
Sagittal Stratum (SS) *p.207-219 (Oishi et al., 2011)*	• Under *Image tab* move sagittal plane until image looks similar to 'ss' on p.207 around sagittal 170 for right, 80 for left • Under *Fiber tab* use POLY shape	

	with CUT operation to select lower green area • Move plane 5 slices down for RIGHT and up for LEFT using *Image tab* and you should see an image similar to p.219 • Under *Fiber tab* press CUT again and select large lower green area under hook End result: 5 sagittal slice isolated piece of sagittal stratum	

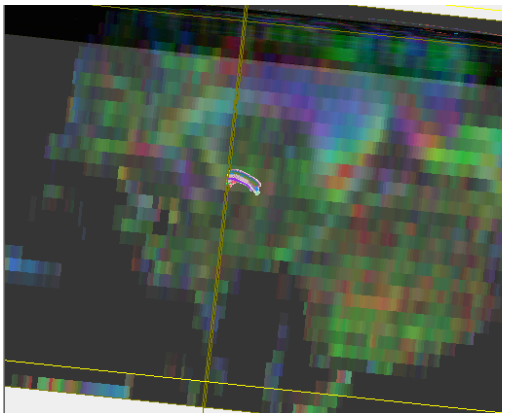
The same protocol was used to isolate each of the secondary tracts as well. The following tables show the sample size and protocol for each tract. Table 4 shows the sample size and the percent of each tract measured. Table 5 presents the measurement protocol used and a visual display of an exemplar for the portion of each tract measured.

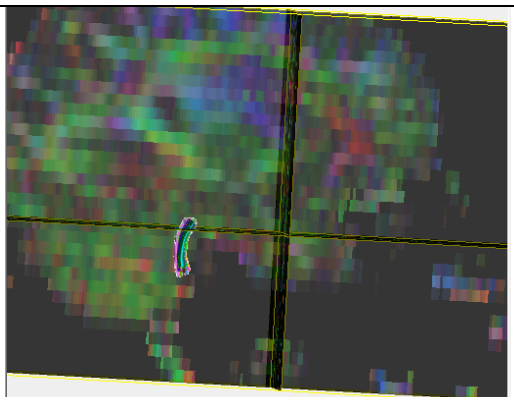
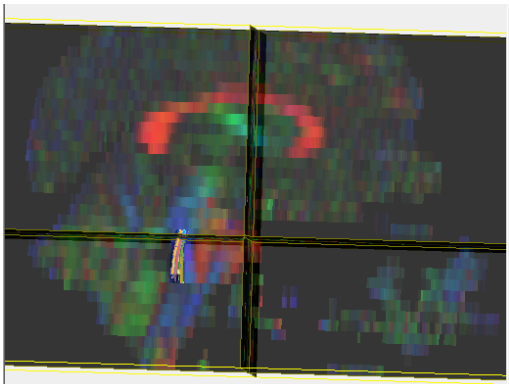
Table 4. *Sample size for secondary tracts.*

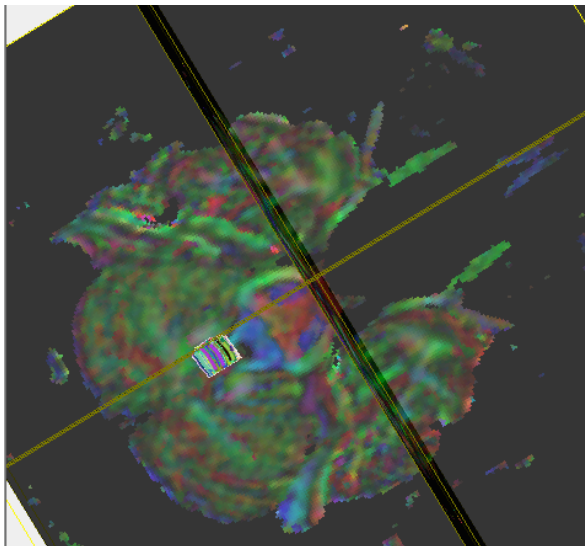
Tract	Sample Size	% of Tract
Uncinate Fasiculus (UNC)	10 slices coronally (naturally approximately 5 sagittally)	14% of entire UNC
Medial Lemniscus (ML)	5 slices axially (naturally approximally 10 slices sagittally, 5 slices coronally)	36% of entire ML
Pontine Region (P)	5 slices axially (naturally approximately 5 slices coronally, less than 10 slices sagittally)	> 90% of entire P
Corpus Callosum (CC)	3 x 10	60% of splenium

(splenium)	Axial x coronal	region of CC 9% of whole CC

Table 5 . *Secondary tract selection and visual exemplar of the tracts identified.*

Secondary Tracts		
Tract	**Tract Selection**	**Visual Exemplar**
Uncinate Fasiculus (UNC) *p.141-145 (Oishi et al., 2011)*	• Under *Image tab* move coronal plane until image looks similar to 'unc' on p.145 • Under Fiber tab use POLY shape with CUT operation to select cut select bluish area on left side below green dot (ifo), • go down by 10 following with eyes using Image tab and you should see an image similar to p.141 • Under *Fiber tab* press CUT again and select dark area under green ifo End result: 10 slice coronal isolated piece of superior uncinate fasciculus	 *naturally 5 slices sagitally. Did a 10 slice coronal isolated piece because of the curvature of the UNC; 5 slices weren't adequate.
Medial Lemniscus (ML)	• Under *Image tab* move axial plane until	

p. 41-51 (Oishi et al., 2011)	image looks similar to 'ml' on p.41 • Under Fiber tab use POLY shape with CUT operation to select blue/purple area under red pct • Go up by 5 following with eyes using Image tab and you should see an image similar to p.51 • Under *Fiber tab* press CUT again and select POLy top round part of blue ML under red pct End result: 5 axial slice isolated piece of medial lemniscus	 *naturally 5 slices coronally *naturally ~ 10 slices sagittally - just leave it as is, nice bulky area
Pontine Region (P) *p.41-57 (Oishi et al., 2011)*	• Under *Image tab* move axial plane until image looks similar to 'pon' on p.41 • Under Fiber tab use POLY shape with CUT operation to select dark blue area under ML, whole round-looking thing • Go up by 5	

	following with eyes using Image tab and you should see an image similar to p.57 • Under *Fiber tab* press CUT again and select POLY top round blue area End result: 5 axial slice of pontine region	
Corpus Callosum (CC) *(Splenium) p.245-251 (Oishi et al., 2011)*	• Under *Image tab* move sagittal plane until image looks similar to 'scc' on p.245 • Place axial plane line in sagittal view so that it's it right at the beginning of the scc (bottom part of cc in sagittal view), move axial plane UP by 3. • Under Fiber tab use RECTANGLE shape with CUT operation to select splenium end of CC by starting right below axial plane line down to bottom of scc	 *this gave me the most consistent view, without including the cingulate gyrus. Nice bulky piece. Axial by sagittal piece

	• Go up sagitally by 10 following with eyes using Image tab and you should see an image similar to p.251 • Under *Fiber tab* press CUT again and select RECTANGLE orange area below axial plane line one again End result: 3 x 10 isolated piece of splenium of corpus callosum	

From each tract, two measures of DTI were taken: FA and MD. These were calculated based on the following statistical output from DTI Studio: Eigen value-0, Eigen value-1, Eigen value-2, and Anisotropy-FA. These measurements served as the dependent variables for the current study. Mean diffusivity is represented by the average of the three Eigen values listed above. White matter fibre tracts are composed of high FA and low MD values, indicating a high degree of orientation and integrity.

Statistical Analysis

The statistical methods chosen for this study were based on a hypothesis-driven approach. Based on previous research, certain outcomes were chosen *a-priori.* This study used t-tests, one-way analysis of variance (ANOVA) and their nonparametric equivalents when appropriate, to compare means (or medians)

between and within groups. It was hypothesized that facial reanimation surgery may cause changes in white matter structural organization; however no cause-effect hypothesis could be made due to the lack of control over the manipulation of independent variables. The independent variables examined in the hypotheses of the study were 1) Group of participants (facial reanimation group, control group); 2) Hemisphere (right, left) and 3) Group by Tract (posterior limb of the internal capsule, cerebral peduncle, corona radiate, sagittal stratum, cingulum). Differences between tracts were not examined. A total of 10 comparisons were made for each dependent variable. A Bonferroni correction was not used for multiple comparisons. This less conservative statistical approach was employed because of the exploratory nature of this study and the desire not to overlook any potentially important neural pathways associated with this particular clinical group. Moreover, visual and statistical trends observed in these data will inform future studies about potential cortical tracts and DTI variables that may be useful in determining underlying neural mechanisms of change in this population.

CHAPTER IV: RESULTS

Reliability

In the current study, each measurement was repeated three or four times for each tract in each participant and used to assess intra-rater reliability. The mean of the first three measurements from each tract were used in the statistical analysis. Intra-rater reliability measures were performed on a random selection of 20% of all primary tracts for FA and MD variables. A Spearman rho statistic resulted in a significant correlation of r = 0.99, $p<.01$ for FA (mean 1 = .59, mean 2 = .59) and r = .90, $p<.01$ for MD (mean 1 = .00079 , mean 2 = .00079). A second researcher performed reliability measures after being trained to the specific DTI protocol used in the current study. Inter-rater reliability measures were performed on a random selection of 20% of all the primary tracts for FA and MD variables. A Spearman rho statistic resulted in r =. 939, $p<.01$ for FA (mean 1 = .59, mean 2 = .58) and r = .918, $p < .01$ for MD (mean 1 = .00078, mean 2 = .00079), suggesting excellent inter-rater reliability.

Primary Tracts

Table 6 shows the control and facial reanimation group means and standard deviations for FA and MD derived from each of the primary tracts in the right and left hemispheres (right, left) when appropriate. These data were submitted to statistical analysis based on the study's hypotheses.

