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Sensory Nerve Injuries: Advances in Diagnosis and Novel Therapy to Enhance Sensory Recovery in Humans

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Abstract

The unique difference between central and peripheral nervous systems is that regeneration actually occurs in the periphery. However, the functional recovery after surgical repair is highly variable and return to pre-injury state despite surgical advances is rare. When specifically looking at sensory regeneration, the functional recovery is even worse, where less than half of patients who receive operative repair have satisfactory recovery. Another problem lies in the paucity of knowledge regarding diagnosis of sensory nerve injury. This thesis reviews the current literature regarding sensory nerve regeneration, and subsequently investigates two critical voids in the literature: first, the diagnostic precision of several available sensory tests are described when looking at complete nerve transection; and second, the effect of novel post-surgical electrical stimulation on human sensory nerve recovery is reported in a randomized controlled trial.

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List of Abbreviations:

2PD	two-point discrimination
ANOVA	analysis of variance
AUC	area under the curve
BDNF	brain derived neurotrophic factor
cAMP	cyclic adenosine monophosphate
CAD	Canadian dollar
CASE IV	Computer Aided Sensory Evaluator version IV
CDT	cold determination threshold
CGRP	calcitonin-gene related peptide
CNS	central nervous system
COPM	Canadian Occupational Performance Measure
DASH	Disability of the Arm, Shoulder and Hand
DRG	dorsal root ganglion
ECM	extracellular matrix
EGF	endothelial growth factor
EMG	electromyography
Epo	erythropoietin
EpoR	erythropoietin receptor
ES	electrical stimulation
EURO	European Union currency dollar
FGF	fibroblast growth factor
FGF-2	basic fibroblast growth factor
GAP43	growth associated protein 43
GDNF	glia-cell-line derived neurotrophic factor
GGF	glial growth factor
GOT	grating orientation test
HPT	heat pain threshold
HPT1	intermediate heat pain threshold
HPT2	heat pain determination threshold
HSD	honestly significant difference
ICC	intra-class correlation coefficient
IFN-ϒ	interferon gamma
IGF 1 and 2	insulin like growth factor 1 and 2
IL-1	interleukin-1
IL-2	interleukin-2
IL-6	interleukin-6
J	Youden index
JND	just noticeable difference unit
LIF	leukemia inhibitory factor
MAG	myelin associated glycoprotein
MAPK	mitogen activated protein kinase

MBP	myelin basic protein
MRC	medical research council
N-CAM	neural cell adhesion molecule
NGF	nerve growth factor
NRG1	neuregulin-1
NT-3	neurotrophin-3
NT3R	nuclear triiodothyronine receptor
NT-4/5	neurotrophin-4/5
P0	myelin protein zero
PDGF	platelet derived growth factor
PMP-22	peripheral myelin protein-22
PNS	peripheral nervous system
PTHrP	parathyroid hormone-related peptide
QST	quantitative sensory testing
ROC	receiver operator characteristic
SNOSE	sequential numbered opaque sealed envelope
SPC	sham placebo control
STI-test™	shape-texture identification test
SWMT	Semmes Weinstein monofilament test
WDT	warm determination threshold
WEST	Weinstein Enhanced sensory test

Chapter 1: Epidemiology and pathophysiology of peripheral nerve injury, interventions to augment regeneration, and the challenge of sensibility testing

Introduction

Peripheral nerve injuries are common and affect a wide range of individuals on a daily basis. They can range from repetitive compression such as carpal tunnel syndrome to obstetrical brachial plexus injury brought on by vaginal delivery in the newborn. As such, there are a variety of etiologies that lead to a spectrum of nerve injury: compression, crush, partial axotomy, and complete transection. In this study, we shall be dealing with complete nerve transection usually secondary to trauma – accidental or intentional.

In contrast to the central nervous system, peripheral nerves are more capable of regeneration. Much of the last century has been devoted to technical advances to improve the repair of injured peripheral nerves in order to achieve ideal functional recovery (Slutsky & Hentz, 2006). However, as technology and coaptation techniques have reached a plateau in the past few decades, the functional outcomes have reached an impasse as well (Slutsky & Hentz, 2006). As a result, investigators are increasingly focusing on adjuvant treatments such as pharmaceuticals, stem cells, and other methods to stimulate nerve regeneration with concomitant surgery. One such technique that shows great promise is post-surgical electrical stimulation. Electrical stimulation has been trialed in animal models for augmenting nerve regeneration post-surgical coaptation. Advanced

techniques for measuring the speed, density and precision of nerve regeneration in the rat model have shown that post-surgical electrical stimulation improves regeneration of both motor and sensory nerves (Al-Majed *et al.*, 2000c;Geremia *et al.*, 2007). These promising findings have stimulated the translation of this new intervention to the study of the median nerve at the wrist for human patients with carpal tunnel syndrome (Gordon *et al.*, 2009).

However, there exists a realm that has yet to be explored and tested in the human. In comparison to motor function, sensory nerve recovery is much more difficult to characterize in the human. While radioactive nerve labeling can trace the speed and precision of recovery in the animal model, no such test is possible in the human. In comparison to motor nerves that can be measured objectively with electromyography (EMG), there are a multitude of sensory functions that are difficult to objectively test in the human. The most frequented tests used in previous studies of surgical outcomes for sensory nerve repair include Semmes-Weinstein Monofilament testing (SWMT) and two-point discrimination (2PD) (Lundborg & Rosen, 2004). However, those tests do not usefully reflect the functional capacity of the hand such as nociception, protective sensation, and vibration.

The purpose of this paper is to review the fundamentals of nerve injury and regeneration, the current outcomes with surgical repair, the evidence behind post-surgical electrical stimulation, and the available methods for comprehensive sensory testing. Based on those observations, I will address two

critical voids in the current literature: accuracy of diagnostic testing for transection injury of sensory nerves and the effects of post-surgical electrical stimulation on functional recovery following digital nerve laceration.

Epidemiology of Nerve Injury in the Upper Extremity

Peripheral nerve injuries in the upper extremity are common. In North America, they have a diverse range of etiologies including blunt and penetrating injuries. While assaults are less common in Canada, in some countries with military conflicts, upper extremity nerve injuries are often associated with far more serious tissue damage.

Good quality data on the incidence and prevalence of peripheral nerve injuries is limited. In Canada, a survey of all trauma patients seen in an urban hospital revealed that 3% had a major component of peripheral nerve injury (Noble *et al.*, 1998). Obstetrical brachial plexus injuries have an incidence in the US of 0.8-1 cases for every 1000 live births with resulting permanent impairment ranging from 3-25% (Gilbert *et al.*, 1999). Nerve transections constitute around 3% of all hand injuries that presented to a major hand trauma unit (Rosberg *et al.*, 2005). The estimated cost of a median nerve injury in the forearm was 51,238 euros, 90% of which was due to a "loss of production or sick leave" (Rosberg *et al.*, 2005). Hence, the cost of nerve injuries extends well beyond the suffering of the individual patients to one that carries major socioeconomic consequences.

A common but relatively minor nerve injury is carpal tunnel syndrome, which is associated with a compression injury of the median nerve at the wrist. The prevalence of this is 2-3% in the general population. Approximately 10% of these patients have to quit their occupations even after surgery. Upper extremity visits at outpatient clinics in the US that are nerve-related total 2.7 million per year, accounting for about 13% of all outpatients visits (Slutsky & Hentz, 2006). Thus, nerve injuries in the upper-extremity consume a tremendous amount of health-care resources, in addition to the huge societal burden.

Digital nerve injuries are also extremely common. In fact, they are the most commonly lacerated peripheral nerve in the western world (Buncke, 1972) at home and at the workplace. A recent epidemiological study on digital nerve injuries showed an estimated incidence of ~1 in 10,000 inhabitants per year with the index finger being the most commonly injured (Thorsen *et al.*, 2012). Perhaps, more importantly, is to note the clinical importance of these nerves as they supply sensation to the fingertips that is amongst the most densely innervated sensate region in the entire body (Purves, 2008). The functional importance of the hand is well reflected by the larger representation on the somatosensory cortex compared to the rest of the body. Not only that, hand and digital pulp sensation is vital to most vocational and recreational tasks. In addition to having significant negative impact on quality of life, the economic cost of a digital nerve injury estimated in the range of 3000 euro is substantial with a median 59 days of sick leave (Thorsen *et al.*, 2012). All these form a

compelling argument that improvements in the repair, regeneration and functional recovery of these injuries are urgently needed.

Anatomy and Pathophysiology of Nerve Injury and Regeneration

Anatomy of the Nervous System

The human nervous system is divided into the central (spinal cord and cerebrum) and peripheral nervous systems. The function of the peripheral nervous system (PNS) is to connect the central nervous system to muscles and sensory organs. It includes the cranial nerves, the spinal nerves with their roots and rami, the peripheral nerves, and the peripheral components of the autonomic nervous system. Sensory nerves convey information via receptors in the skin, muscle, tendon and joints toward the brain. Motor fibers carry information from the brain to the end plates of skeletal muscle to affect motion.

The functional unit in the nervous system is a neuron, which consists of a cell body with its dendrites and an elongated axon projecting out to a target organ. The cell bodies of motor neurons exist in the ventral horn of the spinal cord, whereas those of the sensory neuron reside in the dorsal root ganglia (DRG) just outside the spinal cord. Myelinated axons are enveloped by Schwann cell (SC) sheaths contained within a basal lamina and basement membrane. This allows marked acceleration and synchronization of conduction through "saltatory conduction" (see Figure 1-1). Demyelination leads to decreased conduction velocity and in some cases conduction blockade.

Neurons are surrounded by supporting cells which are called neuroglia. In the peripheral nervous system these are the SCs, whose best known function is to myelinate peripheral axons. In the central nervous system (CNS) that role is performed by oligodendrocytes. Other neuroglia that are present in the CNS are the astrocytes and microglia, that have a role in chemical maintenance and immune response, respectively.

Finally, there is the connective tissue that serves as the glue for a peripheral nerve branch. The endoneurium is the innermost layer that provides support for nerve fibres. The perineurium is responsible for maintaining the physiologic balance of the conducting elements in the axon. When this layer is breached, conduction is impeded or blocked altogether. The epineurium contains blood vessels and protects the nerve against compression. It accounts for 60-85% of the cross-sectional area of the nerve (Birch, 2011). Finally, the adventitial mesoneurium conveys external blood supply to the nerve branches and is responsible for enabling gliding of the nerve (see Figure 1-2).

The Somatosensory System

The human senses encompass a multitude of organs that transduce and enable gustation, hearing, olfaction, vision, somatosensation and vestibular balance. We shall focus on somatosensory functions in the glabrous and hairy skin in the human. It is known that the density of skin somatosensory receptors is highest in the human hand so that refined texture identification and delicate precise tasks can be performed.

The somatic senses include touch, vibration, pressure, proprioception, pain and temperature. Each sense function utilizes a different sensory receptor located within different depths of the skin.

Touch or “cutaneous displacement” is conveyed in the non-glabrous skin of the hand by four main receptors: Meissner corpuscle and Merkel cells in the epidermis, Ruffini endings in the dermis and the Pacinian corpuscle in the subdermal tissue (see Figure 1-3). Whereas the Meissner corpuscle and the Merkel cells have a small receptive field size the Ruffini ending and Pacinian corpuscle have large receptive field sizes as they are situated deeper in the skin. The Meissner and Pacinian corpuscles are called rapidly adapting receptors while the Merkel cell and Ruffini ending are slowly adapting. Sensations are transmitted via these receptors to nerves, the spinal cord and the brain to convey information about the external environment. The axons that carry information regarding touch, pressure and vibration are the Aβ fibres with axon diameters of 6-12 microns and relatively fast conduction velocity of 35-75 m/s (See Figure 1-4).

In contrast, there are no specific receptors that transduce nociceptive information (pain and temperature sense) to their respective afferents. They merely rely on free nerve endings of unmyelinated C fibres in the epidermis and myelinated Aδ fibres in the dermis. There are a variety of nociceptors including mechanical nociceptors (Aδ) fibres, polymodal (sensitive to mechanical, thermal and chemical stimuli) nociceptors (C fibres), other mechanical nociceptors (C

fibres), mechanoheat nociceptors (Aδ fibres), and cold nociceptors (Aδ or C fibres) (Zochodne, 2008). Whereas the small myelinated Aδ fibres have an axon diameter of 1-5 microns and conduction velocity of 5-30m/s, the nonmyelinated C fibres have axon diamters of 0.2-1.5 microns and even slower conduction velocity of 0.5-2m/s.

In muscles and joints there are other afferents that transduce information regarding proprioception. Muscle spindle afferents (Group Ia, II) fibres carry somatosensory information for muscle length and velocity of movement and Golgi tendon organs carry information regarding muscle force (Group Ib). These fibres are amongst the largest caliber afferents with 13-20 micron axon diameter and extremely fast conduction velocity of 80-120 m/s in some animal species.

This fundamental knowledge of the somatosensory system is required to understand the different methods of sensory testing that are employed to measure deficits and follow recovery of sensation after nerve injury.

Nerve Injury

Nerve injury classification was popularized by Sunderland and Seddon. Seddon classified nerve injury into neurapraxia, axonotmesis, and neurotmesis. Neurapraxia is described as an injury secondary to blunt nerve trauma that results in focal demyelination of several internodes at the injury site. In order for there to be a disruption of nerve function, there must be enough demyelination

or ion channel dysfunction to produce a conduction block. Prolonged mechanical compression of the nerve at the injury site can often cause focal demyelination. Following this injury the local SCs and circulating macrophages phagocytose the damaged myelin. The resultant gap of demyelination produces a conduction block that results in nerve palsy symptomatology (Zochodne, 2008). Subsequently, the neighboring SCs remyelinate the demyelinated site and remodel it to return to previous internodal distance.

Axonotmesis is defined as a blunt injury that results in disruption of axons in the nerve branch but no disruption of the epineurium and connective tissues surrounding it. This means that the portion of nerve proximal to the injury site and the portion distal remain continuous. In this case, because the axons have been disrupted the portion distal to the injury site undergoes Wallerian degeneration, which is break-down of the distal myelin and axons (Zochodne, 2008).

Neurotmesis is the most severe of injuries, whereby the entire nerve trunk (including epineurium and connective tissue) has been severed (Birch, 2011). The etiology of this injury is usually from a sharp penetrating injury, an adjacent fracture fragment or a surgical iatrogenic injury (Slutsky & Hentz, 2006). The issue with this injury is that there is no continuity between the proximal and distal segments of the injury site and as such, the nerve ends retract and produce a significant nerve gap. In order for this nerve to successfully regenerate and

reinnervate native target receptors or muscles, connective tissue and axons must regrow to fill the gap or surgery must be performed to coapt the nerve ends.

Sunderland similarly classified nerve injuries into five degrees of severity based on the specific layer of nerve involved. A first-degree injury is similar to neurapraxia in that there is no disruption of neuronal integrity and thus, no Wallerian degeneration. Second-degree injury is similar to axonotmesis in that there is axonal damage but the endoneurium and basal lamina are not disrupted. Third-degree injuries involve endoneurial disruption but the perineurium is unscathed. Fourth-degree injury disrupts all layers of the nerve except for the epineurium. Finally, fifth degree injury is similar to neurotmesis in that there is complete nerve transection (Zochodne, 2008). Recent investigators have suggested a modification of a sixth degree injury: either a combination of any of the first five degrees or otherwise classified as a neuroma in-continuity (Mackinnon *et al.*, 1992).

Cell Body Response to Nerve Injury

After nerve crush injury or axotomy the neuron cell body undergoes a series of structural changes termed “chromatolysis” which consists of nuclear eccentricity, nucleolar swelling and dissolution of Nissl bodies (Zochodne, 2008). Following this, the neuron may either undergo apoptosis or survive and regenerate. However, the underlying molecular mechanism whether the neuron

survives or undergoes cell death is not fully known. If the neuron survives, it is the response of the cell body that allows it to ultimately regenerate.

Neurotrophic factors have been implicated in both neuron survival as well as regeneration. These factors can come from the distal nerve stump, the injured neuron or the surrounding glial cells. These neurotrophic factors induce the production of many regeneration associated proteins including tubulin, actin, calcitonin-gene related peptide (CGRP), growth associated protein 43 (GAP 43), and other growth associated proteins (Fu & Gordon, 1997).

In addition, axonal injury upregulates several signaling neuropeptides including CGRP in regenerating motor neurons and vasoactive intestinal peptide (VIP) in sensory axons (Grafstein, 1975). VIP has been shown to increase blood supply to regenerating axons and CGRP is involved in sustaining the inflammatory response required for regeneration (Said & Mutt, 1970). In addition to this, both VIP and CGRP may be involved in supporting glial cell function by increasing cyclic adenosine monophosphate (cAMP) that potentiates the effects of mitogenic growth factors on SCs and blood vessels (Cheng *et al.*, 1995). These factors include fibroblast growth factor (FGF), glial growth factor (GGF), and platelet derived growth factor (PDGF), which are released from damaged axons, platelets, SCs, and circulating macrophages.

Axomotized neurons also synthesize and release cytokines that potentiate the inflammatory response and act synergistically with other cytokines released by macrophages and non-neuronal cells. These include

interleukin-1 (IL-1), IL-2, IL-6, transforming growth factor-β (TGF-β), and interferon-gamma (IFN-ϒ) (Kilmer & Carlsen, 1987;Murphy *et al.*, 1995). All these cytokines are mitogenic for SCs and help to determine their phenotype (See Figure 1-5).

Macrophages and microglia are other sources of cytokine release in nerve injury. These two cell lines proliferate following nerve injury and may participate in the cell body reaction (Perry *et al.*, 1987). Macrophages induce the release of leukemia inhibitory factor (LIF) from glial cells via IL-1 expression and astro/microglia upregulate neurotrophic factors via IL-6 and TGF-β expression (Rao *et al.*, 1993;Kiefer *et al.*, 1993;Murphy *et al.*, 1995).

Wallerian Degeneration and the Distal Nerve Environment

After an irreversible axonal injury, the nerve segment distal to the injury site undergoes degeneration termed Wallerian degeneration. The evolutionary reason for this is that in order for successful regeneration to occur, a permissive environment with disposed debris and appropriate biochemical support must exist. In Wallerian degeneration (See Figure 1-6), a specific signal triggers the breakdown of axons and myelin. This was initially characterized by Augustus Waller in 1850 when he observed axotomized hypoglossal and glossopharyngeal nerves of frogs and noticed that the myelin, which he termed “medulla,” disintegrated in the distal segment shortly after injury (Zochodne, 2008). A cascade of events involving calcium-dependent proteases leads to disruption of

microtubules, neurofilament dissolution, axon and myelin breakdown, and finally phagocytosis of debris by circulating macrophages and local SCs (Zochodne, 2008). This is essential because the nerve and myelin debris are inhibitory to nerve regrowth and must be removed.

In the first two days after nerve injury, SCs are the major agents of phagocytosis. Within the first few hours after injury, SCs express a myelin protein called Mac-2, which mediates *non-immune* opsonin-dependent phagocytosis (Reichert *et al.*, 1994). At two to three days after injury, macrophages invade the distal environment and express Mac-1, Mac-2 and Fc receptor, which mediate *immune* opsonin-dependent phagocytosis (Reichert *et al.*, 1994). In addition to phagocytosis, macrophages release multiple growth factors and cytokines that stimulate SCs and fibroblasts, cell-adhesion molecule production, and endothelial cells. Previous studies have shown that exogenous macrophages increases axonal outgrowth, which speaks to the integral role they play in successful nerve regeneration (Stolz *et al.*, 1991). In addition, macrophages secrete cAMP dependent cytokines including PDGF, FGF, and TGF-β. TGF-β has been shown to induce SCs to switch to their pre-myelinating status via the down-regulation of p75 and regulation of myelin related proteins (Carey *et al.*, 1986;Jessen *et al.*, 1991;Mews & Meyer, 1993;Morgan *et al.*, 1991;Raff *et al.*, 1978). SCs will go beyond initial phagocytosis and proliferate when macrophages are stimulated and there is a disruption of axonal contact. In this proliferation process, they multiply and fill the once-emptied distal endoneurial sheath

forming longitudinal bands called Bands of Büngner (Zochodne, 2008) (See Figure 1-7). Subsequently, SCs switch from a myelination state to a non-myelination state because myelin associated genes such as myelin protein zero (P0), myelin basic protein (MBP), myelin associated glycoprotein (MAG), and peripheral myelin protein-22 (PMP-22) are down-regulated (De *et al.*, 1991;LeBlanc & Poduslo, 1990;Trapp *et al.*, 1988). In this non-myelination state, SCs upregulate multiple growth factors including nerve growth factor (NGF), neurotrophin 4/5 (NT-4/5), brain derived neurotrophic factor (BDNF), endothelial growth factor (EGF), insulin like growth factor (IGF) 1 and 2 and glia-derived neurotrophic factor (GDNF) (Fu & Gordon, 1997). Once regenerating axons from the proximal stump enter the distal stump, there is a second-phase of SC proliferation which results in a 3-fold increase in numbers to remyelinate the elongating axon (Pellegrino & Spencer, 1985).

There are several growth factors active in the distal nerve stump that prevent apoptosis and assist with SC migration and adhesion to the regenerating axon projections. These include NGF, BDNF, neurotrophin-3 (NT-3), and NT-4/5 (Funakoshi *et al.*, 1993;Meyer *et al.*, 1992;Boyd & Gordon, 2003). In addition, IL-6 activates macrophages to enhance nerve regeneration and IGF 1 and 2, FGF, PDGF, and GDNF all play a neurotrophic role to enhance the distal environment for regeneration.

