Distribution Agreement

In presenting this thesis or dissertation as a partial fulfillment of the requirements for an advanced degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis or dissertation in whole or in part in all forms of media, now or hereafter known, including display on the world wide web. I understand that I may select some access restrictions as part of the online submission of this thesis or dissertation. I retain all ownership rights to the copyright of the thesis or dissertation. I also retain the right to use in future works (such as articles or books) all or part of this thesis or dissertation.

Signature:

_____ _____

Claire Emma McGregor Date

The effect of the Val66Met BDNF polymorphism on axon regeneration after peripheral nerve injury

By

Claire Emma McGregor
Doctor of Philosophy

Graduate Division of Biological and Biomedical Science
Neuroscience

Arthur W. English, Ph.D.

Advisor

Francisco J. Alvarez, Ph.D.
Committee Member

Shannon L. Gourley, Ph.D.
Committee Member

Yue Feng, Ph.D.
Committee Member

Shawn Hochman, Ph.D.
Committee Member

Kathryn J. Jones, Ph.D.
Committee Member

Accepted:

Lisa A. Tedesco, Ph.D.
Dean of the James T. Laney School of Graduate Studies

Date

The effect of the Val66Met BDNF polymorphism on axon regeneration after peripheral nerve injury

By

Claire Emma McGregor

B.A. Neuroscience (High Honors), Oberlin College, 2011

Advisor: Arthur W. English, Ph.D.

An abstract of a dissertation submitted to the Faculty of the James T. Laney School of Graduate Studies of Emory University in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Neuroscience

2018

The effect of the Val66Met BDNF polymorphism on axon regeneration after peripheral nerve injury

By

Claire Emma McGregor

Although peripheral nerves can spontaneously regenerate after injury, outcome is generally very poor. Activity-dependent treatments to enhance peripheral nerve regeneration after injury have shown great promise, and clinical trials implementing them have begun. Success of these treatments requires activity-dependent release of brain derived neurotrophic factor (BDNF). A single nucleotide polymorphism (SNP) in the *BDNF* gene known as Val66Met found in nearly one third of the human population results in defective activity-dependent BDNF secretion and could impact the effectiveness of these therapies. We used a mouse model of this SNP to test the efficacy of treadmill exercise in enhancing axon regeneration in animals both heterozygous (V/M) and homozygous (M/M) for the SNP. Axon regeneration was studied four weeks after complete transection and repair of the sciatic nerve, using both electrophysiological and histological outcome measures. Regeneration was enhanced significantly without treatment in V/M mice, compared to wild type (V/V) controls. Unlike V/V mice, treatment of both V/M and M/M mice with treadmill exercise did not result in enhanced regeneration. These results were recapitulated *in vitro* using dissociated neurons containing the light-sensitive cation channel, channelrhodopsin. Three days after plating, neurites of neurons from V/M and M/M mice were longer than those of V/V neurons. In neurons from V/V mice, but not those from V/M or M/M animals, longer neurites were found after optical stimulation. Taken together, Met carriers possess an intrinsically greater capacity to regenerate axons in peripheral nerves, but this cannot be enhanced further by activity-dependent treatments. This enhanced axon regeneration can be blocked by application of a trkB antagonist, ANA-12. In culture, application of ANA-12 to adult dorsal root ganglion (DRG) neurons blocks enhanced neurite outgrowth in cells from M/M mice. Application of the fusion protein trkB-Fc which sequesters trkB ligands also results in decreased neurite outgrowth in all genotypes. Inhibiting the pan-neurotrophin receptor, p75NTR, resulted in a modest improvement in V/V axon regeneration, and no effect in Met-carriers. Thus, the enhanced axon regeneration in Met-carriers is trkB-dependent.

The effect of the Val66Met BDNF polymorphism on axon regeneration after peripheral nerve injury

By

Claire Emma McGregor

B.A. Neuroscience (High Honors), Oberlin College, 2011

Advisor: Arthur W. English, Ph.D.

A dissertation submitted to the Faculty of the James T. Laney School of Graduate Studies of Emory University in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Neuroscience

2018

Table of Contents

Figure Index

Chapter 1:

Introduction to the role of BDNF in peripheral nerve injury and regeneration

Currently under review at Frontiers in Cellular Neuroscience: McGregor CE & English AW (2018) The role of BDNF in peripheral nerve regeneration: Activity-dependent treatments and Val66Met. Front Cell Neurosci: submitted.

Peripheral nerves connect our central nervous system to our sensory organs, which allow us to perceive the world, and the musculature that allows us to navigate it. The sciatic nerve, originating from L3-L5 segments of the spinal cord and extending as far as the lumbrical muscles of the foot, can contain axons as long as 3 feet in a human. If severed, a peripheral nerve can regenerate at a rate of about 1-5 mm/day (Jacobson and Guth, 1965, Grafstein, 1971, Hoffman and Lasek, 1980, Gutmann et al., 1942). Thus, after a very proximal sciatic nerve transection, axons could take two and a half years to fully regenerate. In reality, peripheral nerve injuries can take much longer than this—consider the case of Dr. Henry Head, who in 1905 intentionally transected his own radial nerve to observe regeneration. Though the injured nerve needed to regenerate a distance of no more than 30cm, it took two years for his sensory recovery (Compston, 2009, Mundie, 1920). In others, injured peripheral nerves often fail to regenerate completely.

Millions of peripheral nerve injuries occur every year, which in the United States can result in approximately $150 billion spent annually (Taylor et al., 2008). Common causes include car accidents, gunshot wounds, surgical transection, and work-related injuries (Kouyoumdjian, 2006, Noble et al., 1998). Nerve injury results in loss of motor function and sensory input. Unlike spinal cord injuries, peripheral nerves can spontaneously regenerate. This regeneration is slow and inefficient, however, and as mentioned above, most patients never regain full motor function after a peripheral nerve injury (Portincasa et al., 2007). Currently the only treatment for peripheral nerve transection is surgical—placing the two ends of the injured nerve together or using a graft from a different nerve to bridge the gap (Grinsell and Keating, 2014). For those with more minor injuries, no treatments are available. Therapies that enhance the speed of regeneration are sorely needed.

Peripheral Nerve Structure

The structure of peripheral nerves was first described by Greek physician Herophilus in 300 BCE, who determined the nervous system was continuous (Lee and Wolfe, 2000). Spinal nerves contain the axons of motoneurons, whose cell bodies are located in the ventral horn of spinal cord, and sensory neurons, whose cell bodies reside in the dorsal root ganglia (DRG) (Rexed, 1952). Cranial nerves also can be mixed but may contain only sensory or motor axons. Neurons in sensory ganglia are pseudo-unipolar, with one axon that extends centrally into the central nervous system (CNS) and one axon that extends peripherally into the nerve. Motor axons exit the spinal cord through the ventral root and fuse with the peripheral processes of DRG axons to form a spinal nerve. Glial Schwann cells make up approximately 80% of the cells in a peripheral nerve and can either be myelinating or non-myelinating (Griffin and Thompson, 2008, Brosius Lutz and Barres, 2014). Unlike central oligodendrocytes, Schwann cells only myelinate one axon. Individual myelinated axons are surrounded by multiple Schwann cells, arranged in series, and the space between myelinating Schwann cells forms the Nodes of Ranvier. Non-myelinating Schwann cells ensheath multiple small axons in bundles. Together, Schwann cells and axons are enclosed by three layers of connective tissue. Individual axons and myelinating Schwann cells are surrounded by the endoneurium. The tubes formed by the endoneurium are organized both spatially and by function into fascicles surrounded by the perineurium, and the outermost layer of connective tissue enclosing the nerve is the epineurium (Topp and Boyd, 2012).

Motoneurons synapse onto muscle fibers, forming the neuromuscular junction. In mammals, these synapses are cholinergic, and the motor endplate is characterized by highly organized nicotinic acetylcholine receptors. Release of acetylcholine from neuromuscular synapses produces a muscle action potential that results in contraction of the muscle fiber. In adults, muscle fibers receive synaptic inputs from only one motoneuron, but individual motoneurons innervate multiple muscle fibers. Together the motoneuron and every muscle fiber

it innervates is called a motor unit (Sherrington, 1925). The average number of muscle fibers per motoneuron is called the innervation ratio (Heckman and Enoka, 2012), and this differs in different muscles. Muscles that require fine motor control, such as those in the hand and fingers, tend to have smaller innervation ratios compared to large muscles with fewer degrees of freedom, such as trunk muscles. The fibers that comprise an individual motor unit are generally interspersed throughout the muscle body (Bodine et al., 1988).

Unlike motoneurons, sensory neurons are a heterogeneous population. They respond to numerous sensory modalities, such as proprioceptors in skeletal muscle and mechanoreceptors or free nerve endings in the skin. Sensory neurons are traditionally categorized by cell body size, fiber type/axonal conduction velocity, and protein expression (Horch et al., 1977, Ju et al., 1987). For example, small diameter DRG neurons are associated with unmyelinated and slow-firing C-fibers or thinly myelinated Aδ fibers, which are responsible for nociceptive, thermal, and high threshold mechanoreceptive signals. These cells are further classified by expression of either a carbohydrate on the cell surface that specifically binds isolectin B4 (non-peptidergic), or different peptides such as calcitonin gene-related peptide (CGRP, peptidergic). Large diameter DRG neurons are associated with Aβ fibers, respond to low threshold mechanoreceptive and proprioceptive stimuli and express neurofilament 200 (Wang et al., 1994, Li et al., 2016, Alvarez and Fyffe, 2000).

Peripheral Nerve Injury

In 1943, Seddon classified various peripheral nerve injuries in a simple three class system: Neuropraxia, in which nerve conduction is lost, but axonal integrity is not; axonotmesis, in which axons and myelin are damaged but the surrounding connective tissue remains continuous, such as would be found in a nerve crush; and neurotmesis, a complete transection of the nerve (Kaya and Sarikcioglu, 2015). In 1951, Sunderland further expanded the third class, neurotmesis, into three separate classes to specify levels of damage to the three types of connective tissues described above (Grinsell and Keating, 2014). Once a nerve has been

injured, whether through crush or a more severe transection injury, multiple cellular processes take place, including both central and peripheral changes within the injured cells as well as changes in glial signaling and activity.

First described in 1850 by Waller, after a peripheral nerve transection, the distal portion of the nerve is degraded in a process dubbed “Wallerian Degeneration” (Waller, 1850). This process is carried out by invading immune cells and Schwann cells which dedifferentiate into a pre-myelinating/pro-regenerative phenotype (Stoll et al., 1989, Liu et al., 1995). Over the course of 48 hours in rodents, and up to a week in humans, distal axons are degraded retrogradely from the site of injury in a progressive wave (Beirowski et al., 2005, Kato and Ide, 1994, Chaudhry and Cornblath, 1992). Axons in the proximal stump are also degraded as far back as the closest Node of Ranvier (Kato and Ide, 1994). The clearing of this cellular debris associated with axon degeneration can take over a week, and the distal axon portion can remain active during that time, firing action potentials and activating muscle (Pan et al., 2003, Mackenzie et al., 2012). Schwann cells initiate the degeneration process in the first 48 hours and act as the main phagocytic cells for the first five days following injury (Perry et al., 1995). Neutrophils are the first invading immune cells to aid in the phagocytosis of cellular and myelin debris, and they recruit other immune cells (Nathan, 2006). Macrophages are present in uninjured nerves, but after injury their population increases, and both resident and invading hematogenous macrophages are responsible for the later phases of Wallerian degeneration (Perry et al., 1987, Mueller et al., 2003).

Dedifferentiated Schwann cells proliferate and populate the empty endoneurial tubes, forming Bands of Büngner to guide the regenerating axons (Webber and Zochodne, 2010). The Schwann cells secrete growth factors to attract the axons, including nerve growth factor (NGF), brain derived neurotrophic factor (BDNF), and glial cell line-derived neurotrophic factor (GDNF) (Glenn and Talbot, 2013, Brosius Lutz and Barres, 2014, Arthur-Farraj et al., 2012). Once dedifferentiated Schwann cells make contact with the regenerating axons, they redifferentiate

into myelinating Schwann cells, forming new myelin sheaths around axons that are permanently thinner than the original sheaths that formed during development (Gomez-Sanchez et al., 2017, Schroder, 1972). Myelinating Schwann cells do not secrete growth factors, and the conversion of Schwann cells as the growth cone reaches them may create a natural gradient of growth-promoting molecules in the nerve that encourages forward growth through the endoneurial tubes. In chronic denervation conditions, Schwann cells die off by six months (Ebenezer et al., 2007, Sulaiman and Gordon, 2000). Once the endoneurial tubes have lost their Schwann cells, regeneration is severely hampered (Fu and Gordon, 1995b, Fu and Gordon, 1995a). Lack of Schwann cell support has been blamed for failure of regenerating axons to regenerate into conduits and acellular grafts. Although Schwann cells in the proximal segment of cut nerves are able to invade these constructs, regeneration is generally very poor.

The initiation of distal axon degeneration triggers the blood-nerve barrier to become permeable. This is different from any breaches that might occur during the trauma that caused the PNI. This change can last up to four weeks post-injury and is caused by invading macrophages (Gray et al., 2007). Dedifferentiated Schwann cells begin expressing pro-inflammatory cytokines which recruit monocytes to the site of injury (Tofaris et al., 2002). Monocytes can differentiate into reparative macrophages which, in addition to phagocytosing cellular debris, also secrete neurotrophins (Heumann et al., 1987b, Perry et al., 1987, Hikawa and Takenaka, 1996) which could aid in axon regeneration. This role of monocytes was utilized in an experiment in which fractalkine, a chemokine that preferentially recruits reparative monocytes, was added to a synthetic nerve conduit used to repair a cut nerve and enhanced axon regeneration (Mokarram et al., 2017).

Immediately following an injury, a calcium influx propagates a retrograde action potential. Once this injury-evoked action potential reaches the cell body it triggers the process of changing the injured neurons into a regenerative state (Mar et al., 2014). Other injury signals are retrogradely transported to the nucleus—these proteins act as sentinels, ready to inform the

nucleus of injuries to the axon. This system was first described in *Aplysia*. Intact neurons injected with axoplasm from an injured cell shifted into a regenerative state (Ambron et al., 1995). Over time, a number of retrogradely transported molecules were identified, including extracellular signal-regulated kinase (ERK), c-Jun, the c-Jun NH_2-terminal kinase (JNK), and signal transducer and activator of transcription 3 (STAT3) (Mar et al., 2014, Perlson et al., 2005, Cavalli et al., 2005, Raivich et al., 2004, Ben-Yaakov et al., 2012). These molecules are all involved in complex intracellular signaling. For example, STAT3 is a transcription factor important in development, which in adulthood is activated by cytokines and promotes neural survival and regeneration through upregulating pro-regeneration genes, such as GAP-43 (Qiu et al., 2005, Dziennis and Alkayed, 2008, Takeda et al., 1997). Proteins related to retrograde axonal transport are found in higher levels after axon injury and can be locally translated in the injured axon (Perlson et al., 2005, Cavalli et al., 2005, Hanz et al., 2003). As the cell changes to a regenerative state, the cell body swells, the nucleus shifts position within the cell body, and protein synthesis increases (Watson, 1968, Lieberman, 1971). Specifically, regeneration associated genes such as GAP-43 are upregulated whereas genes associated with neurotransmission, such as choline acetyltransferase in motoneurons are downregulated (Fu and Gordon, 1997, Zigmond et al., 1997).

The first challenge for a regenerating axon in an injured nerve is to cross the site of injury and enter one or more endoneurial tubes in the nerve distal to the site of injury. Within two hours of complete transection, a new growth cone forms at the Node of Ranvier most proximal to the site of injury (Kato and Ide, 1994). The injured axon possesses all the machinery necessary to form a growth cone with no input from the cell body, relying on local protein synthesis and degradation, allowing for this process to occur on a short time scale (Kato and Ide, 1994, Verma et al., 2005). Despite the relatively quick formation of new growth cones, crossing the site of injury to invade the distal nerve stump can take multiple days (Sunderland, 1947, Danielsen et al., 1986). Many axons “wander” the site of injury, sometimes coiling or

turning around completely (Gordon and English, 2016, Witzel et al., 2005). This wandering of axons results in a slow and temporally staggered navigation past the injury site— after nerve transection and surgical repair, only 25% of axons cross the repair site within the first week, and 3-4 weeks are required for all axons to enter distal endoneurial tubes (Witzel et al., 2005, Brushart et al., 2002).

The ability of growth cones to cross the site of injury depends on a balance of inhibitory and pro-regeneration signals. The Schwann cell basal lamina contains many molecules that function as pro-regenerative signals, such as laminin and the neurotrophins, as well as inhibitory signals. In the first week following an injury, Schwann cells deposit chondroitin sulfate proteoglycans (CSPGs) around the endoneurial tubes in the distal nerve segment (Krekoski et al., 2001). The glycosaminoglycan side chains of CSPGs inhibit axon growth (Zuo et al., 1998, Braunewell et al., 1995, Shen et al., 1998). These CSPGs are upregulated in injured nerves, with levels peaking at 7 days after injury (Zuo et al., 1998). Matrix metalloproteinases (MMPs), which break down CSPGs, are found in increased levels in in the Schwann cell basal lamina of the distal stump as Wallerian degeneration takes place, starting at day 3 and peaking at day 14 in rodents (Ferguson and Muir, 2000). This coincides with peak Schwann cell secretion of growth factors in rodents at 15 days after injury (Hoke et al., 2006). The initial increase in inhibitory signals followed by disinhibition through upregulated MMPs and growth signaling through growth factors could explain the delay in axons crossing the site of injury. This is supported by studies which enhanced the ability of axons to invade endoneurial tubes during the first week of regeneration by treating injured nerves with enzymes that degrade the glycosaminoglycan side chains of CSPGs (Krekoski et al., 2001, Sabatier et al., 2012, Groves et al., 2005, Zuo et al., 2002, Udina et al., 2010). However, despite the inhibitory effect of CSPGS, they may play an important role in targeting the regenerating axons into endoneurial tubes and preventing them from invading the area around the tubes. Blocking all CSPG activity results in inappropriate reinnervation of peripheral targets (English, 2005).

Mistargeting of regenerating axons is a common feature of peripheral nerve regeneration, and can result in poor functional recovery even when regeneration has been successful (Brushart and Mesulam, 1980). Regenerating axons form a number of new growth cones, a process called sprouting, described a century ago by Ramón y Cajal (Ramon y Cajal, 1928). Sprouts from individual axons invade multiple endoneurial tubes with varied results. For example, sensory axons can regenerate into endoneurial tubes previously occupied by motor axons, resulting in decreased motor function as well as a degradation of sensory feedback. In addition, motoneurons reinnervating inappropriate muscle can result in dyskinesia and poor functional recovery, particularly when reinnervating functionally antagonistic muscles (i.e. a motoneuron previously innervating an extensor muscle now innervating a flexor) (Gillespie et al., 1986, English, 2005). Similarly, two sprouts from the same motoneuron can innervate fibers in antagonistic muscles (Brushart and Mesulam, 1980, Gordon et al., 2003).

Mistargeting of axons occurs as a result of newly formed growth cones entering inappropriate endoneurial tubes (Höke and Brushart, 2010). Once in the tube, the axon is committed to the pathway formed by that tube, and cannot switch, regardless of end organ. This problem is much more common in transection injuries than nerve crush injuries, because in a nerve crush, endoneurial tubes remain intact, and injured axons remain in their original tube (Brown and Hardman, 1987, Nguyen et al., 2002). In transection injuries, however, axons often lose endoneurial tube specificity. During the process of regeneration, axons of motoneurons spontaneously sprout numerous collaterals that can end up in both motor tubes and sensory tubes. Once the motor axons reach the target muscle, the incorrect collaterals in the sensory tubes are pruned, demonstrating a preference for motor tubes. Motoneurons that only innervate sensory tubes, however, never correct and remain mistargeted (Brushart, 1993). Moreover, the specificity of sprouts reaching the endoneurial tubes later in the process of regeneration appears to be higher. The same number of mistargeted motoneurons is found at two weeks as is found at eight weeks (Brushart, 1993). This could be a result of changes taking place at the

site of injury—in rodents, in approximately 10 days, Schwann cells have formed Bands of Büngner—linear arrays to support sprouts entering the endoneurial tubes (Fu and Gordon, 1997, Gordon and English, 2016). At 15 days, rodent Schwann cells have peaked in growth factor secretion, and this secretion may be sensory/motor pathway specific (Brushart et al., 2013, Hoke et al., 2006, Gordon, 2015). Thus, temporal staggering of regeneration may actually increase the specificity of regenerating axons targeting their appropriate end organs.

In addition to cellular changes at the site of injury, numerous central changes occur after a peripheral nerve injury. Central reorganization of synapses occurs after a peripheral nerve injury. In mice, two weeks after injury axotomized neurons have lost approximately 35% of their overall synaptic coverage on the cell body (Krakowiak et al., 2015). Both excitatory and inhibitory synapses onto motoneurons are degraded (Alvarez et al., 2011, Brannstrom and Kellerth, 1998, Blinzinger and Kreutzberg, 1968, Linda et al., 2000, Oliveira et al., 2004). Reinnervation of target muscles results in a recovery of lost synapses (Brannstrom and Kellerth, 1999). A slower and more extensive die-back of the central branches of IA afferent neurons results in permanent synaptic loss on the motoneurons, primarily at the soma and most proximal dendrites (Alvarez et al., 2011, Rotterman et al., 2014). This permanent loss of IA afferent signaling is hypothesized to be the mechanism behind failure of muscle stretch reflex restoration even after successful nerve regeneration (Bullinger et al., 2011).