Table 6. *FA and MD for primary white matter tracts (continued onto next page)*

	FA			
	Control		Facial Reanimation	
	left	right	left	right
PLIC	0.69 ± 0.01	0.71 ± 0.01	0.66 ± 0.01	0.65 ± 0.01*
CP	0.75 ± 0.01	0.79 ± 0.02	0.71 ± 0.01	0.75 ± 0.01
CR	0.40 ± 0.01	0.40 ± 0.01	0.43 ± 0.02	0.39 ± 0.01
SS	0.60 ± 0.01	0.62 ± 0.01	0.61 ± 0.01	0.60 ± 0.01
CG	0.51 ± 0.01	0.49 ± 0.01	0.48 ± 0.03	0.47 ± 0.01
	MD			
	Control		Facial Reanimation	
	left	right	left	right
PLIC	0.00075 ± .00002	0.00076 ± 0.00001	0.00076 ± 0.00002	0.00076 ± 0.00003
CP	0.00080 ± .00001	0.00079 ± 0.00002	0.00087* ± 0.00002	0.00081 ± 0.00002
CR	0.00071 ± 0.00001	0.00070 ± 0.00001	0.00076* ± 0.00001	0.00075 ±0.00001
SS	0.00084 ± 0.00000	0.00085 ± 0.00003	0.00089 ± 0.00002	0.00089 ± 0.00001
CG	0.00084 ± 0.00000	0.00084 ± 0.00002	0.00083 ± 0.00002	0.00085 ± 0.00001

Values represent means and standard deviation for FA and MD for 5 primary tracts (PLIC = Posterior Limb of the Internal Capsule, CP = Cerebral Peduncle, CR = Corona Radiata, SS = Sagittal Stratum, CG = Cingulum). * Significant group differences ($p \leq .05$) when compared to control.

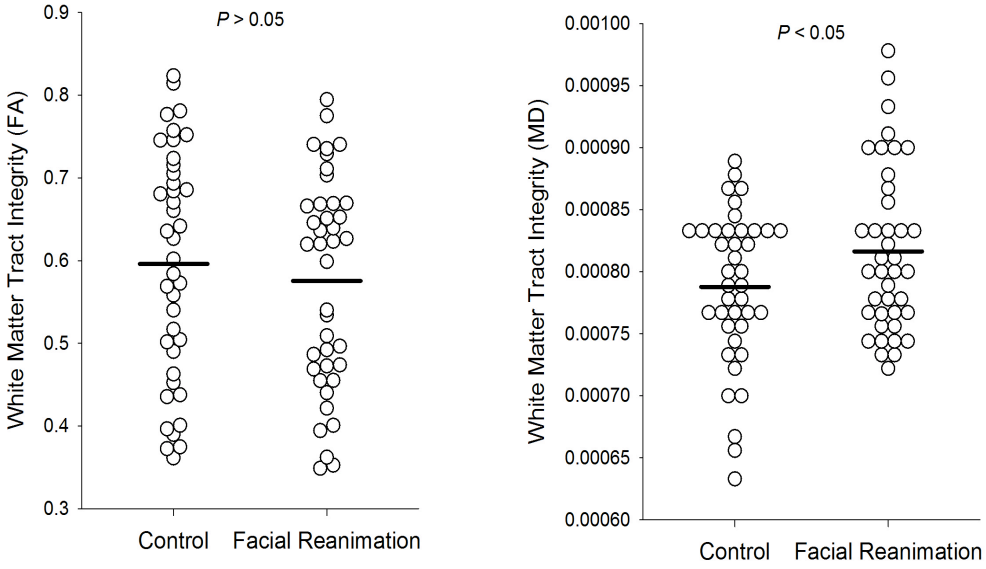
Between-Groups Comparisons

FA and MD by Group

To answer the first hypothesis, the facial reanimation group will have lower FA and higher MD when compared to the control group, a between-group analysis was completed using a one-way Analysis of Variance (ANOVA) for each dependent variable (FA & MD). Overall FA and MD values were

compared between the control group and facial reanimation group. Figure 1 shows the data points averaged for each individual by tract and hemisphere.

Figure 1. *Individual Data Points Showing Group Difference in FA and MD*



Fractional anisotropy (FA) values are depicted in the left panel and MD values are depicted in the right panel for control and facial reanimation groups. Open circles represent individual means associated with each tract (i.e., 5 tracts, 2 hemispheres, and 4 participants per group) for a total of 40 data points per group. The horizontal line running through each data set represents the mean. Only the values associated with MD reached a statistically significant between-group difference.

Values for FA were in the predicted direction, however statistical analysis revealed that no significant group differences were found ($F_{(1,79)} = 0.474$, $p = .25$). The facial reanimation group did however, have significantly higher mean diffusivity values (x = .00082) than the control group (x = .00079) ($F_{(1,79)} = 3.977$, $p \leq .03$).

The next series of statistical analyses were conducted to answer hypotheses related to group differences in specific tracts. The primary focus

of these analyses was on the brain regions that are known to contain fibres of the CBT.

Corticobulbar Tract Analysis by Group

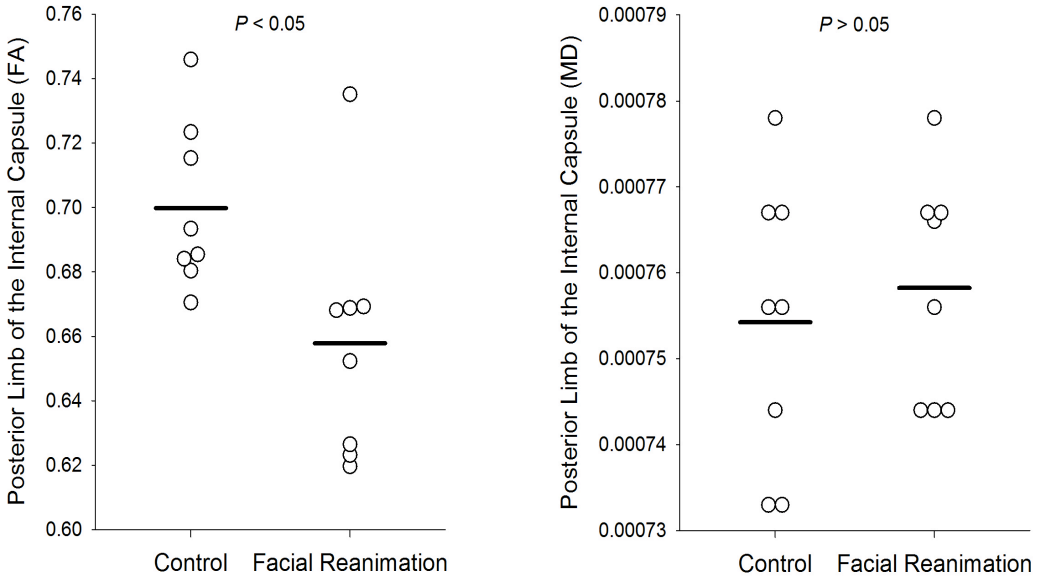
Since the CBT could not be assessed directly, the following tracts were analyzed based on their containment of corticobulbar fibres: posterior limb of the internal capsule, cerebral peduncle, and corona radiata, It was hypothesized that there would be lower FA and higher MD corresponding to these areas of suspected damage, regardless of hemisphere, in the facial reanimation group when compared to the control group. Table 7 shows the results from the statistical analysis for FA and MD and Figures 2, 3 and 4 show the data points averaged for each individual by hemisphere.

Table 7. *One-way ANOVA for Group Differences for FA and MD in Selected Tracts of Interest*

FA				
Tracts	Control Group	Facial Reanimation Group	F- Statistic	*p*-value
PLIC	0.70	0.66*	6.722	.01
CP	0.77	0.73*	3.511	.04
CR	0.40	0.41	0.069	.40
MD				
Tracts	Control Group	Facial Reanimation Group	F- Statistic	*p*-value
PLIC	0.00075	0.00076	0.298	.38
CP	0.00080	0.00084*	3.737	.04
CR	0.00070	0.00076*	7.354	.01

Values represent collapsed means across tracts, irrespective of hemisphere, in the control group and facial reanimation group. PLIC = Posterior Limb of the Internal Capsule, CP = Cerebral Peduncle, CR = Corona Radiata. *Significant difference ($p<.05$) when compared to the control group.

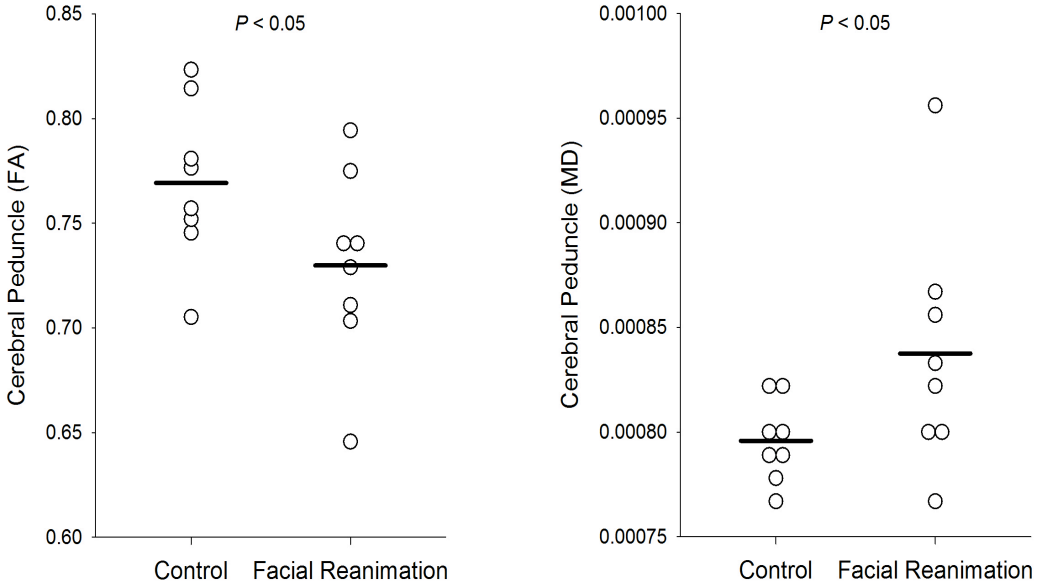
Figure 2. Vertical plot showing mean and variance of individual data for FA and MD for the posterior limb of the internal capsule



Fractional anisotropy values are depicted in the left panel and MD values are depicted in the right panel for control and facial reanimation groups. Open circles represent individual means associated with each hemisphere (i.e., 2 hemispheres, and 4 participants per group) for a total of 8 data points per group. The horizontal line running through each data set represents the mean. The values associated with FA measured in the posterior limb of the internal capsule reached a statistically significant between-group difference.