Finally, cell adhesion molecules and basement membrane components are integral in contact guidance of growth cones (Davis *et al.*, 1987;Davis *et al.*,

1986;Manthorpe *et al.*, 1983). Molecules such as glycoprotein L1, neural cell adhesion molecule (N-CAM), N-cadherin, laminin, and tenascin are upregulated by SCs and aid in axon adhesion and neuronal outgrowth (Doherty *et al.*, 1995;Martini & Schachner, 1986).

The Proximal Nerve Stump

Following nerve injury, the nerve stump proximal to the injury site degenerates back to at least the first or second node of Ranvier in a process called "traumatic degeneration" (Zochodne, 2008). After this, growth cones begin to form with somewhere between fifty to one hundred axon branches arising from the node of Ranvier. The development of the growth cone is not dependent on the cell body but on local factors and elements surrounding the axon (See Figure 1-7). In vitro studies have shown that if there is continued axonal transport, even isolated axons can continue to support the formation of growth cones (Bray *et al.*, 1978). However, the building blocks required for elongation of the axon beyond the growth cone comes from the proteins synthesized in the cell body and transported down the regenerating axon (Davis *et al.*, 1992). The initial axon elongation is slow for the first three days in a period called slow-staggered regeneration but increases to a constant rate by the third day post-injury. The rate of axonal elongation is limited by the rate that cytoskeletal proteins can be transported down the axon to the growth cone (Cleveland & Hoffman, 1991;Grafstein, 1971;Hoffman & Lasek, 1980;Hoffman &

Lasek, 1980). The fate of the growth cone is dictated by the environment distal to the injury site. If the environment is not supportive, the growth cone grows in a spiral formation yielding a neuroma (a swollen nerve ending as a result of ineffective uncontrolled regeneration) (Sunderland, 1978). On the flipside, if it is supportive, growth cones grow toward the distal nerve stump and once reaching it form numerous fine nerve fibers that grow distally. These axonal sprouts are termed “regenerating units”, which remain in the distal stump until they make connections with target receptors (Morris *et al.*, 1972). In a process that can take months to years, all other regenerating units are withdrawn once a single unit makes a target connection. Until the regenerating axon reaches a functional target connection, it does not reach its normal diameter (Gordon & Stein, 1982). In humans, the rate of axonal regeneration is between 1-3mm/day after the first three days of slow-staggered regeneration (Grafstein, 1971;Hoffman & Lasek, 1980;Hoke, 2006). Once the growth cone reaches the distal nerve stump it stimulates SCs to proliferate and myelinate the newly formed regenerating axon. The ultimate extent of myelination depends on the size of the outgrowing axon (Hildebrand *et al.*, 1986;Simpson & Young, 1945). Although SCs are primarily responsible for myelination, they initially myelinate with short inter-nodal distances that result in slower conduction speeds. Over time, these distances are rearranged and remodeled to become longer and resume normal conduction speeds.

Neurotrophic Factors and Axonal Growth

Neurotrophic factors such as NGF, BDNF, and NT-3 are critical in contributing to neuron survival after injury as well as inducing axon growth via a "conserved cell polarity signaling pathway in vitro" (Koliatsos *et al.*, 1993;Miyata *et al.*, 1986). These factors induce a tightly regulated activation of PI3-kinase at the growth cone, which phosphorylates GSK-3β and promotes axon growth via cytoskeleton protein-binding regulation (Yoshimura *et al.*, 2005;Zhou *et al.*, 2004). This effect on axonal growth is similar to the signaling pathway of laminin (Arimura & Kaibuchi, 2005;Menager *et al.*, 2004) and in the case of NGF, laminin works synergistically to enhance axon growth of dorsal root ganglion neurons (Lentz *et al.*, 1999;Liu *et al.*, 2002). Neurotrophins such as BDNF and NGF are upregulated after peripheral nerve injury but their role in axon regeneration is still not fully understood (Makwana & Raivich, 2005). Previous studies by Boyd and Gordon showed that low-dose application of BDNF promoted axon regeneration in chronic axotomy lesions but not acute nerve lesions (Boyd & Gordon, 2002a). However, high-dose BDNF in fact inhibited regeneration in both chronic and acute nerve injuries (Boyd & Gordon, 2002a).

Schwann Cell Response

As already mentioned, SCs dedifferentiate and assume a non-myelination state, proliferate, then resume myelination state during peripheral nerve

regeneration. There are three major agents that regulate these events: extra-cellular matrix (ECM) proteins, neurotrophic factors, and hormones.

i) ECM Proteins

Laminin is one of the most important ECM proteins involved in axon remyelination after nerve injury. Laminins are primary components of the SC basal lamina and are required for proper ensheathing during myelination. Disruption of laminins has been shown to result in severe hypomyelination (Yang *et al.*, 2005;Yu *et al.*, 2005). In fact, laminin-deficiency results in SC arrest in the premyelination state and ultimately, impaired SC proliferation and survival (Yu *et al.*, 2005). As SCs dedifferentiate immediately after nerve injury, laminins are also downregulated. In the later stages of axon regeneration, as SCs proliferate to remyelinate the regenerated axons, the laminins are also progressively upregulated (Masaki *et al.*, 2000). Studies have also suggested that remyelinating SCs upregulate the expression of laminin receptors β1-integrin and dystroglycan (Masaki *et al.*, 2000). Feltri and Saito independently studied these receptors concluding that β1-integrin is critical for axonal sorting at the promyelinating state, whereas dystroglycan is required later for maintenance of the myelin sheath (Saito *et al.*, 2003;Feltri *et al.*, 2002).

The other ECM protein that is involved in the SC response is the plasminogen-activator (PA) cascade known best in the process of fibrinolysis. Two types of PAs exist in mammals: tissue-type (tPA) and urokinase-type (uPA).

There are specific serine proteases that convert zymogen plasminogen to plasmin. Peripheral neurons and SCs secrete plasminogen activators (Krystosek & Seeds, 1984). Administration of exogenous tPA to injured sciatic nerves resulted in improved axon regeneration, remyelination and functional recovery (Zou *et al.*, 2006). This suggests that tPA plays a protective role after nerve injury and the potential mechanism is via the fibrinolytic activity of plasmin. After nerve injury, fibrinogen is deposited into peripheral nerve endoneurium (where normally it does not exist) and is converted to fibrin. This fibrin deposition inhibits SC migration and remyelination (Akassoglou *et al.*, 2002). Additionally, fibrin in the SC endoneurium triggers ERK1/2 phosphorylation and downregulates gene expression involved in myelin production resulting in SCs arrested in predifferentiation proliferation state (Akassoglou *et al.*, 2002). Hence, tPA produced by SCs activates fibrinolysis and allows SCs to return to their remyelinating state.

ii) Neurotrophic Factors

Neurotrophic factors and their receptors are involved in nerve survival, axon regeneration, as well as SC differentiation and remyelination. The neurotrophin family includes NGF, BDNF, NT-3, NT-4/5, and NT-6 (Notterpek, 2003). Neurotrophins bind with high affinity to tyrosine receptor kinases (Trk) and with low affinity to the nerve growth factor receptor p75 (Chao, 2003). p75 binds all the neurotrophins with similar affinity and acts as a coreceptor for the

Trk receptors. There are three different types of Trk receptors, each of which binds a particular neurotrophin. TrkA selectively binds NGF, TrkB is specific for BDNF and NT-4/5, and TrkC preferentially binds NT-3.

Most knowledge is known regarding NGF binding TrkA from the original studies done by Levi-Montalcini in 1953 showing enhanced neuronal survival and outgrowth in sensory neurons (Cohen *et al.*, 1954). In fact, TrkA receptors are only present on sensory neurons and not on motor neurons. NGF is usually in low concentrations in a healthy nerve environment, but is substantially upregulated in the distal nerve stump when there is nerve injury. It is thought that NGF exerts a direct influence on sensory nerve survival and regeneration but is also an indirect influence on motor neuron regeneration via non-neuronal cells. NGF also potentiates the migration of SCs in the regeneration process as well as increasing angiogenesis to the site of regeneration (Chen *et al.*, 1989).

BDNF is a neurotrophin known to preferentially support the survival of motor neurons following axotomy (Lundborg, 2000). BDNF acts via binding TrkB, TrkC and p75 receptors. Studies have shown increased regeneration of motor neurons with application of exogenous BDNF in low doses but inhibition of regeneration in high doses (Boyd & Gordon, 2002a). This effect is due to differential effects when BDNF binds different receptors. It was found that when antibodies were introduced to block the p75 receptor, the inhibitory effects of high-dose BDNF were ameliorated, indicating that p75 has an inhibitory role in motor neuron regeneration (Boyd & Gordon, 2002a).

When NT-3 binds TrkC receptors, there is enhanced motor neuron survival and growth in vitro (Henderson *et al.*, 1993). When NT-4/5 binds TrkB receptors, studies have shown an increased ability to innervate skeletal muscle fibers (Yin *et al.*, 1998).

One interesting point is the role of neurotrophins in axon regeneration can be summated by their differential expression after nerve injury. Following nerve injury, p75 mRNA and BDNF are upregulated in the distal nerve stump, whereas NT-3 mRNA levels return to baseline levels at two weeks (Funakoshi *et al.*, 1993;Meyer *et al.*, 1992). As was mentioned before, it was classically thought that neurotrophins affect primarily the survival and differentiation of regenerating neurons. However, recent studies have revealed that neurotrophins play a large role in regulating SC myelination as well (Cosgaya *et al.*, 2002;Chan *et al.*, 2001). Results from Chan et *al.* confirmed that exogenous BDNF enhances myelination and exogenous NT-3 inhibits myelination (Chan *et al.*, 2001). This would explain the necessary increase of BDNF and decrease in NT-3 post-injury to allow for an environment conducive to remyelination of the regeneration axon before neurotrophin levels return to baseline equilibrium. Song et al. and Zhang et al. have subsequently showed depletion of BDNF results in smaller and less axon regrowth and deficiency in p75 results in less myelinated axons and a thinned myelin sheath (Zhang *et al.*, 2000;Song *et al.*, 2006).

Other neurotrophic factors involved in nerve regeneration include basic fibroblast growth factor (FGF-2), GDNF, neuregulin-1 (NRG1), and TGF-β.

In addition to supporting neurite outgrowth and sensory neuron survival, FGF-2 is a potent SC mitogen and inhibitor of P0 contributing to arrest of SC differentiation and inducing proliferation after nerve injury (Davis & Stroobant, 1990;Morgan *et al.*, 1994).

GDNF is also upregulated in the distal nerve segment post-injury and is involved in enhancing neuron survival and axon outgrowth (Naveilhan *et al.*, 1997). However, Hoke and Iwase also revealed that GDNF stimulated SC proliferation and migration to enhance myelination (Hoke *et al.*, 2003;Iwase *et al.*, 2005).

NRG1 are a family of proteins that are also involved in nerve regeneration and SC myelination. Out of the three major types of NRG1, type III is the class that is responsible for SC tropism which is mediated by the tyrosine receptor kinase ErbB2 and ErbB3. After nerve injury, NRG1 and their receptors ErbB2/B3 are upregulated and result in increased SC migration and neurite outgrowth (Carroll *et al.*, 1997;Mahanthappa *et al.*, 1996).

TGF-β is required for the maintenance of the nonmyelinating, proliferating state of SCs during axon development. However, it is upregulated post-injury to prevent early myelination by blocking expression of its downstream protooncogene Ski (Atanasoski *et al.*, 2004).

iii) Hormones

Progesterone, thyroid hormone and erythropoietin (Epo) are several hormones that are implicated in SC regulation of nerve regeneration. Progesterone is thought to enhance myelination via stimulaton of promoters for PMP22 and P0 genes (Desarnaud *et al.*, 1998) as well as activating transcription factors for myelination (Guennoun *et al.*, 2001;Mercier *et al.*, 2001). Thyroid hormone increases axon number, diameter of remyelinated axons, and myelin thickness due to increased expression of the nuclear triiodothyronine receptors (NT3R) (Barakat-Walter *et al.*, 1992;Voinesco *et al.*, 1998). In addition, parathyroid hormone-related peptide (PTHrP) stimulates SC migration but it is unknown what its exact role in nerve regeneration is. Finally, Epo and erythropoietin receptors (EpoR) are expressed in increased concentrations after nerve injury in rats (Li *et al.*, 2005). Exogenous Epo stimulates SC proliferation via the MAPK (mitogen-activated protein kinase) (Li *et al.*, 2005).

Reinnervation of the Skin from the Regenerating Axon

The mechanisms that dictate the reinnervation of the skin and it's receptors by the regenerating axon are not fully understood. It is assumed that once the axon reaches the vicinity of the skin, certain guidance cues and trophic factors allow precise reinnervation throughout the dermis and epidermis. However, from the outcomes of nerve reinnervation, it is obvious that there are some barriers to accurate and complete reinnervation. This is true because

reinnervation of skin is never complete after transections and abnormal sensory function may persist for long periods of time (Dubovy & Aldskogius, 1996). Examples of this abnormal function include parathesias or a tingling sensation, cold hypersensitivity or misrepresentations of innocuous stimuli termed allodynia. There are likely differences in the regeneration rate of different types of skin afferents. The small myelinated and non-myelinated fibres seem to reinnervate much more effectively than the large myelinated fibres. There is a large amount of redundancy in the reinnervation of the Pacinian corpuscle because of its large receptive field size. Additionally, Meissner corpuscles appear deeper in the skin than their original position (Munger, 1988). The reasons for these differences are likely due to differences in trophic factors and guidance cues that are necessary for each fiber type to find its native receptor. All in all, sensory nerve recovery after axotomy is not perfect and there are many gaps that exist in the literature regarding the reasons why.

History and Current Surgical Nerve Repair

Technological advances in the surgical repair of nerve transections have improved a great deal over the last 75 years. Preliminary findings by the British neurologist Henry Head in the early 1900s showed that surgical coaptation of severed nerve ends facilitated the speed and precision of peripheral nerve recovery and regeneration (Purves, 2008). After World War I, the fundamental goal in nerve repair was coaptation of the two nerve ends at any cost. As a

result, many maneuvers were utilized to achieve this end such as lengthy joint immobilization, bone shortening, extreme joint flexion and the use of high-calibre suture material (Slutsky & Hentz, 2006). The functional outcomes of this time were very poor due to the excessive tension at the repair site.

Hence, during and after World War II, there was a focus on improving outcomes following surgery. The timing of repair and techniques were modified in order to improve outcomes, which spurred on research and advancement in the area of primary nerve repair up to the present day (Slutsky & Hentz, 2006).

Primary nerve repair is defined as suturing a transected nerve within 2 weeks after initial injury. Immediate primary repair involves suturing the severed nerve at the time of diagnosis. Delayed primary repair is performing the nerve repair as an elective procedure within 2 weeks after the initial diagnosis. Delayed primary repair offers many advantages including allowing the patient to be counseled regarding the nature of the injury and proposed treatment, performing the procedure with the preferred anesthetic, excision of any devitalized tissue, and a decreased chance of infection (Slutsky & Hentz, 2006).

There are two anatomical structures that are pertinent to a nerve repair: fascicles and the epineurium. The fascicles include the axons and their SC sheaths. This includes the perineurium that binds each fascicle and provides a diffusion barrier and pressure gradient. On the outside of the fascicles is the epineurium, which contains collagen and larger blood vessels that provide structural integrity to the nerve. The epineurium is the medium by which surgery

is preferably performed. It can be incised, excised or sutured in order to prepare nerve ends or complete the coaptation. This way, the fascicle is minimally damaged so as to avoid rendering the nerve non-functional due to a conduction blockade (Slutsky & Hentz, 2006) (see Figure 1-8).

The indications for primary nerve repair include a clean wound with tidy nerve endings that have been sharply transected. If the nerve endings are untidy, trimming them can allow a tension-free repair to still be performed. However, if the mechanism of injury is due to crush or stretch then it is usually wise to perform a wound cleansing with debridement and perform the nerve repair several days later.

The current gold standard for an ideal nerve repair is described by Slutsky as primary repair – immediate or delayed – in a well-vascularized bed with no scarring. The nerve ends are preferably viable and sharply transected. There should be no hematoma present. Accurate fascicular alignment should be achieved with loupe or surgical microscopic magnification and the fewest number of small caliber (9-0 to 11-0) nylon sutures placed in the epineurium only. Finally, there should be minimal tension and minimal joint flexion to achieve this (Slutsky & Hentz, 2006).

When there is excessive tension upon approximating the cut nerve ends, the surgeon can trial moderate flexion at a joint in order to relieve the tension. However, if the tension is still excessive, other techniques must be used to bridge the gap and relieve the tension. The two most common methods of

achieving this are by way of nerve grafts and nerve conduits. However, these techniques are beyond the scope of this study.

Outcomes of Current Surgical Nerve Repair

Despite advances in surgical technique, suturing material and microscopic assistance, the functional outcomes of current surgical nerve repair are still limited. Most nerves that are repaired in the upper extremity have mixed origin and include both sensory and motor innervation. From a surgical standpoint, a grading scale called the Medical Research Council System is used to follow nerve recovery after repair (See Table 1-1). The grading of these results are then transmitted to a score of good, fair, poor or bad (Birch, 2011) (See Table 1-2). In addition to motor and sensory recovery, Rosen and Lundborg included a third parameter to measure recovery, which was pain (Rosen & Lundborg, 2000). This is referred to as hypersensitivity or cold intolerance experienced by the patient during recovery.

In general, several criteria affect the overall prognosis of nerve repair. It has been shown that near normal function has been achieved with immediate repair of median or ulnar nerves in infants or young children. The more distal the injury, the smaller the distance the nerve needs to reinnervate to reach the target organ, and the better the recovery. The same applies to nerve fibres that innervate less muscles or skin. Omer showed that for every six day delay after surgery, 1% of maximal functional recovery is lost (Omer, Jr., 1974).

Birch et al. described 165 ulnar nerves that were repaired with only 42% of patients achieving excellent or good sensory and motor recovery (Birch, 2011). In the same study, 134 median nerves were repaired with only 44% of patients achieving excellent or good recovery (Birch, 2011). Birch and Raji discussed 108 median and ulnar nerve repairs and compared results showing that on the whole, the primary nerve repair achieved better results (Birch & Raji, 1991). Repairs of the radial nerve show no better results. Shergill et al. described 242 radial nerve repairs with only 30% of patients achieving a good recovery and 28% achieving fair results (Shergill *et al.*, 2001).

While palmar cutaneous digital nerves are mostly sensory in nature, no known nerve is purely motor or sensory. In addition to sensory afferents from the median and ulnar distribution, digital nerves also have post-ganglionic sympathetic efferent fibers. Nevertheless, there is much less complexity of reinnervation when there is no motor component. Hence, we should expect that the results of digital cutaneous nerve repairs to be quite good due to the former reason and the short distance of axonal regeneration required for them to reach their target skin receptors. However, Coates et al. showed a series of 74 adults with digital nerve repaired within 48 hours of injury and only 45% of patients achieved good or better recovery. If the repair was performed two weeks or later only 32% of patients achieved a good result (Goldie *et al.*, 1992). Forty percent of patients sustained persistent hyperesthesia after two years and the authors stated that they think that “normal sensation will never be regained” (Birch,

2011). Kallio did report some better results as 80% of primary suture repairs achieved "useful" levels and most children did much better than adults (Kallio, 1993). However, in a review of studies dating 1985 to 2000, Allan described how satisfactory sensation (two-point discrimination $\leq$ 10mm) was achieved in only 50% of patients (Allan, 2004a). It is surprising that on the whole, an anatomic median nerve repair at the wrist achieves better sensory recovery than a similarly repaired digital nerve, which is further distal.

Adjuvants to Surgery that Augment Nerve Regeneration

As functional results with surgery alone have reached an impasse, investigators are looking at adjuvants to surgery that may augment axon regeneration. There are two main molecular pathways that promote the process of regeneration: Trk Receptor signaling events and cAMP signaling.

Early research on the rat pheochromocytoma PC12 cell line was fundamental in establishing the effects of neurotrophin signaling events (Boyd & Gordon, 2003). In this model, nerve growth factor (NGF) binds trkA receptor to affect differentiation into cells resembling adult sympathetic neurons. When neurotrophins bind their corresponding trk receptor, the receptor dimerizes and activates several intracellular signaling cascades that stimulate neuronal survival, growth and regeneration. Some of these pathways include the cAMP, P13K-Akt and Ras-Erk signaling cascades (Boyd & Gordon, 2003).

Cyclic AMP is an important mediator for many neuronal processes. Recently, it was shown that neurite outgrowth was increased in motoneurons when intracellular cAMP is upregulated (Udina *et al.*, 2010). Also, downregulating cAMP results in a profound decrease in neurite outgrowth (Aglah *et al.*, 2008) (See Figure 1-9).

In the following paragraphs, I will describe some of the different interventions that have been investigated in order to promote peripheral nerve regeneration. Chondroitinase is an enzyme that degrades chondroitin sulfate proteoglycans (CSPGs), which are myelin proteins in the PNS and CNS that inhibit axonal outgrowth (Hamel *et al.*, 2008). Udina et al. showed that chondroitinase administration following repair of transected rat peroneal nerve increased the number of regenerating motor and sensory neurons across the repair site (Udina *et al.*, 2010)

Fibroblast growth factors (FGF), which exist in the acidic (FGF-1) and basic form (FGF-2), are multifunctional growth factors with a wide variety of effects including angiogenesis, wound healing, nerve development and for our purposes, nerve regeneration. Grothe et al. showed that FGF-2 protein and mRNA was upregulated following nerve injury (Grothe *et al.*, 2006). Haastert et al. showed that rats treated with genetically modified Schwann cells expressing low levels of FGF-2 had increased levels of regeneration associated proteins (GAP43 and SYN-1) as well as increased myelinated axons (Haastert *et al.*, 2008).