Following a peripheral nerve injury, there is substantial DRG neuron death. In adults, motoneurons are spared and do not undergo cell death in response to axotomy. In contrast, over the course of up to 32 weeks post-injury, there is substantial loss of DRG neurons—up to 50% of all cells (Tandrup et al., 2000). The bulk of cell death occurs in non-peptidergic (IB4+) small DRG cells, with large DRG neurons and muscle afferents spared (Welin et al., 2008, Hu and McLachlan, 2003). Immediate repair of the injured nerve prevents the bulk of cell death, but not entirely (McKay Hart et al., 2002). Treatment with the neurotrophins, NGF or NT3, but not BDNF, can rescue some of these DRG neurons from death (Ljungberg et al., 1999).

There is also a reorganization in the innervation patterns of motoneurons in muscles after regeneration. The first regenerating motor axons to reach the muscle capture a large number of fibers (Rafuse and Gordon, 1998). In contrast to uninjured conditions, these fibers are generally located close to one another. Once more axons reach the muscle, there is pruning of innervation by the larger motor units, and innervation ratios return to pre-injury sizes. While denervated, muscle fibers lose their phenotype specificity—slower muscles become faster and faster muscles become slower (Pette and Staron, 2001, Buller et al., 1960). Once reinnervated, the muscle fiber changes phenotype to reflect the motoneuron innervating it. This can result in a reorganization of fiber types in the reinnervated muscle (Wang et al., 2002b). There is significant atrophy of the muscles after a peripheral nerve injury, and this atrophy can persist for long periods after the muscle has been reinnervated (Boudriau et al., 1996).

Brain Derived Neurotrophic Factor

The neurotrophins have emerged as an important modulator of axon regeneration, particularly brain derived neurotrophic factor (BDNF) (Boyd and Gordon, 2003). BDNF is a member of the neurotrophin family, which also includes nerve growth factor (NGF), neurotrophin 3 (NT3), and neurotrophin 4/5 (NT4/5). BDNF is required for normal development—BDNF knockout (KO) is embryonic lethal (Jones et al., 1994, Schwartz et al., 1997). In adulthood, BDNF is involved in synaptic plasticity, long term potentiation (LTP), learning and memory as well as hippocampal neurogenesis and regeneration after injury (Lindsay, 1988, Richner et al., 2014, Lu et al., 2014, Lewin and Barde, 1996). In the subsequent paragraphs, we review how BDNF is regulated at the level of mRNA transcripts, protein trafficking, and receptor binding, following with its role in peripheral nerve regeneration.

Regulation of BDNF Transcripts - The human *BDNF* gene resides on the short arm of the 11th chromosome (Maisonpierre et al., 1991). It consists of 9 exons—eight 5’ untranslated exons and one protein coding 3’ exon (Figure 1) (Aid et al., 2007, Pruunsild et al., 2007, Liu et al., 2006). Through alternative splicing, 17 distinct mRNA transcripts for BDNF have been

identified in humans and 11 in rodents (Pruunsild et al., 2007). Additionally, the 3'UTR of the gene contains two polyadenylation sites, resulting in both a long 3'UTR and a short 3'UTR, doubling the possible splice variants. The entire protein-coding region resides on exon IX, so the mature BDNF protein is identical regardless of mRNA splicing. Splice variants allow for spatial and temporal control of the BDNF transcript.

Spatial control of the 5'UTRs can be seen in exon expression throughout the body. BDNF transcripts containing exons I, II, and III are found exclusively in the brain, and transcripts containing exon IV are predominantly found peripherally in the lung and heart, but can also be found in brain tissue (Timmusk et al., 1993). Even within brain tissue, different promoters can be found in different cell types. For example, exon IV transcripts are required for proper GABAergic interneuron function in the prefrontal cortex (Sakata et al., 2009).

Many different stimuli exert temporal control over BDNF transcription. In cultured cortical neurons, Ca^{2+} influx results predominantly in transcription of exon IV containing mRNA (Tao et al., 1998). This promoter contains a cAMP/Ca^{2+}-response element-like element (CaRE3/CRE) that is required for activity-dependent transcription (Hong et al., 2008, Tao et al., 1998). The transcription factor CREB binds this element, is phosphorylated by calcium-regulated kinase cascades, and recruits transcriptional machinery resulting in Ca^{2+} dependent transcription of exon IV-containing BDNF mRNA (Lonze and Ginty, 2002, West et al., 2001). Other stimuli have been identified in modulating BDNF expression. In motoneurons, exon VI transcripts are androgen sensitive, despite no known androgen response element on the *bdnf* gene (Ottem et al., 2010, Sabatier and English, 2015). There is, however, an estrogen response element (Sohrabji et al., 1995). SRY-box containing gene 11 (Sox11), a transcription factor involved in neuronal survival, axon growth, and regeneration after injury, increases exon I containing BDNF mRNA transcripts specifically in peripheral DRG neurons, but not in CNS neurons (Salerno et al., 2012, Jankowski et al., 2006, Struebing et al., 2017). Exons II and VI are sensitive to tricyclic and atypical antidepressants (Vaghi et al., 2014).

A further role for 5' promoter exons regulating BDNF mRNA may lie in mRNA trafficking. In both cortical and hippocampal neurons, BDNF mRNA is found in dendrites and activity induces trafficking of BDNF mRNA to distal dendrites (Tongiorgi et al., 1997, Chiaruttini et al., 2009, Chiaruttini et al., 2008, Capsoni et al., 1999). Interestingly, only certain splice variants are found in dendrites—those containing exons IIB, IIC, and VI (Chiaruttini et al., 2008, Pattabiraman et al., 2005). Transcripts containing exons I, III, and IV are restricted to the cell body.

Further spatial and temporal translational control of BDNF mRNA may come via the 3'UTR. The *bdnf* 3' UTR contains two polyadenylation sites. This allows for both a long 3'UTR and a short 3'UTR to be transcribed (Pruunsild et al., 2007, Aid et al., 2007, Timmusk et al., 1993). These different 3'UTRs are thought to determine mRNA trafficking within the cell. Both the long and short 3'UTR transcripts can be found in the dendrites under different conditions (Vicario et al., 2015). However, in general, the long 3'UTR transcripts are trafficked to the dendrites, where local BDNF synthesis can regulate pruning and enlargement of synapses, whereas the short 3'UTR transcripts stay in the cell body (An et al., 2008). Both depolarization of the neuron as well as BDNF itself increase the number of BDNF mRNA transcripts targeted to the dendrites (Tongiorgi et al., 1997, Righi et al., 2000). Remarkably, the short 3'UTR transcripts account for the majority of BDNF translation, whereas the long 3'UTR transcripts are translationally repressed by RNA binding proteins which stabilize the mRNA until neural activity elicits rapid local translation (Lau et al., 2010, Allen et al., 2013, Vaghi et al., 2014). Calcium influx associated with neuronal activity also results in increased stabilization of BDNF mRNA (Fukuchi and Tsuda, 2010). Conversely, the 3'UTR also contains regions which interact with microRNAs, which are short, non-coding RNA strands that complement mRNA transcripts and result in transcript degradation (Bartel, 2004). Numerous microRNAs have been identified as regulators of BDNF, and microRNA access to binding sites depends on the presence of the long or short 3'UTR (Varendi et al., 2014, Mellios et al., 2008).

BDNF Trafficking and Secretion - BDNF, like the other neurotrophins, is synthesized as a pre-proprotein. The pre-domain functions as a signaling peptide that directs synthesis to the endoplasmic reticulum (ER) for future packaging as a secretory protein. It is immediately cleaved to form proBDNF upon sequestration in the ER (Lessmann et al., 2003). Within the ER, proBDNF forms homodimers (Kolbeck et al., 1994). Both proBDNF and a further cleaved form, mature BDNF, can be packaged into vesicles and secreted. ProBDNF is approximately 29kDa, and once cleaved, mature BDNF is approximately 14kDa (Seidah et al., 1996a). Intracellularly, cleavage can occur within the trans-Golgi network or secretory vesicles by furin, a protease, and proprotein convertases PCSK6 and PC5-6b. Extracellularly, cleavage is executed by tissue plasminogen activator or matrix metalloproteinases (Mowla et al., 2001, Nagappan et al., 2009, Hwang et al., 2005, Keifer et al., 2009, Lee et al., 2001, Yang et al., 2009, Seidah et al., 1996b, Seidah et al., 1996a). These proteins are secreted in an activity-dependent manner (Krystosek and Seeds, 1981, Gualandris et al., 1996). Once cleaved, the prodomain is not immediately degraded, and can be secreted with mature BDNF (Anastasia et al., 2013).

From the trans-Golgi network, BDNF is directed to two different secretory pathways: a constitutive pathway and a regulated, Ca^{2+} dependent pathway (Mowla et al., 1999, Lessmann et al., 2003, Kuczewski et al., 2009). The constitutive pathway consists of small granules (50-100nm) that fuse with the cell membrane near the neuronal somata and proximal processes. The regulated secretory pathway consists of larger granules (300nm) that fuse in distal processes and axon terminals (Brigadski et al., 2005, Dieni et al., 2012, Kohara et al., 2001, Conner et al., 1997). The dual pathway for release is distinctive of BDNF. The other neurotrophins are preferentially secreted through a constitutive pathway. Under normal conditions, most neuronal BDNF is packaged into the regulated pathway (Lu et al., 2014).

Two important interactions have been identified in the sorting of BDNF into the regulatory pathway. The first to be discovered was the interaction between the sorting receptor carboxipeptidase E (CPE) and a three-dimensional motif on the mature domain of BDNF (Lou et

al., 2005). Knocking out CPE in cortical neurons blocks activity-dependent release of BDNF and increases constitutive release. Similarly, adding this motif to NGF redirected its release to the regulated secretory pathway (Lou et al., 2005). The second interaction is between the prodomain and the protein sortilin (Chen et al., 2005). Sortilin is localized predominantly to the Golgi apparatus and interacts with the BDNF prodomain to direct it to the regulated secretory pathway (Nielsen et al., 2001, Chen et al., 2005). When sortilin is unable to interact with the prodomain of BDNF, regulated release is decreased, but there is no compensatory increase in constitutive release (Chen et al., 2005, Lu et al., 2005). This has led to the hypothesis that the interaction between sortilin and the prodomain of BDNF is necessary for proper protein folding, which allows CPE to interact with the mature domain and sort BDNF into one of the two pathways (Lu et al., 2005). When NT-4/5, which is secreted constitutively, is modified to contain the BDNF prodomain, it is trafficked to the regulated secretory pathway (Brigadski et al., 2005). Similarly, blocking the cleavage of the prodomain of NGF, which is also released constitutively, results in its sorting into regulated secretory pathways (Mowla et al., 1999).

BDNF Receptors - Once secreted, BDNF can bind to one of two receptors—tropomyosin receptor kinase B (trkB) or the common neurotrophin receptor, $p75^{NTR}$. Mature BDNF preferentially binds trkB, resulting in pro-growth signaling, whereas proBDNF (as well as the other proneurotrophins) preferentially binds $p75^{NTR}$, resulting in antigrowth signaling (Lee et al., 2001). BDNF is primarily secreted as proBDNF (Chen et al., 2004, Mowla et al., 2001, Mowla et al., 1999). Thus the availability of proteins that cleave the prodomain may regulate which receptor is activated by BDNF release, providing another mechanism for control of BDNF signaling.

The trkB receptor is a typical tyrosine kinase. When ligand is bound, it dimerizes and autophosphorylates. In addition to BDNF, trkB can also bind NT-4/5. Several isoforms of trkB have been discovered, including isoforms that change its sensitivity to NT-4/5, as well as a truncated form that lacks an intracellular kinase domain (Eide et al., 1996). The truncated form

acts as a dominant negative receptor, forming heterodimers with full length trkB receptors and blocking neurotrophin signaling (Eide et al., 1996, Fryer et al., 1997). Another possible role for truncated trkB on astrocytes and Schwann cells may be to act to control the pool of available neurotrophins, preventing them from degrading or signaling until released into the extracellular space (Alderson et al., 2000). In its full-length form, trkB has several intracellular tyrosine residues that can be phosphorylated (Huang and Reichardt, 2003). Three possible signaling cascades are then activated: phospholipase C gamma (PLCɣ); phosphotidyl-inositol-3 kinase (PI3K); and mitogen activated protein kinase/extracellular receptor kinase (MAPK/ERK) (Reichardt, 2006).

The phosphorylation of residue Y490 creates a binding site for adaptor protein Shc (Patapoutian and Reichardt, 2001). Shc binding trkB allows for activation of Ras and further activation of the MAPK/ERK pathway. Downstream of this pathway is mechanistic target of rapamycin (mTOR). Shc binding residue Y490 also results in the recruitment of PI3K and activation of protein kinase B (Akt) (Reichardt, 2006). The phosphorylation of residue Y785 creates a binding site for PLCɣ, which is then phosphorylated by trkB (Patapoutian and Reichardt, 2001). This phosphorylation activates PLCɣ, which then hydrolizes phosphotidylinositides to generate diacylglyerol (DAG), which activates protein kinase C (PKC), and inositol 1,4,5 triphosphate (IP_3), which results in an influx of intracellular Ca2+ stores from the ER. These signaling cascades all converge at the level of the nucleus, where transcription is affected through CREB and other transcription factors (Minichiello, 2009).

Once bound to ligand, trkB is endocytosed to form a signaling endosome (Reichardt, 2006, Delcroix et al., 2003). Both ligand and receptor are contained within the endosome, allowing trkB to continue signaling as it is trafficked through the cell. In this way, trkB can be moved closer to the nucleus, where it can affect gene transcription, as well as brought into closer proximity to signaling effectors (Delcroix et al., 2003). However, not all actions of trkB happen at the level of the soma-- BDNF-trkB activation has been shown to affect local protein

synthesis in the growth cone as well (Yao et al., 2006).

The second receptor for BDNF is the pan-neurotrophin receptor, $p75^{NTR}$ (Rodríguez-Tébar et al., 1992). Generally thought of as a pro-death receptor, $p75^{NTR}$ is a member of the tumor necrosis factor receptor super family and contains a cytosolic death domain (Locksley et al., 2001, Liepinsh et al., 1997). It is expressed primarily during development, but sensory neurons and spinal motoneurons maintain low expression through adulthood (Wyatt et al., 1990, Ernfors et al., 1989, Ibáñez and Simi, 2012, Heuer et al., 1990). Its cytosolic domain is non-enzymatic, so its actions depend entirely on associations with cytoplasmic proteins (Nagata, 1997). Despite its canonical role, $p75^{NTR}$ can mediate both pro-death and pro-survival signals depending on its cytosolic partners. For example, $p75^{NTR}$ is required during development for normal neuron growth and ramification (Yamashita et al., 1999). Multiple adaptor complexes interact with its cytosolic domain to mediate downstream effects (Dechant and Barde, 2002).

Additionally, $p75^{NTR}$ has multiple membrane-bound and extracellular binding partners which can alter whether its signaling is pro-survival or pro-death. Through extracellular pairing with sortilin, $p75^{NTR}$ is able to bind the proneurotrophins (Teng et al., 2005, Nykjaer et al., 2004), resulting in pro-death or anti-growth signaling through downstream JNK activation or caspase activation (Reichardt, 2006). However, neurotrophin binding to $p75^{NTR}$ can also result in NFκB activity, which is a pro-survival signal (Hamanoue et al., 1999, Middleton et al., 2000, Carter et al., 1996). One key protein regulated by $p75^{NTR}$ is RhoA, a small GTPase that regulates the actin cytoskeleton and inhibits axon elongation (Schmidt and Hall, 2002, Walsh et al., 1999). Through such interactions with the so-called death domain of $p75^{NTR}$, neurotrophin binding inhibits Rho (Yamashita et al., 1999, Roux and Barker, 2002). Through forming a receptor complex with the Nogo receptor, NgR1, $p75^{NTR}$ can act as a receptor for myelin-associated glycoprotein (MAG) (Wang et al., 2002a), which enhances Rho activation and results in neurite collapse (Mi et al., 2004). Curiously, $p75^{NTR}$ can act as a binding partner for the trks, including trkB, and increases affinity and selectivity of binding and thus enhancing trk signaling (Bibel et

al., 1999).

Like trkB, truncated forms of $p75^{NTR}$ have been identified. One short $p75^{NTR}$ isoform lacks an extracellular ligand binding domain, but contains its intracellular machinery (Roux and Barker, 2002). This form is unable to bind the neurotrophins (Dechant and Barde, 1997). The extracellular domain of $p75^{NTR}$ can also be cleaved by extracellular metalloproteinases (Roux and Barker, 2002). These isoforms could act as modulators of neurotrophin signaling.

The two receptors for BDNF are generally thought to have opposing roles and may mediate a balance between growth and death. trkB has a higher affinity for mBDNF, but as levels of neurotrophin increase, $p75^{NTR}$ will also bind mBDNF and activate signals in direct opposition to trkB. Because of the different affinities for pro- and mature BDNF, cleavage of BDNF becomes another mechanism to control its downstream signaling effects (Lee et al., 2001). Depolarization of a neuron, which results in secretion of BDNF, also results in secretion of tissue plasminogen activator which cleaves proBDNF to create mature BDNF (Gualandris et al., 1996). BDNF-trkB signaling increases expression of matrix metalloproteinase 9, which also cleaves BDNF (Kuzniewska et al., 2013).

Role of BDNF in Peripheral Nerve Injury

In peripheral nerves, BDNF is synthesized by motoneurons, a subset of DRG neurons, and Schwann cells (Apfel et al., 1996, Cho et al., 1997, Michael et al., 1997). After nerve crush or complete transection, BDNF mRNA increases in all three cell types, including in trkB- and trkC-expressing DRG neurons not found previously to express BDNF (Al Majed et al., 2000a, Michael et al., 1999, Kobayashi et al., 1996, Meyer et al., 1992, Funakoshi et al., 1993, English et al., 2007). BDNF mRNA can be found in low levels in the sciatic nerve, and after injury, that expression is upregulated. This upregulation is sustained over the course of weeks and can be attributed to both neuronal and non-neuronal sources (Meyer et al., 1992, Funakoshi et al., 1993).

Following sciatic nerve injury, a transient increase in both BDNF and full length trkB

mRNA is found in motoneurons (Al Majed et al., 2000a, Kobayashi et al., 1996). Unlike sensory neurons, NGF and trkA are not expressed by motoneurons, nor are they upregulated after injury (Funakoshi et al., 1993, Escandon et al., 1994). There is a small and short-lived upregulation of NT3 and NT4/5 in motoneurons (Funakoshi et al., 1993). TrkC is expressed by adult motoneurons, but it is not upregulated after injury (Johnson et al., 1999). Thus, the rapid upregulation of BDNF and trkB make it likely that BDNF is the main neurotrophin mediating early motoneuron response to nerve injury (Boyd and Gordon, 2003).

Schwann cells express only the truncated form of trkB, which has the potential to act as a dominant negative receptor for BDNF and NT4/5. Schwann cell truncated trkB mRNA levels decrease significantly after sciatic nerve injury (Frisén et al., 1993). This could be viewed as pro-regenerative, enabling available BDNF to bind to trkB receptors on regenerating neurites and enhance their growth. Conversely, after injury, Schwann cells upregulate $p75^{NTR}$, which has been suggested to result in sequestration of neurotrophins and inhibit regeneration (Scott and Ramer, 2010, Taniuchi et al., 1986, Bibel et al., 1999).

trkB in Peripheral Nerve Injury - Axons regenerate through the formation of growth cones, which need cytoskeletal proteins, such as actin and tubulin, to extend and stabilize the new growth. Beta actin mRNA is localized to peripheral axons, and peripheral nerve injury triggers actin mRNA to be transported down the axon for local protein synthesis (Willis et al., 2011, Koenig et al., 2000, Sotelo-Silveira et al., 2008). BDNF/trkB signaling triggers local translation of transported mRNAs through a Ca^{2+}-dependent mechanism, and this is required for bidirectional turning toward BDNF (Yao et al., 2006). BDNF application to injured axons increases the number of actin waves (transport of actin filaments and associated proteins toward the growth cone) per hour (Difato et al., 2011, Inagaki and Katsuno, 2017). Neurotrophins also stimulate growth cone sprouting and actin accumulation in the sprouts (Gallo and Letourneau, 1998). Both of these processes are mediated through the PIP3/PI3K signaling pathway described above (Asano et al., 2008). When an actin wave reaches the growth cone,

the growth cone enlarges, branches, and undergoes forward expansion (Flynn et al., 2009). Application of BDNF to growth cones results in microtubule reorganization to form lamellipodial as well as filopodial elongation (Gibney and Zheng, 2003).

In addition to local protein synthesis, trkB signaling has effects on cyclic AMP (cAMP) production, which may be important for the initial extension of growth cones across the site of injury. The MAPK/ERK pathway of BDNF/trkB signaling results in inhibition of phosphodiesterases (PDE) which normally degrade cAMP (Gao et al., 2003). As such, BDNF/trkB signaling results in increased levels of cAMP (Souness et al., 2000, Gao et al., 2003). This pathway has been shown to be necessary to overcome inhibition by MAG, and therefore PDE inhibition has been most thoroughly studied in models of spinal cord injury, where MAG inhibition of axon growth creates a substantial barrier to regeneration (Gao et al., 2003, Cai et al., 1999, Batty et al., 2017). Although injured peripheral nerves do not suffer inhibition by MAG to the same extent as that seen in the central nervous system, early in the regeneration process, inhibitory proteoglycans and myelin debris form an impermissible environment for axon regeneration (Shen et al., 1998). Increasing cAMP through PDE inhibition enhances peripheral regeneration after injury, and it is likely that trkB activation contributes to this cAMP-mediated effect on regeneration (Udina et al., 2010, Gordon et al., 2009).

The different neurotrophin signaling pathways activated through trk receptors converge at the level of transcription in the nucleus. CREB, resulting from trkB-generated PI3K-Akt activation, increases sensory neurite outgrowth (White et al., 2000). Inhibiting phosphatase and tensin homolog (PTEN), an endogenous inhibitor of the PI3K pathway, through genetic knock out or pharmacology, enhances peripheral nerve regeneration *in vivo* and neurite outgrowth *in vitro* (Christie et al., 2010, Park et al., 2008). Numerous other transcription factors downstream of MAPK/ERK signaling, such as c-jun, STAT3, and ATF-3, have all been associated with changes in gene expression after injury that enhance survival and regeneration (Makwana and Raivich, 2005). mTOR, also downstream of MAPK/ERK signaling and repressed by PTEN,

regulates protein synthesis and is also beneficial for DRG regeneration after injury (Abe et al., 2010, Park et al., 2008).