In the primary area of interest, the posterior limb of the internal capsule, there was statistically significant lower FA in the facial reanimation group (x = .66) when compared to the control group (x = .70) ($F_{(1,15)} = 6.722$, $p = <.01$). No between-group differences were found for MD.

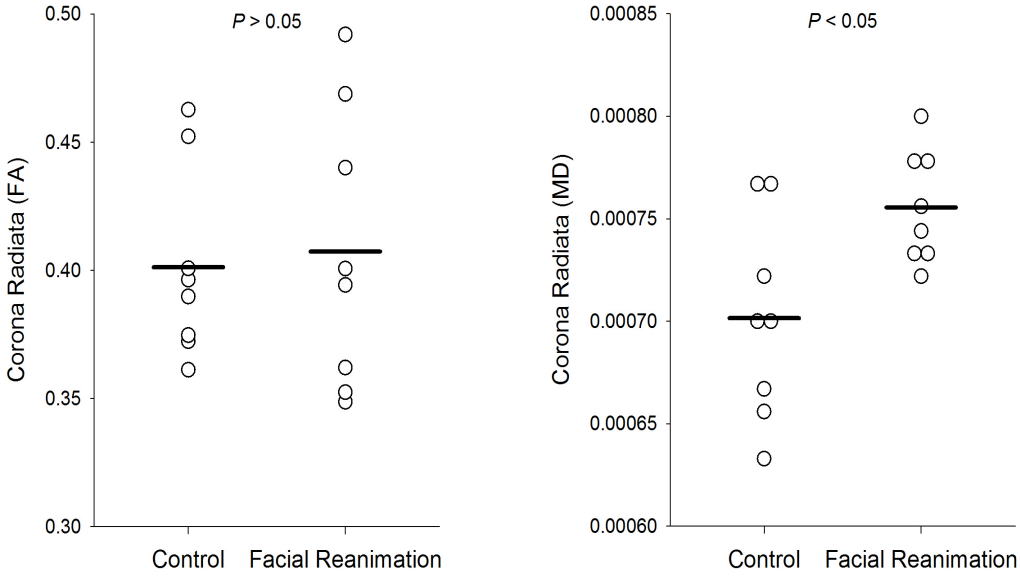
Figure 3. Vertical plot showing mean and variance of individual data for FA and MD for the cerebral peduncle.



Fractional anisotropy (FA) values are depicted in the left panel and MD values are depicted in the right panel for control and facial reanimation groups. Open circles represent individual means associated with each hemisphere (i.e., 2 hemispheres, and 4 participants per group) for a total of 8 data points per group. The horizontal line running through each data set represents the mean. The values associated with FA and MD measured in the cerebral peduncle reached a statistically significant between-group differences.

There also was significantly lower FA in the facial reanimation group (x = .73) when compared to the control group (x = .77) in the cerebral peduncle ($F_{(1, 15)} = 3.511$, $p \leq .04$). Mean diffusivity in the cerebral peduncle was significantly higher in the facial reanimation group (x = .00084) when compared to the control group (x = .00080) as well ($F_{(1,15)} = 3.737$, $p \leq .04$).

Figure 4. Vertical plot showing mean and variance of individual data for FA and MD for the corona radiata.



Fractional anisotropy (FA) values are depicted in the left panel and MD values are depicted in the right panel for control and facial reanimation groups. Open circles represent individual means associated with each hemisphere (i.e., 2 hemispheres, and 4 participants per group) for a total of 8 data points per group. The horizontal line running through each data set represents the mean. The values associated with MD measured in the corona radiata reached a statistically significant between-group difference.

Finally, in the third area of predicted difference, the corona radiata, there were no significant group differences in FA. However, there was significantly higher MD in the corona radiata with participants who had facial reanimation surgery (x = .00076), when compared to the control group (x = .00070), ($F_{(1,15)} = 7.354$, $p \leq .01$).

Using independent samples *t*-tests, significant between group differences were found when left vs. right hemisphere tracts were compared (see Table 6 for means). There was a significant group difference in FA of the right posterior limb of the internal capsule ($t = 2.589$, df = 6, $p \leq .02$, one-

tailed). There was also a significant group difference in MD of the left cerebral peduncle (t = -1.921, df = 6, $p \leq .05$, one-tailed) as well as a significant group difference in the MD of the left corona radiata (t= -0.713 , df = 6, $p \leq .05$, one-tailed). General hemispheric differences were examined in the following section.

Within-Groups Comparisons

A third set of hypotheses addressed right and left hemisphere differences within each group. It was hypothesized that there would be asymmetry between the left and right hemisphere of the control group; specifically the left hemisphere will have higher FA and lower MD than the right hemisphere, due to its dominance for language and detection of asymmetry using DTI, in other studies (Rodrigo et al., 2007). Also, it was predicted that there would be symmetry between the left and right hemisphere of the facial reanimation group.

Hemisphere Asymmetry

Asymmetry between the left and right hemisphere of the control group was predicted, in terms of FA and MD measurements. Using a one-way ANOVA for each measurement (FA and MD), no asymmetries were found between the left and right hemisphere of the control group, for FA ($F_{(1,39)}$ = 0.049, p = .83) and MD ($F_{(1,39)}$ = .013, p = .91). When tracts were examined separately through paired samples t-tests, there was a significant difference in FA between the left (x =0 .75) and right (x =0 .79) cerebral peduncle in the control group (t = 2.617, df = 3, $p \leq .04$, one-tailed). This result was not in the

predicted direction (i.e., the left hemisphere did not have higher FA than the left).

Symmetries were predicted in FA and MD values between the left and right hemispheres of the facial reanimation group. There was no difference between the left and right hemisphere of the facial reanimation group in FA ($F_{(1,39)} = .027$, $p = .87$) and MD ($F_{(1,39)} = .156$, $p = .70$). When examining tracts, there was a significant difference in FA between the left (x = 0.43) and right (x = 0.39) hemisphere in the corona radiata ($t = -3.550$, df = 3, $p \leq .04$, two-tailed). There was also was a significant difference in MD in the left (x = 0.00087) and right (x = 0.00081) hemisphere in the cerebral peduncle ($t = -5.047$, df = 3, $p \leq .02$, two-tailed).

Although some right and left hemisphere contrasts were statistically significantly different, there was not a clear pattern of asymmetry for tracts and group.

Visual Trends in Facial Reanimation Group

All participants who underwent facial reanimation surgery were matched to controls in terms of age and gender. A visual display of means and standard deviations for each tract was created for FA values derived for each individual participant. These figures are presented in Appendix A.

Participants with Unilateral Paralysis

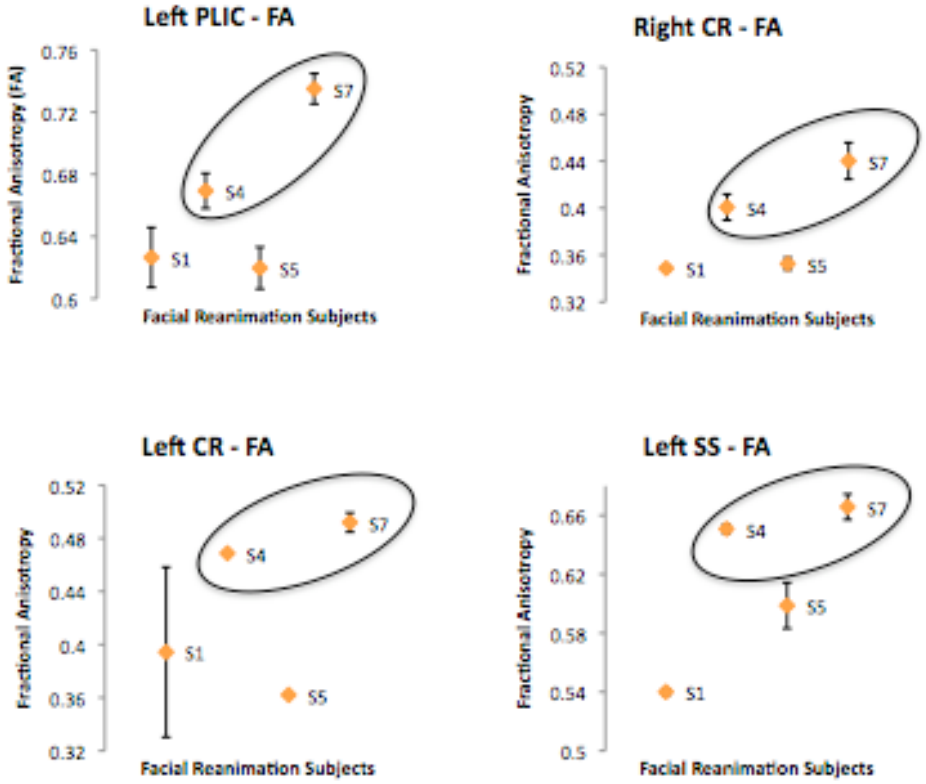
Participants S1 and S5 both presented with right-sided paralysis and received a cross-facial nerve graft with a gracilis muscle transfer. They appeared to have lower FA values than the other facial reanimation

participants in the left sagittal stratum, left corona radiata, right corona radiata, and left posterior limb of the internal capsule.