Following peripheral nerve injury, Rho A GTPase and Rho kinase are proteins that negatively modulate neurite outgrowth. Madura showed that post axotomy and surgical repair of rat peroneal nerves, administration of the Rho-kinase inhibitor Fasudil increased the density, caliber and number of regenerating nerve fibres as well as improved the functional recovery according to the peroneal functional index (Madura *et al.*, 2007). The theory is that Fasudil prevents the collapse of growth cones to promote the regenerative process and prevents the migration of neutrophils to protect the proximal nerve stump from inflammatory damage (Madura *et al.*, 2007).

As was mentioned above, cAMP is critical in mediating neurite outgrowth. Rolipram is an inhibitor of phosphodiesterase-4 (PDE-4), which is the most common PDE found in neural tissue. In preventing the decline of cAMP, this agent has been shown to increase myelination and improve functional recovery post-nerve injury and repair (Pearse *et al.*, 2002). Most recently, Rolipram treated rats with transected and repaired common peroneal nerves had increased numbers of motor and sensory nerves regenerating across the repair site (Udina *et al.*, 2010).

Agents have also been investigated to promote the survival of neurons targeting the P13K-Akt and Ras-Erk signaling pathways. These treatments include acetyl-L-carnitine to enhance NGF binding capacity, erythropoietin (EPO) to prevent apoptosis and N-acetyl cysteine (NAC) as a free radical scavenger in neuronal cells (Taglialatela *et al.*, 1992;Hoke & Keswani, 2005;Yan *et al.*, 1995).

Amongst all of the interventions described to promote neuronal regeneration, one of the most promising is post-surgical low-frequency electrical stimulation.

Electrical Stimulation to Enhance Nerve Regeneration

Electrical stimulation (ES) of repaired transected motor and sensory nerves has been studied extensively in the rat model. ES promotes nerve regeneration by activating pathways in three regions: Schwann cells, inflammatory cells and the cell body of nerves. Wang et al. showed that neurotrophic factors BDNF, NT-3 and NT-4 were upregulated following ES (Wang *et al.*, 2009). Other molecules that play a crucial role in nerve regeneration such as tubulin, GAP-43 and cAMP are also increased post-ES (Al-Majed *et al.*, 2004;Udina *et al.*, 2008) (See Figure 1-9). Interestingly, it seems that without an effect on the cell body the potency of ES to promote regeneration is eliminated. This was demonstrated when the sodium channel blocker tetrodotoxin (TTX) was administered and completely blunted the growth effects of ES (Al-Majed *et al.*, 2000c).

Al-Majed et al. showed that following repair of transected rat femoral nerves, one hour of 20Hz electrical stimulation on the proximal nerve stump accelerated the slow-staggered regeneration across the repair site as well as preferential motor reinnervation (Al-Majed *et al.*, 2000c;Al-Majed *et al.*, 2000a). In 2000, he subsequently demonstrated that up to 2 weeks of electrical

stimulation post-rat femoral nerve repair could accelerate the staggered regeneration phase and preferential motor reinnervation. In that study, they were able to deduce that the effects of ES were mediated by the cell body to affect an enhanced growth program (Al-Majed *et al.*, 2000c). In a companion study of the same year, the same investigators then showed that brief ES post-transected rat femoral nerve repair upregulated BDNF and its receptor trkB within the first 2 days after surgery (Al-Majed *et al.*, 2000b). Subsequently, Brushart et al. demonstrated in the same model that ES does not increase the speed of regeneration of axons but promotes an earlier onset of axon regeneration (Brushart *et al.*, 2002). A follow-up study in 2004 confirmed that the upregulation of BDNF and trkB from Al-Majed's study had downstream effects of downregulating medium-molecular-weight neurofilament (NFM) which leads to a smaller axon diameter and upregulating Tα1 tubulin and GAP43 via regeneration associated gene expression.

With the early studies predominantly focused on motor reinnervation, in 2005 Brushart et al. showed that brief ES substantially alters the distribution of regenerating sensory nerves so that the random behavior of reinnervation between axon and target tissue is replaced with much more specific reinnervation (Brushart *et al.*, 2005). Geremia et al. performed a pivotal study showing that there was an increase in axonal regeneration in sensory neurons if the period of ES was 1hr, and this effect was blunted if there was ES longer than 1 hr (Geremia *et al.*, 2007). There was also a similar increase in BDNF expression

in the sensory neurons similar to what was found in motor neurons (Geremia *et al.*, 2007;Al-Majed *et al.*, 2000b).

The potential mechanism for the increased regeneration associated gene expression post-ES may lie in the cell body response. For one, Al-Majed and Geremia showed that a sodium channel blockade would null the regenerative effects (Al-Majed *et al.*, 2000c;Geremia *et al.*, 2007). This would eliminate any increased cAMP, BDNF or trkB expression that is seen with increased neuronal activity and blunt neurite outgrowth. The molecular pathways that are upregulated and likely causing the promotion of regeneration with ES are BDNF and cAMP via the cell body as well as the local Schwann cell response (Geremia *et al.*, 2007). However, this SC response is likely not helpful if the cell body response is obliterated, as was seen with tetrodotoxin.

The differential response to ES by motor and sensory nerves has led to subsequent study regarding sensory reinnervation. Udina et al. confirmed that ES in fact does upregulate intracellular cAMP and this leads to promoting axon outgrowth into the repair site but not elongation across the repair site (Udina *et al.*, 2008). One author suggests that ES stimulates nerve cell activity and this upregulates BDNF and the pro-regenerative associated genes (Asensio-Pinilla *et al.*, 2009).

Recently, Geremia et al. showed that endogenous BDNF is necessary to induce the cell body response to promote neurite outgrowth in peripheral nerve injury. However, the *induction* of the cell body response is all that is necessary to

sustain an increased sensory regenerative response, as BDNF does not need to be persistently upregulated in order for the regenerative response to be sustained (Geremia *et al.*, 2010). This could explain why a short period of ES immediately post-nerve repair may benefit the regenerative response in sensory nerve but not if it is extended past 1 hr. To explain this Geremia proposed that the key difference in mechanisms is that whereas in motor nerves upregulation of BDNF leads to persistently increased trkB expression, prolonged exposure to high levels of BDNF results in an overall decrease in trkB expression in sensory neurons (Geremia *et al.*, 2007).

Gordon and colleagues translated all this groundwork in animal models to human patients with median nerve compression at the wrist. Brief post-surgical ES showed significant axonal regeneration to thenar muscles by 3 months post-operatively, whereas such results were not seen until 1 year in the controls (Gordon *et al.*, 2009). Furthermore, motor unit number estimation was shown to be significantly greater in stimulated patients by 6 months to 1 year compared to controls (Gordon *et al.*, 2009). Studies on motor function and recovery are possible with the technology of electromyography, motor unit number estimation and nerve conduction studies. To date, there are no studies that investigate the effect of ES on sensory recovery in humans. One reason for this is because testing of sensory nerve function in humans is difficult due to its subjectivity, lack of standardization and broad range of functions.

The Challenge of Testing Sensory Nerves

Objective analysis of sensory recovery is difficult due to the sheer number of sensory modalities and the paucity of conventional standardized tests to measure them. Unlike motor reinnervation, which can be measured objectively and precisely with electrophysiologic studies, sensory studies are subject to patient variables and are overall much harder to measure. Although Gordon did follow sensory recovery in the carpal tunnel study, these were via the Semmes Weinstein Monofilament test (SWMT) for fine touch and sensory nerve conduction studies (Gordon *et al.*, 2009). Whereas in animal models retrograde labeling and microscopic cell body analysis can be used to measure the direct number of regenerating sensory neurons, the same obviously cannot be done in humans. The tests that are available frequently have inter-rater variability and often only measure one sensory function out of many. Such is the case in the former study where sensory nerve conduction tests and SWMT only measure the function of large myelinated Aβ fibres but neglect nociception and higher level recognition. As was explained earlier, there are many sensory receptors that serve important functions beyond just touch such as temperature, pain, vibratory, and gnosis.

The additional challenge in measuring a comprehensive range of sensory functions is to combine them for a sense of overall clinical or functional disability related to the deficit. The importance of how each deficit relates to the patients' activities of daily living must be correlated and compared.

Naturally, this leads us to investigate the current available methods of sensory testing and explore less conventional or novel techniques.

Current Methods of Sensory Testing

The current methodology in sensory testing requires a detailed understanding of the different somatosensory receptors that are present in the skin of the human hand. As techniques for improving sensory nerve recovery post-injury are being investigated with well-designed randomized control trials, authors have suggested a revised armament of sensory testing to comprehensively measure meaningful recovery over time (Rosen & Lundborg, 2000;Sunderland, 1978). Tests included in this battery of examinations need to be reliable (reproducible), responsive (sensitive to detect small changes over time), and validated (Streiner D & Norman G, 1989). In addition, the tests need to be clinically relevant, practical to use, and cost-efficient (Jerosch-Herold, 2005a).

The classification of sensibility tests has been a challenge due to the many sensory functions that exist. However, a simple way is to divide sensibility into “protective” and “discriminative” sensation (Jerosch-Herold, 2005a;Sunderland, 1978). The most useful classification, Jerosch-Herold purports, is that of Fess (Fess, 1995), which divides the tests into three categories based on hierarchy: (1) tests which assess detection thresholds such as light touch, deep pressure and dynamic vibration; (2) tests of spatial

discrimination such as two-point discrimination; and (3) tests requiring identification of objects, shapes and textures. Jerosch-Herold's systematic review of sensibility tests outlines the main tests available in each category above (Jerosch-Herold C, 2005a).

Tests for Detection Threshold

Tests that are used to measure "static" touch detection are most commonly measured by von Frey hairs or later revised as the Semmes-Weinstein nylon monofilament test (SWMT) (Weinstein, 1993). The Weinstein enhanced Sensory Test (WEST) was a revised version of the SWMT that improved portability, tip geometry and calibration (Weinstein, 1993). This test measures the force applied to the skin at which detection of the pressure stimulus takes place and should be reported as such. There are some studies that show a moderately strong correlation coefficient between touch threshold and tests of tactile gnosis (Dellon & Kallman, 1983;Novak *et al.*, 1993b), but none show that touch threshold is predictive of function. The validity of this test as a measure of touch threshold is well documented due to its widespread use in literature. Two studies demonstrated that SWMT has good responsiveness and large effect sizes (ES>0.8) (Jerosch-Herold C, 2003a;Rosen *et al.*, 2000). Inter-rater reliability was also established to be very high with an intra-class correlation coefficient (ICC) of 0.965 indicating good repeatability in nerve-injured patients (Novak *et al.*, 1993a).

Dynamic detection threshold is measured most commonly with tuning forks or vibrometers. Tuning forks are not well-controlled and have no standardized protocol for use. For this reason, they are not the preferred choice for dynamic threshold detection (Bell-Krotoski *et al.*, 1993). Vibrometers, on the other hand, are commercially available and usually come with fixed or variable frequency and amplitude. The downside is that they are extremely expensive and are not widely used. In Novak's study, the correlation with tactile gnosis (texture and shape recognition) was not high (Novak *et al.*, 1993b;Novak *et al.*, 1993a). The inter-tester reliability was found to be very good with an ICC=0.982 (Novak *et al.*, 1993a). However, Jerosch-Herold concludes that vibrometry does not fulfill standardization criteria and is not as good as SWMT (Jerosch-Herold, 2005a).

Another way to test dynamic threshold is via vibration threshold quantitative sensory testing (QST). This is a method that utilizes a computer generated stimulus and uses a method of levels to allow the patient to respond. A vibration stimulator is applied to the skin on the area that is to be measured and a 4,2,1 stepping algorithm developed by PJ Dyck is used to determine the threshold the patient can detect vibration (Dyck *et al.*, 1993). This methodology will be explained in more detail below but is mentioned here because it is a method to detect dynamic large myelinated (Aβ) fiber transduction. Limitations exist in this methodology for assessing distal nerve function in the extremities. It seems that activation of normal nerves adjacent to the nerve of interest as well

as proprioceptive changes in nearby joints leads to a higher rate of false negatives. In fact, in our experience, this leads to a near normal result despite a completely transected sensory nerve.

Tests for Spatial Discrimination

These tests constitute a higher level of sensibility and are designed to quantify the threshold at which distinction of different spatial stimuli exist. The smaller the distance represents a higher degree of native receptor and receptive field density, which varies in the human body. In fact, it has been purported that spatial discrimination is twenty times more accurate in the fingertips than it is in the forearm.

Two-point discrimination (2PD) is the most commonly used method and is done with either a bent paper-clip, calipers or the calibrated Dellon-Mackinnon Disk-Criminator™ (Dellon *et al.*, 1992). In most cases, an ascending method of levels is employed in which at least 75% of responses need to be correct to determine the lowest threshold distance that is discriminable. In 1981, Dellon revised this test to move the calipers so that it would become a dynamic test. The validity of 2PD is questionable even though it is the most widely used test and part of the MRC classification of sensory recovery. Contrary to studies stating the opposite, Marsh demonstrated that the relationship between 2PD and tactile gnosis is weak when other variables are controlled (Marsh, 1990). Dressler showed that 2PD is subject to a "learning effect" and that over time

control patients will detect lower and lower thresholds (Johnson K *et al.*, 1994). Novak showed that inter-rater reliability is high for both static and dynamic 2PD (Novak *et al.*, 1993b). The responsiveness of 2PD has been and shown to be poor – especially in patients with complete nerve transections (Jerosch-Herold, 2000;Jerosch-Herold, 2003a;Rosen B & Jerosch-Herold, 2000;Rosen *et al.*, 2000).

Other tests that are less common but also detect spatial discrimination are the grating orientation test (GOT) and the Renfrew ridge (Johnson & Phillips, 1981). The GOT improves on 2PD in that there is a constant surface area of testing and the only change is the spatial threshold. However, this has only been tested in the trigeminal nerve and healthy hands of controls so there is limited evidence on validity, reliability and responsiveness. The Renfrew ridge (Renfrew, 1969) lacks test specificity as depth and distances are not controlled well. Finally, another test to detect spatial threshold is point localization where the distance between an actual and perceived stimulus is measured or the number of correctly localized stimuli in a predetermined zone is quanitified (Jerosch-Herold, 1993a). This has reasonable validity, unknown inter-tester reliability and high responsiveness (Marsh, 1990;Jerosch-Herold, 2003a).

Tests for Object, Shape and Texture Recognition

Two tests that are classically used to measure stereognosis and tactile gnosis are the Moberg pickup test (picking up objects with and without vision) and the Modified Dellon-Moberg pickup test (identifying objects by touch

without vision) (Moberg, 1958;Dellon, 1981). Unfortunately, both tests rely on control of the thumb, index and long fingers, limiting the test to median nerve injuries only. Also, in addition to testing tactile gnosis, another factor that confounds results from the test is the amount of motor reinnervation. The validity of these two tests is quite reasonable but neither are standardized with varying protocols and objects for testing (Dellon & Kallman, 1983;Jerosch-Herold, 1993a;Jerosch-Herold, 2003a;Marsh, 1990;Novak *et al.*, 1993b).

A novel test for detecting tactile gnosis was developed by Rosen and Lundborg in 1998 called the shape-texture identification tests (STI-test™). This test requires patients to identify three shapes and textures in three different sizes without vision. This test can be used to assess finger pulp sensation at the index and little fingers, but not the ring finger (mixed median-ulnar distribution). It is commercially available and has a standardized protocol. Construct validity has been argued with factor analysis and test-retest as well as inter-tester reliability has been shown to be good (Rosen & Lundborg, 2000;Rosen B. & Lundborg G., 1998). Finally, the responsiveness is also very good as shown with patients with 6-month follow-up and large effect size of 0.73 ((Rosen & Jerosch-Herold, 2000).

Sensory Nerve Conduction Studies

All the aforementioned tests assess touch threshold, which are mainly the four receptor subtypes in the skin related to large myelinated Aβ fibers.

Another test that also looks at large myelinated fibers is sensory nerve conduction studies. This may be the only objective sensory measurement by allowing measurement of conduction velocity along a sensory nerve. The method involves calculating the latency period between a stimulating and recording electrode and the distance between the two electrodes. As well, the magnitude of sensory nerve action potential across a set distance along a nerve can be measured. Potential setbacks of this technique include needing to apply electrodes to skin around fresh incisions, potential discomfort due the same reasons, and potential contamination from nearby adjacent nerves. As well, all tests explained so far only measure for the large myelinated fibers and neglect the small myelinated and unmyelinated fibers that transduce temperature, pain, and parasthesias.

Testing for Pain, Parasthesias, Temperature and Protective Sensation

One method to measure the small sensory Aδ and C fibers is to measure the thermal and heat pain thresholds in patients with peripheral nerve injuries. Although this only encapsulates a portion of the functions conveyed by small sensory fibers, it provides an accurate means of evaluating the function and recovery of these fibers has been studied in multiple disease states such as diabetic neuropathy and neuropathic pain (Backonja *et al.*, 2009;Dyck *et al.*, 1983).

Historically, it was very difficult to measure temperature sensation in the skin because of the difficulty in controlling a constant temperature stimulus and the lack of technology to achieve this. Investigators attempted originally to test this by submerging the area of skin in question into a water bath with a constant temperature and testing thresholds. However, there was large difficulty maintaining a constant temperature due to temperature flux from evaporative losses (Neff WD, 1970). An alternative method was to use a brass cylinder submerged in hot or cold water and applied to skin to determine the areas that could detect warm and cold. This procedure was unreliable and difficult to perform consistently (Neff WD, 1970).

By far the most promising technique of measuring heat pain and thermal detection is quantitative sensory testing (QST) by use of equipment that could heat a thermal stimulator via circulating water from hot and cold water tanks. The temperature of the stimulus would be calibrated by a thermostat and varied by switching between water tanks. Later on, this technology was advanced to apply the "Peltier" principle, which applies thermoelectric heating and cooling between two conductors of different materials when current is passed through them in alternating directions. Computer assistance was then applied later in the century to allow for automation and further technical refinement.

The first of the automated versions of this QST was utilized by Fruhstorfer et *al.* with Marstock's method of limits to compare 100 patients with neuropathies to controls (Fruhstorfer *et al.*, 1976). They concluded that this

method was practical, reliable and efficient in determining thermal thresholds in skin.

Technically, QST is a method to apply standardized stimuli (light touch/pressure, vibration, thermal stimuli, and pain) and elicit a quantifiable level of response (Gruener & Dyck, 1994). This tests a range of sensory modalities (large and small sensory fibers) in an objective fashion that is not covered by conventional methods of testing. Automation removes a large portion of human error that is inherent in conventional methods of testing (Jerosch-Herold, 2005a).

Two methods of administering QST have been used to determine thermal thresholds. The first is the "method of limits," first described by Marstock, that involves varying the temperature of the thermode (thermal stimulator) until the patient can feel a switch from warm to cold and subsequently in the reverse direction as well. The weakness of this method is that it relies upon patient reaction time and constant vigilance to achieve consistent results, which is confounded by age and cognitive ability (Siao & Cros, 2003).

The second method used is the "method of levels", which applies a set stimulus and requires a response from the subject of whether the stimulus was detectable. This eliminates the element of reaction time and hence decreases the variability amongst patients. A revision to this is the "forced-choice" method that requires the subject to choose from two different stimuli which stimulus was detectable for the thermal threshold being measured. The disadvantages of

this method are that it is difficult for subjects to follow and it takes a long time to administer (Siao & Cros, 2003).

By far most efficient and accurate method of determining thermal threhold is via Dyck's method of a "4,2 and 1 stepping algorithm with null stimuli" (See Figure 1-10) (Dyck *et al.*, 1993). This is administered with the computer aided sensory evaluator version IV (CASE IV; WR Medical Electronics Inc.) (Dyck *et al.*, 1993). Predetermined intensity of stimuli are given in a specific algorithmic order that changes according to the patient response interspersed with null stimuli so as to decrease patient error (Siao & Cros, 2003). Stimuli are graded by preset intervals that have been calibrated to units called "just noticible difference" units. The reproducibility of this method has been established by Peltier in a multicenter trial and shown to have a high ICC=0.81 (Peltier *et al.*, 2009). This algorithmic approach has been tested and recommended as sufficiently robust for clinical use in detection of thermal thresholds for controlled clinical and epidemiologic trials (Dyck *et al.*, 1993). In addition to testing negative sensory phenomena, this testing of small sensory fibers also includes detection of positive phenomena such as allodynia and hyperesthesia through heat pain detection (Verdugo & Ochoa, 1992). Dyck studied a related algorithm for testing heat pain thresholds called the non-repeating ascending algorithm with random null stimuli and it was found to have good validity, reliability and responsiveness (Dyck *et al.*, 1996). The ultimate

strength of QST is that it is quantifiable (as its name suggests) so that statistical methodology can be applied for the analysis of results (McAllister RM, 1994).

Functional Disability

In addition to measuring sensory deficits, there is a need to measure how they are functionally disabling. This was recommended by Jerosch-Herold because he stated that the conventional battery of tests does not accurately represent a patient's ability to carry out functional activities and that a measure of everyday activities is necessary (Jerosch-Herold, 1993a). Classically, this has been difficult to determine and compare due to subjectivity and variability between subjects. Nevertheless, several questionnaires have been devised to elicit this functional aspect that translates the sensory deficit to effects on activities of daily living. Two such tests include the Disability of the Arm, Shoulder and Hand (DASH) questionnaire and the Canadian Occupational Performance Measure (COPM). The DASH is a 30-item questionnaire that is used to measure physical functions and symptoms related to any upper extremity injury or illness. It is quantified by a score in one of three modules (disability, vocation, and recreation) that can be compared between subjects. However, one of its criticisms is that there is no grading of importance of functional disability. Nevertheless, this has been shown to be valid, reliable and responsive in many previous studies (Gabel *et al.*, 2009;Westphal, 2007;Fayad *et al.*, 2009). The COPM is an individualized measure designed to detect change in a patient's

perception of occupational performance (Law *et al.*, 1990). It was developed in 1991 and has been established as a valid, reliable, practical and responsive tool for following patient performance of ADLs and iADLs over time (Law *et al.*, 1994;Pan *et al.*, 2003;Cup *et al.*, 2003;Kjeken *et al.*, 2005). The potential downfall of the COPM is that scores are based on individualized activities that patients have difficulty performing, and therefore is not likely a valid score for comparison between patients or treatment groups.