$p75^{NTR}$ in Peripheral Nerve Injury - Because of its roles in both pro-death and pro-survival signaling, it is not surprising that the role of $p75^{NTR}$ in regeneration after injury has been controversial. Although generally considered an anti-growth signal, its role is far more complex as evidenced by conflicting results using $p75^{NTR}$ knock-out mice.

Although expression is high during development, mature Schwann cells do not express $p75^{NTR}$. Schwann cell expression of $p75^{NTR}$ increases after injury (Heumann et al., 1987a, Heumann et al., 1987b, Taniuchi et al., 1986). Deletion of $p75^{NTR}$ in Schwann cells mediates improved regeneration in DRG neurons (Scott and Ramer, 2010). Conversely, for motoneurons, Schwann cell $p75^{NTR}$ deletion results in diminished functional recovery and axonal growth (Tomita et al., 2007). Expression of $p75^{NTR}$ is thought to mediate remyelination through a BDNF-dependent mechanism. Disruption of endogenous BDNF signaling impairs myelination (Zhang et al., 2008, Cosgaya et al., 2002), as does $p75^{NTR}$ knockout from Schwann cells (Song et al., 2006, Cosgaya et al., 2002, Tomita et al., 2007).

In motoneurons, $p75^{NTR}$ levels rise dramatically after injury, returning to baseline levels by 30 days (Ernfors et al., 1989, Gschwendtner et al., 2003, Koliatsos et al., 1991, Saika et al., 1991, Rende et al., 1995, Raivich and Kreutzberg, 1987, Yan and Johnson, 1988). This upregulation in $p75^{NTR}$ does not result in motoneuron cell death, however (Kuzis et al., 1999, Bueker and Meyers, 1951). Treating injured motoneurons with low-levels of recombinant human BDNF enhances their regeneration. Higher doses, however, result in failure to regenerate, which can be reversed by $p75^{NTR}$ blockade (Boyd and Gordon, 2002). There is currently no motoneuron-specific $p75^{NTR}$ knockout model, but conflicting results have been found with regard to motoneuron regeneration in $p75^{NTR}$ pan-knockout mice. Boyd and Gordon found improved motor axon regeneration in knockout mice after peroneal nerve transection (Boyd and Gordon, 2001). Gschwendtner et al. found no effect of knocking out $p75^{NTR}$ on facial nerve axon

regeneration (Gschwendtner et al., 2003). Ferri et al. found worse axon regeneration but improved functional recovery after facial nerve crush (Ferri et al., 1998). Song et al. found fewer regenerating axons in $p75^{NTR}$ knockout mice using both sciatic nerve and facial nerve crush injuries (Song et al., 2009). Most recently, Zhang et al. found worse axonal regeneration among $p75^{NTR}$ knockout mice using a facial nerve crush model (Zhang et al., 2010). Using cell-type specific knockout of $p75^{NTR}$ or targeting the binding partners of $p75^{NTR}$ that result in different signaling could provide clarity to these conflicting results in how $p75^{NTR}$ is affecting motoneuron regeneration.

Sensory neurons decrease expression of $p75^{NTR}$ after injury (Zhou et al., 1996). Unlike adult motoneurons, there is significant cell death of DRG neurons after an injury, but this is restricted to small-diameter, mainly cutaneous afferent neurons (Wiberg et al., 2018, Welin et al., 2008). Cell death in these smaller DRG neurons can be blocked by application of NGF (Rich et al., 1987, Ljungberg et al., 1999). Likewise, in injured DRG neurons, BDNF acts in an autocrine loop to prevent cell death, and disruption in BDNF expression increases cell death (Acheson et al., 1995). A decrease in expression of $p75^{NTR}$ could act as a survival signal, such that the endogenous increased neurotrophin secretion would be more likely to bind trk receptors and result in pro-survival signaling. In support of this hypothesis, in cultures of DRG neurons in which $p75^{NTR}$ has been rendered inactive, cell survival is higher, and increased neurotrophin concentration does not result in increased cell death (Zhou et al., 2005). Similarly, disrupting the NT binding domain of $p75^{NTR}$ results in increased sprouting after injury in DRG neurons (Scott et al., 2005).

Despite the upregulation of BDNF and its receptors after injury, neither BDNF nor the other neurotrophins (NGF, NT3, and NT4/5), is required for spontaneous regeneration of peripheral neurons (Streppel et al., 2002, Wilhelm et al., 2012, Diamond et al., 1987, Diamond et al., 1992). However, application of exogenous BDNF enhances axonal regeneration, functional recovery and decreases synaptic stripping (Lewin et al., 1997, Boyd and Gordon,

2002, Boyd and Gordon, 2003, Davis-Lopez de Carrizosa et al., 2009). Recently, small molecule trkB agonists have been developed, and these also enhance regeneration after injury (English et al., 2013).

Activity Dependent Treatments Enhance Regeneration

The first published report using activity-dependent treatments to enhance peripheral nerve regeneration was from Hines in 1942. He tested both electrical stimulation (ES) and different exercise paradigms in enhancing functional outcome in rats with tibial nerve transections (Hines, 1942). Since then, there has been great interest in treatments which activate injured neurons, collectively known as activity-dependent treatments, to enhance nerve regeneration (Udina et al., 2011a). These treatments include ES (Table 1), exercise (Tables 2-4), and more recently, optogenetic stimulation (Table 5). One benefit of activity-dependent treatments is the potential for easy translation from bench to bedside—using ES and exercise in human patients would require meeting far fewer regulatory requirements than the use of an experimental drug. Moreover, for nerve injuries that require surgical intervention, ES could be performed easily at the time of surgical repair of the nerve, as has already begun with clinical trials for patients requiring surgery for carpal tunnel release and complete digital nerve transection (Gordon et al., 2010, Wong et al., 2015). Exercise has the advantage of being low cost and allowing patients to take control of their recovery. However, in the case of extensive injuries, exercise may not be an option for a recovering patient. For this reason, finding treatments that mimic the effects of exercise, such as optogenetic stimulation, may be advantageous in treating patients. To accomplish such a goal, an understanding of the biological basis for the enhancement seen with these treatments is necessary.

Electrical Stimulation - Immediately after a peripheral nerve injury, a calcium wave propagates along the cut axons toward their cell bodies. Blocking this calcium wave through inhibition of voltage gated calcium channels or inhibition of calcium release from the neuronal endoplasmic reticulum blocks regeneration (Ghosh-Roy et al., 2010). It has been proposed that

ES mimics the retrograde calcium wave that propagates at the time of injury in order to elicit cell-autonomous mechanisms that initiate regeneration (Mar et al., 2014). This hypothesis is supported by evidence that ES enhances early regeneration by accelerating the process of axons crossing the site of injury to enter endoneurial tubes in the segment of the nerve distal to the injury (Brushart et al., 2002). ES results in a doubling of the number of motoneurons crossing the site of injury into the distal nerve at one week after nerve injury (Brushart et al., 2002). Without treatment, axons can take as long as four weeks to cross the site of injury, but by three weeks after injury, Al Majed et al. found all electrically stimulated motoneurons had already regenerated to their target muscle compared to 8 weeks for untreated controls (Al Majed et al., 2000b, Brushart et al., 2002).

The first applications of ES focused on the functional recovery of the affected muscles. In 1983, Nix and Hopf described that as early as 2 weeks after injury, treatment with 4Hz stimulation 24 hours daily increased twitch force, tetanic tension, and muscle action potentials (Nix and Hopf, 1983). In 1985, Pockett and Gavin found earlier return of the plantar extensor reflex with just 15-60 minutes of 20Hz stimulation (Pockett and Gavin, 1985). Al Majed et al. chose their 20Hz regimen based on the mean physiological frequency of motoneuron discharge and tried numerous stimulation regimens, stimulating continuously for one hour, one day, one week, and two weeks. They were the first to examine the effect of ES on the regenerating axons (Al Majed et al., 2000b, Loeb et al., 1987). Just one hour of 20Hz stimulation resulted in long-lasting enhancement of peripheral nerve regeneration. Following publication of this paper, 20Hz stimulation became the standard for studying ES (Table 1).

Without treatment, axon regeneration into motor or sensory pathways in the distal segment of a cut nerve is random for the first two weeks following injury (Brushart, 1993). Motoneurons whose axons have entered only sensory pathways (endoneurial tubes previously occupied by cutaneous axons) remain permanently mistargeted (Brushart, 1993). Enhancing the speed of regeneration but increasing mistargeting could result in poorer functional recovery.

Table 1.1 Electrical Stimulation.

Regimen		Model	Result		Reference
Unspecified	3min/day	Rat tibial nerve crush	Functional recovery	⬆	(Hines, 1942)
4Hz	24hrs/4wks	Rabbit soleus nerve crush	Functional recovery	⬆	(Nix and Hopf, 1983)
20hz	15-60min	Rat sciatic nerve crush	Functional recovery	⬆	(Pockett and Gavin, 1985)
20Hz	60min, 24hrs, 1 week,2 weeks	Rat femoral nerve transection	Axonal growth	⬆	(Al Majed et al., 2000b)
20Hz	8hrs/day/4 weeks	Rat sciatic nerve avulsion	Functional Recovery	⬇	(Tam et al., 2001)
100hz	10 pulses/2minutes	Cultured rat retinal ganglion cell	Neurite outgrowth	⬆	(Goldberg et al., 2002)
20Hz	1hr	Rat femoral nerve transection	Axonal growth	⬆	(Brushart et al., 2002)
20Hz	1hr	Rat femoral nerve transection	Axonal growth	⬆	(Brushart et al., 2005)
20Hz	1hr	Thy1-H-YFP mouse fibular nerve transection	Axonal growth	⬆	(English et al., 2007)
20Hz	1hr, 3hrs, 24hrs, 1 week, 2 weeks	Rat femoral nerve transection	1hr-Axonal growth Others—no change	⬆	(Geremia et al., 2007)
20Hz	1hr	Mouse femoral nerve transection	Functional recovery	⬆	(Ahlborn et al., 2007)
?	30min/day until recovery	Rat facial nerve transection	Functional recovery	⬆	(Lal et al., 2008)
20Hz	1hr	Rat sciatic nerve transection	Functional Recovery Axonal growth	⬆	(Vivo et al., 2008)
20hz	1hr	Human carpal tunnel syndrome release surgery	Functional recovery	⬆	(Gordon et al., 2010)
20Hz	3 days	Rat adult cultured DRG neurons	Neurite outgrowth	⬇	(Enes et al., 2010)
20Hz	1hr	Rat sciatic nerve crush	Myelination and myelin thickness	⬆	(Wan et al., 2010)
20Hz	1hr	Thy1-H-YFP mouse sciatic nerve transection	Axonal growth	⬆	(Singh et al., 2012)
20Hz	1hr	Rat cultured DRG neurons	Neurite outgrowth	⬆	(Singh et al., 2012)
20Hz	30min/day 1-7days	Rat facial nerve crush	Functional recovery	⬆	(Foecking et al., 2012)
20Hz	20min	Rat sciatic nerve Delayed repair 2hr-24 weeks	Axonal growth Functional recovery	⬆	(Huang et al., 2013)
20Hz	1hr	Human digital nerve transection	Functional sensory recovery	⬆	(Wong et al., 2015)
20Hz	1hr	Rat common peroneal nerve transection Delayed repair 3 months	Axonal growth	⬆	(Elzinga et al., 2015)

Table 1. Effect of Electrical Stimulation on Peripheral Nerve Regeneration. Regimen specifies stimulation paradigm. Model specifies which animal and injury model was used. Result specifies what outcome measure was analyzed. ⬆ Denotes improvement in outcome measured, ⬇ denotes worse outcome.

However, ES has been shown to increase the sensorimotor specificity of regenerating axons after peripheral nerve injury. More motoneurons regenerate exclusively into motor pathways in

rats treated with ES (Al Majed et al., 2000b). Fewer than 40% of injured DRG neurons reinnervated sensory pathways in controls compared to 75% in ES-treated animals (Brushart et al., 2005). However, innervating a motor endoneurial tube does not necessitate reaching the appropriate muscle target. Indeed, topographic analysis of motoneuron regeneration after ES revealed *increased* misdirection of regenerating motor axons to functionally inappropriate targets by over 500% (English, 2005). This misdirection resulted in motoneurons previously innervating flexor muscles reinnervating antagonistic extensor muscles.

ES increases Schwann cell neurotrophin secretion (Koppes et al., 2014). This could account for the effect of ES on earlier appropriate pathway preference for motor and sensory neurons. Schwann cells in the cutaneous nerves express high levels of NGF after injury, whereas Schwann cells in ventral roots express high levels of glial cell line-derived neurotrophic factor (GDNF) (Hoke et al., 2006, Brushart et al., 2013). This difference in growth factor expression has been proposed to be the mechanism through which preferential motor reinnervation occurs, with DRG neurons choosing paths with high NGF, and motoneurons entering paths with high GDNF. With no treatment, in rodents, neurotrophin expression peaks 15 days after injury and declines back to baseline by day 30. The day 15 peak coincides with the onset of pathway preference for regenerating axons (Gordon, 2015). ES, however, dramatically increases NGF secretion from Schwann cells for three days following stimulation (Koppes et al., 2014), possibly providing an earlier signal to regenerating sensory axons as to which pathways to take, and thus improving pathway targeting.

Exercise Treatment - For years, the evidence for exercise enhancing regeneration was not as clear as the evidence for ES. Over the years, many different types of exercise with varying intensities applied at different times prior to or after injury have resulted in conflicting results. It was hypothesized that increased neuronal activity through exercise would enhance regeneration as early as 1979, but early studies utilizing treadmill training, voluntary wheel

Table 2. Treadmill Training.

Regimen	Duration	Delay	Model	Result	Reference
6.5-27m/min 10-40 minutes/day	1-2/day	2-3 weeks	Rat sciatic nerve crush ♀	Functional recovery ⬇	(Herbison et al., 1980a)
27m/min 1-2/day 5 days/week	3-4 weeks	2-3 weeks	Rat sciatic nerve crush ♀	No change	(Herbison et al., 1980b)
1hr/day 26.8m/min	10 weeks	Prior to injury	Rat L4 root transection ♀	Increased sprouting	(Gardiner et al., 1984)
10m/min 30min/twice/day	21 days	None	Rat sciatic nerve crush ♂	Functional recovery ⬇	(van Meeteren et al., 1998)
10m/min 1.5hr/twice/day 5 days/week	10 weeks	1 week	Rat peroneal nerve transection ♀	Functional recovery ⬆	(Marqueste et al., 2004)
18m/min 30min/twice/day	2 weeks	12hrs	Rat sciatic nerve crush ♂	Axonal growth ⬆	(Seo et al., 2006)
10m/min*1hr/day 20m/min Or 2min*4/day 5 days/week	2 weeks	3 days	Thy1-H-YFP mouse sciatic nerve transection	Axonal growth ⬆	(Sabatier et al., 2008)
8m/min 30min/twice/day	2 weeks	12hrs	Rat sciatic nerve crush ♂ DRG culture ♂	Axonal growth ⬆ Neurite length	(Seo et al., 2009)
10m/min*1hr/day 20m/min 2min*4/day 5 days/week	2 weeks	3 days	Mouse sciatic nerve transection	Axonal growth ⬆	(English et al., 2009)
20Hz 1hr ES + 5m/min 2hr/day	4 weeks	5 days	Rat sciatic nerve transection ♀	Axonal growth ⬆	(Asensio-Pinilla et al., 2009)
20cm/sec-54cm/sec 60min/day 5 days/week	5 or 52 days	3 days	Mouse sciatic nerve chronic constriction injury ♂	Functional recovery ⬆	(Cobianchi et al., 2010)
4.6m/min 30min/twice/day	4 weeks	5 days	Rat sciatic nerve transection ♀	Functional Recovery ⬆ Axonal growth	(Udina et al., 2011b)
1.8-3m/min 20min/day	3 weeks	7 days	Rat ulnar nerve crush ♂	Functional recovery ⬆	(Pagnussat et al., 2012)
10m/min 1hr/5days/week	2 weeks	3 days	Rat sciatic nerve transection ♀	Functional recovery ⬆	(Boeltz et al., 2013)
10m/min 1hr/5days/week	2 weeks	3 days	Mouse sciatic nerve transection ♀ ♂	Synaptic stripping ⬆	(Liu et al., 2014)
10m/min 1hr/5 days/week	6 weeks	3 days	Mouse median nerve transection ♂	Functional recovery ⬆	(Park and Höke, 2014)
20m/min 2min*4/day 5 days/week	2 weeks	3 days	SLICK::BDNF$^{f/f}$ mouse sciatic nerve transection ♀	Synaptic stripping ⬆	(Krakowiak et al., 2015)

Table 2. Effect of Treadmill Training on Peripheral Nerve Regeneration. Regimen is the speed of running and for how long each day. Duration is how many days TT was performed. Delay refers to how long after injury before exercise was performed. Model specifies what type of injury and in what animal. Sex of animals is specified by ♀ or ♂. If this is not listed, it was not specified. Result specifies what outcome measure was analyzed. ⬆ Denotes improvement in outcome measured, ⬇ denotes worse outcome.

running, and swimming found unfavorable results (Hoffer et al., 1979, Herbison et al., 1980a, Gardiner et al., 1984, Badke et al., 1989, van Meeteren et al., 1998, Tam et al., 2001). These experiments largely focused on the effect of exercise on muscle fiber alterations and muscle function, and did not probe the effect of exercise on axon regeneration.

Table 3. Swimming

Regimen	Delay	Model	Result	Reference
1hr/day	Unspecified	Rat tibial nerve transection	Functional recovery ⬆	(Hines, 1942)
10min/day 10 days	4, 11, 18 days	Rabbit sciatic nerve crush	Myelination ⬆	(Sarikcioglu and Oguz, 2001)
30min/day 2 weeks	None 2 weeks	Rat sciatic nerve crush ♂	Decreased sprouting	(Teodori et al., 2011)
10-30min/day 3 days/week 3 weeks	7 days	Rat sciatic nerve transection	No change	(Liao et al., 2017)

Table 3. Effect of Swimming Exercise on Peripheral Nerve Regeneration. Regimen specifies how long swimming exercise lasted each day and how many days swimming was performed. Delay refers to how long after injury before exercise was performed. Model specifies what type of injury and in what animal. Sex of animals is specified by ♀ or ♂. If this is not listed, it was not specified. Result specifies what outcome measure was analyzed. ⬆ Denotes improvement in outcome measured, ⬇ denotes worse outcome.

The change in emphasis from the effect of exercise on denervated muscle to the effect of exercise on injured spinal motoneurons and DRG neurons encouraged scientists to continue researching exercise, despite previous underwhelming results. In 2008, English and colleagues tested the efficacy of interval training (short high-speed sprints followed by periods of rest) in enhancing regeneration as a model that resembles how mice voluntarily run (Sabatier et al., 2008, De Bono et al., 2006). They found the surprising result that this regimen was effective only in female mice, and in fact the more commonly used training regimen of slow continuous treadmill walking was effective only in males (Wood et al., 2012). This previously unknown sex difference could have affected outcomes in numerous exercise experiments. For example, Seo et al. treated intact male rats with either high or low intensity treadmill training before culturing their DRGs and found only low intensity treadmill training increased neurite outgrowth (Seo et al., 2009). Their treadmill training regimen was very similar to the one used by Wood et al. that proved effective only in male mice, and the results of this experiment could have been different

had females been included. Many of the prior experiments mentioned used animals of only one sex, and this could explain some of the variability in the effects of exercise (Table 3).

Table 4. Other exercise.

Exercise	Regimen	Delay	Model	Result		Reference
Forced Wheel Running	2hrs/day	Unspecified	Rat tibial nerve transection	Functional Recovery	⬆	(Hines, 1942)
Overwork	Chronic	none	Rat sciatic nerve crush ♀	Functional recovery	⬆	(Herbison et al., 1973)
Voluntary wheel running	4 weeks	none	Mouse tibial nerve transection	Functional recovery Axonal growth	⬆ ⬇	(Badke et al., 1989)
Stretch Training	24 days	none	Rat sciatic nerve crush ♀	Functional recovery	⬆	(van Meeteren et al., 1997)
Voluntary wheel running	8hr/day	none	L4 and L5 avulsion ♀	Axonal growth Functional Recovery	⬇ ⬇	(Tam et al., 2001)
Voluntary wheel running	3 or 7 days	Prior to injury	Rat DRG culture Rat sciatic nerve crush	Neurite outgrowth Axonal growth	⬆	(Molteni et al., 2004)
Passive bicycle training	45rpm 30min/twice/day 4weeks	5 days	Rat sciatic nerve transection ♀	Functional Recovery Axonal growth	⬆	(Udina et al., 2011b)
Skilled Motor Task	20min/day 3 weeks	7 days	Rat ulnar nerve crush ♂	Functional recovery	⬆	(Pagnussat et al., 2012)

Table 4. Effect of Other Exercise Paradigms on Peripheral Nerve Regeneration. Exercise refers to what type of paradigm was used. Regimen specifies how long exercise lasted each day and how many days exercise was performed. Delay refers to how long after injury before exercise was performed. Model specifies what type of injury and in what animal. Sex of animals is specified by ♀ or ♂. If this is not listed, it was not specified. Result specifies what outcome measure was analyzed. ⬆ Denotes improvement in outcome measured, ⬇ denotes worse outcome.