Participants with Bilateral Paralysis

Participants S4 and S7 both presented with Moebius syndrome, which was treated with a gracilis muscle transfer with masseteric nerve innervation on both sides of the face for participant S7, and one side of the face for participant S4. It was hypothesized that in these participants with bilateral paralysis, both hemispheres would be affected. Thus, it would be predicted that the left and right hemispheres in these participants would not be significantly different and perhaps these two brains would look similar to each other. Upon visual examination of the FA figures (Appendix A), participant S4 S7 appeared to group together in terms of FA values. For example, higher FA values were derived from these two individuals compared to the other two participants for tracts in the left sagittal stratum, the left and right corona radiata, and the left posterior limb of the internal capsule (see Figure 5).

Figure 5. Grouping together of FA for participants with bilateral paralysis for the left posterior limb of the internal capsule (PLIC), left and right corona radiata (CR), left sagittal stratum



Participants with Moebius syndrome (S4, S7) had similar values for FA in the regions depicted in the panels shown.

Visual Discrepancies in Participant S1

Fractional anisotropy in selected tracts for participant S1 appeared to be quite varied from the rest of the participants (refer to Appendix A for FA figures). Her FA values were much lower in the right posterior limb of the internal capsule, right sagittal stratum, left sagittal stratum, and the right pontine tracts when compared to the other facial reanimation participants.

Secondary Tracts

For exploratory purposes, four secondary tracts were examined that were thought to be unaffected by facial reanimation procedures. These tracts included: (1) the uncinate fasciculus , (2) the medial lemniscus , (3) pontine region and (4) the corpus callosum. These tracts were examined to further explore the possibility of neuroplasticity and to determine if any differences unrelated to facial reanimation would be present. Table 8 shows the group means and standard deviations for each tract for the control group and facial reanimation group.

Table 8. *FA and MD for Secondary White Matter Tracts*

	FA			
	Control		Facial Reanimation	
	left	right	left	right
UNC	0.53 ± .01	0.53 ± .03	0.60 ± .02	0.57 ± .01
ML	0.60 ± .01	0.64 ± .01	0.59 ± .02	0.62 ± .02
P	0.57 ± .01	0.54 ± .02	0.58 ± .02	0.55 ± .02
CC	0.78 ± .02		0.78 ± .01	
	MD			
	Control		Facial Reanimation	
	left	right	left	right
UNC	0.00081 ± .00001	0.00078 ± .00002	0.00078 ± .00001	0.00080 ± .00001
ML	0.00080 ± .00001	0.00076 ± .00001	0.00079 ± .00001	0.00080 ± .00001
P	0.00098 ± .00004	0.00115 ± .00006	0.00100 ± .00005	0.00110 ± .00008
CC	0.00081 ± .00001		0.00085 ± .00001	

Values represent means and standard deviations FA and Mean Diffusivity for 4 secondary tracts (UNC = Uncinate Fasciculus, ML = Medial Lemniscus, P =

Pontine Area, CC = Corpus Callosum). No statistically significant results were found (p>.05).

Between-Groups Comparisons

FA and Mean Diffusivity by Group

Two separate one-way ANOVAs were conducted to determine group differences for FA and MD. There was no significant group difference for these secondary white matter tracts in terms of FA ($F_{(1,55)} = .235$, $p = .63$) and MD ($F_{(1,55)} = .012$, $p = .91$).

Tract Analysis by Group

One-way ANOVA was used to examine group differences in these secondary tracts. Table 9 shows that no significant group differences were found in any of the secondary tracts, which were unrelated to facial movement. Of the secondary tracts, the uncinate fasciculus came closest to showing significant group differences.

Table 9. *One-way ANOVA for Group Differences in Secondary Tracts*

		FA		
Tracts	Control Group	Facial Reanimation Group	F-Statistic	*p*-value
UNC	0.53	0.58	2.554	.13
ML	0.62	0.61	.277	.61
P	0.56	0.56	0.075	.79
CC	0.40	0.41	0.069	.40
		MD		
Tracts	Control Group	Facial Reanimation Group	F-Statistic	*p*-value
UNC	0.00079	0.00079	0.042	.84
ML	0.00078	0.00080	.268	.61
P	0.00106	0.00105	.014	.91
CC	0.00081	0.00085	2.254	.18

Values represent collapsed means across tracts, irrespective of hemisphere, in the control group and facial reanimation group. UNC = Uncinate Fasciculus, ML = Medial Lemniscus, P = Pontine Area, CC = Corpus Callosum. No significant group differences were found (p>.05).

Within-Groups Comparisons

Hemisphere Asymmetry

There was no significant difference between the left and right hemisphere in of the control group for FA ($F_{(1,23)} = .009$, $p = .93$) and MD ($F_{(1,23)} = .209$, $p = .65$), when tracts were collapsed. There was a significant difference in MD between the left (MD = .00080) and right (MD = .00076) hemisphere of the medial lemniscus ($t = -2.81$, df = 3, $p < .05$, one-tailed). The left hemisphere had higher MD, so it was not in the predicted direction (p=.07).

A one-way ANOVA also was conducted to compare the left and right hemisphere of the facial reanimation group for these secondary tracts. There was no significant difference between the left and right hemisphere for the facial reanimation group in terms of FA ($F_{(1,23)} = .142$, $p = .71$) and MD ($F_{(1,23)} = .35$, $p = .559$).

Visual Trends in Facial Reanimation Group

Limited trends were observed in the secondary tracts when examining the figures containing means and standard deviations for each tract (See Appendix A). There did not appear to be any specific trends within participants in the facial reanimation group for these tracts.

CHAPTER V: DISCUSSION

The purpose of study was to determine if DTI, could be used as a cortical neuroimaging technique for detecting differences in white matter fibre tract organization between patients with facial reanimation surgery and their matched controls. Specifically, this study examined cortical white matter fibre tracts in four patients who had facial reanimation surgery following facial paralysis. Fractional anisotropy (FA) and mean diffusivity (MD) were derived from tractography using DTI Studio software and values were compared to healthy age and sex matched control participants.

Water diffusion tensor images were completed on the patient group, following facial reanimation surgery. Surgeries involved a gracilis muscle transfer either with a cross-facial nerve graft or masseteric nerve innervation, depending on the type of paralysis. It was hypothesized that patients with facial reanimation surgery would have lower FA and higher MD values than their matched controls as a result of facial paralysis, facial reanimation surgery, or both. More specifically, it was hypothesized that lower FA and higher MD values would be observed in regions of the brain containing CBT fibres because of their role in sensorimotor control of the face. Specific cortical regions were selected for study because they contained white matter fibres of the CBT.

The main finding from the current study was that FA and MD measures derived from DTI tractography differentiated the patients with facial reanimation from their control counterparts specifically in regions of

the brain containing white matter fibres of the CBT. The current results support previous studies that have shown that decreases in FA and increases in MD are related to disease progression (i.e., supranuclear plasy), and likely indicate a reduction in myelin (Wang et al., 2010). Facial paralysis is a result of injury to the seventh cranial nerve, which may be associated with demyelination of the facial nerve (Edstrom et al., 1987). Therefore, the (CBT) which includes the facial nerve pathway (Yildiz et al., 2005), is likely severely affected in patients with facial paralysis. The CBT also may be one of the main tracts affected by intrinsic and/or extrinsic neuroplasticity following facial reanimation surgery (Anastakis, Chen, Davis & Mikulis, 2005). The interpretation of the results, specific to each of the current study's hypotheses, is described below.

Group Differences in White Matter Integrity in Patients with Facial Reanimation Surgery and their Matched Controls

The first hypothesis was that people with facial reanimation surgery would have lower FA and higher MD than age and sex matched controls in regions of the brain containing the CBT. Overall, a trend of lower FA and statistically significant higher MD was found in reanimation group when all tracts were collapsed. The neurologic determinants of FA are not fully understood, but associated with myelination, axon size, axon density, path geometry and crossing fibre effects (Schaechter et al., 2009). Previous studies have found that FA values derived from the CST appeared to be associated with motor outcomes. Stroke patients with reduced motor skills

had lower FA than controls, whereas patients with better motor skills had elevated FA of the bilateral CST compared to the lower functioning motor group (Schaechter, et al., 2009). Diffusion tensor imaging was used to confirm the findings in this study, suggesting that the level of motor skill recovery reached in patients with stroke, related to the microstructural status of the CST. The previous observations support the findings from the current study, where compared to healthy controls, this small sample of patients with facial reanimation with lower motor control of facial movements may have compromised white matter organization and integrity, possibly due to reduced myelination, axon size, axon density, or aberrant path geometry in the CST.

Although FA was not statistically significant between groups, the trend of the group results was in the predicted direction. Of the two dependent variables, FA was more variable. Therefore, more participants will need to be included to establish whether or not overall FA values are lower in the CBT in this patient population. However, the trend found in the lower FA value and the statistically significant difference in the MD value suggested that although facial paralysis patients had facial reanimation surgery to restore or develop facial movements, the CBT organization still remains structurally distinct from their matched controls. These results are consistent with previous findings suggesting that reorganization occurs following paralysis as well as surgery (Klebuc & Shenaq, 2004; Franchi & Veronesi, 2004; Li et al., 2002).