In addition, several symptoms that are important to patients after sensory nerve injury include pain and cold intolerance (Allan, 2004a). Questionnaires such as the McGill Pain Score and Cold Sensitivity Severity Scale have been developed to quantify and qualify these symptoms to grade recovery after nerve injury (Melzack, 1975;McCabe *et al.*, 1991). However, the list of tests is vast, and there is no conclusive evidence on which ones are best for certain clinical scenarios.

A Note on Diagnosis of Sensory Nerve Injuries

In reviewing the literature on sensory nerve testing in humans, it became evident that the diagnostic accuracies of the aforementioned tests represent a critical void. Although multiple studies have looked at the diagnostic acumen in diseases such as diabetic polyneuropathy and leprosy, no studies have looked at the diagnosis of sensory nerve transection (Villarroel *et al.*, 2007a;Villarroel *et al.*, 2007b;Dyck *et al.*, 1983).

This point is of practical importance before the efficacy of new treatments (surgery and adjuvant therapies) can be meaningfully interpreted. Currently, clinical diagnosis of a laceration in the emergency room involves the patient's subjective ability to perceive light touch and painful stimuli (Mielke *et al.*, 1996). With that, any level of sensory dysfunction would warrant surgical exploration. While this minimizes the risk of missing any fully transected nerves, it also results in erroneous over-inclusion of patients who might not otherwise need surgery.

Diagnostic Accuracy

The metrics classically used to define diagnostic accuracy are binary classification tables that yield sensitivity, specificity, positive and negative predictive values based on a certain test outcome used as a diagnostic cutoff point (along the range of available outcomes). Sensitivity and specificity are a test's ability to identify truly diseased and non-diseased subjects respectively. However, if an optimized test outcome has not been established in the literature, one would have a range of corresponding sensitivity and specificity outcomes for each test outcome (if the range of potential outcomes is greater than one degree of freedom). The method of origin to establish diagnostic accuracy and select an optimized cutoff point in this scenario is via receiver operating characteristic (ROC) curves first used in the Second World War for evaluating the success of radar detection of submarines (Kumar & Indrayan,

2011). The ROC curve plots sensitivity versus false positive rate (1-specificity) so as to yield a curve that describes a test's diagnostic performance (See Figure 1-11). The area under the curve (AUC) has been used to determine general diagnostic accuracy where a larger area signifies higher accuracy (Cleves, 2002). Another measure used has been the Youden Index (J) which is the maximal distance that a point on the curve sits from the reference line (a line denoting a test based on chance only) (Perkins & Schisterman, 2006). Though many details and permutations exist for further evaluation, the general idea is to compare the accuracies of different tests using AUC or optimized sensitivity and specificity values. With this knowledge, rational decisions can be made about what tests or combination of tests should be used to best deliver clinical care.

Formulation of Thesis

A thorough review of literature has revealed two major knowledge gaps in sensory nerve injuries. First, the diagnosis of sensory nerve lacerations is not well defined. Secondly, the efficacy of electrical stimulation after repair of transected sensory nerves in humans has not been studied.

The remainder of this thesis will address these two issues by the following papers:

I. Comparing Quantitative Sensory Testing, Monofilament Testing, and Two-Point Discrimination in Diagnosing Digital Nerve Transection

Current methods of sensibility testing are by tests developed for grading the level of nerve dysfunction in disease and recovery. Minimal literature exists regarding their accuracy for diagnosis of sensory neurotmesis (complete transection). As an addition to current sensory examination, the knowledge of diagnostic accuracy in these tests may help to improve health-care efficiency and lower patient risk without sacrificing clinical sensitivity. The purpose of this study is to determine the diagnostic accuracies of pressure threshold testing (SWMT), spatial discrimination (2PD), and nociception (temperature and pain threshold via QST) in detecting complete digital nerve transection.

II. Novel Electrical Stimulation Therapy to Enhance Sensory Nerve Regeneration: a Double-Blind, Randomized, Placebo-Controlled, Clinical Trial

Brief post-surgical electrical stimulation has shown multiple benefits to motor and sensory axonal regeneration in the rat femoral nerve model (Al-Majed *et al.*, 2000c;Geremia *et al.*, 2007). In fact, its effects have even been shown in human patients with carpal tunnel compression injury based on motor unit recovery (Gordon *et al.*, 2009). One of the missing pieces of the puzzle is establishing its effect on sensory neurons in humans. The purpose of this study is to investigate the effect of post-surgical ES on humans with complete digital nerve transection. Outcomes will include a comprehensive

range of sensory functions including touch, spatial discrimination, nociception, as well as functional disability.

THE BRITISH MEDICAL RESEARCH COUNCIL SYSTEM

DELLON MODIFIED OF HIGHET SCALE

Sensory Recovery

S0 – Absence of sensibility in the autonomous area

S1 – Recovery of deep cutaneous pain sensibility within the autonomous area of the nerve

S2 – Return of some degree of cutaneous pain and tactile sensibility within the autonomous area

S3 – Return of some degree of superficial cutaneous pain and tactile sensibility within the autonomous area with disappearance of any previous overreaction (2PD>15mm)

S3+ - Return of sensibility as in stage 3 with the addition that there is some recovery of two-point discrimination (2PD (7-15mm)

S4 – Complete recovery (2PD<7mm)

Table 1-1: British Medical Research Council System for Grading Nerve Dysfunction

This system is the most commonly used classification to classify recovery of neurologic function (motor and sensory) after injury and repair. Notice that two-point discrimination is used as a higher level of sensory recovery (Birch *et al.*, 1998;Dellon *et al.*, 1974).

GRADING OF RESULTS

MOTOR	***RESULT***
M4 or better	Good
M3	Fair
M2	Poor
M1 and 0	Bad
SENSORY	
S4 or S3+	Good
S3	Fair
S2	Poor
S1 and 0	Bad

Table 1-2: Grading of MRC Results

Most articles publish with results that have been translated to good, fair, poor or bad. In addition, some studies have added the category of excellent, which signifies indistinguishable from normal (Birch *et al.*, 1998).

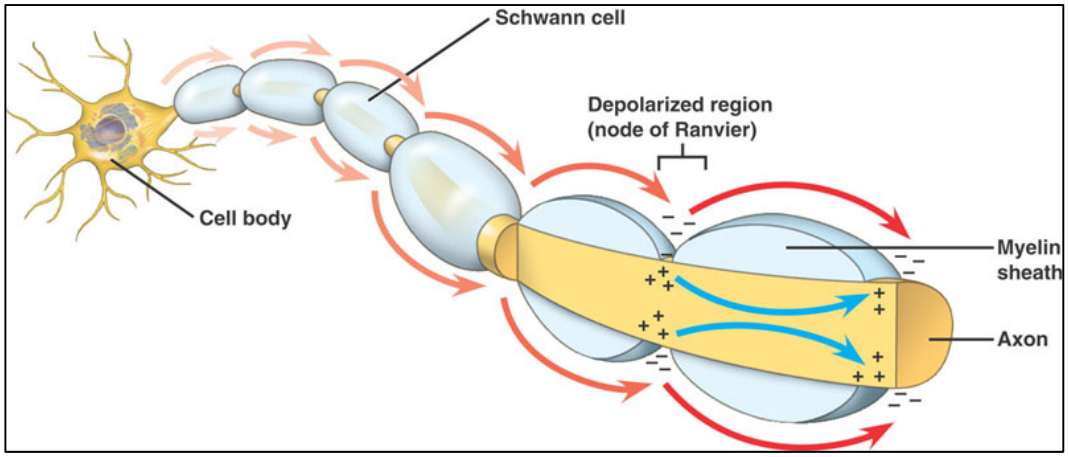

Figure 1-1: Saltatory propagation/conduction

The velocity of depolarization and conduction is significantly increased because myelination leaves only small gaps termed nodes of Ranvier that can be depolarized. This results in a jumping of the wave of depolarization from one node of Ranvier to the next (Brown, 2011).

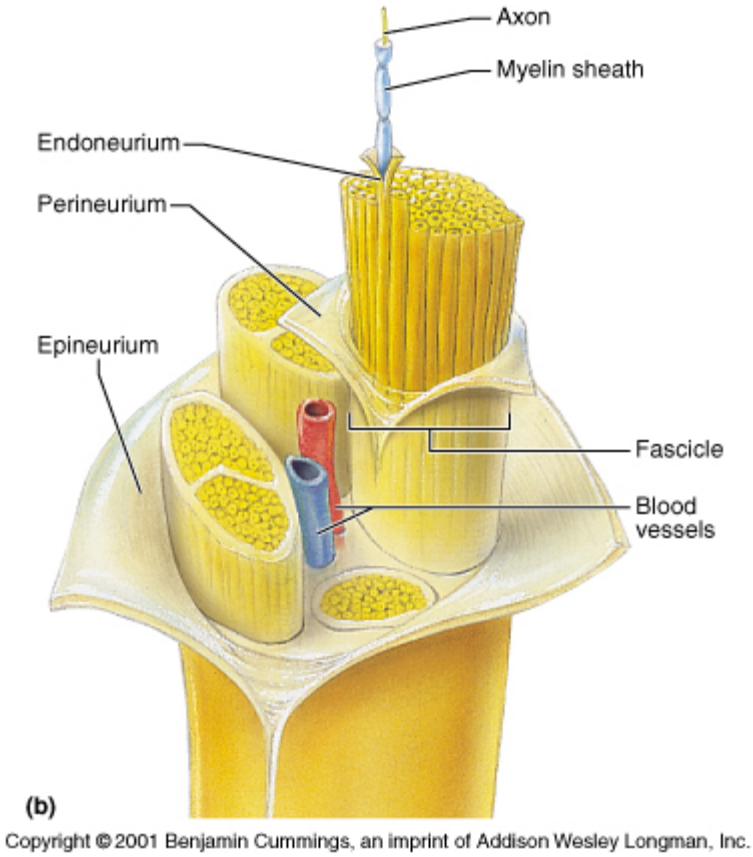

Figure 1-2: Layers of connective tissue surrounding peripheral nerves

The epineurium surrounds a bundle of fascicles, the perineurium surrounds a single fascicle (bundle of axons), and the endoneurium surrounds a single axon (Cummings, 2011).

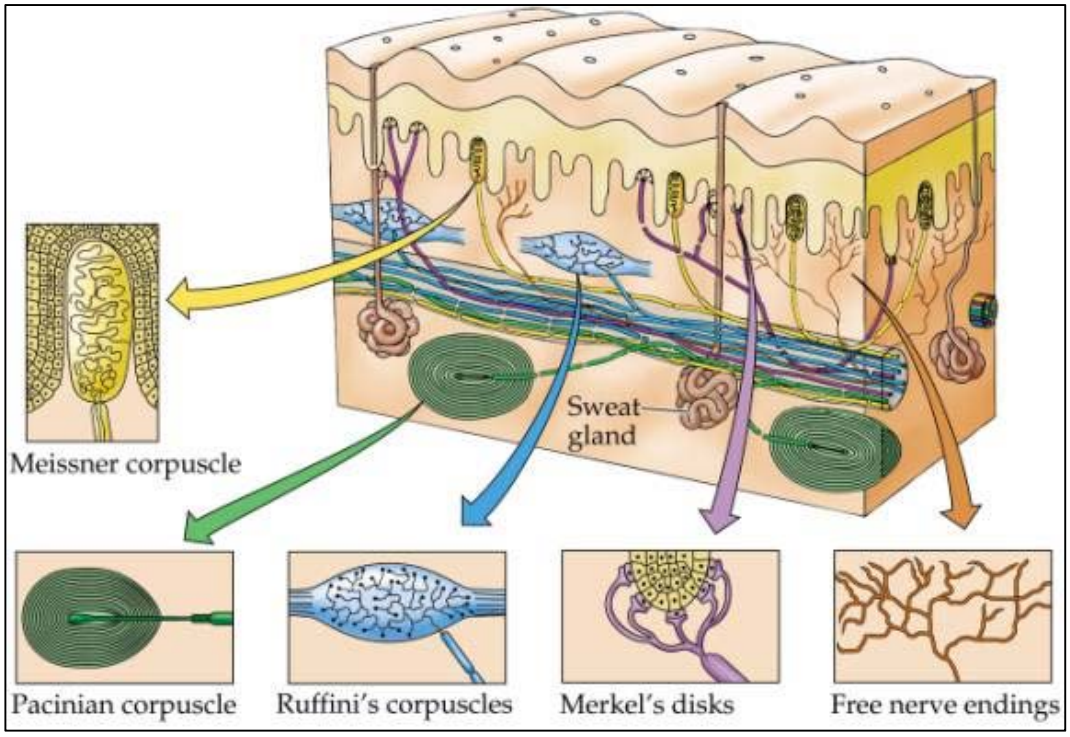

Figure 1-3: Touch receptors in human skin

The Merkel cells/Disks, Meissner Corpuscle, Ruffini endings/Corpuscle, and Pacinian Corpuscle transduce sensory information via large myelinated Aβ fibers. The small sensory Aδ and C fibers do not have specific receptors but utilize free nerve endings for sensory transduction (Joseph, 2000).

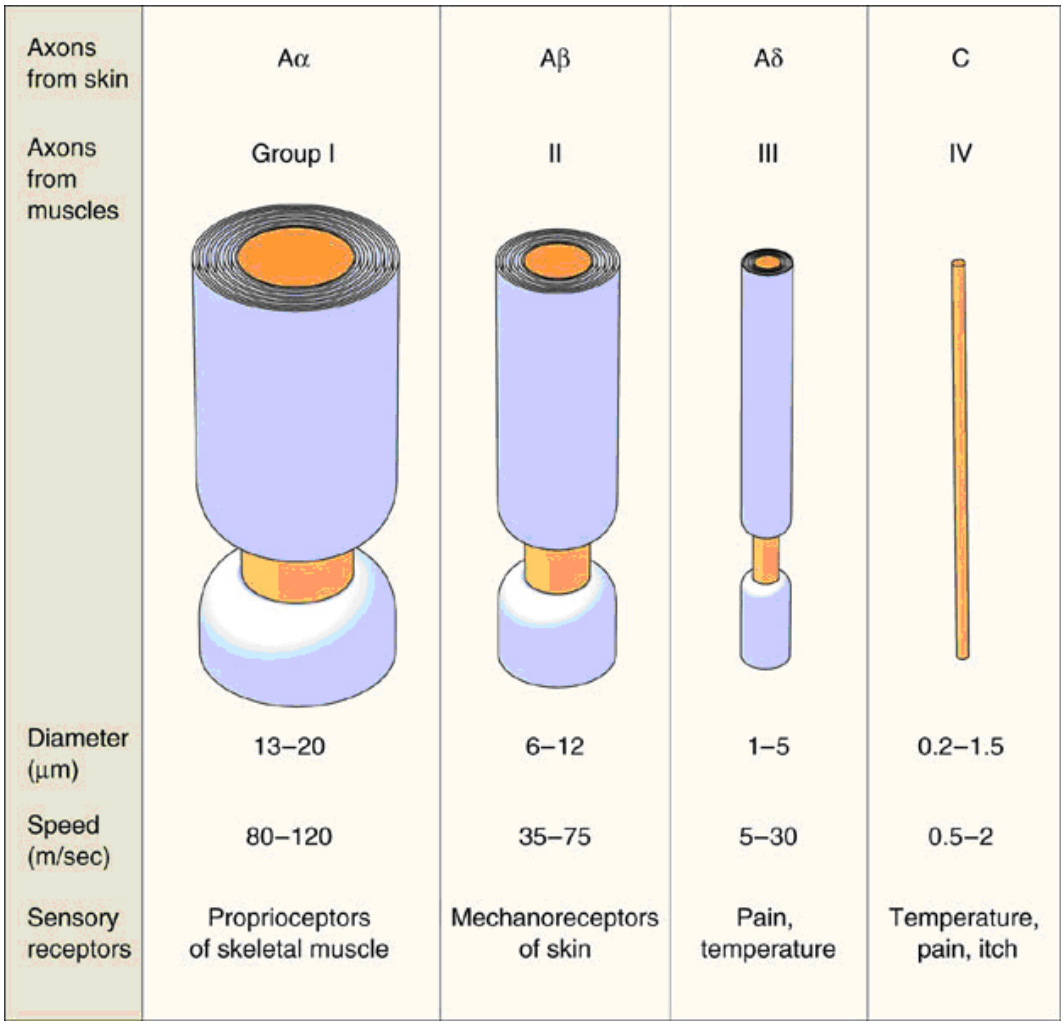

Figure 1-4: Somatic sensory afferents

A comparison of axon diameter and conduction velocity between the four main sensory afferent fiber types is shown. Notice that there are two classification systems: alpha numeric for skin afferent and Roman numeral for muscle afferents (Lafontaine, 2008).

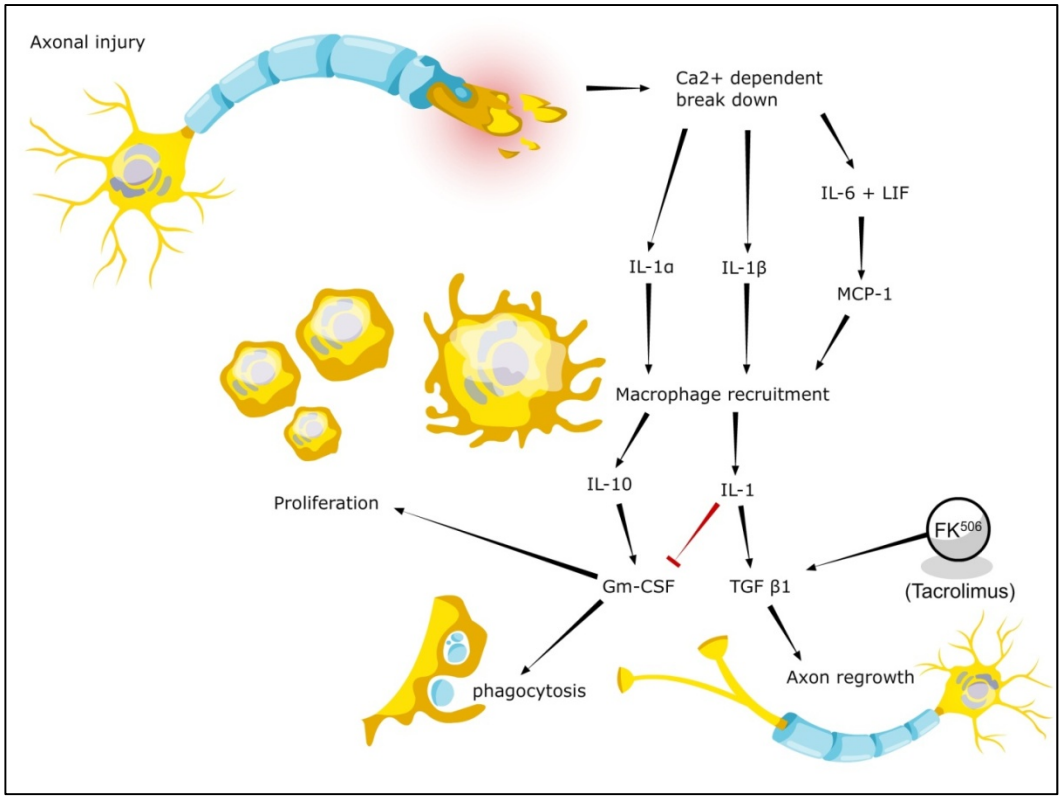

Figure 1-5 Inflammatory pathways induced by axonal injury

Axonal injury induces an inflammatory reaction incited by a slew of inflammatory cytokines such as IL-1, IL-6, IL-10, TGF-β that ultimately leads to phagocytosis of axonal debris, axonal regrowth and regeneration, as well as SC proliferation.