There are a few advantages to exercise over ES. For example, while ES may increase misdirection of motoneurons reaching target muscles, treadmill training enhances motoneuron regeneration without decreasing topographic specificity (English et al., 2009). The mechanism of ES is to accelerate crossing the site of injury by regenerative sprouts; exercise does the same but also sustains pro-growth signaling throughout the process of regeneration (Gordon and English, 2016). There is also evidence that the enhancing effects of two weeks of exercise are more robust than that of a single bout of ES (Sabatier et al., 2008, Wood et al., 2012, Gordon and English, 2016). In 2009, Asensio-Pinilla et al. combined treadmill training with a single bout of ES given at the time of injury, and found greater enhancement of muscle reinnervation in the initial phase of recovery compared to either treatment alone (Asensio-Pinilla

et al., 2009). Thus, after inauspicious beginnings, exercise has shown great promise as a treatment in the field of peripheral nerve regeneration.

Optogenetic stimulation - The advent of optogenetics enabled cell-specific neuronal activation with the use of the light-sensitive cation channel, Channelrhodopsin (ChR2) (Krook-Magnuson et al., 2014). Whereas ES stimulates all cells within the nerve (including Schwann cells and various immune cells) and exercise likely affects cells throughout the entire body, specific neuronal activation can be achieved using optogenetics by expressing ChR2 only in neurons. Park et al. were the first to demonstrate the efficacy of light stimulation in enhancing regeneration by replicating the common ES protocol using light stimulation of 20Hz for one hour on explanted neonatal DRGs (Park et al., 2015). Although they tested a number of different stimulation regimens, the one hour of 20Hz stimulation provided the largest effect on neurite outgrowth. Ward et al. recapitulated this *in vivo,* finding that one hour of 20Hz light stimulation of light-sensitive neurons enhanced axon regeneration only in the light-sensitive cells (Ward et al., 2016, Ward et al., 2018).

Table 5. Optogenetic Stimulation

Regimen	Mouse	Model	Result		Reference
1hr 20Hz 5ms pulse	Thy1ChR2	Neonate DRG explant	Neurite outgrowth	⬆	(Park et al., 2015)
1hr 20Hz 1ms pulse	Thy1ChR2	Sciatic nerve transection	Axonal outgrowth	⬆	(Ward et al., 2016)
1-2hr 10-20Hz (72k pulse total) 1ms pulse	Avil-Cre::ChR2-YFP$^{f/f}$ Chat-ChR2-YFP	Sciatic nerve transection	Axonal outgrowth	⬆	(Ward et al., 2018)

Table 5. Effect of Optogenetic Stimulation on Peripheral Nerve Regeneration. Regimen specifies stimulation paradigm. Mouse specifies what transgenic mouse model was used. Result specifies what outcome measure was analyzed. ⬆Denotes improvement in outcome measured, ⬇denotes worse outcome.

Mechanisms

Neurotrophins - Activity-dependent treatments require neuronal neurotrophin production. ES increases neurotrophin expression in Schwann cells, DRG neurons, and motoneurons (Al Majed et al., 2000a, English et al., 2007, Koppes et al., 2014, Wan et al., 2010). Electrically

stimulating Schwann cells increases their secretion of NGF specifically, and not BDNF (Koppes et al., 2014). While Schwann cell NGF is sufficient to promote axon growth, the study of axon regeneration through nerve grafts made acellular by repeated freezing and thawing has demonstrated that stimulation of Schwann cells (and other cell types) is not required for the efficacy of ES to enhance axon regeneration (Koppes et al., 2014, English et al., 2007). Moreover, the use of optogenetics to stimulate neurons selectively has shown that specific neuronal activation is sufficient to enhance regeneration (Ward et al., 2016). ES is also effective in promoting regeneration in nerves that have been repaired months after injury, when Schwann cells have stopped secreting neurotrophins and have started to die off (Elzinga et al., 2015, Huang et al., 2013, Sulaiman and Gordon, 2000, Brushart et al., 2013, Hoke et al., 2006). Thus, while activity-dependent treatments may increase Schwann cell neurotrophin secretion, this is not required for their enhancing effects.

Unlike non-neuronal cells*, neuronal* neurotrophin secretion *is* required for the efficacy of activity-dependent treatments. Genetically deleting NT4/5 or BDNF from Schwann cells does not alter the efficacy of ES or treadmill training in enhancing axon growth, but deleting these neurotrophins from neurons abolishes the effectiveness of these activity-dependent treatments (English et al., 2007, Wilhelm et al., 2012). Both exercise and ES have been shown to increase neuronal BDNF and its receptor, trkB (Al Majed et al., 2000a, Park and Höke, 2014, Park et al., 2015, English et al., 2007, Gomez-Pinilla et al., 2002). Through co-culturing light-sensitive DRG explants with wild type DRGs, Park et al. demonstrated that the BDNF secreted in response to light stimulation was sufficient to increase neurite outgrowth not only from cells in a light-sensitive (ChR2-expressing) DRG, but also in neighboring ganglia derived from wild type mice. Protein analysis of the media revealed increased BDNF and NGF secretion in response to optical stimulation from the light-sensitive DRGs only (Park et al., 2015).

There is a dose-dependence in activity-dependent treatments for enhancing nerve regeneration. Whereas one hour of 20Hz stimulation has been shown to enhance DRG

regeneration after injury, an increase to just 3 hours of ES decreased sensory neuron regeneration, and was associated with a downregulation in expression of the regeneration associated gene, GAP-43 (Geremia et al., 2007). *In vitro*, neurites from DRG explants containing ChR2 had higher rates of growth with stimulation paradigms resulting in 72k pulses of light (1 hour 20Hz, 2 hours 10Hz, 4 hours 5Hz) than stimulation paradigms that resulted in a higher number of pulses (20Hz for 1-3 days) or much lower number of pulses (20Hz for 15 minutes) (Park et al., 2015). Three days of continuous depolarization through ES or high concentrations of KCl results in complete failure of dissociated DRG neurons to grow neurites in culture (Enes et al., 2010). For motoneurons, high intensity exercise or repeated bouts of ES results in decreased sprouting and fewer synaptic contacts at neuromuscular junctions (Tam et al., 2001). Application of one hour of 20 Hz ES every third day for two weeks after sciatic nerve transection and repair did not enhance the regeneration of motor axons in mice (Park et al., 2018). Interestingly, exogenous application of BDNF resulted in a dose-dependent enhancement of axon regeneration as well (Boyd and Gordon, 2002). Low to modest doses produced enhances axon regeneration, but higher doses inhibited regeneration. Treatments with high doses of BDNF caused $p75^{NTR}$ activation, which prevented DRG neurite outgrowth (Boyd and Gordon, 2002).

Neuronal Activity - The success of activity-dependent treatments in promoting axon regeneration requires activation of the injured neurons. Treating the neurons proximal to the stimulation site with tetrodotoxin (TTX) to block their ability to conduct antidromic action potentials abolishes the effect of ES, despite the continued orthodromic firing of distal axons and muscle fibers (Al Majed et al., 2000b). Similarly, inhibition of motoneuron activity during treadmill training, using bioluminescent optogenetics (BL-OG), abolishes the enhancing effect of exercise on motoneuron regeneration (Jaiswal et al., 2017). Whether the increased activation needed to promote axon regeneration requires action potential generation is not entirely clear. Enhancement of regeneration of axons of many more motoneurons than are likely to be brought

into full activity is found after treatments with exercise at a slow treadmill speed (Gordon and English, 2016). Simply increasing the excitability of injured neurons using chemogenetics could be sufficient to enhance regeneration (Jaiswal et al., 2018).

Although Park et al. found that BDNF secretion from neighbors can stimulate regeneration in neurons that were not activated *in vitro*, optogenetic stimulation *in vivo* of only motoneuron axons did not enhance DRG axon regeneration, nor vice versa (Ward et al., 2018). When BDNF is knocked out in only a subset of neurons, those specific neurons do not benefit from exercise treatment (Wilhelm et al., 2012). Thus, it appears neuronal BDNF is acting as an autocrine signal facilitating enhanced regeneration (English et al., 2014, Gordon, 2016).

Androgens - The sex difference found in response to different exercise regimens led to the hypothesis that androgen receptor signaling was involved in activity-dependent treatments. The effect of androgens in enhancing peripheral nerve regeneration had already been thoroughly explored several years prior (Fargo et al., 2009). All motoneurons contain androgen receptors, though testosterone is not required for spontaneous regeneration—treating animals of both sexes with the androgen receptor blocker flutamide does not inhibit regeneration, nor does castration of males (Thompson et al., 2013, Freeman et al., 1995). Application of exogenous androgens in males and females, however, enhances axon regeneration in both cranial and spinal nerve injuries (Kujawa et al., 1991, Kujawa et al., 1989, Brown et al., 1999, Freeman et al., 1995, Tanzer and Jones, 1997, Jones, 1993). This effect is androgen receptor-dependent, and blocking the androgen receptor with flutamide prevents testosterone-induced enhanced regeneration (Kujawa et al., 1995). In females, treating mice with anastrazole, an aromatase inhibitor which blocks the conversion of testosterone into estradiol, also dramatically enhanced axon regeneration, without increasing serum androgen levels (Thompson et al., 2013).

The sex difference in response to exercise regimens was evidence that androgen receptor signaling and activity-dependent treatments were linked. Slow, continuous treadmill

training resulted in an increase in serum testosterone levels in males, though no similar increase was found for females with interval training (Wood et al., 2012). Castrating males prior to treadmill training abolishes its enhancing effect, which cannot be rescued with interval training (Wood et al., 2012). Treating both sexes with flutamide before appropriate exercise regimens abolishes the effectiveness of this treatment in enhancing peripheral nerve regeneration (Thompson et al., 2013). Testosterone is also necessary for the beneficial effects of ES—castrated rats treated with ES have poorer regeneration compared to littermates who are treated with exogenous testosterone (Hetzler et al., 2008, Sharma et al., 2009). As with exercise, flutamide blocks the enhancing effects of ES in both males and females (Thompson et al., 2013). Conversely, combined exogenous androgen treatment with ES enhances facial nerve regeneration in gonadally intact rats (Sharma et al., 2010b).

Androgens regulate BDNF and its receptor, trkB, in motoneurons (Osborne et al., 2007, Ottem et al., 2010, Sharma et al., 2010a, Verhovshek et al., 2010). Exercise elicits an upregulation of testosterone that is sustained and could result in an increased duration of BDNF and trkB expression (Thompson et al., 2013, English et al., 2014). ES elicits an early increase in BDNF expression, whereas exogenous androgen application results in a later and longer-duration increase in expression. Combining the two treatments results in an additive upregulation of BDNF and could explain the improved recovery over either treatment alone (Sharma et al., 2010a).

Synaptic Rearrangements

After peripheral nerve injury both excitatory and inhibitory synaptic inputs onto injured motoneurons are withdrawn (Blinzinger and Kreutzberg, 1968, Linda et al., 2000, Brannstrom and Kellerth, 1998, Oliveira et al., 2004). If motor axons regenerate and reinnervate muscle targets, many of these inputs are restored but, for those expressing the vesicular glutamate transporter 1 (VGLUT1) and arising from muscle spindles, a gradual withdrawal of their central axonal processes from the ventral horn follows, resulting in a permanent loss of these synaptic

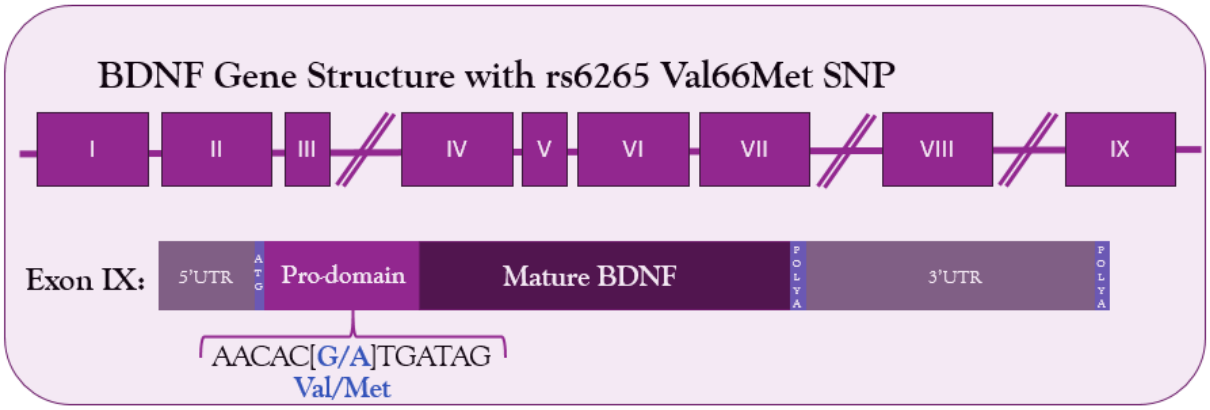
inputs (Alvarez et al., 2011, Rotterman et al., 2014). In animals treated with exercise during the first few days following sciatic nerve transection and repair, the extent of synaptic contacts between these important sources of proprioceptive feedback and motoneurons is not reduced (English et al., 2011, Liu et al., 2014, Krakowiak et al., 2015). This robust connectivity by VGLUT1+ inputs is retained at least 12 weeks later. No similar effect is found if the onset of the exercise treatment is delayed (Brandt et al., 2015). Application of one hour of 20 Hz ES had no effect on synaptic coverage after nerve injury, but repeated applications every third day for two weeks resulted in an effect similar to that observed using exercise (Park et al., 2018). It is not clear whether this effect of these activity-dependent therapies is the prevention of the original synaptic withdrawal, a stimulation of new synapse formation to replace the withdrawn inputs, or some combination of both. More studies are needed.

It is clear that BDNF plays a role in maintaining and preserving synaptic inputs on motoneurons. Without exercise, axotomized motoneurons lose approximately 35% of their overall synaptic coverage (Krakowiak et al., 2015). This effect is BDNF-dependent—knocking out BDNF in a subset of motoneurons reduces synaptic coverage in those specific cells in intact animals, and this synapse loss cannot be rescued with exercise (Krakowiak et al., 2015). Wild-type motoneurons within an animal maintain their synaptic contacts after nerve injury with exercise, but those in which BDNF has been knocked out do not (Krakowiak et al., 2015).

BDNF Val66Met Polymorphism

Given the relationship between activity-dependent treatments and BDNF, any genetic mutations altering BDNF signaling among the human population could affect the success of these treatments. Such a mutation exists—a common single nucleotide polymorphism in the *bdnf* gene. The G to A mutation at site 196 results in a Valine to Methionine substitution in the 66th codon (Figure 1). This polymorphism was first described by Egan et al. in 2003 and was quickly identified as incredibly common—the met allele of the *bdnf* gene is present in 25% of the American population and up to 50% of East Asian populations (Egan et al., 2003, Shimizu et al.,

2004). Carrying the met allele was originally described as a risk factor for schizophrenia (Egan



et al., 2003). It has since been linked to numerous other disorders and diseases, including Alzheimer's disease, obsessive compulsive disorder, anorexia nervosa, and bipolar disorder (Sklar et al., 2002, Hall et al., 2003, Ribases et al., 2003, Neves-Pereira et al., 2002, Notaras et al., 2015, Egan et al., 2003). Physiologically, Met-carriers have been found to have decreased hippocampal volume, and cells transfected with the Met allele have altered activity-dependent secretion of BDNF (Egan et al., 2003).

Figure 1. Structure of BDNF gene and location of Val66Met SNP in the coding exon IX. The G to A substitution in the prodomain results in a valine to methionine substitution and decreased Ca^{2+}- dependent release of BDNF

Testing for deficient activity-dependent secretion of BDNF in humans can be tricky. Generally, BDNF secretion is measured through serum as an indirect measure of neuronal BDNF, and exercise is a reliable method to increase serum BDNF levels (Elfving et al., 2010, Klein et al., 2011, Berchtold et al., 2005, Szuhany et al., 2015). Although one study has found that healthy adult Met-carriers did have increased serum BDNF after exercise (Helm et al., 2017), others have found serum levels of BDNF did not increase after high intensity exercise in elderly (Nascimento et al., 2015), spinal cord injured (Leech and Hornby, 2017), or healthy Met-carriers (Lemos et al., 2015). In mice expressing the met allele, exercise results in deficient mRNA production as well as decreased protein expression of BDNF (Ieraci et al., 2016). These deficiencies in exercise-induced BDNF secretion mirror the findings in cultured neurons

expressing the Met allele. The use of cells transfected with the met allele *in vitro* as well as the development of a transgenic mouse have allowed researchers to elucidate the mechanism behind this deficient secretion (Egan et al., 2003, Chen et al., 2006, Chen et al., 2004).

The valine to methionine substitution in this SNP occurs in the prodomain of the BDNF protein (Egan et al., 2003). Although it does not affect the ability of mature BDNF to bind its receptor, this substitution results in disorganized folding of the prodomain, resulting in abnormal interactions with sortilin (see above) (Chen et al., 2004, Anastasia et al., 2013). $BDNF_{Met}$ is thus packaged inefficiently into calcium-sensitive secretory vesicles trafficked to the distal dendrites and axon, accounting for the deficient activity-dependent secretion that has been reported (Chen et al., 2004). Being heterozygous for the met allele does not protect from this deficient BDNF secretion—BDNF forms homodimers, and in cells heterozygous for the met allele, $BDNF_{Met}$ dimerizes with $BDNF_{Val}$ and prevents its packaging into Ca^{2+}-regulated secretory vesicles (Kolbeck et al., 1994, Chen et al., 2004). Analysis of activity-induced BDNF secretion from cultured hippocampal cells bears this out—those cells heterozygous for the Met allele have deficient activity-dependent secretion despite the presence of one copy of the $BDNF_{Val}$ allele (Chen et al., 2006). This effect appears to be dose-dependent. Heterozygous cells have more activity-dependent secretion of BDNF than homozygous cells (Chen et al., 2006). Furthermore, once secreted, BDNF availability may be affected by binding with the cleaved prodomain. The prodomain binds BDNF with high affinity, and the met allele results in enhanced BDNF binding and slower dissociation once bound (Uegaki et al., 2017). This could limit the availability of BDNF to bind its receptors.

Activity-dependent secretion of BDNF relies not only on packaging into calcium-sensitive vesicles, but also on the spatial targeting of mRNA into distal processes where BDNF can be locally translated (Chiaruttini et al., 2008). This targeting is achieved through binding of BDNF mRNA with translin, a DNA/RNA binding protein involved in dendritic trafficking of mRNAs (Li et al., 2008). The G to A mutation at site 196 disrupts translin binding of BDNF mRNA, and thus

Met-carriers have deficient trafficking of BDNF mRNA to distal processes (Chiaruttini et al., 2009). Moreover, the transcripts containing exon VI, which is upregulated by exercise, and exon IV, which is calcium-sensitive, are found in reduced levels in the hippocampus of mice homozygous for the met allele (Baj et al., 2012, Mallei et al., 2015, Tao et al., 1998). These transcripts, along with those containing exon II, are generally trafficked to distal processes (Baj et al., 2011).

In addition to deficient activity-dependent BDNF secretion, the met allele may result in increased $p75^{NTR}$ activation. Unlike $BDNF_{Val}$, when the prodomain is cleaved from $BDNF_{Met}$, it is bioactive and able to activate $p75^{NTR}$ with the help of SorCS2, a member of the sortilin family of receptors (Anastasia et al., 2013, Deinhardt et al., 2011). *In vitro*, application of exogenous prodomain protein results in growth cone collapse and dendritic spine disassembly (Anastasia et al., 2013, Giza et al., 2018). Stimulating cells with high KCl concentration results in activity-dependent secretion of both Val and Met prodomains, though secretion is deficient in Met-carriers (Anastasia et al., 2013). Although endogenous secretion of the Met prodomain has yet to be linked to alterations in dendrites, decreased arborization has been found in hippocampal and cortical neurons (Chen et al., 2006, Liu et al., 2012).

Dissertation Overview

The deficit in activity-dependent release of BDNF led to the hypothesis that activity-dependent treatments to enhance axon regeneration after peripheral nerve injury would be ineffective in this population. Using a mouse model of this polymorphism which recapitulates certain phenotypic aspects of the human population such as decreased hippocampal volume and increased anxiety-like behavior (Chen et al., 2006), we tested the efficacy of two weeks of treadmill training in enhancing sensory and motoneuron regeneration (Chapter 2). We also cultured adult DRG neurons and tested the effect of optogenetic stimulation in enhancing neurite outgrowth (Chapter 2).

Peripheral axon regeneration in Met-carriers was surprisingly enhanced without any

treatment (Chapter 2). To ascertain the mechanism behind the enhanced regeneration, we treated the Met-carriers with pharmacological agents to block trkB receptor activity and $p75^{NTR}$ receptor activity (Chapter 3). These treatments were administered both *in vivo* at the time of injury as well as *in vitro* to cultured DRG neurons. Additionally, we treated cultured DRG neurons with trkB-Fc, a fusion protein that sequesters all trkB ligands.

In summary, the main goal of this thesis was to analyze the axon regeneration of Met-carriers after peripheral nerve injury as well as probe the translational potential of activity-dependent treatments in enhancing axon regeneration.

References

ABE, N., BORSON, S. H., GAMBELLO, M. J., WANG, F. & CAVALLI, V. 2010. Mammalian target of rapamycin (mTOR) activation increases axonal growth capacity of injured peripheral nerves. *J Biol Chem,* 285**,** 28034-43.

ACHESON, A., CONOVER, J. C., FANDL, J. P., DECHIARA, T. M., RUSSELL, M., THADANI, A., SQUINTO, S. P., YANCOPOULOS, G. D. & LINDSAY, R. M. 1995. A BDNF autocrine loop in adult sensory neurons prevents cell death. *Nature,* 374**,** 450-3.

AHLBORN, P., SCHACHNER, M. & IRINTCHEV, A. 2007. One hour electrical stimulation accelerates functional recovery after femoral nerve repair. *Exp Neurol,* 208**,** 137-44.

AID, T., KAZANTSEVA, A., PIIRSOO, M., PALM, K. & TIMMUSK, T. 2007. Mouse and rat BDNF gene structure and expression revisited. *J Neurosci Res,* 85**,** 525-535.