Consideration of Corticobulbar Tract Changes following Facial Reanimation Surgery

The second hypothesis in the current study was related to whether or not FA and MD values would be different between the patient and control groups relative to different regions in the brain containing the CBT. It was predicted that differences in white matter organization along the CBT would exist following facial paralysis or facial reanimation surgery as a function of long-term neuroplasticity, as many studies have demonstrated cortical change following peripheral nerve injuries (Taylor, Anastakis, & Davis, 2009; Yogarajah et al., 2010; Navarro et al. , 2007, Anastakis et al., 2005). Recent studies have determined that functional reorganization in the brain for patients with Bell's Palsy continues to evolve, even after complete functional recovery has been observed (Klingner et al., 2011).

In the current study, specific areas of the brain known to contain corticobular fibres, were examined. These areas included the posterior limb of the internal capsule, the cerebral peduncle, and the corona radiata. Significantly lower FA was found in the posterior limb of the internal capsule, and cerebral peduncle in the patient group. As well, significantly higher MD values were found in the cerebral peduncle and corona radiata. These findings indicated that regions of the brain containing rich concentrations of CBTs were significantly different in the patient population compared to the controls. These results support previous studies using a rat model to identify cortical changes following repair of the facial nerve (Franchi, 2000). Animal

studies by Lu et al. (2002) also found that regeneration occurs among motoneurons innervating different branches following facial nerve re-innervation in rats.

The posterior limb of the internal capsule has consistently been shown to contain CBT fibres, specifically in its dorsal third quarter (Wang & Melhem, 2005; Ulag, et al., 1999; Johansen-Berg & Behrens, 2009). In the current study, this tract showed significantly lower FA for the facial reanimation group when compared to the control group, suggesting reduced white matter integrity specific to area of the CBT in the facial reanimation group. This result was likely due to a significant group difference in the right posterior limb of the internal capsule.

The cerebral peduncle showed robust differences between groups for both FA and MD values. The cerebral peduncle was chosen as a tract of interest, because it also contains CBT fibres (Mori, et al., 2005; Johansen-Berg & Behrens, 2009). Supra-nuclear lesions found in or near the cerebral peduncle, have possibly led to dissociation of emotional and voluntary facial movements (Victor & Ropper, 2001). Moreover, the significant group difference found for MD values in the left cerebral peduncle may be indicative of the need to voluntarily control facial movements used to express emotion. However, caution should be taken when linking structural DTI anomalies to function.

The corona radiata was selected as it also contains parts of the CBT and has been reliability assessed in previous studies (Mori, et al., 2005;

Nagae et al., 2007). The corona radiata had significantly higher MD in the facial reanimation group when compared to the control group, suggesting less neural integrity following facial reanimation surgery. Specifically, the left corona radiata showed significant group differences.

Taken together, these findings suggest that the CBT appears to be different in facial reanimation group when compared to controls. The current findings are commensurate with those from previous studies showing that the amount of movement performed by individuals, as measured by an actigraph worn on participants' wrist for 24 hours, was related to the function and structure of their white matter motor tracts (Walther et al., 2010). Walther and colleagues (Walther, et al., 2010) found a positive correlation in motor activity level and FA in relevant motor areas and negative correlations in areas unrelated to motor areas. This provides evidence for the association of FA in motor tracts and quantitative motor behaviour, such as facial movement. The white matter integrity in the brains of patients with facial reanimation surgery is reduced in areas related to facial movement which remains reduced or aberrant after surgery. It should also be noted that the posterior limb of the internal capsule, cerebral peduncle and corona radiata regions potentially sampled more CBTs than other regions measured in the current study. Therefore, group differences may have been robust because of the ability to sample a larger portion of CBT fibres in these regions. It is difficult to know whether group differences are truly a function of CBT integrity, sample size or both.

Other areas that were predicted to show group differences were the sagittal stratum and cingulum. There were no significant group differences observed in these areas possibly because of fibre tract orientation and DTI tractography protocol used. The sagittal stratum was selected because some CBTs run through this region (Mori, et al., 2005). Sampling size and orientation of these fibres minimized the chances of finding group differences. The cingulum was chosen as a tract of interest due to its involvement in facial movements associated with emotion (Tate & Tollefson, 2006). It is possible that this tract did not show significant group differences, because this tract is associated with involuntary motor control associated with emotional expression, in contrast to the cerebral peduncle which appears to voluntarily control facial expression. Perhaps the structural aspects of pathways related to emotional expression are not different in patients with facial reanimation, whereas the structural integrity of the motor execution pathways such as those found in the cerebral peduncle are affected by facial paralysis and reanimation sequelae.

To further substantiate the group differences found in regions containing CBT fibres, the current data indicated no significant group differences in secondary tracts. However, the uncinate fasciculus may have come close to a region of difference between the two groups. Recall that the uncinate fasciculus is involved in limbic connectivity, suggesting that it may be involved in some form of facial expression (Taylor, MacFall, Gerig, & Krishnan, 2007). Therefore, this tract may be sensitive cortical change in

people with facial reanimation surgery. These four tracts were examined for exploratory purposes and did not contain and fibres related to the CBT. It is possible to consider the secondary tracts as control tracts against which to compare observations in FA and MD values from the CBT. Consequently, these comparative data suggest that areas of the brain directly thought to relate to facial muscle movement, were affected in this group of facial reanimation patients.

Hemisphere Symmetry and Asymmetry

Analysis of within-group comparisons was completed to determine if differences between the left and right hemisphere existed in both the control group and facial reanimation group. Asymmetry was predicted between the left and right hemisphere of the control group, because the left hemisphere is dominant for fine motor control of speech (Kang, Herron, & Woods 2011). Previous studies have shown asymmetry in regions of white matter in the healthy adult population and therefore it was predicted that structural differences (i.e., FA and MD) would differentiate the regions of interest in the left and right hemispheres of the control group (Park et al., 2004). In the current study hemispheric differences were examined, regardless of tracts. For exploratory reasons, specific tracts were further examined for left and right hemisphere differences in the primary tracts: posterior limb of the internal capsule, cerebral peduncle, corona radiata, sagittal stratum, cingulum and secondary tracts uncinate fasciculus, pons, medial lemniscus and corpus callosum.

No white matter asymmetry was found between the left and right hemisphere of control group, when tracts were collapsed. When tracts were examined separately, there was a statistically significant difference in FA between the left and right cerebral peduncle. The corticobulbar fibres in this region of the right hemisphere may have more integrity, however these results may not link to function.

Symmetry was predicted to be present between the left and right hemispheres of the facial reanimation group. Because of the suspected neural reorganization associated with facial paralysis and reanimation surgical procedures it was predicted that a lack of asymmetry in white matter fibre tract integrity would be detectable (Anastakis et al., 2005). When all tracts were collapsed, no significant difference between the left and right hemisphere of the facial reanimation group in FA and MD were found. However, when examining specific tracts by hemisphere, significant between hemisphere differences were found in the corona radiata for FA and cerebral peduncle for MD.

Although some hemispheric differences may have been detected in the current data, no clear pattern of asymmetry was observed. To establish whether or not asymmetries exist in white matter fibre tracts as they relate to facial reanimation and healthy controls, more participants are needed and homogeneous clinical groups may be required.

Grouping Trends in Participants with Unilateral Paralysis and Bilateral Paralysis

The facial reanimation group for this study included four patients who had undergone facial reanimation surgery as treatment for their varying degrees and types of paralysis. Values for FA derived from each tract were plotted on a scatter plot and examined for visual trends based on clinical sequelae.

Two participants, participant S1 and participant S5, presented with unilateral facial paralysis, which was surgically treated with a cross-facial nerve graft with a gracilis muscle transfer. It was hypothesized that these patients would look visually similar in terms of their FA values, when compared to participant S4 and participant S7, as their types of paralysis and surgery were similar. Participant S1 and participant S5 appeared to group together and had lower FA values than participant S4 and participant S7 in the left posterior limb of the internal capsule, left corona radiata, right corona radiata and left sagittal stratum (See Figure 5 for visual display).

It is possible that the utilization of a cross-facial nerve graft along with a gracilis muscle transfer, leads to the expansion of motor and sensory representations in the contralateral side of the brain in response to the acquisition of the new motor skill (Anastakis et al., 2005). Recent studies have shown that reorganization was found in the hemisphere contralateral to the paretic facial nerve (Klingner et al, 2011). This organization was detectable even after complete clinical recovery. It is apparent that

reorganization may have occurred in the facial reanimation group, with cortical differences in participants who had unilateral paralysis vs. bilateral paralysis.

Higher FA values in the left posterior limb of the internal capsule, left corona radiata, right corona radiata and left sagittal stratum in the participants with gracilis muscle transfer with masseteric nerve innervation, agree with studies that have shown that the masseteric nerve leads to more movement than the cross-facial nerve transfer (Bienstock & Aly, 2009), as it is presumed higher white matter integrity is associated with more motor movement (Walther et al., 2010). Furthermore, participant S4 had lower FA values than participant S7 in these areas. As participant S4 received only stage 1 of the two-part surgery, it is possible that the white matter integrity in this participant was organized to a lesser degree than participant S7, suggesting less movement in the face for this participant.

Some individual variation was seen in the visual trends, as participant 1 appeared to have lower FA values than the others in the right posterior limb of the internal capsule, right sagittal stratum, left medial lemniscus, and right pontine area. Some possible explanations for the discrepancies observed in participant 1 include her age and length of time post surgery. Although the current study did not examine the effects of age on white matter structural organization following facial reanimation surgery, it may be important to note that the capacity for plasticity is age-dependent; hence, more changes in the younger patients may have been present (Plowman &

Kleim, 2009). Animal studies related to the current study have demonstrated that following the severing of a facial nerve, new neurocranial pathways do develop and age differences between the motor representations and cortical reorganization in newborns and adult rats have been found (Klebuc & Shenaq, 2004; Franchi & Veronesi, 2004; Li et al., 2002) . Perhaps participant 1 recovered slower than the other three participants, and thus had lower white matter integrity at the time these neuroimages were taken.