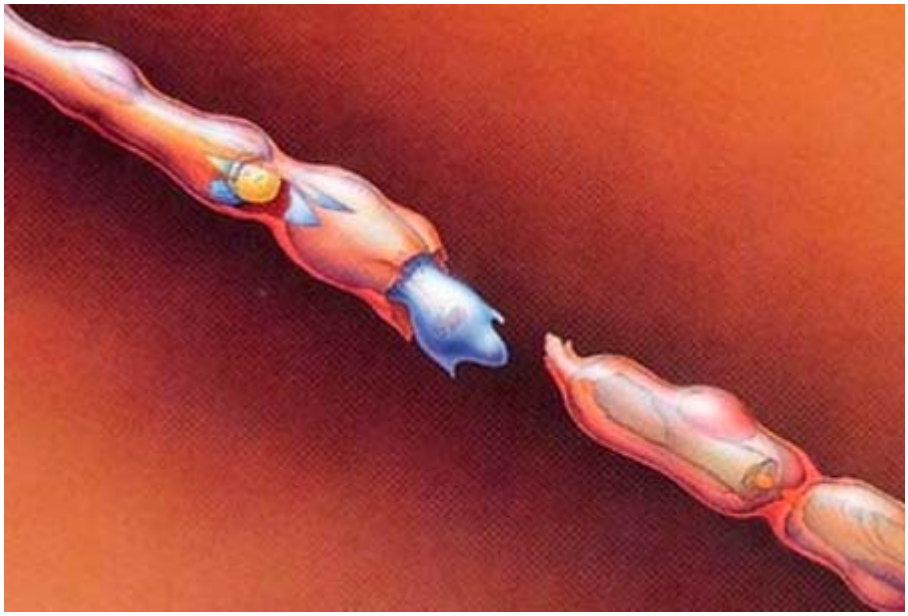

Figure 1-6a: The beginning of Wallerian degeneration after axotomy

The nerve axon is seen just shortly after axotomy (nerve transection). The axon can be seen in yellow, the SC in blue and the basal lamina in orange (Mackinnon & Dellon, 1988)

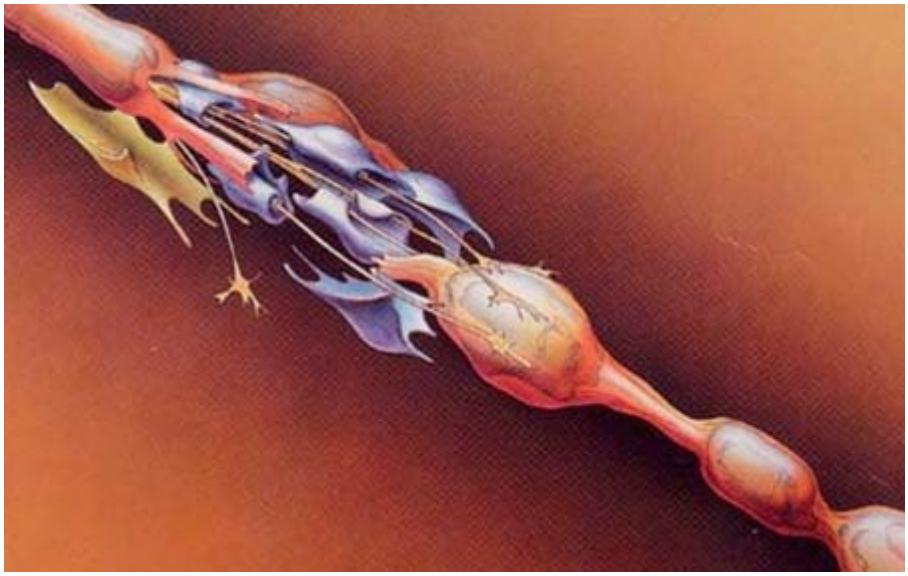

Figure 1-6b: Wallerian degeneration

Shortly after axotomy, the distal portion (left) undergoes dissolution and digestion of axons and myelin. This is assisted mediated by both SCs (blue) and macrophages (Mackinnon & Dellon, 1988).

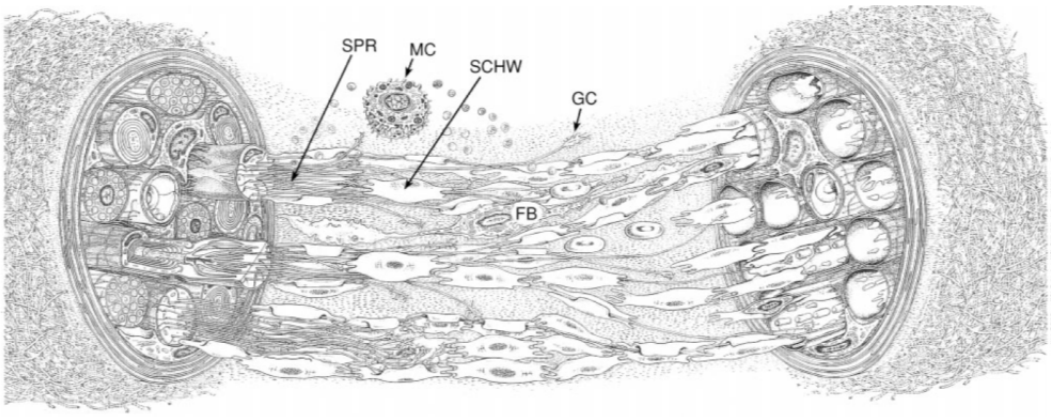

Figure 1-7: The emerging growth cone after axotomy

The proximal nerve stump (left) with numerous axonal sprouts (SPR) emerging towards the distal stump (right). At the tip of the sprout is the growth cone (GC) and the regenerative support cells such as SCs (SCHW), mast cells (MC), and fibroblasts (FB) are identified. Also, the axonal sprouts attach to the columns of SCs that develop a formation called bands of Büngner (Ladak, 2009).

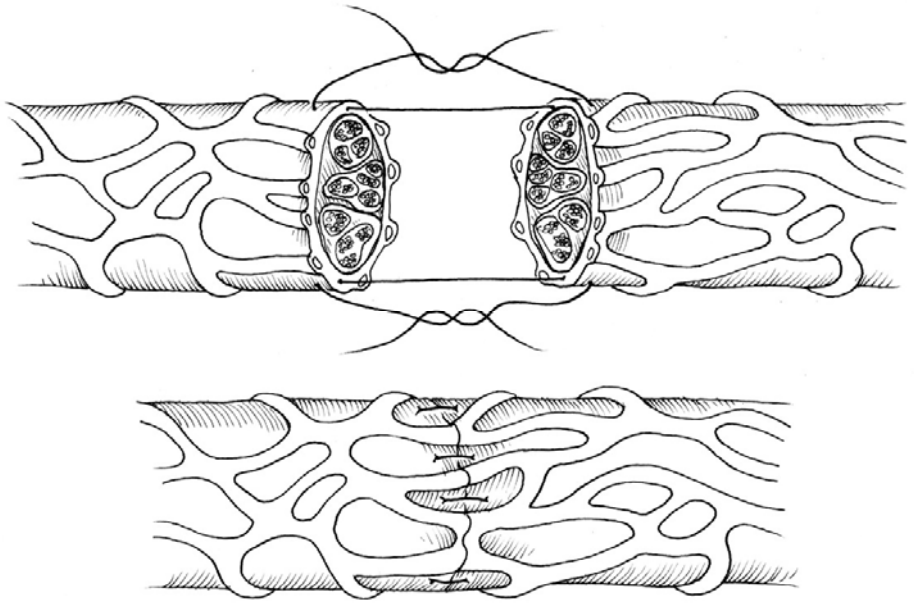

Figure 1-8: Epineurial nerve repair

Sutures are placed only in the epineurium to reappose the severed nerve ends. Note that there is no suture in the perineurium or fascicles deep to the epineurium (Lee & Wolfe, 2000).

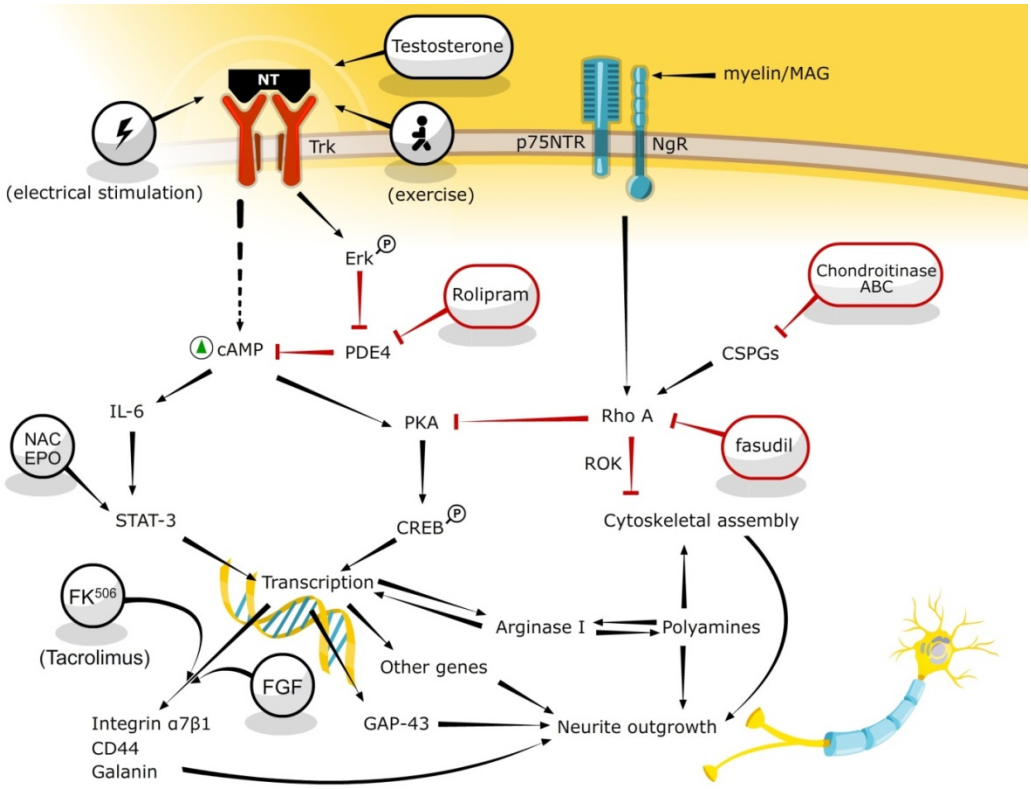

Figure 1-9: The downstream effects of upregulation of cAMP

Shown here is schematic that demonstrates the effects of neurotrophin binding its Trk receptor. As can be seen cAMP is upregulated and the ultimate effect is increased neurite outgrowth. Notice also, that multiple interventions can potentiate this process including those at the neurotrophin binding level (in black) and agents working downstream (in red).

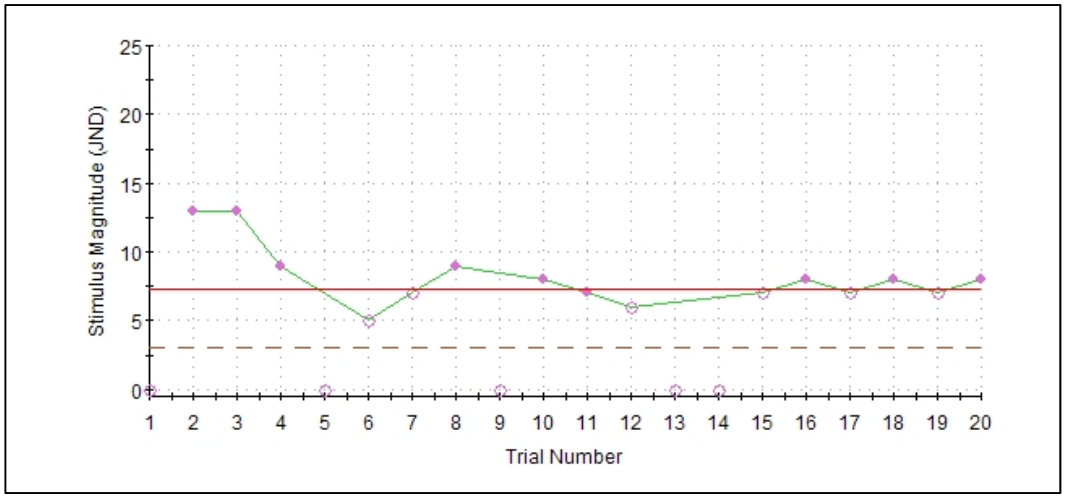

Figure 1-10: An example of data using the 4,2 and 1 Stepping Algorithm with null stimuli

The x-axis showns 20 trials of stimuli given using the algorithm and the y-axis demonstrates the level of stimuli delivered. The graph point indicates the response given (solid = stimulus detected; hollow = stimulus not detected). As can be seen, a null stimulus is delivered every 4 stimuli to reduce patient error. The procedure starts with increasing or decreasing the stimulus intensity by 4 JND (just noticeable difference units) until a turnaround is seen (trial 6 in this case). Then the stimulus intensities are finetuned at 2 JND until the next turnaround (trial 8 in this case). Finally, the stimulus is adjusted by just 1 JND per stimulus until the threshold is detected at 20 trials and results averaged (red line).

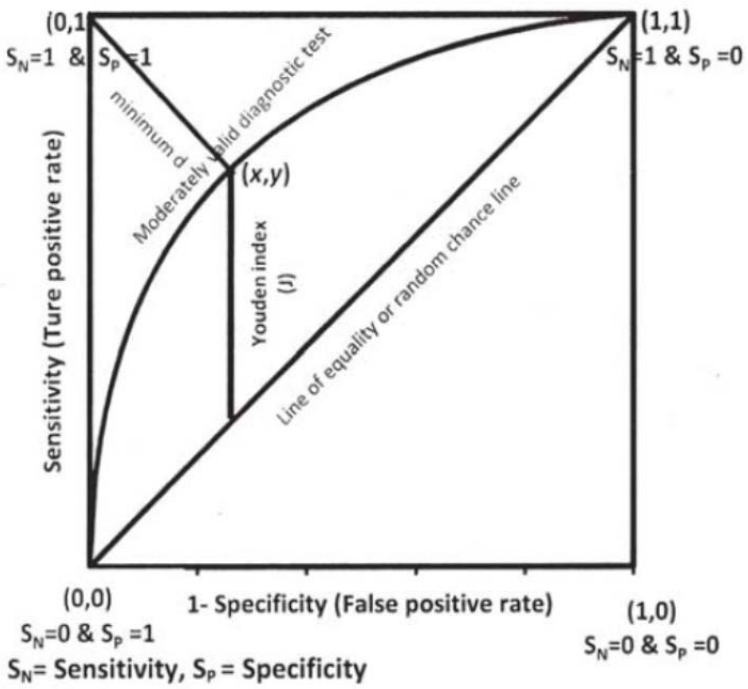

Figure 1-11: The Receiver Operator Characteristic Curve

A theoretical plot of sensitivity over false positive rate is the ROC curve. Notice how an ideal test will approach a point of maximum sensitivity with the lowest false positive rate (0,1). Also, the reference or chance line (line drawn from (0,0) to (1,1)) denotes a test that would perform only based on chance alone. The area under the curve and distance from the chance line are different measures of diagnostic accuracy.

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Chapter 2: Comparing QST, Monofilament and Two-point Discrimination in Diagnosing Sensory Nerve Transection

Introduction

Glabrous skin on the finger tips is among the most densely innervated cutaneous tissues supplied by sensory nerve fibers in the human somatosensory system (Purves, 2008). Well-endowed sensory feedback is essential in many daily activities such as buttoning a shirt, entering a text message or playing the guitar. Therefore, even the loss of a single digital nerve can be highly debilitating considering the wide range of vocational, recreational and daily activities that would be abruptly disturbed. Unfortunately, digital nerves are the most commonly severed peripheral nerves in the Western world (Buncke, 1972). Common culprits include domestic glass, knives, industrial table-saws and sheet metal.

To minimize functional loss, accurate diagnosis of digital nerve injuries is crucial to permit timely operative repair. Although partially injured digital nerves do have the ability to regenerate and re-innervate the skin, prognosis for functional recovery in the case of complete laceration is much poorer (Wang *et al.*, 1996). Therefore, it is imperative that those patients are not missed when presenting to surgeons, so that coaptation of the severed nerve ends can be carried out as expeditiously as possible. To that end, a diagnostic test with high sensitivity is needed. Conversely, to avoid unnecessary surgery and make the

best use of limited healthcare resources, a test so selected also needs to be highly specific (Irwin & Irwin, 2011).

At present, the Semmes-Weinstein Monofilament Test (SWMT) and static two point discrimination (s2PD) are the most commonly used sensory assessment tools in this setting (Mielke *et al.*, 1996). However, despite their simplicity and convenience there are concerns over their validity, reliability and responsiveness (Jerosch-Herold, 2005). Furthermore, their sensitivity and specificity in the diagnosis of digital nerve laceration have not been established. With that in mind, there is a strong need to test their diagnostic accuracies for this particular application.

For the use of diagnosing digital nerve laceration, quantitative sensory testing (QST) holds potential promise. In addition to being able to measure the function of a wide range of sensory nerve fibers, it has a number of advantages over SWMT and s2PD. Firstly, QST not only measures negative sensory deficits, but by using heat-pain threshold determination it can also detect positive sensory phenomena such as allodynia and hyperesthesia (Verdugo & Ochoa, 1992). Secondly, QST is automated and thus not easily influenced by tester and subject bias (Dyck *et al.*, 1993). Thirdly, since the results are continuous interval variables, more powerful statistical analysis methodology can be applied (McAllister RM, 1994). However, in spite of these potential advantages and its established use in conditions such as diabetic polyneuropathy, its diagnostic utility in digital nerve laceration has not been tested.

Therefore, the goal of this study is to compare the diagnostic accuracies of SWMT, s2PD, and QST in determining complete digital nerve laceration based on sensitivity, specificity and receiver operator characteristic (ROC) analysis. This will provide the necessary rationale to support the selection of a single test, or combination of tests, that will maximize diagnostic precision.

Research Design and Methods

Subjects: Patient recruitment and follow-up were conducted in a prospective fashion at the University of Alberta Plastic Surgery Hand Trauma Clinic in Edmonton, Canada. Eligible patients were those aged 18-65 years with suspected complete digital nerve laceration. Those with previous digital nerve injury, preexisting neuropathy, cognitive impairment that precludes the ability to provide informed consent, and concomitant bone injury to the affected digit were excluded. Verbal and written consent were obtained from all patients. The study was approved by the Human Research Ethics Board at the University of Alberta.

Test protocols: After enrollment, subjects were seen in a neuro-rehabilitation facility for sensibility testing of the hand.

1. Static two-point discrimination was performed using a Dellon-MacKinnon Disk-Criminator to assess spatial discrimination. Employing an ascending method of levels, the pins were aligned longitudinally on the affected side of the finger pulp. Pressure was applied for 1.5 s just prior to skin blanching and then released

(Klein, 2007). Patients would then be asked to give a response of one or two pin sensations felt. The smallest distance where 75% of responses correctly identified 2 pin sensations would be accepted as the detection threshold.

2. Semmes-Weinstein Monofilament Test: The full SWMT kit with 20 nylon monofilaments (Sammons Preston Rollyan, Canada) was applied to the same area of the finger pulp in increasing order of monofilament size until sensation of pressure was identified. Again, the method of levels was used so that 75% correct responses for the smallest filament had to be achieved to represent the pressure detection threshold (Klein, 2007).

3. Quantitative Sensory Testing: This was conducted using a CASE IV System (Computer Aided Sensory Evaluator version 4, WR Medical Electronics Co., Maplewood, MN) developed by Dyck et al (Dyck *et al.*, 1993). Warm determination threshold (WDT) and cold determination threshold (CDT) were detected with the affected digital pulp placed on the thermal stimulator using the 4, 2, 1 stepping algorithm with random null stimuli. The thermal stimulator utilizes a thermoelectric diode to regulate its temperature. Once the target temperature is reached, a closed circuit water reservoir is used for cooling the detection area on the thermode. For each stimulus, the subject was asked to respond with yes or no whether they detected a temperature change. The reverse was used in cold determination where the temperature is first lowered and then normalized by thermoelectricity. The algorithm uses “just noticeable difference” (JND) units defined as the smallest detectable differences in

temperature for both the cold and warm tests (Gruener & Dyck, 1994). Null stimuli are inserted randomly in each block of four responses so that error from guessing can be eliminated (the test would be abolished when a random guess error is detected). Heat pain threshold (HPT) was assessed using the modified non-repeating ascending algorithm with null stimuli (Dyck *et al.*, 1996). The subject was asked to report the intensity of the heat stimuli with a 10 point scale, based on the level of discomfort from the stimulus. These yield two heat pain outcomes: HPT1 represents the intermediate heat-pain response and HPT2 represents the heat-pain determination threshold (Dyck *et al.*, 1996).

Study protocol: All sensory examination data was collected by the same investigator (JNW) trained in hand sensibility testing. There was no blinding of results as the technician was aware of the outcomes from each test. Once the diagnostic studies had been completed, the subjects were taken to the operating room. Under general anesthesia, the digital nerve was exposed. The reference standard for disease status was established by the exploring hand surgeon with direct inspection under loop or microscopic magnification in the operating room. Nerve injuries were classified as either completely lacerated or intact.

Statistical Analysis

Patient characteristics were reported as mean and standard deviation or number and percentage. Patient demographics in each group were compared using two-

sample Student's t-test or Fisher's exact test. The diagnostic accuracy of each sensory test was analyzed using non-parametric Receiver Operating Characteristic (ROC) curve analysis yielding the area under the curve (AUC) with derived standard error based on Delong's method (DeLong *et al.*, 1988). Non-parametric analysis was chosen as a more prudent approach because the data was not normally distributed in all cases and not all test outcomes are continuous variables (Cleves, 2002;Kumar & Indrayan, 2011). Equality of the AUCs was compared using the Delong method, a technique similar to utilizing a variation of the Mann-Whitney U test to determine variance. As well, sensitivity and specificity values at each cutoff threshold were calculated and the Youden (J) Index was tabulated for comparison of accuracy across tests. All analyses were done using STATA 12 (StataCorp LP, College Station, TX).

Results

During the recruitment period of July 2011 to June 2012, sixty patients were suspected to have complete digital nerve laceration based on clinical exam requiring surgical exploration. Of those, forty-one (68%) indeed turned out to have complete transection while 19 (32%) had intact nerves. The demographic data of all patients stratified into completely lacerated and intact groups are presented in Table 2-1. All sensibility tests were performed in the morning approximately 2.5 hours prior to surgical exploration. All diagnostic tests were performed in all subjects by the same investigator with no missing values.

Demographic characteristics

The intact group was significantly older than the complete laceration group ($p<0.05$). Otherwise, there was otherwise no difference between the groups based on gender, hand-dominance, and side of injury. To evaluate whether age could be a potential confounding factor, linear regression correlation analysis was done on the results of all the diagnostic tests. When the outcomes were regressed based on age, none of the coefficients of determination (r^2) were greater than 0.30, indicating that there was no significant effect of age on test performance (Figure 2-1).