AL MAJED, A., BRUSHART, T. & GORDON, T. 2000a. Electrical stimulation accelerates and increases expression of BDNF and trkB mRNA in regenerating rat femoral motoneurons. *Eur J Neurosci,* 12**,** 4381-4390.

AL MAJED, A., NEUMANN, C., BRUSHART, T. & GORDON, T. 2000b. Brief electrical stimulation promotes the speed and accuracy of motor axonal regeneration. *J Neurosci,* 20**,** 2602-2608.

ALDERSON, R. F., CURTIS, R., ALTERMAN, A. L., LINDSAY, R. M. & DISTEFANO, P. S. 2000. Truncated TrkB mediates the endocytosis and release of BDNF and neurotrophin-4/5 by rat astrocytes and Schwann cells in vitro. *Brain Res,* 871**,** 210-222.

ALLEN, M., BIRD, C., FENG, W., LIU, G., LI, W., PERRONE-BIZZOZERO, N. I. & FENG, Y. 2013. HuD promotes BDNF expression in brain neurons via selective stabilization of the BDNF long 3'UTR mRNA. *PLoS One,* 8**,** e55718.

ALVAREZ, F. J. & FYFFE, R. E. W. 2000. Nociceptors for the 21st Century. *Curr Rev Pain,* 4**,** 451-458.

ALVAREZ, F. J., TITUS-MITCHELL, H. E., BULLINGER, K. L., KRASZPULSKI, M., NARDELLI, P. & COPE, T. C. 2011. Permanent central synaptic disconnection of proprioceptors after nerve injury and regeneration. I. Loss of VGLUT1/IA synapses on motoneurons. *J Neurophysiol,* 106**,** 2450-2470.

AMBRON, R. T., DULIN, M. F., ZHANG, X. P., SCHMIED, R. & WALTERS, E. T. 1995. Axoplasm enriched in a protein mobilized by nerve injury induces memory-like alterations in Aplysia neurons. *J Neurosci,* 15**,** 3440-6.

AN, J. J., GHARAMI, K., LIAO, G. Y., WOO, N. H., LAU, A. G., VANEVSKI, F., TORRE, E. R., JONES, K. R., FENG, Y., LU, B. & XU, B. 2008. Distinct role of long 3' UTR BDNF mRNA in spine morphology and synaptic plasticity in hippocampal neurons. *Cell,* 134**,** 175-87.

ANASTASIA, A., DEINHARDT, K., CHAO, M., WILL, N., IRMADY, K., LEE, F., HEMPSTEAD, B. & BRACKEN, C. 2013. Val66Met polymorphism of BDNF alters prodomain structure to induce neuronal growth cone retraction. *Nat Commun,* 4**,** 2490.

APFEL, S. C., WRIGHT, D. E., WIIDEMAN, A. M., DORMIA, C., SNIDER, W. D. & KESSLER, J. A. 1996. Nerve growth factor regulates the expression of brain-derived neurotrophic factor mRNA in the peripheral nervous system. *Mol Cell Neurosci,* 7**,** 134-42.

ARTHUR-FARRAJ, PETER J., LATOUCHE, M., WILTON, DANIEL K., QUINTES, S., CHABROL, E., BANERJEE, A., WOODHOO, A., JENKINS, B., RAHMAN, M., TURMAINE, M., WICHER, GRZEGORZ K., MITTER, R., GREENSMITH, L., BEHRENS, A., RAIVICH, G., MIRSKY, R. & JESSEN, KRISTJÁN R. 2012. c-Jun reprograms Schwann cells of injured nerves to generate a repair cell essential for regeneration. *Neuron,* 75**,** 633-647.

ASANO, Y., NAGASAKI, A. & UYEDA, T. Q. 2008. Correlated waves of actin filaments and PIP3 in Dictyostelium cells. *Cell Motil Cytoskeleton,* 65**,** 923-34.

ASENSIO-PINILLA, E., UDINA, E., JARAMILLO, J. & NAVARRO, X. 2009. Electrical stimulation combined with exercise increase axonal regeneration after peripheral nerve injury. *Exp Neurol,* 219**,** 258-65.

BADKE, A., IRINTCHEV, A. P. & WERNIG, A. 1989. Maturation of transmission in reinnervated mouse soleus muscle. *Muscle Nerve,* 12**,** 580-586.

BAJ, G., D'ALESSANDRO, V., MUSAZZI, L., MALLEI, A., SARTORI, C. R., SCIANCALEPORE, M., TARDITO, D., LANGONE, F., POPOLI, M. & TONGIORGI, E. 2012. Physical exercise and antidepressants enhance BDNF targeting in hippocampal CA3 dendrites: further evidence of a spatial code for BDNF splice variants. *Neuropsychopharmacology,* 37**,** 1600-1611.

BAJ, G., LEONE, E., CHAO, M. V. & TONGIORGI, E. 2011. Spatial segregation of BDNF transcripts enables BDNF to differentially shape distinct dendritic compartments. *Proc Natl Acad Sci U S A,* 108**,** 16813-8.

BARTEL, D. P. 2004. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell,* 116**,** 281-97.

BATTY, N. J., FENRICH, K. K. & FOUAD, K. 2017. The role of cAMP and its downstream targets in neurite growth in the adult nervous system. *Neurosci Lett,* 652**,** 56-63.

BEIROWSKI, B., ADALBERT, R., WAGNER, D., GRUMME, D. S., ADDICKS, K., RIBCHESTER, R. R. & COLEMAN, M. P. 2005. The progressive nature of Wallerian degeneration in wild-type and slow Wallerian degeneration (WldS) nerves. *BMC Neurosci,* 6**,** 6.

BEN-YAAKOV, K., DAGAN, S. Y., SEGAL-RUDER, Y., SHALEM, O., VUPPALANCHI, D., WILLIS, D. E., YUDIN, D., RISHAL, I., ROTHER, F., BADER, M., BLESCH, A., PILPEL, Y., TWISS, J. L. & FAINZILBER, M. 2012. Axonal transcription factors signal retrogradely in lesioned peripheral nerve. *EMBO J,* 31**,** 1350-1363.

BERCHTOLD, N. C., CHINN, G., CHOU, M., KESSLAK, J. P. & COTMAN, C. W. 2005. Exercise primes a molecular memory for brain-derived neurotrophic factor protein induction in the rat hippocampus. *Neuroscience,* 133**,** 853-61.

BIBEL, M., HOPPE, E. & BARDE, Y. A. 1999. Biochemical and functional interactions between the neurotrophin receptors trk and p75NTR. *EMBO J,* 18**,** 616-22.

BLINZINGER, K. & KREUTZBERG, G. 1968. Displacement of synaptic terminals from regenerating motoneurons by microglial cells. *Z Zellforsch Mikrosk Anat,* 85**,** 145-57.

BODINE, S. C., GARFINKEL, A., ROY, R. R. & EDGERTON, V. R. 1988. Spatial distribution of motor unit fibers in the cat soleus and tibialis anterior muscles: local interactions. *J Neurosci,* 8**,** 2142-52.

BOELTZ, T., IRELAND, M., MATHIS, K., NICOLINI, J., POPLAVSKI, K., ROSE, S. & ENGLISH, A. 2013. Effects of treadmill training on functional recovery following peripheral nerve injury in rats. *J Neurophysiol,* 109**,** 2645-2657.

BOUDRIAU, S., CÔTÉ, C. H., VINCENT, M., HOULE, P., TREMBLAY, R. R. & ROGERS, P. A. 1996. Remodeling of the cytoskeletal lattice in denervated skeletal muscle. *Muscle Nerve,* 19**,** 1383-1390.

BOYD, J. G. & GORDON, T. 2001. The neurotrophin receptors, trkB and p75, differentially regulate motor axonal regeneration. *J Neurobiol,* 49**,** 314-25.

BOYD, J. G. & GORDON, T. 2002. A dose-dependent facilitation and inhibition of peripheral nerve regeneration by brain-derived neurotrophic factor. *Eur J Neurosci,* 15**,** 613-26.

BOYD, J. G. & GORDON, T. 2003. Neurotrophic factors and their receptors in axonal regeneration and functional recovery after peripheral nerve injury. *Mol Neurobiol,* 27**,** 277-324.

BRANDT, J., EVANS, J. T., MILDENHALL, T., MULLIGAN, A., KONIECZNY, A., ROSE, S. J. & ENGLISH, A. W. 2015. Delaying the onset of treadmill exercise following peripheral

nerve injury has different effects on axon regeneration and motoneuron synaptic plasticity. *J Neurophysiol,* 113**,** 2390-9.
BRANNSTROM, T. & KELLERTH, J. O. 1998. Changes in synaptology of adult cat spinal alpha-motoneurons after axotomy. *Exp Brain Res,* 118**,** 1-13.
BRANNSTROM, T. & KELLERTH, J. O. 1999. Recovery of synapses in axotomized adult cat spinal motoneurons after reinnervation into muscle. *Exp Brain Res,* 125**,** 19-27.
BRAUNEWELL, K. H., PESHEVA, P., MCCARTHY, J. B., FURCHT, L. T., SCHMITZ, B. & SCHACHNER, M. 1995. Functional involvement of sciatic nerve-derived versican- and decorin-like molecules and other chondroitin sulphate proteoglycans in ECM-mediated cell adhesion and neurite outgrowth. *Eur J Neurosci,* 7**,** 805-14.
BRIGADSKI, T., HARTMANN, M. & LESSMANN, V. 2005. Differential vesicular targeting and time course of synaptic secretion of the mammalian neurotrophins. *J Neurosci,* 25**,** 7601-14.
BROSIUS LUTZ, A. & BARRES, B. A. 2014. Contrasting the glial response to axon injury in the central and peripheral nervous systems. *Dev Cell,* 28**,** 7-17.
BROWN, M. C. & HARDMAN, V. J. 1987. A reassessment of the accuracy of reinnervation by motoneurons following crushing or freezing of the sciatic or lumbar spinal nerves of rats. *Brain,* 110 (Pt 3)**,** 695-705.
BROWN, T. J., KHAN, T. & JONES, K. J. 1999. Androgen induced acceleration of functional recovery after rat sciatic nerve injury. *Restor Neurol Neurosci,* 15**,** 289-295.
BRUSHART, T. M. 1993. Motor axons preferentially reinnervate motor pathways. *J Neurosci,* 13**,** 2730-8.
BRUSHART, T. M., ASPALTER, M., GRIFFIN, J. W., REDETT, R., HAMEED, H., ZHOU, C., WRIGHT, M., VYAS, A. & HOKE, A. 2013. Schwann cell phenotype is regulated by axon modality and central-peripheral location, and persists in vitro. *Exp Neurol,* 247**,** 272-81.
BRUSHART, T. M., HOFFMAN, P. N., ROYALL, R. M., MURINSON, B. B., WITZEL, C. & GORDON, T. 2002. Electrical stimulation promotes motoneuron regeneration without increasing its speed or conditioning the neuron. *J Neurosci,* 22**,** 6631-8.
BRUSHART, T. M., JARI, R., VERGE, V., ROHDE, C. & GORDON, T. 2005. Electrical stimulation restores the specificity of sensory axon regeneration. *Exp Neurol,* 194**,** 221-229.
BRUSHART, T. M. & MESULAM, M. M. 1980. Alteration in connections between muscle and anterior horn motoneurons after peripheral nerve repair. *Science,* 208**,** 603.
BUEKER, E. D. & MEYERS, C. E. 1951. The maturity of peripheral nerves at the time of injury as a factor in nerve regeneration. *Anat Rec,* 109**,** 723-743.
BULLER, A. J., ECCLES, J. C. & ECCLES, R. M. 1960. Interactions between motoneurones and muscles in respect of the characteristic speeds of their responses. *J Physiol,* 150**,** 417-439.
BULLINGER, K. L., NARDELLI, P., PINTER, M. J., ALVAREZ, F. J. & COPE, T. C. 2011. Permanent central synaptic disconnection of proprioceptors after nerve injury and regeneration. II. Loss of functional connectivity with motoneurons. *J Neurophysiol,* 106**,** 2471-85.
CAI, D., SHEN, Y., DE BELLARD, M., TANG, S. & FILBIN, M. T. 1999. Prior Exposure to Neurotrophins Blocks Inhibition of Axonal Regeneration by MAG and Myelin via a cAMP-Dependent Mechanism. *Neuron,* 22**,** 89-101.
CAPSONI, S., TONGIORGI, E., CATTANEO, A. & DOMENICI, L. 1999. Dark rearing blocks the developmental down-regulation of brain-derived neurotrophic factor messenger RNA expression in layers IV and V of the rat visual cortex. *Neuroscience,* 88**,** 393-403.
CARTER, B. D., KALTSCHMIDT, C., KALTSCHMIDT, B., OFFENHAUSER, N., BOHM-MATTHAEI, R., BAEUERLE, P. A. & BARDE, Y. A. 1996. Selective activation of NF-

kappa B by nerve growth factor through the neurotrophin receptor p75. *Science,* 272**,** 542-5.

CAVALLI, V., KUJALA, P., KLUMPERMAN, J. & GOLDSTEIN, L. S. B. 2005. Sunday Driver links axonal transport to damage signaling. *J Cell Biol,* 168**,** 775.

CHAUDHRY, V. & CORNBLATH, D. R. 1992. Wallerian degeneration in human nerves: serial electrophysiological studies. *Muscle Nerve,* 15**,** 687-93.

CHEN, Z., JING, D., BATH, K., IERACI, A., KHAN, T., SIAO, C., HERRERA, D., TOTH, M., MCEWEN, B., HEMPSTEAD, B. & LEE, F. 2006. Genetic variant BDNF (Val66Met) polymorphism alters anxiety-related behavior. *Science,* 314**,** 140-143.

CHEN, Z., PATEL, P., SANT, G., MENG, C., TENG, K., HEMPSTEAD, B. & LEE, F. 2004. Variant brain-derived neurotrophic factor (BDNF) (Met66) alters the intracellular trafficking and activity-dependent secretion of wild-type BDNF in neurosecretory cells and cortical neurons. *J Neurosci,* 24**,** 4401-4411.

CHEN, Z. Y., IERACI, A., TENG, H., DALL, H., MENG, C. X., HERRERA, D. G., NYKJAER, A., HEMPSTEAD, B. L. & LEE, F. S. 2005. Sortilin controls intracellular sorting of brain-derived neurotrophic factor to the regulated secretory pathway. *J Neurosci,* 25**,** 6156-66.

CHIARUTTINI, C., SONEGO, M., BAJ, G., SIMONATO, M. & TONGIORGI, E. 2008. BDNF mRNA splice variants display activity-dependent targeting to distinct hippocampal laminae. *Mol Cell Neurosci,* 37**,** 11-9.

CHIARUTTINI, C., VICARIO, A., LI, Z., BAJ, G., BRAIUCA, P., WU, Y., LEE, F. S., GARDOSSI, L., BARABAN, J. M. & TONGIORGI, E. 2009. Dendritic trafficking of BDNF mRNA is mediated by translin and blocked by the G196A (Val66Met) mutation. *Proc Natl Acad Sci U S A,* 106**,** 16481-16486.

CHO, H. J., KIM, S. Y., PARK, M. J., KIM, D. S., KIM, J. K. & CHU, M. Y. 1997. Expression of mRNA for brain-derived neurotrophic factor in the dorsal root ganglion following peripheral inflammation. *Brain Res,* 749**,** 358-62.

CHRISTIE, K. J., WEBBER, C. A., MARTINEZ, J. A., SINGH, B. & ZOCHODNE, D. W. 2010. PTEN inhibition to facilitate intrinsic regenerative outgrowth of adult peripheral axons. *J Neurosci,* 30**,** 9306.

COBIANCHI, S., MARINELLI, S., FLORENZANO, F., PAVONE, F. & LUVISETTO, S. 2010. Short- but not long-lasting treadmill running reduces allodynia and improves functional recovery after peripheral nerve injury. *Neuroscience,* 168**,** 273-287.

COMPSTON, A. 2009. A human experiment in nerve division by W. H .R. Rivers MD FRS, Fellow of St John's College, Cambridge and Henry Head MD FRS, Physician to the London Hospital, Brain 1908: 31; 323–450. *Brain,* 132**,** 2903-2905.

CONNER, J. M., LAUTERBORN, J. C., YAN, Q., GALL, C. M. & VARON, S. 1997. Distribution of Brain-Derived Neurotrophic Factor (BDNF) protein and mRNA in the normal adult rat CNS: evidence for anterograde axonal transport. *J Neurosci,* 17**,** 2295-2313.

COSGAYA, J. M., CHAN, J. R. & SHOOTER, E. M. 2002. The neurotrophin receptor p75NTR as a positive modulator of myelination. *Science,* 298**,** 1245-8.

DANIELSEN, N., LUNDBORG, G. & FRIZELL, M. 1986. Nerve repair and axonal transport: outgrowth delay and regeneration rate after transection and repair of rabbit hypoglossal nerve. *Brain Res,* 376**,** 125-32.

DAVIS-LOPEZ DE CARRIZOSA, M. A., MORADO-DIAZ, C. J., TENA, J. J., BENITEZ-TEMINO, B., PECERO, M. L., MORCUENDE, S. R., DE LA CRUZ, R. R. & PASTOR, A. M. 2009. Complementary actions of BDNF and neurotrophin-3 on the firing patterns and synaptic composition of motoneurons. *J Neurosci,* 29**,** 575-87.

DE BONO, J. P., ADLAM, D., PATERSON, D. J. & CHANNON, K. M. 2006. Novel quantitative phenotypes of exercise training in mouse models. *Am J Physiol Regul Integr Comp Physiol,* 290**,** R926-34.

DECHANT, G. & BARDE, Y.-A. 1997. Signalling through the neurotrophin receptor p75NTR. *Curr Opin Neurobiol,* 7**,** 413-418.

DECHANT, G. & BARDE, Y. A. 2002. The neurotrophin receptor p75(NTR): novel functions and implications for diseases of the nervous system. *Nat Neurosci,* 5**,** 1131-6.

DEINHARDT, K., KIM, T., SPELLMAN, D. S., MAINS, R. E., EIPPER, B. A., NEUBERT, T. A., CHAO, M. V. & HEMPSTEAD, B. L. 2011. Neuronal growth cone retraction relies on proneurotrophin receptor signaling through Rac. *Sci Signal,* 4**,** ra82.

DELCROIX, J.-D., VALLETTA, J. S., WU, C., HUNT, S. J., KOWAL, A. S. & MOBLEY, W. C. 2003. NGF Signaling in Sensory Neurons: Evidence that Early Endosomes Carry NGF Retrograde Signals. *Neuron,* 39**,** 69-84.

DIAMOND, J., COUGHLIN, M., MACINTYRE, L., HOLMES, M. & VISHEAU, B. 1987. Evidence that endogenous beta nerve growth factor is responsible for the collateral sprouting, but not the regeneration, of nociceptive axons in adult rats. *Proc Natl Acad Sci U S A,* 84**,** 6596-600.

DIAMOND, J., FOERSTER, A., HOLMES, M. & COUGHLIN, M. 1992. Sensory nerves in adult rats regenerate and restore sensory function to the skin independently of endogenous NGF. *J Neurosci,* 12**,** 1467-76.

DIENI, S., MATSUMOTO, T., DEKKERS, M., RAUSKOLB, S., IONESCU, M. S., DEOGRACIAS, R., GUNDELFINGER, E. D., KOJIMA, M., NESTEL, S., FROTSCHER, M. & BARDE, Y.-A. 2012. BDNF and its pro-peptide are stored in presynaptic dense core vesicles in brain neurons. *J Cell Biol,* 196**,** 775-788.

DIFATO, F., TSUSHIMA, H., PESCE, M., BENFENATI, F., BLAU, A. & CHIEREGATTI, E. 2011. The formation of actin waves during regeneration after axonal lesion is enhanced by BDNF. *Scientific Reports,* 1**,** 183.

DZIENNIS, S. & ALKAYED, N. J. 2008. Role of signal transducer and activator of transcription 3 in neuronal survival and regeneration. *Rev Neurosci,* 19**,** 341-361.

EBENEZER, G. J., MCARTHUR, J. C., THOMAS, D., MURINSON, B., HAUER, P., POLYDEFKIS, M. & GRIFFIN, J. W. 2007. Denervation of skin in neuropathies: the sequence of axonal and Schwann cell changes in skin biopsies. *Brain,* 130**,** 2703-14.

EGAN, M., KOJIMA, M., CALLICOTT, J., GOLDBERG, T., KOLACHANA, B., BERTOLINO, A., ZAITSEV, E., GOLD, B., GOLDMAN, D., DEAN, M., LU, B. & WEINBERGER, D. 2003. The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. *Cell,* 112**,** 257-269.

EIDE, F. F., VINING, E. R., EIDE, B. L., ZANG, K., WANG, X. Y. & REICHARDT, L. F. 1996. Naturally occurring truncated trkB receptors have dominant inhibitory effects on brain-derived neurotrophic factor signaling. *J Neurosci,* 16**,** 3123-9.

ELFVING, B., PLOUGMANN, P. H. & WEGENER, G. 2010. Detection of brain-derived neurotrophic factor (BDNF) in rat blood and brain preparations using ELISA: Pitfalls and solutions. *J Neurosci Methods,* 187**,** 73-77.

ELZINGA, K., TYREMAN, N., LADAK, A., SAVARYN, B., OLSON, J. & GORDON, T. 2015. Brief electrical stimulation improves nerve regeneration after delayed repair in Sprague Dawley rats. *Exp Neurol,* 269**,** 142-53.

ENES, J., LANGWIESER, N., RUSCHEL, J., CARBALLOSA-GONZALEZ, M. M., KLUG, A., TRAUT, M. H., YLERA, B., TAHIROVIC, S., HOFMANN, F., STEIN, V., MOOSMANG, S., HENTALL, I. D. & BRADKE, F. 2010. Electrical activity suppresses axon growth through Cav1.2 channels in adult primary sensory neurons. *Curr Biol,* 20**,** 1154-1164.