Clinical Relevance of the Study

Recent studies have indicated that functional recovery of a peripheral nerve palsy may result partly from cortical reorganization (Klingner, et al, 2011). The current study found that DTI derived FA and MD values are sensitive to group differences, suggesting that future studies could use DTI to detect mechanisms of neuroplasticity, increase knowledge on cortical reorganization following facial reanimation surgery, build on neuroplasticity principles to improve function after injury or repair, and guide surgical procedures.

Limitations of the Study

One limitation of the current study was its low number of participants studied (n=8). Although four facial reanimation participants and four control participants were sufficient to indicate significant differences, it is important to be cautious when interpreting the statistical results. In order to have greater statistical power and generalizability of results to other patients in this population, it will be necessary to conduct future studies on a much

larger sample. In addition, clinical subgroups will need to be included based on type of paralysis, age of onset, type of surgery, age when surgical procedures were done, along with clear descriptions of post-surgical rehabilitation.

Although group differences were found in white matter integrity, it is not possible to determine if these structural differences were present as a result of the original facial paralysis or as a result of the facial reanimation surgery. In order to better understand development or change in white matter integrity, it would be necessary to include and study patients with facial paralysis prior to facial reanimation surgery. Pre-post measures would allow for a better understanding of neural tract status related to paralysis and neuroplasticity related changes associated with reanimation. As well, a longitudinal study examining white matter changes over time would help to better understand the neuroplastic potential of the central nervous system.

Finally, a limitation in the method of analysis using DTI may have lead to the large variability in FA measures. Perhaps variability in data could be reduced by having a more consistent methodology for obtaining isolated pieces of whole tracts. As DTI is a newer method of analysis, it is hoped future studies could reproduce the current study's protocol and perhaps expand measurements to include other micro-structural analyses.

Future Directions and Conclusion

This study was successful in determining that DTI is sensitive to group differences in patients post-facial reanimation and their matched controls.

Diffusion tensor imaging can be used as a successful tool to examine differences in white matter tract integrity in this population prior to and after reanimation surgery. To further examine DTI and its sensitivity to change over time, a future study could be designed to include a large homogeneous sample, rather than two small clinical subgroups. It would be beneficial to design a study around participants with Moebius Syndrome, as there would be no need to control for age of onset of facial paralysis. An ideal study would be able to compare function and brain structure between patients with Moebius Syndrome who received facial reanimation surgery, and participants with Moeibius Syndrome who did not receive facial reanimation surgery over the same period of time. As a power statistic was calculated for this study using an alpha level of 0.05 and a beta level of 0.80, a minimum of seven participants per group would be needed to show significant differences. In order to better understand the effects of facial reanimation surgery on people with facial paralysis, it would be ideal to include not only DTI to examine white matter fibre tracts, but also include measurable correlates such as functional resonance imaging (fMRI), facial muscle physiology (i.e., during a voluntary smile and speech), as well as behavioural measures (i.e., speech and non-speech function). Knowledge gained from previous work that measured behavioural and muscular correlates in this population could aide in the development of this future study (Haresam, Rieger, Olson, Wilkes & Boliek, in preparation). Because each group would be measured longitudinally (e.g., pre-surgery, post-

surgery, six months post-surgery, and one year post-surgery and equivalent time points for the untreated group), a repeated measures design would be used. Comparing the structure and function in the treated and untreated groups would aide in achieving a complete picture of neural and behavioural changes associated with facial reanimation in this population. Future studies in this population could be clinically beneficial in helping to answer questions associated with predicting surgical outcomes, guiding surgical techniques and informing rehabilitation therapies designed to enhance function in this population.

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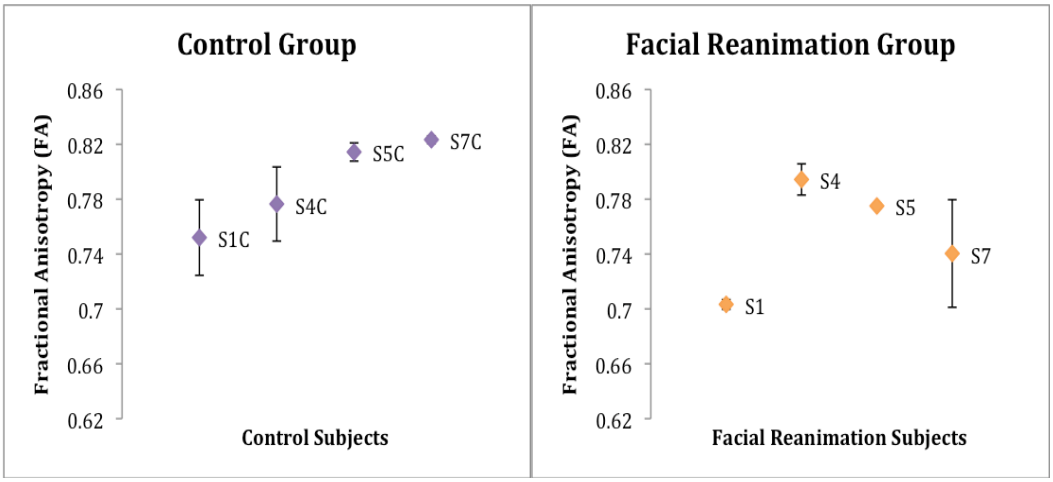
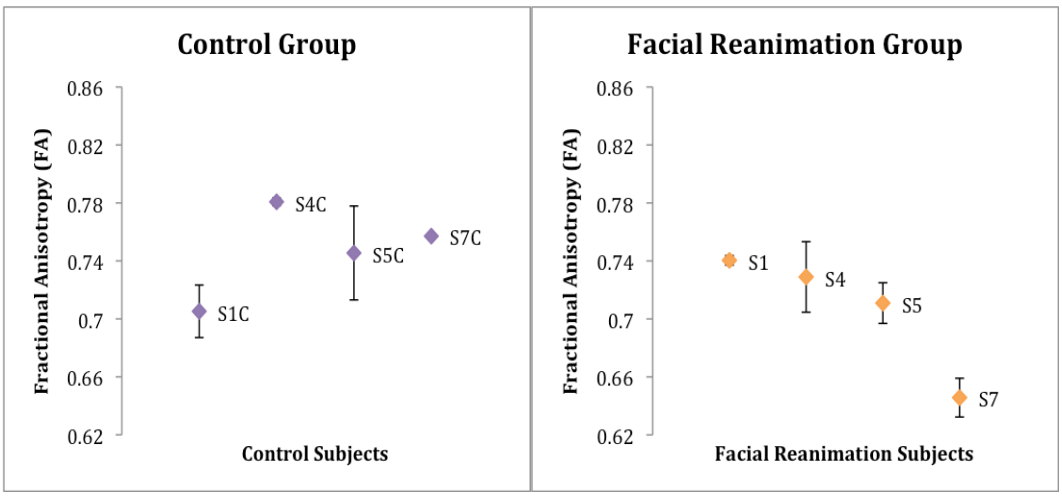
Appendix A