Sensitivity, Specificity, and ROC

The ROC AUC values for SWMT, s2PD, and QST CDT, WDT and HPT are displayed in Table 2-2. Of those tests, the HPT2 showed the greatest AUC (±SEM) at 0.812(±0.067). A non-parametric bootstrap method was used to compare the corresponding AUCs from all tests yielding the probabilities displayed in Table 2-3. The HPT2 AUC was significantly larger than WDT and CDT ($p=0.0229$, 0.0395 respectively). A graphical display of the ROC curves for all the involved tests is shown in Figure 2-2. Of note is that WDT has 100% sensitivity across all observed cutoff points. Consequently, a polynomial regression line could not be fitted. Among the 6 diagnostic tests, the ROC curve of HPT1 had the steepest rising slope compared to the others.

Using non-parametric analysis, we were able to determine the sensitivity and specificity values for each test within their range of cutoff points. The selection of the optimal cutoff point for diagnosis was based on the commonly utilized Youden (J) Index. This is the inflection point on the ROC curve furthest away from the reference line (0,0 to 1,1) (Perkins & Schisterman, 2006). An ideal test would be one with the largest J value. Based on this parameter, the sensitivity and specificity of the optimum cutoff points for each test are tabulated in Table 2-4. Among those, the J value is highest for HPT2 at 0.55. The corresponding sensitivity and specificity of HPT2 at that cutoff point was 90% and 65%, respectively.

Discussion

Current clinical evaluation of a patient suspected of digital nerve laceration is based on a combination of subjective numbness and sometimes a crude test of touch discrimination. While SWMT and s2PD are used in the emergency room and hand clinics, their clinical performance in the setting of digital nerve laceration has not been tested and optimum cutoff points not established. To our knowledge, this is the first study that investigated and directly compared the diagnostic values of a wide range of sensory tests so that inferences can be made as to which tests would confer the greatest accuracy. This comparison is necessary because there are multiple classes of sensory nerve fibers that subserve a wide range of physiological functions.

Which single test is most appropriate for diagnosing complete digital nerve laceration?

One common yardstick used to evaluate the performance of a diagnostic test is the Youden (J) Index. A high J Index infers that the test has good sensitivity with little compromise on specificity. Based on that criterion, HPT2 showed the best performance. With a cutoff point of 22.1 JND, HPT2 had a sensitivity of 90 % and a specificity of 65%. Based on the AUC, another commonly used criterion for judging test performance, HPT2 was also significantly larger than CDT and WDT. On these bases, one might argue that HPT2 would be the logical choice as an ideal tool for diagnosing complete digital nerve laceration. However, given the poor functional outcome of a missed complete nerve laceration that is not repaired, the index of suspicion must be set very high. Therefore, even with a sensitivity of 90%, using HPT2 as the sole diagnostic test would still leave a false negative rate of 10%. Leaving these patients with complete nerve laceration without surgery would not be i clinically acceptable.

An alternative strategy is to consider using a test with the highest sensitivity. Using this scheme, WDT would fulfill the requirements as it had a sensitivity of 100% and specificity of 37% at 25 JND. At that cutoff, WDT correctly identified all 41 patients in our sample with complete digital nerve laceration. With a specificity of 37%, seven patients with intact nerves would be spared of

unnecessary surgery (Table 2-5b), compared to basic examination in the clinic alone (Table 2-5a).

Can a combination of tests further improve diagnostic accuracy?

By knowing the diagnostic profile of different tests at their entire range of cutoff points, one can potentially develop a diagnostic algorithm using a combination of tests to further improve diagnostic performance. Indeed, such strategy is commonly employed in many other clinical scenarios such as prenatal detection for Down's Syndrome using a highly sensitive test for screening followed by a second test with high specificity (Ohno & Caughey, 2013).

In our case, a starting point would be to follow up on the 53 patients with abnormal results on WDT with SWMT. Using the 6.65 gauge monofilament as a cutoff, the SWMT has the highest specificity among all the tests at 95% with a sensitivity of 10%. With these test performance characteristics, forty eight patients would have negative test results – the majority of whom are true negatives (see Table 2-5c). To ensure that no patient with a false negative result after SWMT is erroneously missed, a third test is required. With a cutoff point of 7 mm, static two-point discrimination has a sensitivity of 100% and a specificity of 32%. Based on that, if all subjects who test negative with SWMT are followed with s2PD, all subjects with complete nerve laceration should be correctly identified. However, three more patients with true negative test results would also be eliminated from unnecessary surgery (Table 2-5d). Using this algorithm,

even though a number of patients without complete nerve laceration end up in surgery from our sample, ten of the 19 (7 from WDT, 3 additional after SWMT/s2PD) non-diseased subjects would have been spared of unnecessary surgery. This represents a 53% specificity rate compared to zero if clinical examination was used alone, and 37% if only WDT was employed. The three-tier decision tree algorithm is represented in Figure 2-3.

Clinical implications

The associated functional disability of digital nerve injury is often under-appreciated. A recent review of 194 digital nerve injuries showed that 91% of patients who received surgery were left with reduced function at work, 71% had problems with activities of daily living, 79% suffer from cold hypersensitivity, and 97% had reduced dexterity (Thorsen *et al.*, 2012). The average direct cost (hospital stay, operation, outpatient visits, therapy etc.) of an isolated digital nerve injury in Sweden is 2653 euro (Thorsen *et al.*, 2012). In addition, loss of productivity is even more substantial as 79% of working individuals lost time from work with a median length of 59 days of sick leave (Thorsen *et al.*, 2012).

Therefore, current standard surgical practice is to explore every suspected sensory nerve injury (Farnebo *et al.*, 2013). However, this liberal approach would greatly increase the number of unnecessary surgeries. Indeed, in this study, the number of patients suspected to have complete digital nerve laceration who turned out to have intact nerves was over 30%. This represents a

substantial drain on precious healthcare resources. By adding diagnostic tests like WDT, SWMT and s2PD for screening, that number can be reduced without the risk of missing any diseased subjects.

Other potential diagnostic tests

We did not include several commonly used sensibility tests such as nerve conduction studies, vibration testing, or object recognition. Although these tests are used to grade nerve dysfunction, there are several reasons they are inappropriate for diagnosis of digital nerve injury. First, nerve conduction studies are technically difficult to conduct in the fingers when there are acute lacerations. Even more problematic is the intact digital branch on the contralateral side of the digit would greatly contaminate the test result through volume conduction. Vibration testing is also inappropriate as proprioceptive receptors unaffected by the digital nerve in the proximal joints would also be activated, leading to a high rate of false negatives. Finally, object recognition is more of a functional measure of tactile gnosis rather than a diagnostic tool.

Limitations of the study

Since our sample size is relatively small, it could increase the risk of type II error (failing to detect a difference between the tests when one truly exists). However, despite this, we were still able to reach statistical difference with HPT2 being significantly better than some of the other test parameters.

Since the outcome measures of the tests chosen in this study consist of discrete as well as continuous variables, equal variances and normality of distributions cannot be assumed. Therefore, to be prudent, we chose to use non-parametric techniques for data analysis. As well, although inter-observer and test review bias are eliminated by having a single trained investigator to perform all the tests, the lack of blinding to the test results could potentially introduce biases towards later tests based on the perception of the earlier tests.

Finally, there is also a need to consider the cost-benefit equation. The CASE IV System for QST costs over 15,000 USD and requires 5 to 10 minutes per test. Limited accessibility to the equipment, requiring trained personnel to perform the test, and the time required at a busy clinic would be potential barriers to clinical implementation. However, balancing against the much higher direct and indirect costs leading to the significant socioeconomic burden reported by Thorsen, we believe that the inclusion of QST tests can be justified.

Conclusion

In summary, we have shown that QST heat pain determination threshold is the single best test to diagnose complete digital nerve transection. However, with a sensitivity of 90%, a percentage of patients with complete nerve cut would be erroneously classified as test negative, which is clinically unacceptable. Rather, to ensure that no patient with complete nerve laceration is missed, a more clinically appropriate alternative is to use QST WDT that has 100%

sensitivity as a screening tool. With a specificity of 37%, WDT can still reduce a substantial number of unnecessary surgeries. To further improve test performance, a tiered algorithmic testing scheme that also employs SWMT and s2PD would increase the rate of spared surgery to 53% with no sacrifice to sensitivity.

Demographic	Intact (19)	Complete laceration (41)	P-value
	Mean(SD) or no(%)	Mean(SD) or no(%)	
Age (yrs)	39.9 (16.5)	27.6 (11.6)	0.005 (t-test)
Gender			0.767 (Fisher's exact)
Female	5 (26.3)	13 (31.7)	
Male	14 (73.7)	28 (68.3)	
Handedness			0.705 (Fisher's exact)
Left	2 (10.5)	7 (17.1)	
Right	17 (89.5)	34 (82.9)	
Injury Dominance			0.410 (Fisher's exact)
Non-dominant	13 (68.4)	23 (56.1)	
Dominant	6 (31.6)	18 (43.9)	

Table 2-1: Demographic Data of Patients Parsed by Disease Status

Patient demographics were examined separated by disease status. Statistical comparison was performed to determine whether there was inequality based on any demographic variable in diseased and non-diseased groups. Student's t-test was used for discrete numeric variables and Fisher's Exact test was used to for categorical variables. There was a statistical difference in age in that patients in the non-diseased group were older (p=0.005).

Sensibility Test	AUC
SWMT	0.807 (0.065)
s2PD	0.735 (0.071)
QST CDT	0.660 (0.073)
QST WDT	0.677 (0.060)
QST HPT1	0.778 (0.066)
QST HPT2	**0.812 (0.067)**

Table 2-2: Comparison of Non-parametric AUC of Sensibility Tests

The conservative non-parametric approach was used to determine the area under the curve values for each sensibility test (Stata command roctab). For statistical comparison, the Delong method of determining variance was used for each test. The values reported are mean (SE). HPT2 had the highest AUC with SWMT in close second.

p-values	SW	s2PD	CDT	WDT	HPT1	HPT2
SW	x	0.4649	0.0975	0.2018	0.6841	0.9396
s2PD	x	X	0.4138	0.542	0.5881	0.3295
CDT	x	X	x	0.6267	0.112	0.0229
WDT	x	X	x	x	0.157	0.0395
HPT1	x	X	x	x	x	0.236
HPT2	x	X	x	X	x	x

Table 2-3: Table Showing Statistical Comparison of Sensibility AUCs

A non-parametric bootstrap technique was used to compare the AUCs (Stata command roccomp) yielding the p-values that are displayed. For all tests, a significance level of 0.05 was used. HPT2 AUC is significantly higher than CDT and WDT.

Test	Cutoff	Sensitivity (%)	Specificity (%)	Youden Index J
SWMT	4.31	90.24	57.89	0.48
s2PD	15	82.93	57.89	0.41
QST CDT	24	82.93	47.37	0.30
QST WDT	25	100	36.84	0.37
QST HPT1	30	68.29	82.35	0.51
QST HPT2	**22.1**	**90.24**	**64.71**	**0.55**

Table 2-4: Comparison of Youden Index Optimized Cutoff Points

ROC analysis revealed a range of sensitivity and specificity values for each cutoff point in every test. The Youden (J) Index (the maximum vertical distance on the ROC curve from the chance line) was then determined and the corresponding sensitivity and specificity values are compared between tests. HPT2 was found to have the highest J at 22.1JND cutoff with corresponding sensitivity and specificity of 90 and 65%, respectively.

Disease State	**Diseased**	**Non-Diseased**	**Total**
Test (Exam alone)			
Test Positive	41 (100%)	19	60
Test Negative	0	0 (0%)	**0 spared of surgery**
Total	41	19	N=60

Table 2-5a: Binary Classification Table Describing Hypothetical Results with Clinical Examination Alone

Based on clinical examination alone, the specificity was 0%. All nineteen without complete digital nerve laceration underwent surgery.

Disease State	**Diseased**	**Non-Diseased**	**Total**
Test (WDT)			
Test Positive	41 (100%)	12	53
Test Negative	0	7 (36.84%)	**7 spared of surgery**
Total	41	19	N=60

Table 2-5b: Binary Classification Table Describing Hypothetical Results with WDT

When warm detection threshold testing is added to clinical examination, specificity is increased by ~37%. This would equate to 7/60 patients being spared of unnecessary surgery.

Disease State	**Diseased**	**Non-Diseased**	**Total**
Test (SWMT)			
Test Positive	4 (10%)	1	5 for surgery
Test Negative	37	11 (95%)	**48 for further testing**
Total	41	12	N=53

Table 2-5c: Binary Classification Table Describing Hypothetical Results with SWMT after WDT

ROC classification shows that SWMT has 95% specificity and 10% sensitivity when the 6.65 filament is used as a cutoff. This table shows the results of adding monofilament testing as an intermediate step to improve specificity. The subset of subjects who test positive with WDT would be followed by SWMT. Those testing positive should go ahead for surgery and those who test negative should go for the next stage of testing.

Disease State **Test (s2PD)**	**Diseased**	**Non-Diseased**	**Total**
Test Positive	37 (100%)	8	45 for surgery
Test Negative	0	3 (32%)	**3 more spared of surgery**
Total	41	19	N=48

Table 2-5d: Binary Classification Table Describing Hypothetical Results with s2PD after SWMT

ROC classification shows that s2PD has 100% sensitivity and 32% specificity when the cutoff of 7mm is utilized. When this is employed to those who test negative from SWMT, 100% sensitivity is maintained and 3 more subjects are spared of unnecessary surgery (32% specificity). Added to the 7 spared from WDT alone, this represents a total of 10 of 19 (53%) spared of surgery when all three tests (WDT, SWMT, and s2PD) are implemented.

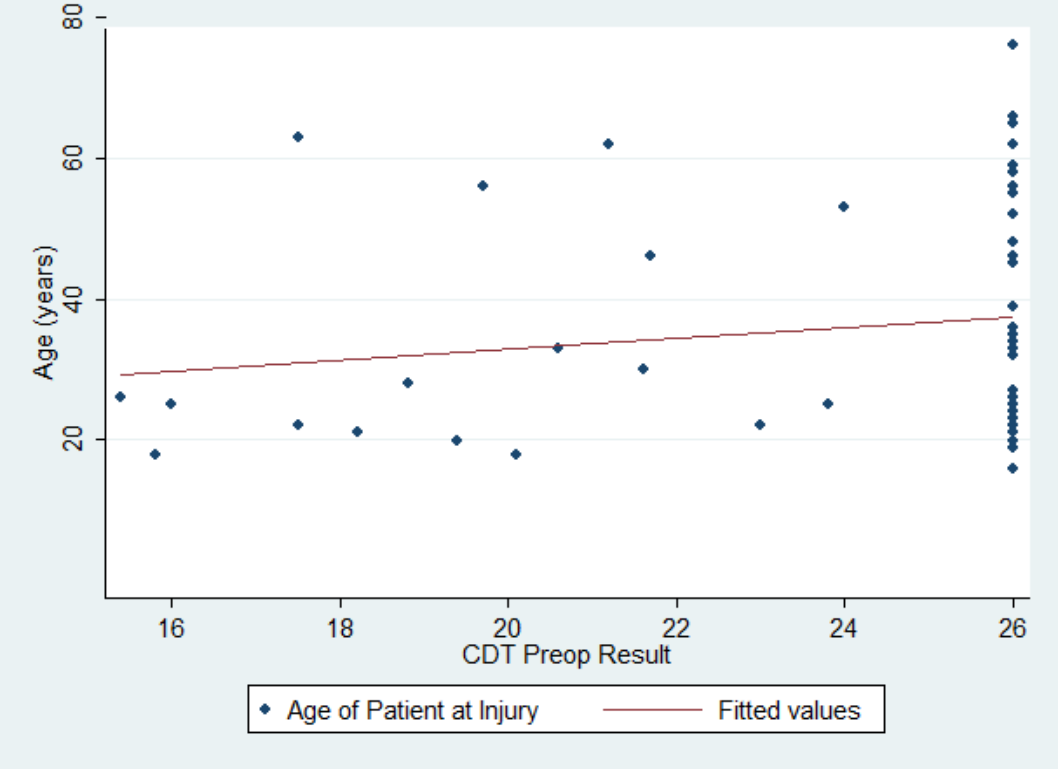

Figure 2-1: Linear Regression scatter plot of Age versus CDT

Linear regression analysis was used to determine if age had a significant effect on test outcome for each test. Here, an example of cold determination thresholds is plotted to confirm the insignificant coefficients of determination that were found. In essence, the regression line is not unlike a horizontal line.

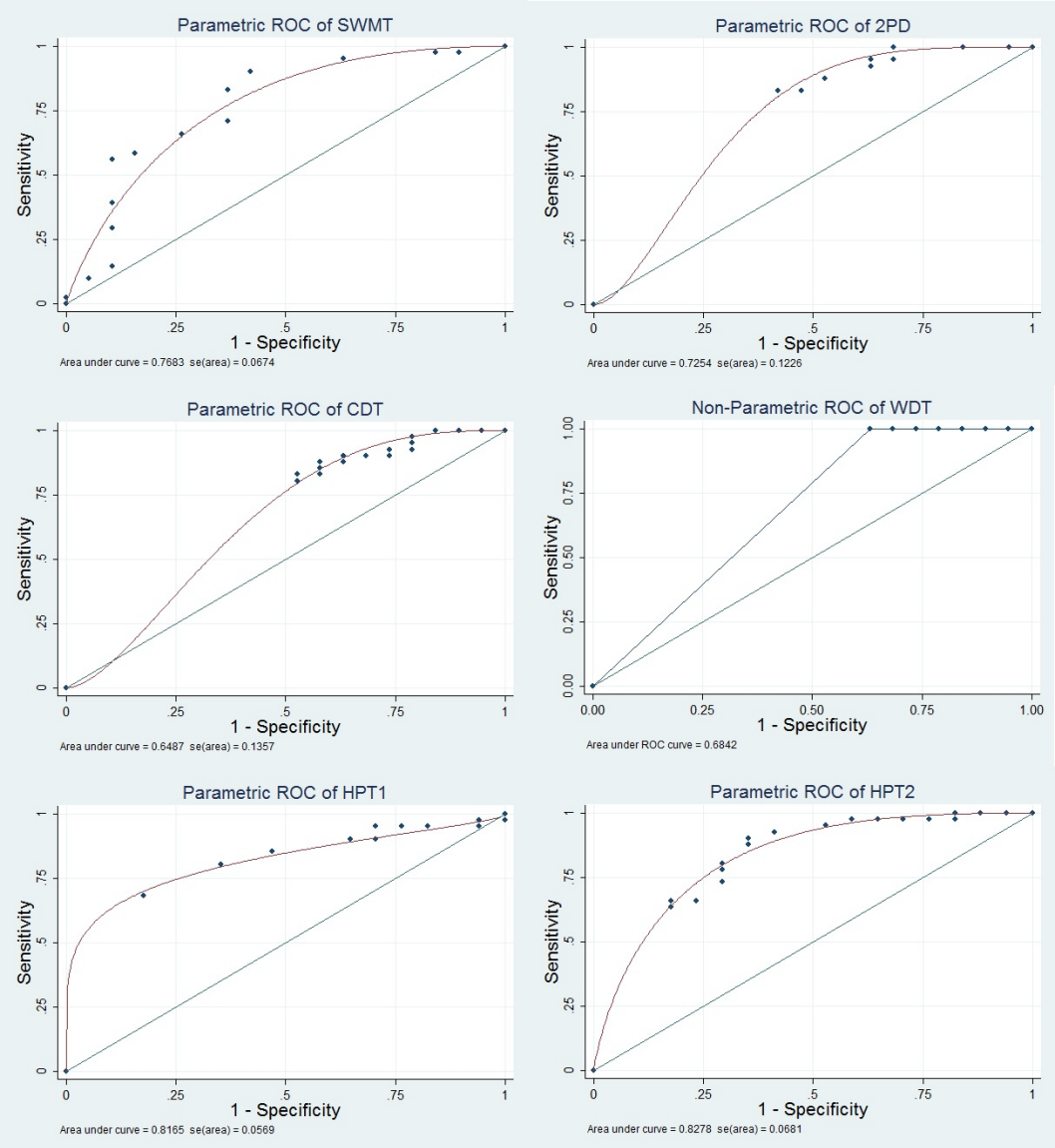

Figure 2-2: Graphical Comparison of Smooth Fit ROC Curves of all Tests

All 6 sensibility tests are plotted with individual maximum-likelihood fit ROC curves. This was done (with exception to WDT because a smooth fit could not be regressed) in order to compare the differences between shape and area of the different ROC curves.