ENGLISH, A. 2005. Enhancing axon regeneration in peripheral nerves also increases functionally inappropriate reinnervation of targets. *J Comp Neurol,* 490**,** 427-441.

ENGLISH, A., CUCORANU, D., MULLIGAN, A. & SABATIER, M. 2009. Treadmill training enhances axon regeneration in injured mouse peripheral nerves without increased loss of topographic specificity. *J Comp Neurol,* 517**,** 245-255.

ENGLISH, A., LIU, K., NICOLINI, J., MULLIGAN, A. & YE, K. 2013. Small-molecule trkB agonists promote axon regeneration in cut peripheral nerves. *Proc Natl Acad Sci U S A,* 110**,** 16217-16222.

ENGLISH, A., SCHARTZ, G., W, M., SABATIER, M. & MULLIGAN, A. 2007. Electrical stimulation promotes peripheral axon regeneration by enhanced neuronal neurotrophin signaling. *Dev Neurobiol,* 67**,** 158-172.

ENGLISH, A., WILHELM, J. & SABATIER, M. 2011. Enhancing recovery from peripheral nerve injury using treadmill training. *Ann Anat,* 193**,** 354-361.

ENGLISH, A. W., WILHELM, J. C. & WARD, P. J. 2014. Exercise, neurotrophins, and axon regeneration in the PNS. *Physiology (Bethesda),* 29**,** 437-45.

ERNFORS, P., HENSCHEN, A., OLSON, L. & PERSSON, H. 1989. Expression of nerve growth factor receptor mRNA is developmentally regulated and increased after axotomy in rat spinal cord motoneurons. *Neuron,* 2**,** 1605-13.

ESCANDON, E., SOPPET, D., ROSENTHAL, A., MENDOZA-RAMIREZ, J., SZONYI, E., BURTON, L., HENDERSON, C., PARADA, L. & NIKOLICS, K. 1994. Regulation of neurotrophin receptor expression during embryonic and postnatal development. *J Neurosci,* 14**,** 2054-2068.

FARGO, K. N., FOECKING, E. M., JONES, K. J. & SENGELAUB, D. R. 2009. NEUROPROTECTIVE ACTIONS OF ANDROGENS ON MOTONEURONS. *Frontiers in neuroendocrinology,* 30**,** 130-141.

FERGUSON, T. A. & MUIR, D. 2000. MMP-2 and MMP-9 Increase the Neurite-Promoting Potential of Schwann Cell Basal Laminae and Are Upregulated in Degenerated Nerve. *Molecular and Cellular Neuroscience,* 16**,** 157-167.

FERRI, C. C., MOORE, F. A. & BISBY, M. A. 1998. Effects of facial nerve injury on mouse motoneurons lacking the p75 low-affinity neurotrophin receptor. *J Neurobiol,* 34**,** 1-9.

FLYNN, K. C., PAK, C. W., SHAW, A. E., BRADKE, F. & BAMBURG, J. R. 2009. Growth cone-like waves transport actin and promote axonogenesis and neurite branching. *Dev Neurobiol,* 69**,** 761-79.

FOECKING, E. M., FARGO, K. N., COUGHLIN, L. M., KIM, J. T., MARZO, S. J. & JONES, K. J. 2012. Single session of brief electrical stimulation immediately following crush injury enhances functional recovery of rat facial nerve. *J Rehabil Res Dev,* 49**,** 451-8.

FREEMAN, L. M., PADGETT, B. A., PRINS, G. S. & BREEDLOVE, S. M. 1995. Distribution of androgen receptor immunoreactivity in the spinal cord of wild-type, androgen-insensitive and gonadectomized male rats. *J Neurobiol,* 27**,** 51-9.

FRISÉN, J., VERGE, V. M., FRIED, K., RISLING, M., PERSSON, H., TROTTER, J., HÖKFELT, T. & LINDHOLM, D. 1993. Characterization of glial trkB receptors: differential response to injury in the central and peripheral nervous systems. *Proc Natl Acad Sci U S A,* 90**,** 4971-4975.

FRYER, R. H., KAPLAN, D. R. & KROMER, L. F. 1997. Truncated trkB receptors on nonneuronal cells inhibit BDNF-induced neurite outgrowth in vitro. *Exp Neurol,* 148**,** 616-27.

FU, S. Y. & GORDON, T. 1995a. Contributing factors to poor functional recovery after delayed nerve repair: prolonged axotomy. *J Neurosci,* 15**,** 3876-85.

FU, S. Y. & GORDON, T. 1995b. Contributing factors to poor functional recovery after delayed nerve repair: prolonged denervation. *J Neurosci,* 15**,** 3886-95.

FU, S. Y. & GORDON, T. 1997. The cellular and molecular basis of peripheral nerve regeneration. *Mol Neurobiol,* 14**,** 67-116.

FUKUCHI, M. & TSUDA, M. 2010. Involvement of the 3'-untranslated region of the brain-derived neurotrophic factor gene in activity-dependent mRNA stabilization. *J Neurochem,* 115**,** 1222-33.

FUNAKOSHI, H., FRISEN, J., BARBANY, G., TIMMUSK, T., ZACHRISSON, O., VERGE, V. M. & PERSSON, H. 1993. Differential expression of mRNAs for neurotrophins and their receptors after axotomy of the sciatic nerve. *J Cell Biol,* 123**,** 455-65.

GALLO, G. & LETOURNEAU, P. C. 1998. Localized sources of neurotrophins initiate axon collateral sprouting. *J Neurosci,* 18**,** 5403.

GAO, Y., NIKULINA, E., MELLADO, W. & FILBIN, M. T. 2003. Neurotrophins elevate cAMP to reach a threshold required to overcome inhibition by MAG through extracellular signal-regulated kinase-dependent inhibition of phosphodiesterase. *J Neurosci,* 23**,** 11770-7.

GARDINER, P. F., MICHEL, R. & IADELUCA, G. 1984. Previous exercise training influences functional sprouting of rat hindlimb motoneurons in response to partial denervation. *Neurosci Lett,* 45**,** 123-127.

GEREMIA, N., GORDON, T., BRUSHART, T., AL MAJED, A. & VERGE, V. 2007. Electrical stimulation promotes sensory neuron regeneration and growth-associated gene expression. *Exp Neurol,* 205**,** 347-359.

GHOSH-ROY, A., WU, Z., GONCHAROV, A., JIN, Y. & CHISHOLM, A. D. 2010. Calcium and cyclic AMP promote axonal regeneration in Caenorhabditis elegans and require DLK-1 kinase. *J Neurosci,* 30**,** 3175-83.

GIBNEY, J. & ZHENG, J. Q. 2003. Cytoskeletal dynamics underlying collateral membrane protrusions induced by neurotrophins in cultured Xenopus embryonic neurons. *J Neurobiol,* 54**,** 393-405.

GILLESPIE, M. J., GORDON, T. & MURPHY, P. R. 1986. Reinnervation of the lateral gastrocnemius and soleus muscles in the rat by their common nerve. *J Physiol,* 372**,** 485-500.

GIZA, J. I., KIM, J., MEYER, H. C., ANASTASIA, A., DINCHEVA, I., ZHENG, C. I., LOPEZ, K., BAINS, H., YANG, J., BRACKEN, C., LISTON, C., JING, D., HEMPSTEAD, B. L. & LEE, F. S. 2018. The BDNF Val66Met Prodomain Disassembles Dendritic Spines Altering Fear Extinction Circuitry and Behavior. *Neuron,* 99**,** 163-178.e6.

GLENN, T. D. & TALBOT, W. S. 2013. Signals regulating myelination in peripheral nerves and the Schwann cell response to injury. *Curr Opin Neurobiol,* 23**,** 10.1016/j.conb.2013.06.010.

GOLDBERG, J. L., ESPINOSA, J. S., XU, Y., DAVIDSON, N., KOVACS, G. T. & BARRES, B. A. 2002. Retinal ganglion cells do not extend axons by default: promotion by neurotrophic signaling and electrical activity. *Neuron,* 33**,** 689-702.

GOMEZ-PINILLA, F., YING, Z., ROY, R. R., MOLTENI, R. & EDGERTON, V. R. 2002. Voluntary exercise induces a BDNF-mediated mechanism that promotes neuroplasticity. *J Neurophysiol,* 88**,** 2187-95.

GOMEZ-SANCHEZ, J. A., PILCH, K. S., VAN DER LANS, M., FAZAL, S. V., BENITO, C., WAGSTAFF, L. J., MIRSKY, R. & JESSEN, K. R. 2017. After nerve injury, lineage rracing shows that myelin and Remak Schwann cells elongate extensively and branch to form repair Schwann cells, which shorten radically on remyelination. *J Neurosci,* 37**,** 9086.

GORDON, T. 2015. Chapter 61 - The Biology, Limits, and Promotion of Peripheral Nerve Regeneration in Rats and Humans. *In:* TUBBS, R. S., RIZK, E., SHOJA, M. M., LOUKAS, M., BARBARO, N. & SPINNER, R. J. (eds.) *Nerves and Nerve Injuries.* San Diego: Academic Press.

GORDON, T. 2016. Electrical stimulation to enhance axon regeneration after peripheral nerve injuries in animal models and humans. *Neurotherapeutics,* 13**,** 295-310.

GORDON, T., AMIRJANI, N., EDWARDS, D. C. & CHAN, K. M. 2010. Brief post-surgical electrical stimulation accelerates axon regeneration and muscle reinnervation without affecting the functional measures in carpal tunnel syndrome patients. *Exp Neurol,* 223**,** 192-202.

GORDON, T., CHAN, K. M., SULAIMAN, O. A. R., UDINA, E., AMIRJANI, N. & BRUSHART, T. M. 2009. Accelerating axon growth to overcome limitations in functional recovery after peripheral nerve injury *Neurosurgery,* 65**,** A132-A144.

GORDON, T. & ENGLISH, A. W. 2016. Strategies to promote peripheral nerve regeneration: electrical stimulation and/or exercise. *Eur J Neurosci,* 43**,** 336-50.

GORDON, T., SULAIMAN, O. & BOYD, J. G. 2003. Experimental strategies to promote functional recovery after peripheral nerve injuries. *J Peripher Nerv Syst,* 8**,** 236-50.

GRAFSTEIN, B. 1971. Role of slow axonal transport in nerve regeneration. *Acta Neuropathol,* 5**,** Suppl 5:144-52.

GRAY, M., PALISPIS, W., POPOVICH, P. G., VAN ROOIJEN, N. & GUPTA, R. 2007. Macrophage depletion alters the blood-nerve barrier without affecting Schwann cell function after neural injury. *J Neurosci Res,* 85**,** 766-77.

GRIFFIN, J. W. & THOMPSON, W. J. 2008. Biology and pathology of nonmyelinating Schwann cells. *Glia,* 56**,** 1518-31.

GRINSELL, D. & KEATING, C. P. 2014. Peripheral nerve reconstruction after injury: A review of clinical and experimental therapies. *BioMed Res Int,* 2014**,** 13.

GROVES, M., MCKEON, R., WERNER, E., NAGARSHETH, M., MEADOR, W. & ENGLISH, A. 2005. Axon regeneration in peripheral nerves is enhanced by proteoglycan degradation. *Exp Neurol,* 195**,** 278-292.

GSCHWENDTNER, A., LIU, Z., HUCHO, T., BOHATSCHEK, M., KALLA, R., DECHANT, G. & RAIVICH, G. 2003. Regulation, cellular localization, and function of the p75 neurotrophin receptor (p75NTR) during the regeneration of facial motoneurons. *Mol Cell Neurosci,* 24**,** 307-322.

GUALANDRIS, A., JONES, T. E., STRICKLAND, S. & TSIRKA, S. E. 1996. Membrane depolarization induces calcium-dependent secretion of tissue plasminogen activator. *J Neurosci,* 16**,** 2220.

GUTMANN, E., GUTTMANN, L., MEDAWAR, P. B. & YOUNG, J. Z. 1942. The Rate of Regeneration of Nerve. *J Exp Biol,* 19**,** 14.

HALL, D., DHILLA, A., CHARALAMBOUS, A., GOGOS, J. A. & KARAYIORGOU, M. 2003. Sequence variants of the brain-derived neurotrophic factor (BDNF) gene are strongly associated with obsessive-compulsive disorder. *Am J Hum Genet,* 73**,** 370-376.

HAMANOUE, M., MIDDLETON, G., WYATT, S., JAFFRAY, E., HAY, R. T. & DAVIES, A. M. 1999. p75-mediated NF-kappaB activation enhances the survival response of developing sensory neurons to nerve growth factor. *Mol Cell Neurosci,* 14**,** 28-40.

HANZ, S., PERLSON, E., WILLIS, D., ZHENG, J. Q., MASSARWA, R., HUERTA, J. J., KOLTZENBURG, M., KOHLER, M., VAN-MINNEN, J., TWISS, J. L. & FAINZILBER, M. 2003. Axoplasmic importins enable retrograde injury signaling in lesioned nerve. *Neuron,* 40**,** 1095-104.

HECKMAN, C. J. & ENOKA, R. M. 2012. Motor unit. *Compr Physiol,* 2**,** 2629-82.

HELM, E. E., MATT, K. S., KIRSCHNER, K. F., POHLIG, R. T., KOHL, D. & REISMAN, D. S. 2017. The influence of high intensity exercise and the Val66Met polymorphism on circulating BDNF and locomotor learning. *Neurobiol Learn Mem,* 144**,** 77-85.

HERBISON, G. J., JAWEED, M. M. & DITUNNO, J. F. 1980a. Effect of activity and inactivity on reinnervating rat skeletal muscle contractility. *Exp Neurol,* 70**,** 498-506.

HERBISON, G. J., JAWEED, M. M. & DITUNNO, J. F. 1980b. Histochemical fiber type alterations secondary to exercise training of reinnervating adult rat muscle. *Arch Phys Med Rehabil,* 61**,** 255-7.

HERBISON, G. J., JAWEED, M. M., DITUNNO, J. F. & SCOTT, C. M. 1973. Effect of overwork during reinnervation of rat muscle. *Exp Neurol,* 41**,** 1-14.

HETZLER, L. E. T., SHARMA, N., TANZER, L., WURSTER, R. D., LEONETTI, J., MARZO, S. J., JONES, K. J. & FOECKING, E. M. 2008. Accelerating functional recovery after rat

facial nerve injury: Effects of gonadal steroids and electrical stimulation. *Otolaryngology–Head and Neck Surgery,* 139**,** 62-67.
HEUER, J. G., FATEMIE-NAINIE, S., WHEELER, E. F. & BOTHWELL, M. 1990. Structure and developmental expression of the chicken NGF receptor. *Dev Biol,* 137**,** 287-304.
HEUMANN, R., KORSCHING, S., BANDTLOW, C. & THOENEN, H. 1987a. Changes of nerve growth factor synthesis in nonneuronal cells in response to sciatic nerve transection. *J Cell Biol,* 104**,** 1623-31.
HEUMANN, R., LINDHOLM, D., BANDTLOW, C., MEYER, M., RADEKE, M. J., MISKO, T. P., SHOOTER, E. & THOENEN, H. 1987b. Differential regulation of mRNA encoding nerve growth factor and its receptor in rat sciatic nerve during development, degeneration, and regeneration: role of macrophages. *Proc Natl Acad Sci U S A,* 84**,** 8735-9.
HIKAWA, N. & TAKENAKA, T. 1996. Myelin-stimulated macrophages release neurotrophic factors for adult dorsal root ganglion neurons in culture. *Cell Mol Neurobiol,* 16**,** 517-28.
HINES, H. M. 1942. Effects of immobilization and activity on neuromuscular regeneration. *JAMA,* 120**,** 515-517.
HOFFER, J. A., STEIN, R. B. & GORDON, T. 1979. Differential atrophy of sensory and motor fibers following section of cat peripheral nerves. *Brain Res,* 178**,** 347-361.
HOFFMAN, P. N. & LASEK, R. J. 1980. Axonal transport of the cytoskeleton in regenerating motor neurons: constancy and change. *Brain Res,* 202**,** 317-33.
HÖKE, A. & BRUSHART, T. 2010. Challenges and Opportunities for Regeneration in the Peripheral Nervous System. *Exp Neurol,* 223**,** 1-4.
HOKE, A., REDETT, R., HAMEED, H., JARI, R., ZHOU, C., LI, Z. B., GRIFFIN, J. W. & BRUSHART, T. M. 2006. Schwann cells express motor and sensory phenotypes that regulate axon regeneration. *J Neurosci,* 26**,** 9646-55.
HONG, E. J., MCCORD, A. E. & GREENBERG, M. E. 2008. A biological function for the neuronal activity-dependent component of Bdnf transcription in the development of cortical inhibition. *Neuron,* 60**,** 610-624.
HORCH, K. W., TUCKETT, R. P. & BURGESS, P. R. 1977. A key to the classification of cutaneous mechanoreceptors. *J Invest Dermatol,* 69**,** 75-82.
HU, P. & MCLACHLAN, E. M. 2003. Selective reactions of cutaneous and muscle afferent neurons to peripheral nerve transection in rats. *J Neurosci,* 23**,** 10559-67.
HUANG, E. J. & REICHARDT, L. F. 2003. Trk receptors: roles in neuronal signal transduction. *Annu Rev Biochem,* 72**,** 609-42.
HUANG, J., ZHANG, Y., LU, L., HU, X. & LUO, Z. 2013. Electrical stimulation accelerates nerve regeneration and functional recovery in delayed peripheral nerve injury in rats. *Eur J Neurosci,* 38**,** 3691-3701.
HWANG, J. J., PARK, M. H., CHOI, S. Y. & KOH, J. Y. 2005. Activation of the Trk signaling pathway by extracellular zinc. Role of metalloproteinases. *J Biol Chem,* 280**,** 11995-2001.
IBÁÑEZ, C. & SIMI, A. 2012. p75 neurotrophin receptor signaling in nervous system injury and degeneration: paradox and opportunity. *Trends Neurosci,* 35**,** 431-440.
IERACI, A., MADAIO, A. I., MALLEI, A., LEE, F. S. & POPOLI, M. 2016. Brain-derived neurotrophic factor Val66Met human polymorphism impairs the beneficial exercise-induced neurobiological changes in mice. *Neuropsychopharmacology*.
INAGAKI, N. & KATSUNO, H. 2017. Actin waves: origin of cell polarization and migration? *Trends Cell Biol,* 27**,** 515-526.
JACOBSON, S. & GUTH, L. 1965. An electrophysiological study of the early stages of peripheral nerve regeneration. *Exp Neurol,* 11**,** 48-60.
JAISWAL, P. B., MISTRETTA, O. C., WARD, P. J. & ENGLISH, A. W. 2018. Chemogenetic enhancement of axon regeneration following peripheral nerve injury in the SLICK-A mouse. *Brain Sci,* 8**,** 93.

JAISWAL, P. B., TUNG, J. K., GROSS, R. E. & ENGLISH, A. W. 2017. Motoneuron activity is required for enhancements in functional recovery after peripheral nerve injury in exercised female mice. *J Neurosci Res*.

JANKOWSKI, M. P., CORNUET, P. K., MCILWRATH, S., KOERBER, H. R. & ALBERS, K. M. 2006. SRY-box containing gene 11 (Sox11) transcription factor is required for neuron survival and neurite growth. *Neuroscience,* 143**,** 501-14.

JOHNSON, H., HOKFELT, T. & ULFHAKE, B. 1999. Expression of p75(NTR), trkB and trkC in nonmanipulated and axotomized motoneurons of aged rats. *Brain Res Mol Brain Res,* 69**,** 21-34.

JONES, K. J. 1993. Recovery from facial paralysis following crush injury of the facial nerve in hamsters: differential effects of gender and androgen exposure. *Exp Neurol,* 121**,** 133-8.

JONES, K. R., FARINAS, I., BACKUS, C. & REICHARDT, L. F. 1994. Targeted disruption of the BDNF gene perturbs brain and sensory neuron development but not motor neuron development. *Cell,* 76**,** 989-99.

JU, G., HOKFELT, T., BRODIN, E., FAHRENKRUG, J., FISCHER, J. A., FREY, P., ELDE, R. P. & BROWN, J. C. 1987. Primary sensory neurons of the rat showing calcitonin gene-related peptide immunoreactivity and their relation to substance P-, somatostatin-, galanin-, vasoactive intestinal polypeptide- and cholecystokinin-immunoreactive ganglion cells. *Cell Tissue Res,* 247**,** 417-31.

KATO, S. & IDE, C. 1994. Axonal sprouting at the node of Ranvier of the peripheral nerve disconnected with the cell body. *Restor Neurol Neurosci,* 6**,** 181-7.

KAYA, Y. & SARIKCIOGLU, L. 2015. Sir Herbert Seddon (1903–1977) and his classification scheme for peripheral nerve injury. *Childs Nerv Syst,* 31**,** 177-180.

KEIFER, J., SABIRZHANOV, B. E., ZHENG, Z., LI, W. & CLARK, T. G. 2009. Cleavage of proBDNF by a tolloid-like metalloproteinase (tTLL) is required for acquisition of in vitro eyeblink classical conditioning *J Neurosci,* 29**,** 14956-14964.

KLEIN, A. B., WILLIAMSON, R., SANTINI, M. A., CLEMMENSEN, C., ETTRUP, A., RIOS, M., KNUDSEN, G. M. & AZNAR, S. 2011. Blood BDNF concentrations reflect brain-tissue BDNF levels across species. *Int J Neuropsychopharmacol,* 14**,** 347-53.

KOBAYASHI, N. R., BEDARD, A. M., HINCKE, M. T. & TETZLAFF, W. 1996. Increased Expression of BDNF and trkB mRNA in Rat Facial Motoneurons after Axotomy. *Eur J Neurosci,* 8**,** 1018-1029.

KOENIG, E., MARTIN, R., TITMUS, M. & SOTELO-SILVEIRA, J. R. 2000. Cryptic peripheral ribosomal domains distributed intermittently along mammalian myelinated axons. *J Neurosci,* 20**,** 8390-400.