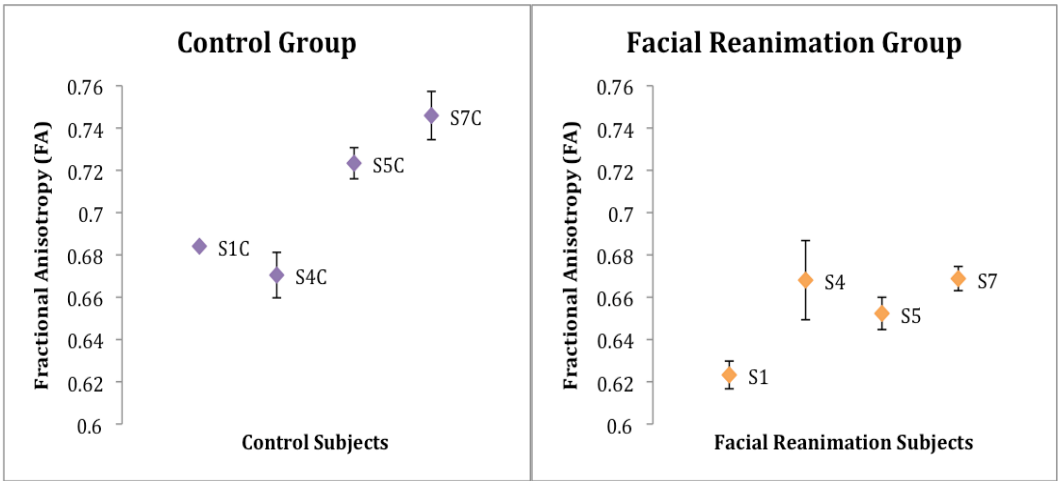
Right Cerebral Peduncle – FA



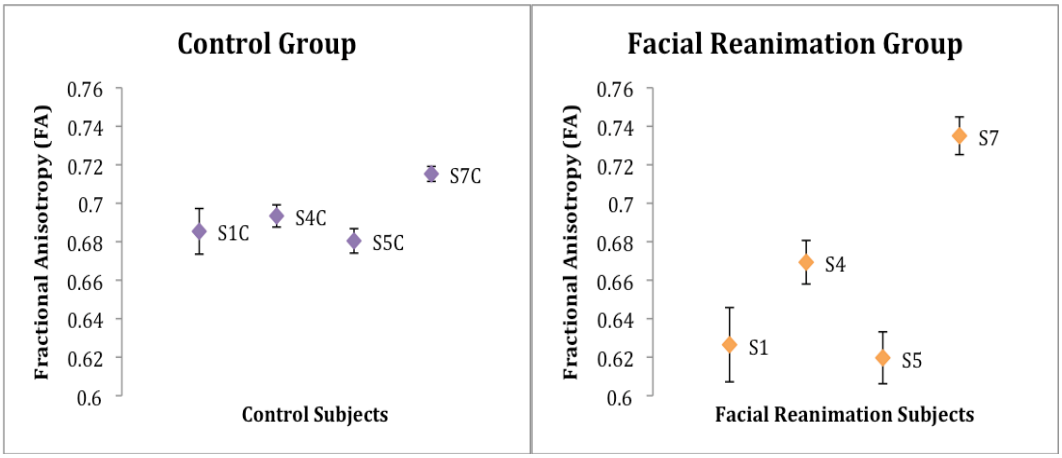
Left Cerebral Peduncle – FA



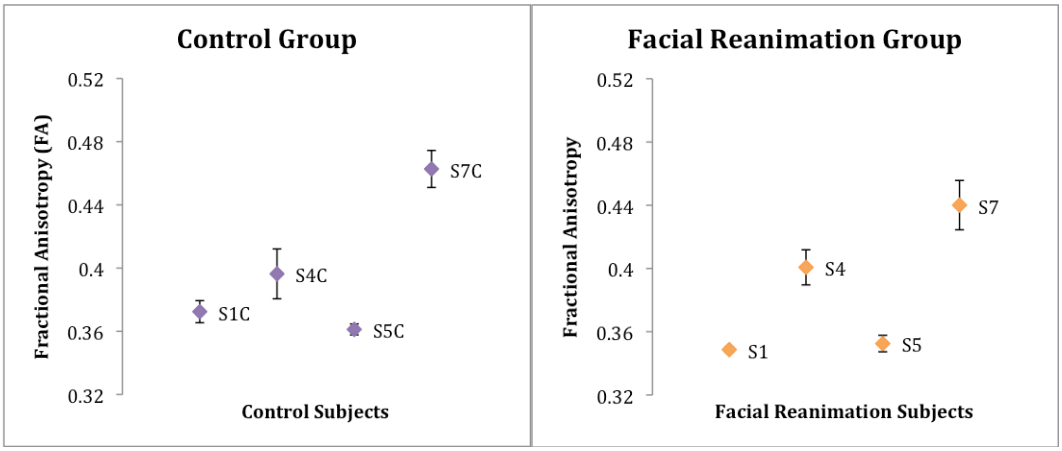
Right Posterior Limb of the Internal Capsule - FA



Left Posterior Limb of the Internal Capsule - FA



Right Corona Radiata - FA



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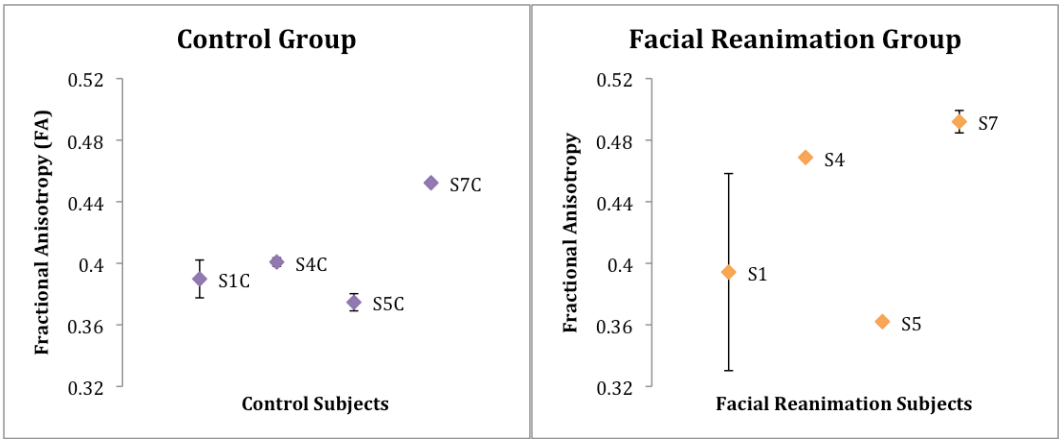
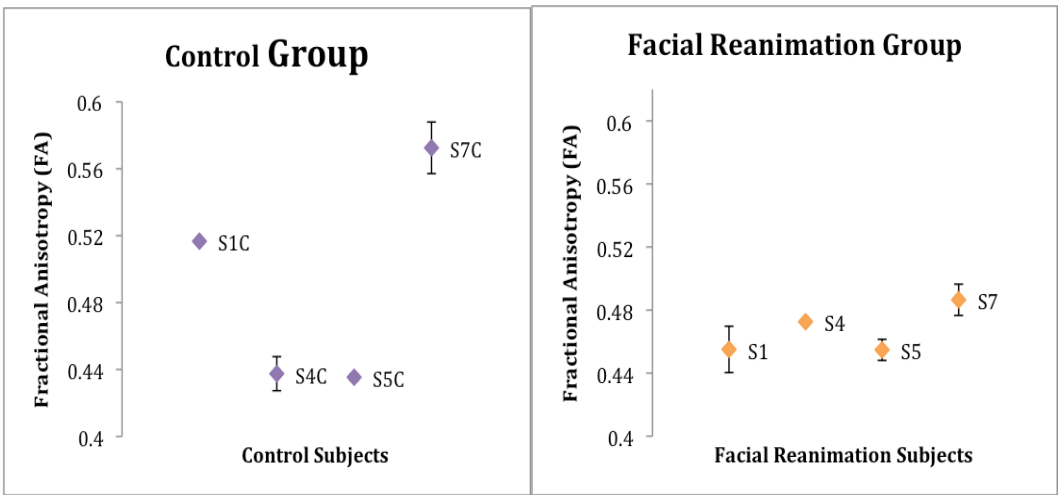
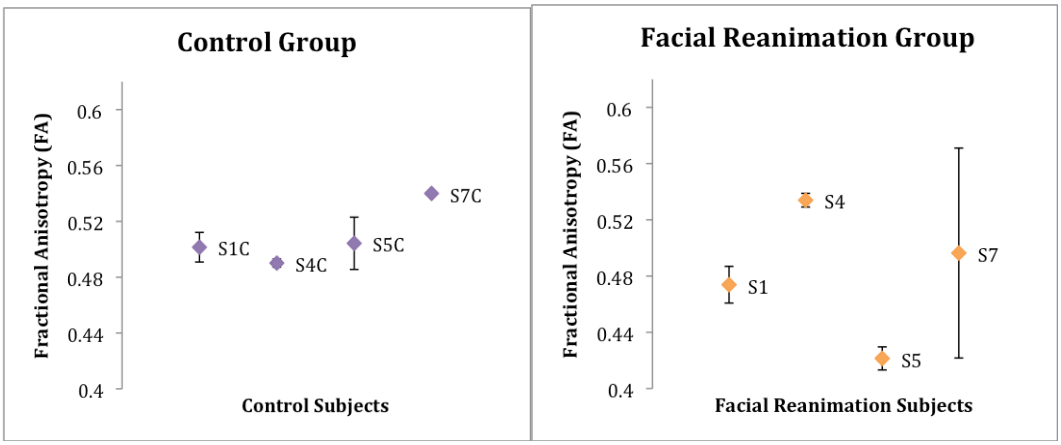