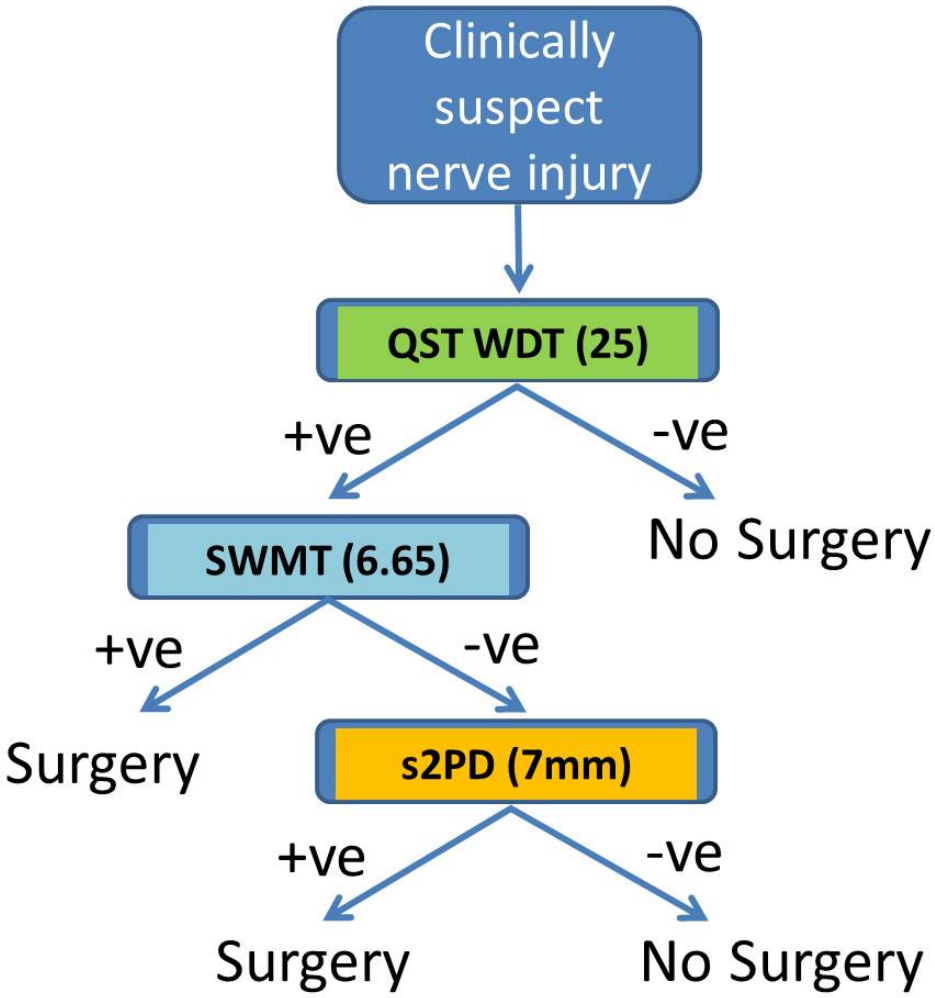

Figure 2-3: Modified Decision Tree Algorithm to Diagnose Digital Nerve Transection Injury

Implementing WDT, SWMT and s2PD in a 3-tiered decision algorithm augments clinical examination by maintaining 100% sensitivity and increasing specificity to 53% (compared to zero with clinical exam alone).

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Chapter 3: Electrical Stimulation Enhances Sensory Recovery: a Double blind, Randomized Control Trial

Introduction

The human hand is a richly endowed sensory instrument and versatile motor tool vital in daily activities. Peripheral nerve injuries in the upper extremity can be highly debilitating. Up to 3% of trauma patients suffer major peripheral nerve injuries (Noble *et al.*, 1998).

Surgeons have refined microsurgical techniques in hopes of removing impediments to axon regrowth. Although some improvements in outcomes have been achieved, major constraints persist. For example, median, ulnar and radial nerve repairs show good outcomes in, at best, only 42% of patients (Birch, 2011;Shergill *et al.*, 2001;Goldie *et al.*, 1992). Therefore, investigators are looking at adjuvants to augment axon regeneration. One promising method is brief post-surgical low-frequency electrical stimulation (ES) (Udina *et al.*, 2010;Madura *et al.*, 2007;Sharma *et al.*, 2010;Li *et al.*, 2005;Sabatier *et al.*, 2008). In a rat model where the femoral nerve was transected and repaired, ES increased regeneration-associated gene expression, accelerated axon growth across the repair site and enhanced specificity of re-innervation in motor and sensory axons (Geremia *et al.*, 2007;Al-Majed *et al.*, 2000;Brushart *et al.*, 2005). When applied to humans, ES significantly accelerated motor unit reinnervation to the thenar muscles after carpal tunnel decompression surgery (Gordon *et al.*, 2009).

In spite of those advancements, the effect of ES on sensory nerve regeneration in humans remains untested. This represents an important gap for 2 major reasons. First, digital nerves are the most commonly lacerated nerves (Buncke, 1972). To restore a high level of sensibility in the digit that is required even in routine daily tasks, vigorous reinnervation of the densely packed sensory end organs is needed. Indeed, even though the distance of regeneration in digital nerves is relatively short and the area of innervation is relatively small, recovery of two-point discrimination is often incomplete and hyperesthesia commonly persists (Kallio, 1993). Therefore, not surprisingly, digital nerve injury carries a substantial socioeconomic burden (Thorsen *et al.*, 2012). Second, in animal studies, the effects of ES on sensory nerve regeneration were shown to be somewhat different to that on motor nerve fibres (Al-Majed *et al.*, 2000;Geremia *et al.*, 2007). Therefore, whether ES also would work on digital nerve injury should not be assumed but, rather, needs to be directly tested.

Therefore, the goal of this study is to test the hypothesis that brief ES would significantly improve digit tip sensation and functional outcomes in patients with complete digital nerve transection compared to surgical repair alone.

Subjects and Methods

This was a randomized, double-blind, parallel-group, placebo-controlled clinical trial. The study was approved by the Human Research Ethics Board at the

University of Alberta. To comply with the CONSORT requirements, we enquired about registration at the clinicaltrials.gov website. However, we were informed that registration was not needed as the study did not involve the use of any pharmaceutical agents or devices that required an IDE license from regulatory agencies.

Participants: Subjects were recruited from plastic surgery hand clinics at the University of Alberta (Edmonton, Canada). Inclusion criteria were (1) ages 18-65 with a completely transected digital nerve, (2) receiving surgery within 14 days of injury, and (3) consenting to general anesthesia for surgery. Exclusion criteria were (1) concomitant bone injury of the affected digit, (2) complete devascularization, (3) those with diabetic or other polyneuropathies, and (4) cognitive impairment. Verbal and written informed consents were obtained from all subjects prior to preoperative testing. Subjects were unaware of treatment allocation.

Pre-operative Evaluation: Subjects with a suspected digital nerve injury underwent a baseline preoperative sensory assessment at a neurophysiology laboratory. Sensations in the territory of the affected nerve as well as the same nerve in the contralateral hand were assessed. Those tests included: i) static two-point discrimination (s2PD) and Semmes-Weinstein monofilaments (SWMT) for large myelinated Aβ fibres and, ii) quantitative sensory testing (QST) to yield

warm (WDT) and cold detection thresholds (CDT) for small Aδ and C fibres. Functional outcomes were assessed using the Disability of the Arm, Shoulder and Hand (DASH) questionnaire. The evaluator was blinded to the treatment assignment.

Surgical Procedure: The subjects underwent surgical exploration under general anesthesia by a plastic surgeon. General anesthesia was required because previous studies showed that propagation of action potentials to the cell body was necessary for ES to be efficacious. Local anesthetics with sodium-channel blockade would render ES ineffective (Al-Majed *et al.*, 2000;Geremia *et al.*, 2007). Intra-operatively, the nerve injury was assessed and those with complete digital nerve transection were included in the study. The nerve ends were debrided and a standard tension-free epineurial repair was performed.

Intervention: Prior to skin closure, two sterilized, Teflon-coated stainless steel wires were placed proximal to the coaptation site. These were secured to the skin with surgical tape before the incision was sutured. The wires were taped to the overlying dressing and splint (Figure 3-1). The operative time required to insert the wires was recorded. In the recovery room, an independent technician blinded to data collection and analysis attached the wires to a Grass SD9 electrical stimulator (Grass Technologies, Warwick, RI). The proximal wire was connected to the cathode and the distal wire to the anode. Delay from the time

of tourniquet release to commencement of post-surgical treatment was approximately 30 minutes. Using computerized random number generation, subjects were assigned (1:1 allocation) to either the experimental (ES) or sham placebo control (SPC) group (Viera & Bangdiwala, 2007). ES subjects received 1 hour of ES as a continuous 20Hz train of balanced biphasic pulses. The stimulation intensity was gradually increased to tolerance limit (<30V, 0.1-0.4ms). In the SPC group, subjects received 5s of low intensity ES and then null stimulation for the remainder of the hour. Since none of the subjects had prior ES, it was difficult for them to gauge the nature of their treatment. The wires were removed immediately after stimulation treatment or at first follow-up within a week.

Blinding and Randomization: Given the subjective nature of sensory response, double blinding was essential. The randomization code was kept at arms-length by a staff not involved in any sensory assessments. The study code was de-identified by an independent investigator removed from testing, treatment allocation and data analysis. The personal information, treatment allocation and test results remained de-identified until all follow-up was completed.

Follow-up Evaluation: Subjects returned for sensory testing at monthly intervals for six months. At each follow-up, all sensory measures were also conducted on the contralateral hand that served as control (Figure 3-2). Sensory examinations

were performed by the same technician who was blinded to the treatment status. The six month duration was chosen based on the assumptions that nerve regrowth should be complete given the relatively short distance for regeneration (2-6 cm) and a predicted growth rate of 1-3mm/day (Sunderland, 1947).

Outcome measures:

(1) Temperature: QST was completed with the Computer Aided Sensory Evaluator version 4 (CASE IV, WR Medical Electronics Co, Maplewood, MN). Quantitative CDT was used as the primary outcome because previous studies showed high reliability and consistency (Moloney *et al.*, 2012). The subject's affected digital pulp was placed on a thermoelectric stimulator using computer delivered stimuli. A 4, 2, 1 stepping algorithm was used to determine the minimum threshold for cold and warm detection measured in just-noticeable difference units (JND) (Dyck *et al.*, 1993). Null stimuli were randomly inserted within the algorithm to eliminate random guessing by the subjects. When an erroneous null response was detected, the test was terminated and restarted.

(2) Spatial discrimination: Static two-point discrimination was assessed using the Dellon-MacKinnon Disk-Criminator (pin distance 1-15mm). Ascending "method of levels" was applied to the side of the affected digital pulp longitudinally. Pressure was applied for 1.5 s just prior to skin blanching. Seventy-five percent of correct responses with the smallest distance was

recorded as the detection threshold (Klein, 2007). The result was used to grade the level of recovery based on Dellon's modification of the MRC Highet Scale (Dellon *et al.*, 1974) (Table 3-1). In this classification, S3 can only be achieved if the subject shows responsiveness to s2PD while S4 is when the s2PD performance is in the normal range (<6mm).

(3) Pressure Threshold: The SWMT 20 monofilament kit (Sammons Preston Rolyan, Canada) was used to assess pressure detection. An ascending "method of levels" was used on the affected digital pulp. Force was applied until the filament was bent for 1.5s. Detection threshold was defined as the smallest fiber with 75% correct identification.

(4) Disability: Functional outcomes were assessed using the disability and work modules of the DASH questionnaire. This instrument has been shown to be reliable, valid and responsive in peripheral nerve injury assessment (Bakhsh *et al.*, 2012;Beaton *et al.*, 2001).

Statistical Analysis: STATA 12 for Windows was used for the statistical analysis (StataCorp LP, College Station, TX). All description results are reported as mean±sd.

Sample size estimates: Since there were no published data on treatment differences with our selected outcome measures, we conducted an interim analysis with 10 patients at 6 months of follow-up using CDT as the primary

outcome. Based on that pilot data, we found that 30 subjects would be sufficient to achieve 80% power.

Student's t-test and Fisher's Exact Test were used to determine demographic differences between treatment groups. Shapiro-Wilk test showed normal distribution ($p>0.05$) in the outcomes for SWMT, s2PD, QST (CDT & WDT). Therefore, one-way ANOVA was used to determine the repeatability of tests by comparing outcomes from the contralateral hand (presumed to be constant) over all time intervals. Homogeneity of sensory outcomes between treatment groups preoperatively was assessed with student's t-test.

To account for inter-subject variability and variability between follow-up exams (time, humidity, temperature etc.), we normalized each test outcome to the outcome from the uninjured hand. To account for inter-subject variability, we also normalized this ratio to the baseline preoperative scores of the same subject.

Treatment effects were evaluated with each sensory test outcome at three physiologic time-points: early (average of 1 & 2 months), mid (3 & 4 months), and late stages (5 & 6 months). This enabled us to incorporate all patient data (intention-to-treat) when follow-ups were missed. Two-way ANOVA was performed on these time-points. When a significant interaction was seen between treatment and follow-up, Tukey's HSD was performed to determine the time points at which a significant change had occurred. For all tests, a 5% (α) level was deemed significant.

Results

Between July 2011 and June 2012, sixty patients with suspected digital nerve injury were identified and agreed to participate in the study. Intra-operatively, thirty-six of the 60 patients turned out to have complete nerve lacerations and went on to receive treatment (sham or ES) and follow-up monthly for six months. Eighteen subjects were randomly allocated to each group: SPC (receiving null stimulation x 1hr) and ES (1 hr continuous ES). Two subjects from each group were lost to follow-up and one additional subject from the ES group withdrew because he felt the treatment was ineffective. In total, 16 from the ES group and 15 from the SPC group completed follow-up (Figure 3-3). The patient demographics and details of the localization of injury are shown in Table 3-2. Fisher's Exact Test and student's t-test revealed no differences between treatment groups for any demographic characteristics or for the nature of injury. The mean operative time required from insertion and anchoring of the wire electrodes to skin closure was 5.68±1.53 mins, which was similar in both groups ($p=0.721$). There were no significant differences in baseline sensory testing results between the groups ($p>0.05$) (Table 3-3).

Consistency of the contralateral control

There was no statistical difference in the function of the contralateral hand over any monthly visit in any of the physiological measures ($p>0.05$). The

high degree of repeatability of these measurements suggests that learning effect did not play a significant role in changes of the longitudinal data. To control for intersubject variability, we normalized all outcomes to the contralateral side.

ES enhances temperature thresholds

QST was measured by means of algorithms developed in previous studies to determine small fiber sensory function (Dyck *et al.*, 1993;Dyck *et al.*, 1996;Gruener & Dyck, 1994). By 6 months, the CDT in ES subjects achieved a near normal mean threshold of 14.33±0.46JND versus 17.22±0.44JND in the control subjects. There was a significant interaction between treatment and follow-up based on two-way ANOVA ($p=0.003$). Tukey's HSD showed a significantly greater recovery with ES over controls at the middle and late stages of recovery ($p<0.001$) (Figure 3-4).

As part of our secondary outcomes, we looked at another facet of small fiber function in the form of WDT. At 6 months, ES patients achieved near normal threshold at 17.44±0.54JND versus 20.23±0.79JND in the control subjects. This difference was reflected by a significant interaction between treatment and follow-up ($p=0.002$) and a significant difference in WDT at the late stage ($p<0.001$).

ES enhances tactile discrimination and pressure detection

Static two-point discrimination and SWMT were utilized to assess large fiber function. The most remarkable effect of ES was seen with s2PD. The control patients recovered an average 8.69±1.05mm, whereas ES patients had near normal 4.71±0.90mm at 6 months. Two-way ANOVA showed a significant interaction between the treatment and follow-up ($p=0.018$) as well as significant advantage over controls at middle and late stage recovery ($p=0.007$, $p<0.001$ respectively). Translating this to the commonly used MRC Modified Highet Scale, 87% of subjects who received ES achieved S4 (normal) recovery versus 44% in the controls (Table 3-4).

When looking at pressure detection, the monofilament threshold in the ES subjects was close to normal (3.38±0.12) at 6 months compared to 3.91±0.11 in the controls. Two-way ANOVA again revealed a significant interaction ($p=0.015$) and significant advantage of ES at the late stage ($p<0.001$). In Figure 3-4, it is clear that control subjects plateaued in recovery after the middle stage while ES subjects continued to improve and approached normal function.

ES enhances disability and return to work scores

The DASH Questionnaire was used to grade and follow functional disability from the nerve injury. At 6 months, control subjects had an average 19.42±6.05 score compared to near normal 3.33±1.21 in ES subjects

(a score of 0 denotes normal function). Two-way ANOVA identified a significant interaction between treatment and follow-up in both disability and work modules (p=0.049, p=0.016 respectively) and a late stage recovery advantage over controls (p=0.014, p=0.027 respectively).

Complications

One control subject had a surgical site infection requiring extended antimicrobial therapy. One ES subject had an irremovable wire in the clinic after surgery. The wire was cut at the level of the skin and the remnant was removed in a tenolysis procedure at 7 months. All patients recruited after this had wires removed immediately post-ES and no further complication was seen (Table 3-5).

Discussion

This is the first human translational study demonstrating that ES in patients with digital nerve transection and repair results in improved physiological and functional sensation compared to surgery alone. Using a double blind randomized control design, the stimulated patients recovered near normal sensation at 6 months while most control patients did not. These physiological results are mirrored by the trends seen with the functional assessments. The DASH scores showed significantly better disability and work recovery at 5 to 6 months. These findings are important as they signify that ES

not only confers physiological benefits, but also has functional implications by enabling ES subjects to return to regular activities and work earlier.

Comparisons to published results in patients with digital nerve injury

In a study by Goldie et al on 27 patients with the majority receiving primary nerve repair within 24 hours, 37% regained normal s2PD after 25 months (Goldie *et al.*, 1992). In another study of 50 patients treated by primary nerve repair within 6 hours, 14% regained normal s2PD while 34% regained 6-10 mm of s2PD (al-Ghazal *et al.*, 1994). Wang et al. found that out of 74 primary digital repairs in 67 adults, forty-nine percent achieved <7 mm s2PD (Wang *et al.*, 1996). In a comprehensive review by Allan, he found that across all studies published between 1985 and 2000, around 50% of subjects could detect 10 mm or less on s2PD stimulation (Allan, 2004). While the outcomes in the control subjects in this study mirrored the aforementioned findings, the ES subjects showed substantially better results with almost double the number (87%) who achieved normal s2PD (Table 3-4).

Correlating results from animal model with this study

The published rate of axonal neurite regrowth at 1-3mm/day is likely overoptimistic (Al-Majed *et al.*, 2000). Recent studies show that it takes up to 8 weeks for the regenerating axons to traverse a 25mm gap – a time-period termed the "slow-staggered regeneration phase" (Al-Majed *et al.*, 2000). ES

decreased this time by 5 weeks (Brushart, 1988;Al-Majed *et al.*, 2000). The results of this study correlate with previous findings in animal studies that earlier and better outcomes were seen with stimulated subjects after 3-4 months post-operatively. The time to detect treatment effect is much longer in humans in part because of longer distances and the fact that outcomes only improved after axons reached the end organ. Static 2PD and SWMT show that ES improves reinnervation of receptors that confer tactile function in the digital pulp skin. QST showed greater recovery in cold and warm detection in the stimulated subjects. In contrast, neuro-labeling was used in animals to estimate the number of regenerating axons at constant distances from the repair site.

Clinical Significance

One of the major reasons for studying digital nerve injury is because it is the most commonly lacerated peripheral nerve, which carries a large health-care burden (Buncke, 1972). In a large study by Thorsen et al. regarding digital nerve injury, the incidence was ~1 in 10,000 per year with a direct cost of 2600-6000 euro per patient (Thorsen *et al.*, 2012). When including the additional 6000-8000 euro due to loss of productivity, the costs are even greater.

Unfortunately, functional outcomes with conventional treatment are poor. A review of surgical outcomes from 1985 and 2000 showed that less than 50% of patients achieved normal MRC recovery of s2PD (Allan, 2004). Hence,

treatments that can enhance recovery like ES are urgently needed to decrease the cumulative socioeconomic impact.

Practicality of ES

The cost of an electrical stimulator is less than 2000 CAD and the implantable wires cost between 5 to 10 CAD. These costs are orders of magnitude lower per patient than the cost savings from earlier rehabilitation and return to productive function. The additional operative time is less than 6 minutes, which can likely be decreased with experience. Adverse events associated with ES are minimal. The single case of infection occurred when the wire electrodes were removed immediately after ES treatment, reflecting an infection rate no different from standard surgery. The revised protocol of immediate wire removal prevents the possibility of adherent wires. Perhaps the most crucial argument for clinical feasibility is the short duration of treatment. Both animal and human research shows that no more than one hour of continuous ES will produce the maximum effect on both sensory and motor axons (Al-Majed *et al.*, 2000;Gordon *et al.*, 2009;Geremia *et al.*, 2007). This requirement should not be onerous as ES can be administered in the recovery room with minimal training at the bedside. This stands in marked contrast to the use of potential pharmacological agents to augment regeneration, where administrating treatment regimens and monitoring side effect profiles are likely

more costly and labor intensive (Sharma *et al.*, 2010;Tetzlaff *et al.*, 2007;Fansa *et al.*, 1999).

Limitations of the study

A constraint of ES is the need for surgery to be done under general anesthesia. Current practice is trending towards local or regional anesthesia that has the benefits of slightly lower perioperative risk, faster changeover, and improved post-operative analgesia. Therefore it may be clinically unacceptable to propose this treatment without investigating methods of administration under local anesthesia. To circumvent this, several alternative techniques have been used in previous studies. One method is to stimulate via a monopolar needle placed near the nerve well proximal to the field of anesthesia (Gordon *et al.*, 2009). A foreseen challenge is that in certain digital nerves, it would be hard to predict which peripheral nerve, median or ulnar, to stimulate (i.e. ring finger).

We did not cover the entire range of sensory functions in assessing treatment effect. As one of the major complaints of patients was inability to identify objects without sight (i.e. picking a dime from a pocket of coins), we could have included the shape-texture identification (STI) test (Rosen B. & Lundborg G., 1998;Rosen B & Jerosch-Herold C, 2000). The reason for its exclusion was because that this test was created for assessment of the index and little finger only. This would have been impossible in 50% of our patients who had nerve injuries in the remaining 3 digits.

In order to better classify functional measures we could go beyond the DASH and investigate voids such as pain and cold sensitivity, two of the most common symptomatic complaints after nerve injury. However, there was a finite time for which we could ensure ideal sensory testing without having a prolonged follow-up. As well, the DASH encompasses pain in both Disability and Work modules.

Finally, the duration of follow-up may have been too short. Although 6 months should theoretically be sufficient to measure reinnervation over the short distance despite "slow staggered regeneration" (Al-Majed *et al.*, 2000), our results show that tests such as WDT have later recovery. Longer follow-up would help to define whether control patients eventually reach normal function or just plateau after 3 to 4 months.