KOHARA, K., KITAMURA, A., MORISHIMA, M. & TSUMOTO, T. 2001. Activity-dependent transfer of brain-derived neurotrophic factor to postsynaptic neurons. *Science,* 291**,** 2419-23.

KOLBECK, R., JUNGBLUTH, S. & BARDE, Y. A. 1994. Characterisation of neurotrophin dimers and monomers. *Eur J Biochem,* 225**,** 995-1003.

KOLIATSOS, V., CRAWFORD, T. & PRICE, D. 1991. Axotomy induces nerve growth factor receptor immunoreactivity in spinal motor neurons. *Brain Res,* 549**,** 297-304.

KOPPES, A. N., NORDBERG, A. L., PAOLILLO, G. M., GOODSELL, N. M., DARWISH, H. A., ZHANG, L. & THOMPSON, D. M. 2014. Electrical Stimulation of Schwann Cells Promotes Sustained Increases in Neurite Outgrowth. *Tissue Engineering. Part A,* 20**,** 494-506.

KOUYOUMDJIAN, J. A. 2006. Peripheral nerve injuries: a retrospective survey of 456 cases. *Muscle Nerve,* 34**,** 785-8.

KRAKOWIAK, J., LIU, C., PAPUDESU, C., WARD, P. J., WILHELM, J. C. & ENGLISH, A. W. 2015. Neuronal BDNF signaling is necessary for the effects of treadmill exercise on synaptic stripping of axotomized motoneurons. *Neural Plast,* 2015**,** 392591.

KREKOSKI, C. A., NEUBAUER, D., ZUO, J. & MUIR, D. 2001. Axonal regeneration into acellular nerve grafts is enhanced by degradation of chondroitin sulfate proteoglycan. *J Neurosci,* 21**,** 6206-13.

KROOK-MAGNUSON, E., LEDRI, M., SOLTESZ, I. & KOKAIA, M. 2014. How might novel technologies such as optogenetics lead to better treatments in epilepsy? *Adv Exp Med Biol,* 813**,** 319-336.

KRYSTOSEK, A. & SEEDS, N. W. 1981. Plasminogen activator release at the neuronal growth cone. *Science,* 213**,** 1532.

KUCZEWSKI, N., PORCHER, C., LESSMANN, V., MEDINA, I. & GAIARSA, J. L. 2009. Activity-dependent dendritic release of BDNF and biological consequences. *Mol Neurobiol,* 39**,** 37-49.

KUJAWA, K. A., EMERIC, E. & JONES, K. J. 1991. Testosterone differentially regulates the regenerative properties of injured hamster facial motoneurons. *J Neurosci,* 11**,** 3898.

KUJAWA, K. A., KINDERMAN, N. B. & JONES, K. J. 1989. Testosterone-induced acceleration of recovery from facial paralysis following crush axotomy of the facial nerve in male hamsters. *Exp Neurol,* 105**,** 80-5.

KUJAWA, K. A., TANZER, L. & JONES, K. J. 1995. Inhibition of the accelerative effects of testosterone on hamster facial nerve regeneration by the antiandrogen flutamide. *Exp Neurol,* 133**,** 138-43.

KUZIS, K., COFFIN, J. D. & ECKENSTEIN, F. P. 1999. Time course and age dependence of motor neuron death following facial nerve crush injury: role of fibroblast growth factor. *Exp Neurol,* 157**,** 77-87.

KUZNIEWSKA, B., REJMAK, E., MALIK, A. R., JAWORSKI, J., KACZMAREK, L. & KALITA, K. 2013. Brain-derived neurotrophic factor induces matrix metalloproteinase 9 expression in neurons via the serum response factor/c-Fos pathway. *Mol Cell Biol,* 33**,** 2149-2162.

LAL, D., HETZLER, L. T., SHARMA, N., WURSTER, R. D., MARZO, S. J., JONES, K. J. & FOECKING, E. M. 2008. Electrical stimulation facilitates rat facial nerve recovery from a crush injury. *Otolaryngology–Head and Neck Surgery,* 139**,** 68-73.

LAU, A. G., IRIER, H. A., GU, J., TIAN, D., KU, L., LIU, G., XIA, M., FRITSCH, B., ZHENG, J. Q., DINGLEDINE, R., XU, B., LU, B. & FENG, Y. 2010. Distinct 3'UTRs differentially regulate activity-dependent translation of brain-derived neurotrophic factor (BDNF). *Proc Natl Acad Sci U S A,* 107**,** 15945-50.

LEE, R., KERMANI, P., TENG, K. K. & HEMPSTEAD, B. L. 2001. Regulation of cell survival by secreted proneurotrophins. *Science,* 294**,** 1945-8.

LEE, S. K. & WOLFE, S. W. 2000. Peripheral nerve injury and repair. *J Am Acad Orthop Surg,* 8**,** 243-52.

LEECH, K. A. & HORNBY, T. G. 2017. High-intensity locomotor exercise increases brain-derived neurotrophic factor in individuals with incomplete spinal cord injury. *J Neurotrauma,* 34**,** 1240-1248.

LEMOS, J. R., ALVES, C. R., DE SOUZA, S. B. C., MARSIGLIA, J. D. C., SILVA, M. S. M., PEREIRA, A. C., TEIXEIRA, A. L., VIEIRA, E. L. M., KRIEGER, J. E., NEGRÃO, C. E., ALVES, G. B., DE OLIVEIRA, E. M., BOLANI, W., DIAS, R. G. & TROMBETTA, I. C. 2015. Peripheral vascular reactivity and serum BDNF responses to aerobic training are impaired by the BDNF Val66Met polymorphism. *Physiol Genomics,* 48**,** 116-123.

LESSMANN, V., GOTTMANN, K. & MALCANGIO, M. 2003. Neurotrophin secretion: current facts and future prospects. *Prog Neurobiol,* 69**,** 341-374.

LEWIN, G. R. & BARDE, Y. A. 1996. Physiology of the neurotrophins. *Annu Rev Neurosci,* 19**,** 289-317.

LEWIN, S. L., UTLEY, D. S., CHENG, E. T., VERITY, A. N. & TERRIS, D. J. 1997. Simultaneous treatment with BDNF and CNTF after peripheral nerve transection and

repair enhances rate of functional recovery compared with BDNF treatment alone. *Laryngoscope,* 107**,** 992-999.

LI, C.-L., LI, K.-C., WU, D., CHEN, Y., LUO, H., ZHAO, J.-R., WANG, S.-S., SUN, M.-M., LU, Y.-J., ZHONG, Y.-Q., HU, X.-Y., HOU, R., ZHOU, B.-B., BAO, L., XIAO, H.-S. & ZHANG, X. 2016. Somatosensory neuron types identified by high-coverage single-cell RNA-sequencing and functional heterogeneity. *Cell Res,* 26**,** 83-102.

LI, Z., WU, Y. & BARABAN, J. M. 2008. The Translin/Trax RNA binding complex: clues to function in the nervous system. *Biochim Biophys Acta,* 1779**,** 479-85.

LIAO, C.-F., YANG, T.-Y., CHEN, Y.-H., YAO, C.-H., WAY, T.-D. & CHEN, Y.-S. 2017. Effects of swimming exercise on nerve regeneration in a rat sciatic nerve transection model. *BioMedicine,* 7**,** 3.

LIEBERMAN, A. R. 1971. The axon reaction: a review of the principal features of perikaryal responses to axon injury. *Int Rev Neurobiol,* 14**,** 49-124.

LIEPINSH, E., ILAG, L. L., OTTING, G. & IBÁÑEZ, C. F. 1997. NMR structure of the death domain of the p75 neurotrophin receptor. *EMBO J,* 16**,** 4999-5005.

LINDA, H., SHUPLIAKOV, O., ORNUNG, G., OTTERSEN, O. P., STORM-MATHISEN, J., RISLING, M. & CULLHEIM, S. 2000. Ultrastructural evidence for a preferential elimination of glutamate-immunoreactive synaptic terminals from spinal motoneurons after intramedullary axotomy. *J Comp Neurol,* 425**,** 10-23.

LINDSAY, R. M. 1988. Nerve growth factors (NGF, BDNF) enhance axonal regeneration but are not required for survival of adult sensory neurons. *J Neurosci,* 8**,** 2394-405.

LIU, C., WARD, P. J. & ENGLISH, A. W. 2014. The effects of exercise on synaptic stripping require androgen receptor signaling. *PLOS ONE,* 9**,** e98633.

LIU, H. M., YANG, L. H. & YANG, Y. J. 1995. Schwann cell properties: 3. C-fos expression, bFGF production, phagocytosis and proliferation during Wallerian degeneration. *J Neuropathol Exp Neurol,* 54**,** 487-96.

LIU, Q. R., LU, L., ZHU, X. G., GONG, J. P., SHAHAM, Y. & UHL, G. R. 2006. Rodent BDNF genes, novel promoters, novel splice variants, and regulation by cocaine. *Brain Res,* 1067**,** 1-12.

LIU, R.-J., LEE, F. S., LI, X.-Y., BAMBICO, F., DUMAN, R. S. & AGHAJANIAN, G. K. 2012. Brain-derived neurotrophic factor Val66Met allele impairs basal and ketamine-stimulated synaptogenesis in prefrontal cortex. *Biol Psychiatry,* 71**,** 996-1005.

LJUNGBERG, C., NOVIKOV, L., KELLERTH, J. O., EBENDAL, T. & WIBERG, M. 1999. The neurotrophins NGF and NT-3 reduce sensory neuronal loss in adult rat after peripheral nerve lesion. *Neurosci Lett,* 262**,** 29-32.

LOCKSLEY, R. M., KILLEEN, N. & LENARDO, M. J. 2001. The TNF and TNF receptor superfamilies: integrating mammalian biology. *Cell,* 104**,** 487-501.

LOEB, G. E., MARKS, W. B. & HOFFER, J. A. 1987. Cat hindlimb motoneurons during locomotion. IV. Participation in cutaneous reflexes. *J Neurophysiol,* 57**,** 563-73.

LONZE, B. E. & GINTY, D. D. 2002. Function and regulation of CREB family transcription factors in the nervous system. *Neuron,* 35**,** 605-23.

LOU, H., KIM, S. K., ZAITSEV, E., SNELL, C. R., LU, B. & LOH, Y. P. 2005. Sorting and activity-dependent secretion of BDNF require interaction of a specific motif with the sorting receptor carboxypeptidase e. *Neuron,* 45**,** 245-55.

LU, B., NAGAPPAN, G. & LU, Y. 2014. BDNF and Synaptic Plasticity, Cognitive Function, and Dysfunction. *In:* LEWIN, G. R. & CARTER, B. D. (eds.) *Neurotrophic Factors.* Berlin, Heidelberg: Springer Berlin Heidelberg.

LU, B., PANG, P. T. & WOO, N. H. 2005. The yin and yang of neurotrophin action. *Nat Rev Neurosci,* 6**,** 603.

MACKENZIE, S. J., SMIRNOV, I. & CALANCIE, B. 2012. Cauda equina repair in the rat: part 2. Time course of ventral root conduction failure. *J Neurotrauma,* 29**,** 1683-90.

MAISONPIERRE, P. C., LE BEAU, M. M., ESPINOSA, R., 3RD, IP, N. Y., BELLUSCIO, L., DE LA MONTE, S. M., SQUINTO, S., FURTH, M. E. & YANCOPOULOS, G. D. 1991. Human and rat brain-derived neurotrophic factor and neurotrophin-3: gene structures, distributions, and chromosomal localizations. *Genomics,* 10**,** 558-68.

MAKWANA, M. & RAIVICH, G. 2005. Molecular mechanisms in successful peripheral regeneration. *Febs j,* 272**,** 2628-38.

MALLEI, A., BAJ, G., IERACI, A., CORNA, S., MUSAZZI, L., LEE, F. S., TONGIORGI, E. & POPOLI, M. 2015. Expression and dendritic trafficking of BDNF-6 splice variant are impaired in knock-in mice carrying human BDNF Val66Met polymorphism. *Int J Neuropsychopharmacol,* 18**,** pyv069.

MAR, F. M., BONNI, A. & SOUSA, M. M. 2014. Cell intrinsic control of axon regeneration. *EMBO Rep,* 15**,** 254-63.

MARQUESTE, T., ALLIEZ, J.-R., ALLUIN, O., JAMMES, Y. & DECHERCHI, P. 2004. Neuromuscular rehabilitation by treadmill running or electrical stimulation after peripheral nerve injury and repair. *J Appl Physiol,* 96**,** 1988-1995.

MCKAY HART, A., BRANNSTROM, T., WIBERG, M. & TERENGHI, G. 2002. Primary sensory neurons and satellite cells after peripheral axotomy in the adult rat: timecourse of cell death and elimination. *Exp Brain Res,* 142**,** 308-18.

MELLIOS, N., HUANG, H.-S., GRIGORENKO, A., ROGAEV, E. & AKBARIAN, S. 2008. A set of differentially expressed miRNAs, including miR-30a-5p, act as post-transcriptional inhibitors of BDNF in prefrontal cortex. *Human Molecular Genetics,* 17**,** 3030-3042.

MEYER, M., MATSUOKA, I., WETMORE, C., OLSON, L. & THOENEN, H. 1992. Enhanced synthesis of brain-derived neurotrophic factor in the lesioned peripheral nerve: different mechanisms are responsible for the regulation of BDNF and NGF mRNA. *J Cell Biol,* 119**,** 45-54.

MI, S., LEE, X., SHAO, Z., THILL, G., JI, B., RELTON, J., LEVESQUE, M., ALLAIRE, N., PERRIN, S., SANDS, B., CROWELL, T., CATE, R. L., MCCOY, J. M. & PEPINSKY, R. B. 2004. LINGO-1 is a component of the Nogo-66 receptor/p75 signaling complex. *Nat Neurosci,* 7**,** 221-8.

MICHAEL, G. J., AVERILL, S., NITKUNAN, A., RATTRAY, M., BENNETT, D. L., YAN, Q. & PRIESTLEY, J. V. 1997. Nerve growth factor treatment increases brain-derived neurotrophic factor selectively in TrkA-expressing dorsal root ganglion cells and in their central terminations within the spinal cord. *J Neurosci,* 17**,** 8476-90.

MICHAEL, G. J., AVERILL, S., SHORTLAND, P. J., YAN, Q. & PRIESTLEY, J. V. 1999. Axotomy results in major changes in BDNF expression by dorsal root ganglion cells: BDNF expression in large trkB and trkC cells, in pericellular baskets, and in projections to deep dorsal horn and dorsal column nuclei. *Eur J Neurosci,* 11**,** 3539-51.

MIDDLETON, G., HAMANOUE, M., ENOKIDO, Y., WYATT, S., PENNICA, D., JAFFRAY, E., HAY, R. T. & DAVIES, A. M. 2000. Cytokine-induced nuclear factor kappa B activation promotes the survival of developing neurons. *J Cell Biol,* 148**,** 325-332.

MINICHIELLO, L. 2009. TrkB signalling pathways in LTP and learning. *Nat Rev Neurosci,* 10**,** 850-860.

MOKARRAM, N., DYMANUS, K., SRINIVASAN, A., LYON, J. G., TIPTON, J., CHU, J., ENGLISH, A. W. & BELLAMKONDA, R. V. 2017. Immunoengineering nerve repair. *Proc Natl Acad Sci U S A,* 114**,** E5077.

MOLTENI, R., ZHENG, J.-Q., YING, Z., GÓMEZ-PINILLA, F. & TWISS, J. L. 2004. Voluntary exercise increases axonal regeneration from sensory neurons. *Proc Natl Acad Sci U S A,* 101**,** 8473.

MOWLA, S. J., FARHADI, H. F., PAREEK, S., ATWAL, J. K., MORRIS, S. J., SEIDAH, N. G. & MURPHY, R. A. 2001. Biosynthesis and post-translational processing of the precursor to brain-derived neurotrophic factor. *J Biol Chem,* 276**,** 12660-6.

MOWLA, S. J., PAREEK, S., FARHADI, H. F., PETRECCA, K., FAWCETT, J. P., SEIDAH, N. G., MORRIS, S. J., SOSSIN, W. S. & MURPHY, R. A. 1999. Differential sorting of nerve growth factor and brain-derived neurotrophic factor in hippocampal neurons. *J Neurosci,* 19**,** 2069-80.

MUELLER, M., LEONHARD, C., WACKER, K., RINGELSTEIN, E. B., OKABE, M., HICKEY, W. F. & KIEFER, R. 2003. Macrophage response to peripheral nerve injury: the quantitative contribution of resident and hematogenous macrophages. *Lab Invest,* 83**,** 175-85.

MUNDIE, G. S. 1920. Peripheral nerve injuries. *Can Med Assoc J,* 10**,** 294-296.

NAGAPPAN, G., ZAITSEV, E., SENATOROV, V. V., YANG, J., HEMPSTEAD, B. L. & LU, B. 2009. Control of extracellular cleavage of ProBDNF by high frequency neuronal activity. *Proc Natl Acad Sci U S A,* 106**,** 1267-1272.

NAGATA, S. 1997. Apoptosis by death factor. *Cell,* 88**,** 355-65.

NASCIMENTO, C., PEREIRA, J., PIRES DE ANDRADE, L., GARUFFI, M., AYAN, C., KERR, D., TALIB, L., COMINETTI, M. & STELLA, F. 2015. Physical exercise improves peripheral BDNF levels and cognitive functions in elderly mild cognitive impairment individuals with different BDNF Val66Met genotypes. *J Alzheimers Dis,* 43**,** 81-91.

NATHAN, C. 2006. Neutrophils and immunity: challenges and opportunities. *Nat Rev Immunol,* 6**,** 173.

NEVES-PEREIRA, M., MUNDO, E., MUGLIA, P., KING, N., MACCIARDI, F. & KENNEDY, J. L. 2002. The brain-derived neurotrophic factor gene confers susceptibility to bipolar disorder: evidence from a family-based association study. *Am J Hum Genet,* 71**,** 651-5.

NGUYEN, Q. T., SANES, J. R. & LICHTMAN, J. W. 2002. Pre-existing pathways promote precise projection patterns. *Nat Neurosci,* 5**,** 861-7.

NIELSEN, M. S., MADSEN, P., CHRISTENSEN, E. I., NYKJAER, A., GLIEMANN, J., KASPER, D., POHLMANN, R. & PETERSEN, C. M. 2001. The sortilin cytoplasmic tail conveys Golgi-endosome transport and binds the VHS domain of the GGA2 sorting protein. *EMBO J,* 20**,** 2180-90.

NIX, W. A. & HOPF, H. C. 1983. Electrical stimulation of regenerating nerve and its effect on motor recovery. *Brain Res,* 272**,** 21-5.

NOBLE, J., MUNRO, C. A., PRASAD, V. S. S. V. & MIDHA, R. 1998. Analysis of upper and lower extremity peripheral nerve injuries in a population of patients with multiple injuries. *J Trauma Acute Care Surg,* 45**,** 116-122.

NOTARAS, M., HILL, R. & VAN DEN BUUSE, M. 2015. The BDNF gene Val66Met polymorphism as a modifier of psychiatric disorder susceptibility: progress and controversy. *Mol Psychiatry,* 20**,** 916-30.

NYKJAER, A., LEE, R., TENG, K. K., JANSEN, P., MADSEN, P., NIELSEN, M. S., JACOBSEN, C., KLIEMANNEL, M., SCHWARZ, E., WILLNOW, T. E., HEMPSTEAD, B. L. & PETERSEN, C. M. 2004. Sortilin is essential for proNGF-induced neuronal cell death. *Nature,* 427**,** 843-8.

OLIVEIRA, A. L. R., THAMS, S., LIDMAN, O., PIEHL, F., HÖKFELT, T., KÄRRE, K., LINDÅ, H. & CULLHEIM, S. 2004. A role for MHC class I molecules in synaptic plasticity and regeneration of neurons after axotomy. *Proc Natl Acad Sci U S A,* 101**,** 17843-17848.

OSBORNE, M. C., VERHOVSHEK, T. & SENGELAUB, D. R. 2007. Androgen regulates trkB immunolabeling in spinal motoneurons. *J Neurosci Res,* 85**,** 303-9.

OTTEM, E. N., POORT, J. E., WANG, H., JORDAN, C. L. & BREEDLOVE, S. M. 2010. Differential expression and regulation of brain-derived neurotrophic factor (BDNF) mRNA isoforms in androgen-sensitive motoneurons of the rat lumbar spinal cord. *Mol Cell Endocrinol,* 328**,** 40-6.

PAGNUSSAT, A. S., MICHAELSEN, S. M., ACHAVAL, M., ILHA, J., HERMEL, E. E. S., BACK, F. P. & NETTO, C. A. 2012. Effect of skilled and unskilled training on nerve regeneration and functional recovery. *Braz J Med Biol Res,* 45**,** 753-762.

PAN, Y. A., MISGELD, T., LICHTMAN, J. W. & SANES, J. R. 2003. Effects of neurotoxic and neuroprotective agents on peripheral nerve regeneration assayed by time-lapse imaging in vivo. *J Neurosci,* 23**,** 11479.

PARK, J.-S. & HÖKE, A. 2014. Treadmill exercise induced functional recovery after peripheral nerve repair is associated with increased levels of neurotrophic factors. *PLoS ONE,* 9**,** e90245.

PARK, K. K., LIU, K., HU, Y., SMITH, P. D., WANG, C., CAI, B., XU, B., CONNOLLY, L., KRAMVIS, I., SAHIN, M. & HE, Z. 2008. Promoting axon regeneration in the adult CNS by modulation of the PTEN/mTOR pathway. *Science,* 322**,** 963-6.

PARK, S., KOPPES, R. A., FRORIEP, U. P., JIA, X., ACHYUTA, A. K. H., MCLAUGHLIN, B. L. & ANIKEEVA, P. 2015. Optogenetic control of nerve growth. *Sci Rep,* 5**,** 9669.

PARK, S., LIU, C., WARD, P. J., JAISWAL, P. B. & ENGLISH ARTHUR, W. 2018. Effects of repeated 20 Hz electrical stimulation on functional recovery following peripheral nerve injury. *Neurorehabil Neural Repair,* submitted.