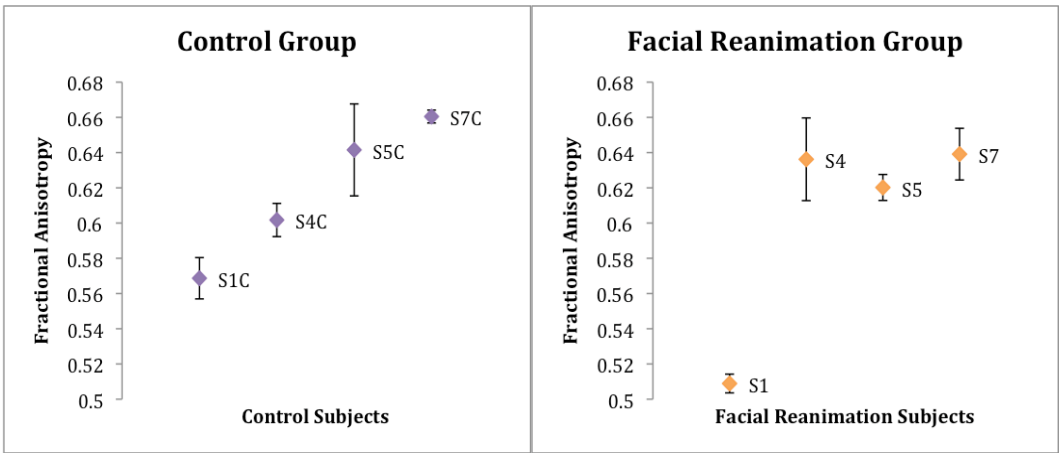
Right Cingulum - FA



Left Cingulum – FA



Right Sagittal Stratum – FA



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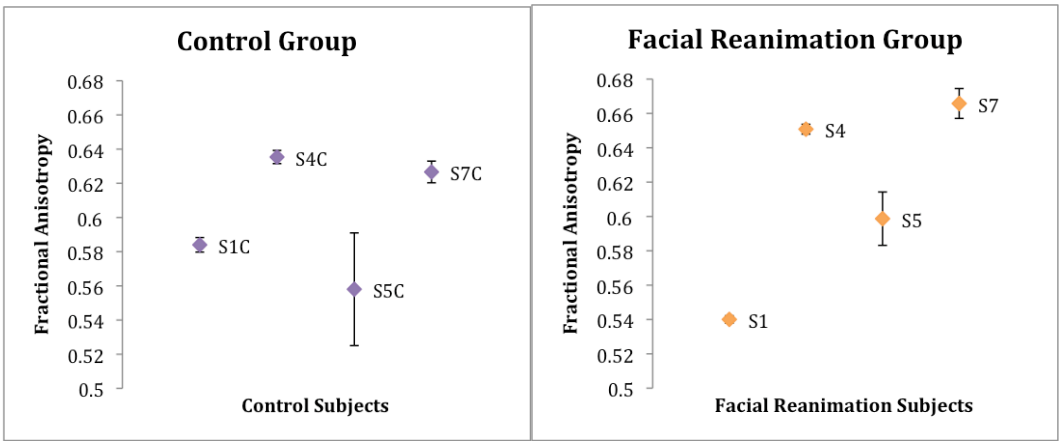
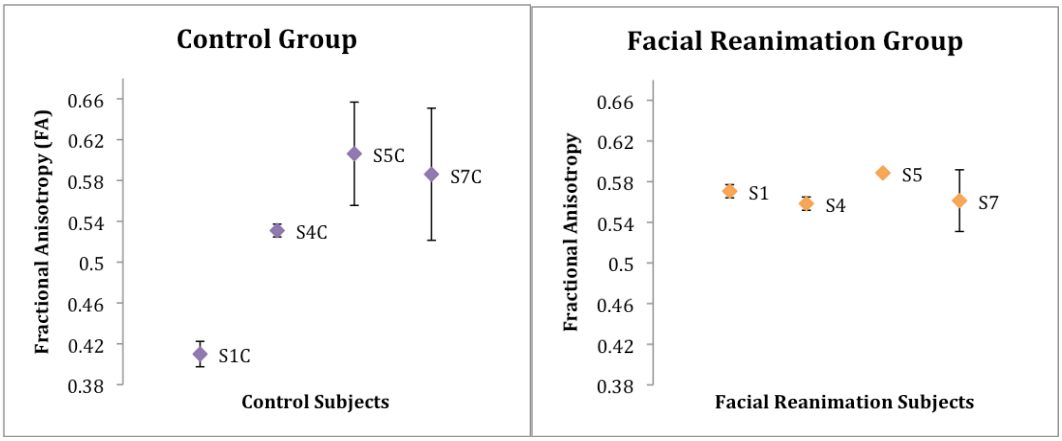
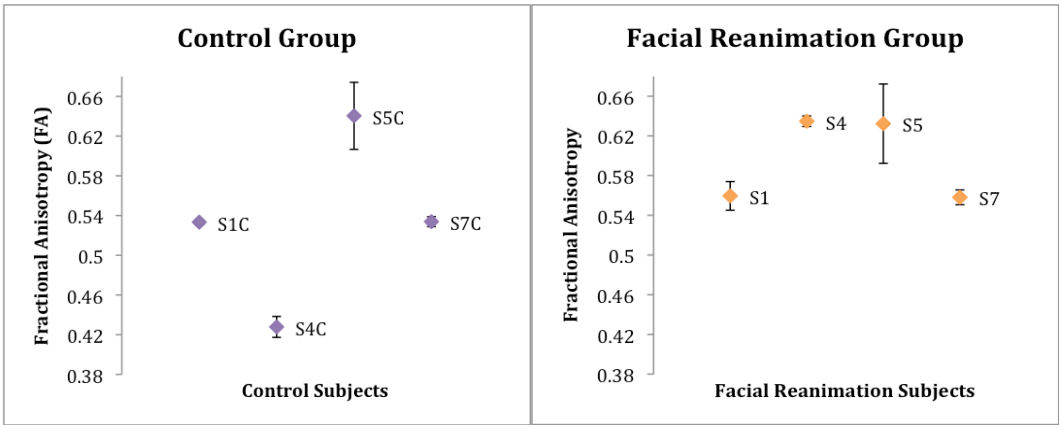


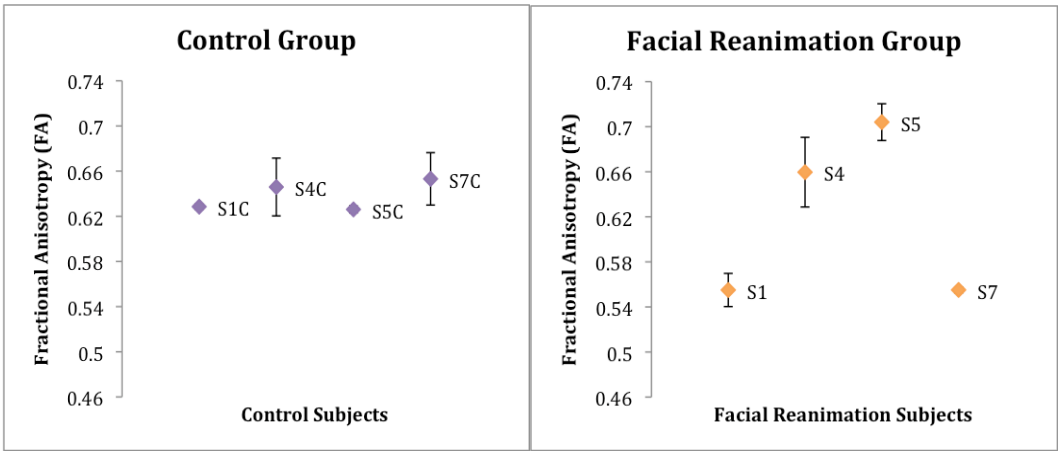
Right Uncinate Fasiculus – FA



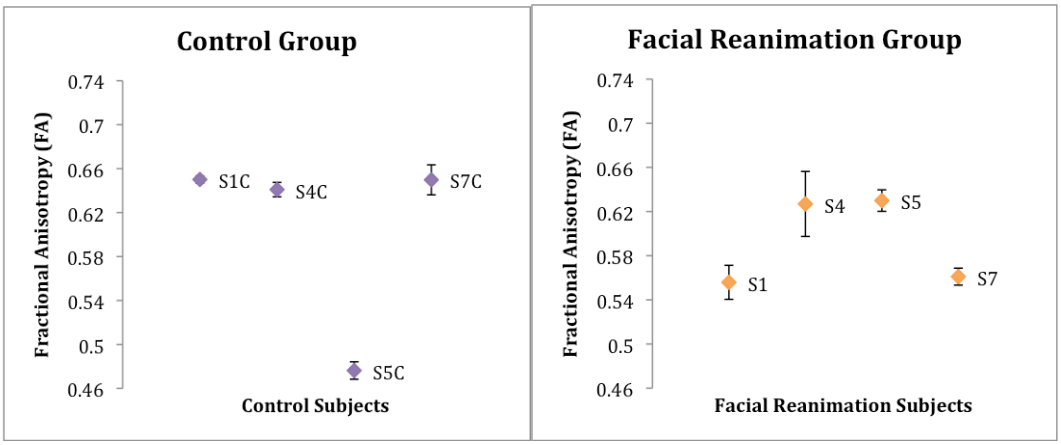
Left Uncinate Fasiculus – FA



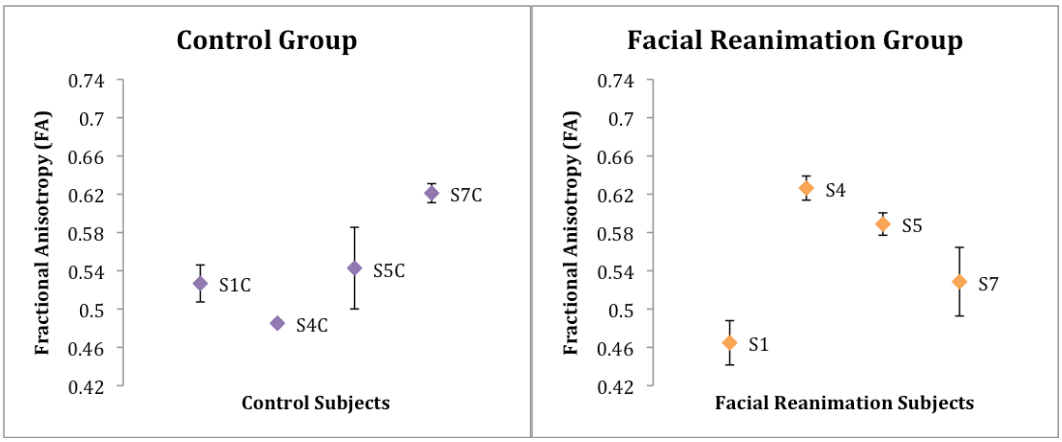
Right Medial Lemiscus – FA



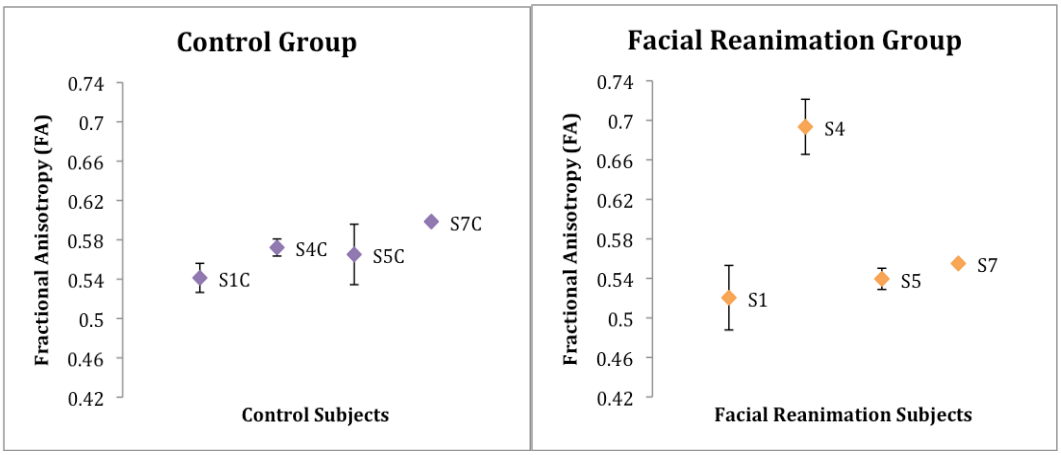
Left Medial Lemniscus – FA



Right Pontine – FA



Left Pontine – FA



`Corpus Collosum – FA