Conclusion

Based on a broad range of physiologic functions and disability assessment, this double blind randomized control trial shows, for the first time that ES of repaired digital nerves results in significantly better temperature distinction, pressure detection, spatial discrimination, and disability recovery in patients with digital nerve injury. This also proves that delivery of ES in the setting of nerve laceration and repair is feasible. When combined with the beneficial effects on motor axonal regeneration, ES can and should be implemented in distal as well as severe proximal nerve injury with motor and

sensory components. The acceleration of recovery will be crucial for injuries that are far from their end organs and the increase in magnitude of recovery will augment recovery of function. Combining ES with nerve transfers, targeted reinnervation, or other nerve injuries would be amongst the potential sites of implementation on the horizon.

Acknowledgements

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Grading	Description
S0	No sensory recovery
S1	Recovery of deep cutaneous sensibility
S2	Recovery of superficial cutaneous pain sensibility
S2+	As in S2, but with overresponse
S3	Recovery of pain and touch sensibility with disappearance of overresponse; static 2-point discrimination > 15mm
S3+	As in S3, but localization of the stimulus is good; static 2-point discrimination 7-15mm
S4	Complete recovery; static 2-point discrimination 2-6mm

Table 3-1: Modified Highet Scale for Grading Nerve Recovery (British Medical Research Council)

The Highet scale for grading nerve dysfunction was modified by Dellon in 1974 (Dellon *et al.*, 1974). Two-point discrimination was used primarily to grade the latter half of sensory recovery. This has been used in multiple studies ever since to grade sensation.

Variable	Total	Placebo	ES	P-value
Age (yrs)	38.3	39.3	37.2	0.698
	(15.5)	(17.9)	(13.3)	
Male Gender	25 (69.4)	15 (83.3)	10 (55.6)	0.070
Smoker	9 (25)	6 (33.3)	3 (16.7)	0.248
Injured Hand Dominant	20 (55.6)	9 (50)	11(61.1)	0.502
Concomitant Tendon Injury	18 (50)	8 (44.4)	10 (55.6)	0.505
Distance (cm)[a]	5.4 (2.2)	5.4 (2.6)	5.4 (1.7)	0.963
Additional OR Time (s)[b]	341.2	335.6	346.8	0.721
	(92.0)	(84.5)	(101.1)	
Right Handed	29 (80.6)	16 (88.9	13 (72.2)	0.402
Right Hand Injured	15 (41.7)	8 (44.4)	7 (38.9)	1.000
Radial Sided Nerve Injured	20 (55.6)	11 (61.1)	9 (50)	0.738

[a] Distance of Laceration from Finger tip
[b] Time required implanting wires, suturing skin and applying

Table 3-2: Patient Demographics Compared Between Treatment Groups

When all patient demographics were examined and compared between treatment groups, there was no significant difference based on Fisher's Exact and Student's t-test. The reported values represent mean (SD).

Test	ES	Placebo	P-value (t-test)
s2PD (mm)	3.23 (0.32)	3.25 (0.45)	0.879
SWMT (size)	5.09 (0.68)	5.08 (0.85)	0.969
CDT (JND)	23.70 (2.44)	24.36 (1.87)	0.369
WDT (JND)	>25 (0)	>25 (0)	1.000
DASH Disability	38.35 (19.53)	40 (18.82)	0.798
DASH Work	68.38 (30.74)	57.03 (34.22)	0.303

s2PD = static two-point discrimination; SWMT = Semmes-Weinstein monofilaments; CDT = quantitative cold detection threshold; WDT = quantitative warm detection threshold; JND = just-noticeable difference units; DASH = Disability of the Arm, Shoulder and Hand questionnaire score

Table 3-3: Comparison of Baseline Sensory Test Scores and Functional Disabilities.

Based on all sensory outcomes measured, there was no significant difference between baseline (pre-operative) test scores between treatment groups. The functional disabilities are also similar. The reported values are mean(SD).

Modified Highet Grading	Placebo Group (n=16)	ES Group (n=15)
<S3+	3 (18.8%)	1 (6.7%)
S3+	6 (37.5%)	1 (6.7%)
S4	7 (43.8)	13 (86.7%)
Lost to Follow-up	2	3

Table 3-4: Comparison of Modified Highet Scale Results

Based on Dellon's modification of the BMRC Highet scale for grading sensory recovery, 44% of subjects had normal recovery of sensation at 6 months (2PD<7mm) in the placebo group. In contrast, almost double this percentage (87%) of patients regained normal sensation in the ES group.

Adverse Event	ES (18)	Placebo (18)
Infection (>5d Abx therapy)	0	1
Irremovable Wire	1	0

Table 3-5: Summary of Adverse Events

Minimal adverse were seen with the ES treatment itself. The only notable event to mention was in the ES group, where an electrode wire was not removable at the first follow-up. The wire was eventually removed in a later tenolysis procedure at 7 months post-operatively. Otherwise, the protocol was modified to include immediate wire removal after this complication was seen (and no further complications occurred thereafter).

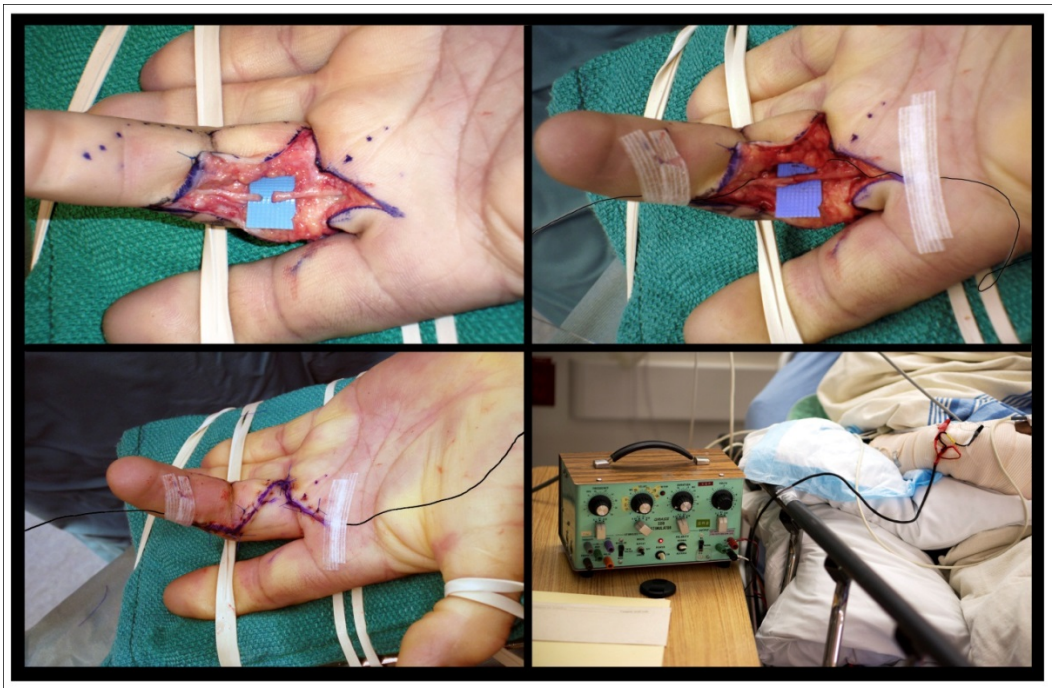

Figure 3-1: Surgical Intervention and Wire Implantation

A standard epineurial repair was performed with 2 to 4 9-0 nylon sutures. Thereafter, two fine-gauge electrode wires were implanted just proximal to the coaptation site and secured to the skin with tape. The wires were then attached to a Grass SD9 electrical stimulator for randomized treatment in the recovery room.

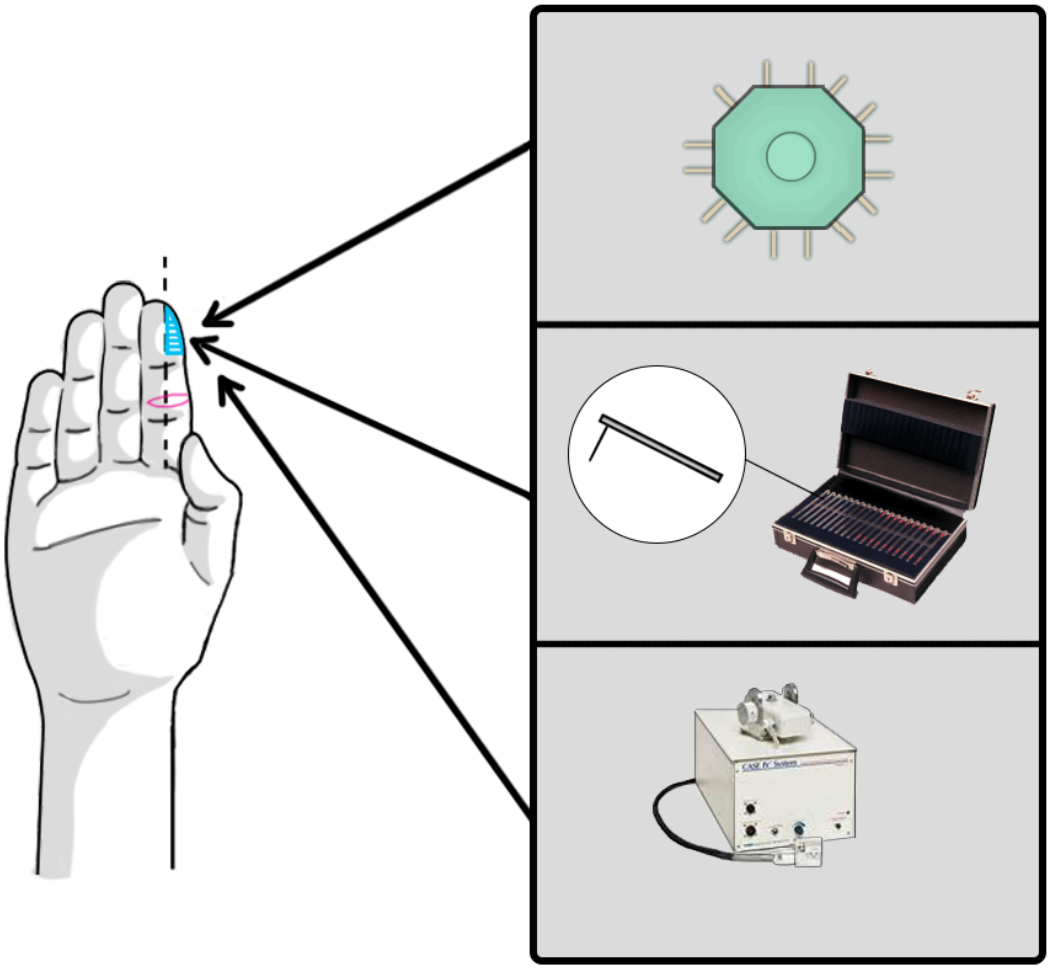

Figure 3-2: Sensory Testing with 2PD, SWMT, and QST

Tests performed preoperatively and post-operatively at monthly intervals for 6 months. Spatial discrimination was assessed with the Dellon-Mackinnon Disk-Criminator, pressure threshold with the Semmes-Weinstein 20 monofilament kit, and QST with the Computer Aided Sensory Evaluator version 4 (yielding CDT and WDT).

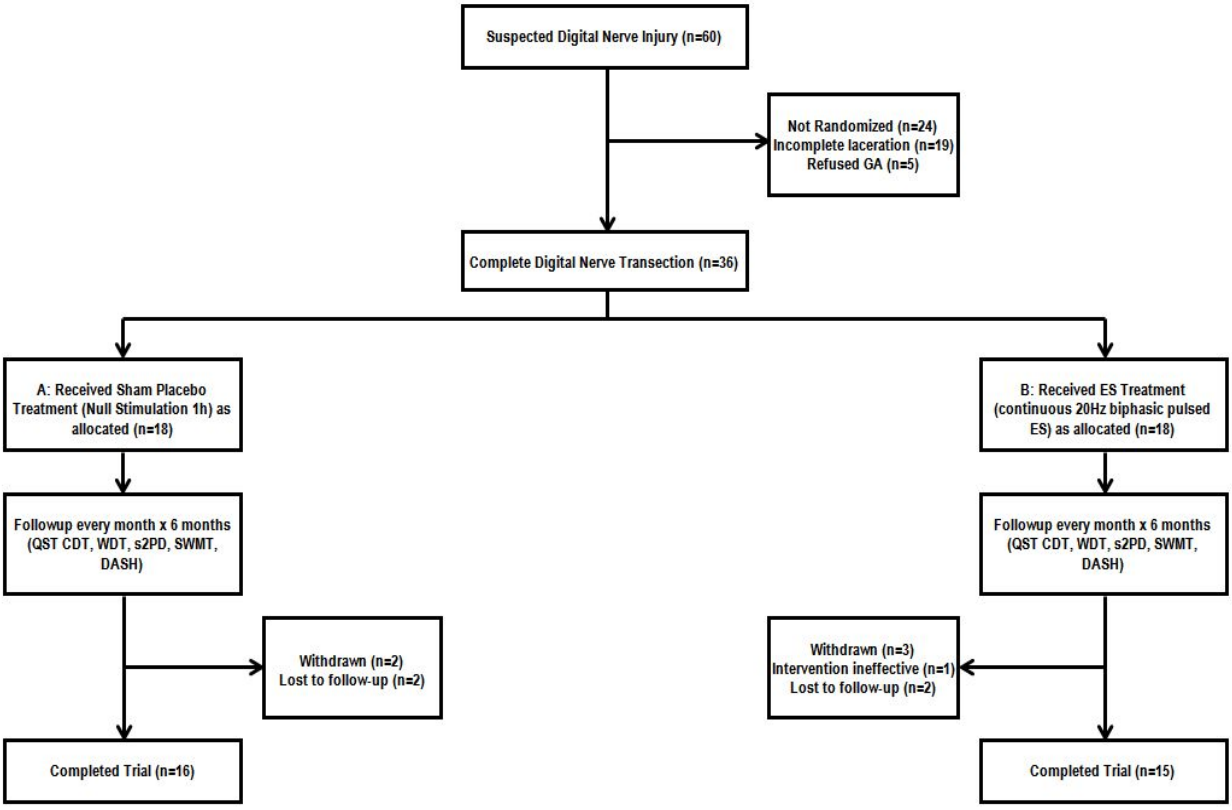

Figure 3-3: Clinical Trial Flow Diagram

This flow chart depicts the recruitment and follow-up of all subjects involved in the study. Out of 60 subjects initially recruited, 36 had complete digital nerve injury seen intra-operatively. 2 subjects in each treatment group were lost to follow-up and an additional subject withdrew from the experimental treatment group because the treatment was perceived to be ineffective.

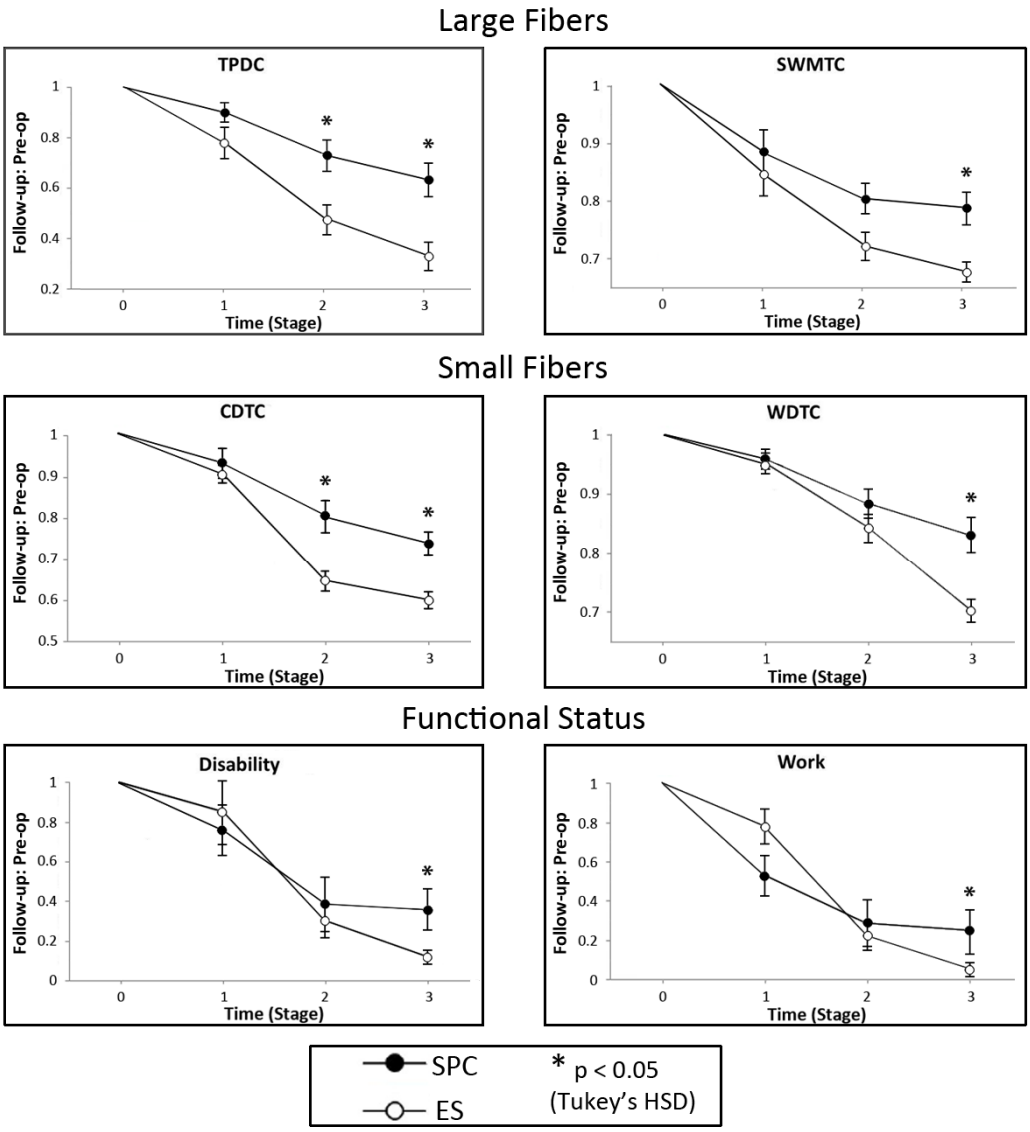

Figure 3-4: Results of Treatment Effect

Compared to the control group, those in the ES group showed significantly greater improvement in cold detection (CDT: primary outcome) at middle and late stages of sensory recovery. This same relationship was seen with static two-point discrimination recovery (s2PD). As well, the remaining secondary outcomes (SWMT, WDT, DASH modules) all showed significant late stage recovery advantage.

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Chapter 4: Sensory Nerve Injuries in the Human: A Final Discussion

Given the high incidence of peripheral nerve injuries, the resultant functional and socioeconomic burden (Rosberg *et al.*, 2005; Thorsen *et al.*, 2012), and most importantly, the fact that outcomes with surgical repair alone are only mediocre (Allan, 2004; Birch, 2011; Shergill *et al.*, 2001; Goldie *et al.*, 1992), a systematic approach to improving functional outcomes is desperately needed. However, a practical barrier that must first be overcome is that even though the methods used to quantify motor nerve physiology and function are relatively straight forward, the testing of the wide range of sensory functions is more difficult in patients. Therefore, it is necessary to take a step-wise approach to first develop better diagnostic tools that quantify the severity of sensory nerve injury and to monitor their recovery following treatment. In other words, if a nerve transection cannot be correctly diagnosed, it would be much harder if not impossible to interpret the effects of treatment that may enhance axonal regeneration and recovery.

An in-depth review of the literature on sensory nerve injuries has revealed two major voids that were addressed in this dissertation. First, although many authors have examined the validity, reliability and responsiveness of available sensibility tests, there is minimal evidence on the diagnostic accuracy of those tests in detecting nerve transection. In the absence of that, the prevalent practical solution is to take a liberal approach and to operatively explore all

suspected transections. This aversion of missing a complete laceration comes with the cost of many unnecessary surgeries. To address that issue, we systematically compared the diagnostic performance of 6 sensibility tests (2PD, SWMT, CDT, WDT, HPT1, HPT2) on 60 subjects with suspected digital nerve laceration. The major findings are that heat pain determination threshold (HPT2) had the highest diagnostic accuracy in detecting digital nerve injury based on AUC analysis as well as optimized sensitivity and specificity (determined by Youden index) (Kumar & Indrayan, 2011; Perkins & Schisterman, 2006). However, even though its specificity is only modest, warm determination threshold (WDT) had 100% sensitivity at every potential cutoff point. Given the paramount importance of correctly identifying all patients with digital nerve transection, we recommend the use of WDT at a cutoff of 25JND. With a specificity of 37%, it would still allow a significant number of patients to be spared from surgery. To optimize the specificity even further, the use of SWMT and s2PD in a revised decision tree algorithm could improve the overall specificity to 53%, representing a substantial health cost saving.

Secondly, although there is convincing evidence to suggest that post-surgical ES results in improved motor axon regeneration in the human, its efficacy in human sensory nerve regeneration remained unproven. This void is critical because most major nerve injuries have a mixed motor and sensory component. Using a comprehensive range of sensory testing, we established the efficacy of ES on digital nerve laceration by conducting a double-blind

randomized controlled trial. Our results showed that patients receiving ES post-operatively had significantly better and faster sensory recovery. This serves as a proof-of-principle study to justify the use of ES as an adjuvant therapy for other nerve transection injuries. In addition, ES may also be useful in other nerve reconstructions such as nerve transfers in targeted reinnervation.

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