PATAPOUTIAN, A. & REICHARDT, L. F. 2001. Trk receptors: mediators of neurotrophin action. *Curr Opin Neurobiol,* 11**,** 272-280.

PATTABIRAMAN, P. P., TROPEA, D., CHIARUTTINI, C., TONGIORGI, E., CATTANEO, A. & DOMENICI, L. 2005. Neuronal activity regulates the developmental expression and subcellular localization of cortical BDNF mRNA isoforms in vivo. *Mol Cell Neurosci,* 28**,** 556-570.

PERLSON, E., HANZ, S., BEN-YAAKOV, K., SEGAL-RUDER, Y., SEGER, R. & FAINZILBER, M. 2005. Vimentin-dependent spatial translocation of an activated MAP kinase in injured nerve. *Neuron,* 45**,** 715-26.

PERRY, V. H., BROWN, M. C. & GORDON, S. 1987. The macrophage response to central and peripheral nerve injury. A possible role for macrophages in regeneration. *J Exp Med,* 165**,** 1218-23.

PERRY, V. H., TSAO, J. W., FEARN, S. & BROWN, M. C. 1995. Radiation-induced reductions in macrophage recruitment have only slight effects on myelin degeneration in sectioned peripheral nerves of mice. *Eur J Neurosci,* 7**,** 271-80.

PETTE, D. & STARON, R. S. 2001. Transitions of muscle fiber phenotypic profiles. *Histochem Cell Biol,* 115**,** 359-72.

POCKETT, S. & GAVIN, R. M. 1985. Acceleration of peripheral nerve regeneration after crush injury in rat. *Neurosci Lett,* 59**,** 221-4.

PORTINCASA, A., GOZZO, G., PARISI, D., ANNACONTINI, L., CAMPANALE, A., BASSO, G. & MAIORELLA, A. 2007. Microsurgical treatment of injury to peripheral nerves in upper and lower limbs: a critical review of the last 8 years. *Microsurgery,* 27**,** 455-462.

PRUUNSILD, P., KAZANTSEVA, A., AID, T., PALM, K. & TIMMUSK, T. 2007. Dissecting the human BDNF locus: bidirectional transcription, complex splicing, and multiple promoters. *Genomics,* 90**,** 397-406.

QIU, J., CAFFERTY, W. B. J., MCMAHON, S. B. & THOMPSON, S. W. N. 2005. Conditioning injury-induced spinal axon regeneration requires signal transducer and activator of transcription 3 activation. *J Neurosci,* 25**,** 1645.

RAFUSE, V. F. & GORDON, T. 1998. Incomplete rematching of nerve and muscle properties in motor units after extensive nerve injuries in cat hindlimb muscle. *J Physiol,* 509**,** 909-926.

RAIVICH, G., BOHATSCHEK, M., DA COSTA, C., IWATA, O., GALIANO, M., HRISTOVA, M., NATERI, A. S., MAKWANA, M., RIERA-SANS, L., WOLFER, D. P., LIPP, H. P., AGUZZI, A., WAGNER, E. F. & BEHRENS, A. 2004. The AP-1 transcription factor c-Jun is required for efficient axonal regeneration. *Neuron,* 43**,** 57-67.

RAIVICH, G. & KREUTZBERG, G. W. 1987. Expression of growth factor receptors in injured nervous tissue. I. Axotomy leads to a shift in the cellular distribution of specific beta-

nerve growth factor binding in the injured and regenerating PNS. *J Neurocytol,* 16**,** 689-700.

RAMON Y CAJAL, S. 1928. *Degeneration and regeneration of the nervous system,* Oxford, England, Clarendon Press.

REICHARDT, L. F. 2006. Neurotrophin-regulated signalling pathways. *Philos Trans R Soc Lond B Biol Sci,* 361**,** 1545-1564.

RENDE, M., GIAMBANCO, I., BURATTA, M. & TONALI, P. 1995. Axotomy induces a different modulation of both low-affinity nerve growth factor receptor and choline acetyltransferase between adult rat spinal and brainstem motoneurons. *J Comp Neurol,* 363**,** 249-263.

REXED, B. 1952. The cytoarchitectonic organization of the spinal cord in the cat. *J Comp Neurol,* 96**,** 415-495.

RIBASES, M., GRATACOS, M., ARMENGOL, L., DE CID, R., BADIA, A., JIMENEZ, L., SOLANO, R., VALLEJO, J., FERNANDEZ, F. & ESTIVILL, X. 2003. Met66 in the brain-derived neurotrophic factor (BDNF) precursor is associated with anorexia nervosa restrictive type. *Mol Psychiatry,* 8**,** 745-51.

RICH, K. M., LUSZCZYNSKI, J. R., OSBORNE, P. A. & JOHNSON, E. M., JR. 1987. Nerve growth factor protects adult sensory neurons from cell death and atrophy caused by nerve injury. *J Neurocytol,* 16**,** 261-8.

RICHNER, M., ULRICHSEN, M., ELMEGAARD, S. L., DIEU, R., PALLESEN, L. T. & VAEGTER, C. B. 2014. Peripheral nerve injury modulates neurotrophin signaling in the peripheral and central nervous system. *Mol Neurobiol,* 50**,** 945-70.

RIGHI, M., TONGIORGI, E. & CATTANEO, A. 2000. Brain-derived neurotrophic factor (BDNF) induces dendritic targeting of BDNF and tyrosine kinase B mRNAs in hippocampal neurons through a phosphatidylinositol-3 kinase-dependent pathway. *J Neurosci,* 20**,** 3165-74.

RODRÍGUEZ-TÉBAR, A., DECHANT, G., GÖTZ, R. & BARDE, Y. A. 1992. Binding of neurotrophin-3 to its neuronal receptors and interactions with nerve growth factor and brain-derived neurotrophic factor. *EMBO J,* 11**,** 917-922.

ROTTERMAN, T. M., NARDELLI, P., COPE, T. C. & ALVAREZ, F. J. 2014. Normal distribution of VGLUT1 synapses on spinal motoneuron dendrites and their reorganization after nerve injury. *J Neurosci,* 34**,** 3475.

ROUX, P. P. & BARKER, P. A. 2002. Neurotrophin signaling through the p75 neurotrophin receptor. *Prog Neurobiol,* 67**,** 203-233.

SABATIER, M. J. & ENGLISH, A. W. 2015. Pathways mediating activity-induced enhancement of recovery from peripheral nerve injury. *Exerc Sport Sci Rev,* 43**,** 163-71.

SABATIER, M. J., REDMON, N., SCHWARTZ, G. & ENGLISH, A. W. 2008. Treadmill training promotes axon regeneration in injured peripheral nerves. *Exp Neurol,* 211**,** 489-493.

SABATIER, M. J., TO, B. N., ROSE, S., NICOLINI, J. & ENGLISH, A. W. 2012. Chondroitinase ABC reduces time to muscle reinnervation and improves functional recovery after sciatic nerve transection in rats. *J Neurophysiol,* 107**,** 747-57.

SAIKA, T., SENBA, E., NOGUCHI, K., SATO, M., YOSHIDA, S., KUBO, T., MATSUNAGA, T. & TOHYAMA, M. 1991. Effects of nerve crush and transection on mRNA levels for nerve growth factor receptor in the rat facial motoneurons. *Brain Res Mol Brain Res,* 9**,** 157-60.

SAKATA, K., WOO, N. H., MARTINOWICH, K., GREENE, J. S., SCHLOESSER, R. J., SHEN, L. & LU, B. 2009. Critical role of promoter IV-driven BDNF transcription in GABAergic transmission and synaptic plasticity in the prefrontal cortex. *Proc Natl Acad Sci U S A,* 106**,** 5942-5947.

SALERNO, K. M., JING, X., DIGES, C. M., CORNUET, P. K., GLORIOSO, J. C. & ALBERS, K. M. 2012. Sox11 modulates brain-derived neurotrophic factor expression in an exon promoter-specific manner. *J Neurosci Res,* 90**,** 1011-9.

SARIKCIOGLU, L. & OGUZ, N. 2001. Exercise training and axonal regeneration after sciatic nerve injury. *Int J Neurosci,* 109**,** 173-7.

SCHMIDT, A. & HALL, A. 2002. Guanine nucleotide exchange factors for Rho GTPases: turning on the switch. *Genes Dev,* 16**,** 1587-609.

SCHRODER, J. M. 1972. Altered ratio between axon diameter and myelin sheath thickness in regenerated nerve fibers. *Brain Res,* 45**,** 49-65.

SCHWARTZ, P. M., BORGHESANI, P. R., LEVY, R. L., POMEROY, S. L. & SEGAL, R. A. 1997. Abnormal cerebellar development and foliation in BDNF-/- mice reveals a role for neurotrophins in CNS patterning. *Neuron,* 19**,** 269-81.

SCOTT, A., L. M., BORISOFF, J., F. & RAMER, M., S. 2005. Deafferentation and neurotrophin-mediated intraspinal sprouting: a central role for the p75 neurotrophin receptor. *Eur J Neurosci,* 21**,** 81-92.

SCOTT, A. L. M. & RAMER, M. S. 2010. Schwann cell p75NTR prevents spontaneous sensory reinnervation of the adult spinal cord. *Brain,* 133**,** 421-432.

SEIDAH, N., BENJANNET, S., PAREEK, S., CHRÉTIEN, M. & MURPHY, R. 1996a. Cellular processing of the neurotrophin precursors of NT3 and BDNF by the mammalian proprotein convertases. *FEBS Lett,* 379**,** 247-250.

SEIDAH, N. G., BENJANNET, S., PAREEK, S., SAVARIA, D., HAMELIN, J., GOULET, B., LALIBERTE, J., LAZURE, C., CHRÉTIEN, M. & MURPHY, R. A. 1996b. Cellular processing of the nerve growth factor precursor by the mammalian pro-protein convertases. *Biochem J,* 314**,** 951-960.

SEO, T. B., HAN, I. S., YOON, J. H., HONG, K. E., YOON, S. J. & NAMGUNG, U. 2006. Involvement of Cdc2 in axonal regeneration enhanced by exercise training in rats. *Med Sci Sports Exerc,* 38**,** 1267-76.

SEO, T. B., OH, M. J., YOU, B. G., KWON, K. B., CHANG, I. A., YOON, J. H., LEE, C. Y. & NAMGUNG, U. 2009. ERK1/2-mediated Schwann cell proliferation in the regenerating sciatic nerve by treadmill training. *J Neurotrauma,* 26**,** 1733-44.

SHARMA, N., COUGHLIN, L., PORTER, R., TANZER, L., WURSTER, R., MARZO, S., JONES, K. & FOECKING, E. 2009. Effects of electrical stimulation and gonadal steroids on rat facial nerve regenerative properties. *Restor Neurol Neurosci,* 27**,** 633-644.

SHARMA, N., MARZO, S. J., JONES, K. J. & FOECKING, E. M. 2010a. Electrical stimulation and testosterone differentially enhance expression of regeneration-associated genes. *Exp Neurol,* 223**,** 183-91.

SHARMA, N., MOELLER, C. W., MARZO, S. J., JONES, K. J. & FOECKING, E. M. 2010b. Combinatorial treatments enhance recovery following facial nerve crush. *Laryngoscope,* 120**,** 1523-30.

SHEN, Y. J., DEBELLARD, M. E., SALZER, J. L., RODER, J. & FILBIN, M. T. 1998. Myelin-associated glycoprotein in myelin and expressed by Schwann cells inhibits axonal regeneration and branching. *Mol Cell Neurosci,* 12**,** 79-91.

SHERRINGTON, C. S. 1925. Remarks on some Aspects of Reflex Inhibition. *Philos Trans R Soc Lond B Biol Sci,* 97**,** 519-545.

SHIMIZU, E., HASHIMOTO, K. & IYO, M. 2004. Ethnic difference of the BDNF 196G/A (val66met) polymorphism frequencies: the possibility to explain ethnic mental traits. *Am J Med Genet B Neuropsychiatr Genet,* 126b**,** 122-3.

SINGH, B., XU, Q. G., FRANZ, C. K., ZHANG, R., DALTON, C., GORDON, T., VERGE, V. M., MIDHA, R. & ZOCHODNE, D. W. 2012. Accelerated axon outgrowth, guidance, and target reinnervation across nerve transection gaps following a brief electrical stimulation paradigm. *J Neurosurg,* 116**,** 498-512.

SKLAR, P., GABRIEL, S. B., MCINNIS, M. G., BENNETT, P., LIM, Y., TSAN, G., SCHAFFNER, S., KIROV, G., JONES, I., OWEN, M., CRADDOCK, N., DEPAULO, J. R. & LANDER, E. S. 2002. Family-based association study of 76 candidate genes in bipolar disorder:

BDNF is a potential risk locus. Brain-derived neutrophic factor. *Mol Psychiatry,* 7**,** 579-93.

SOHRABJI, F., MIRANDA, R. C. & TORAN-ALLERAND, C. D. 1995. Identification of a putative estrogen response element in the gene encoding brain-derived neurotrophic factor. *Proc Natl Acad Sci U S A,* 92**,** 11110-4.

SONG, X. Y., ZHANG, F. H., ZHOU, F. H., ZHONG, J. & ZHOU, X. F. 2009. Deletion of p75NTR impairs regeneration of peripheral nerves in mice. *Life Sci,* 84**,** 61-8.

SONG, X. Y., ZHOU, F. H., ZHONG, J. H., WU, L. L. & ZHOU, X. F. 2006. Knockout of p75(NTR) impairs re-myelination of injured sciatic nerve in mice. *J Neurochem,* 96**,** 833-42.

SOTELO-SILVEIRA, J., CRISPINO, M., PUPPO, A., SOTELO, J. R. & KOENIG, E. 2008. Myelinated axons contain beta-actin mRNA and ZBP-1 in periaxoplasmic ribosomal plaques and depend on cyclic AMP and F-actin integrity for in vitro translation. *J Neurochem,* 104**,** 545-57.

SOUNESS, J. E., ALDOUS, D. & SARGENT, C. 2000. Immunosuppressive and anti-inflammatory effects of cyclic AMP phosphodiesterase (PDE) type 4 inhibitors. *Immunopharmacology,* 47**,** 127-62.

STOLL, G., GRIFFIN, J. W., LI, C. Y. & TRAPP, B. D. 1989. Wallerian degeneration in the peripheral nervous system: participation of both Schwann cells and macrophages in myelin degradation. *J Neurocytol,* 18**,** 671-683.

STREPPEL, M., AZZOLIN, N., DOHM, S., GUNTINAS-LICHIUS, O., HAAS, C., GROTHE, C., WEVERS, A., NEISS, W. F. & ANGELOV, D. N. 2002. Focal application of neutralizing antibodies to soluble neurotrophic factors reduces collateral axonal branching after peripheral nerve lesion. *Eur J Neurosci,* 15**,** 1327-42.

STRUEBING, F. L., WANG, J., LI, Y., KING, R., MISTRETTA, O. C., ENGLISH, A. W. & GEISERT, E. E. 2017. Differential Expression of Sox11 and Bdnf mRNA Isoforms in the Injured and Regenerating Nervous Systems. *Front Mol Neurosci,* 10**,** 354.

SULAIMAN, O. A. & GORDON, T. 2000. Effects of short- and long-term Schwann cell denervation on peripheral nerve regeneration, myelination, and size. *Glia,* 32**,** 234-46.

SUNDERLAND, S. 1947. Rate of regeneration in human peripheral nerves: Analysis of the interval between injury and onset of recovery. *Arch Neurol,* 58**,** 251-295.

SZUHANY, K. L., BUGATTI, M. & OTTO, M. W. 2015. A meta-analytic review of the effects of exercise on brain-derived neurotrophic factor. *J Psychiatr Res,* 60**,** 56-64.

TAKEDA, K., NOGUCHI, K., SHI, W., TANAKA, T., MATSUMOTO, M., YOSHIDA, N., KISHIMOTO, T. & AKIRA, S. 1997. Targeted disruption of the mouse Stat3 gene leads to early embryonic lethality. *Proc Natl Acad Sci U S A,* 94**,** 3801-3804.

TAM, S. L., ARCHIBALD, V., JASSAR, B., TYREMAN, N. & GORDON, T. 2001. Increased neuromuscular activity reduces sprouting in partially denervated muscles. *J Neurosci,* 21**,** 654-67.

TANDRUP, T., WOOLF, C. J. & COGGESHALL, R. E. 2000. Delayed loss of small dorsal root ganglion cells after transection of the rat sciatic nerve. *J Comp Neurol,* 422**,** 172-180.

TANIUCHI, M., CLARK, H. B. & JOHNSON, E. M., JR. 1986. Induction of nerve growth factor receptor in Schwann cells after axotomy. *Proc Natl Acad Sci U S A,* 83**,** 4094-8.

TANZER, L. & JONES, K. J. 1997. Gonadal steroid regulation of hamster facial nerve regeneration: effects of dihydrotestosterone and estradiol. *Exp Neurol,* 146**,** 258-64.

TAO, X., FINKBEINER, S., ARNOLD, D. B., SHAYWITZ, A. J. & GREENBERG, M. E. 1998. Ca2+ influx regulates BDNF transcription by a CREB family transcription factor-dependent mechanism. *Neuron,* 20**,** 709-726.

TAYLOR, C. A., BRAZA, D., RICE, J. B. & DILLINGHAM, T. 2008. The incidence of peripheral nerve injury in extremity trauma. *Am J Phys Med Rehabil,* 87**,** 381-5.

TENG, H. K., TENG, K. K., LEE, R., WRIGHT, S., TEVAR, S., ALMEIDA, R. D., KERMANI, P., TORKIN, R., CHEN, Z. Y., LEE, F. S., KRAEMER, R. T., NYKJAER, A. & HEMPSTEAD, B. L. 2005. ProBDNF induces neuronal apoptosis via activation of a receptor complex of p75NTR and sortilin. *J Neurosci,* 25**,** 5455-63.

TEODORI, R. M., BETINI, J., DE OLIVEIRA, L. S., SOBRAL, L. L., TAKEDA, S. Y. M. & MONTEBELO, M. I. D. L. 2011. Swimming exercise in the acute or late phase after sciatic nerve crush accelerates nerve regeneration. *Neural Plast,* 2011**,** 8.

THOMPSON, N. J., SENGELAUB, D. R. & ENGLISH, A. W. 2013. Enhancement of peripheral nerve regeneration due to treadmill training and electrical stimulation is dependent on androgen receptor signaling. *Dev Neurobiol,* 74**,** 531-540.

TIMMUSK, T., PALM, K., METSIS, M., REINTAM, T., PAALME, V., SAARMA, M. & PERSSON, H. 1993. Multiple promoters direct tissue-specific expression of the rat BDNF gene. *Neuron,* 10**,** 475-89.

TOFARIS, G. K., PATTERSON, P. H., JESSEN, K. R. & MIRSKY, R. 2002. Denervated Schwann cells attract macrophages by secretion of leukemia inhibitory factor (LIF) and monocyte chemoattractant protein-1 in a process regulated by interleukin-6 and LIF. *J Neurosci,* 22**,** 6696-703.

TOMITA, K., KUBO, T., MATSUDA, K., FUJIWARA, T., YANO, K., WINOGRAD, J. M., TOHYAMA, M. & HOSOKAWA, K. 2007. The neurotrophin receptor p75NTR in Schwann cells is implicated in remyelination and motor recovery after peripheral nerve injury. *Glia,* 55**,** 1199-208.

TONGIORGI, E., RIGHI, M. & CATTANEO, A. 1997. Activity-dependent dendritic targeting of BDNF and TrkB mRNAs in hippocampal neurons. *J Neurosci,* 17**,** 9492-505.

TOPP, K. S. & BOYD, B. S. 2012. Peripheral nerve: from the microscopic functional unit of the axon to the biomechanically loaded macroscopic structure. *J Hand Ther,* 25**,** 142-51; quiz 152.

UDINA, E., COBIANCHI, S., ALLODI, I. & NAVARRO, X. 2011a. Effects of activity-dependent strategies on regeneration and plasticity after peripheral nerve injuries. *Ann Anat,* 193**,** 347-353.

UDINA, E., LADAK, A., FUREY, M., BRUSHART, T., TYREMAN, N. & GORDON, T. 2010. Rolipram-induced elevation of cAMP or chondroitinase ABC breakdown of inhibitory proteoglycans in the extracellular matrix promotes peripheral nerve regeneration. *Exp Neurol,* 223**,** 143-152.

UDINA, E., PUIGDEMASA, A. & NAVARRO, X. 2011b. Passive and active exercise improve regeneration and muscle reinnervation after peripheral nerve injury in the rat. *Muscle Nerve,* 43**,** 500-9.

UEGAKI, K., KUMANOGOH, H., MIZUI, T., HIROKAWA, T., ISHIKAWA, Y. & KOJIMA, M. 2017. BDNF binds its pro-peptide with high affinity and the common Val66Met polymorphism attenuates the interaction. *Int J Mol Sci,* 18**,** 1042.

VAGHI, V., POLACCHINI, A., BAJ, G., PINHEIRO, V. L. M., VICARIO, A. & TONGIORGI, E. 2014. Pharmacological profile of brain-derived neurotrophic factor (BDNF) splice variant translation using a novel drug screening assay: a “quantitative code”. *J Biol Chem,* 289**,** 27702-27713.

VAN MEETEREN, N. L., BRAKKEE, J. H., HAMERS, F. P., HELDERS, P. J. & GISPEN, W. H. 1997. Exercise training improves functional recovery and motor nerve conduction velocity after sciatic nerve crush lesion in the rat. *Arch Phys Med Rehabil,* 78**,** 70-7.

VAN MEETEREN, N. L., BRAKKEE, J. H., HELDERS, P. J. & GISPEN, W. H. 1998. The effect of exercise training on functional recovery after sciatic nerve crush in the rat. *J Peripher Nerv Syst,* 3**,** 277-82.

VARENDI, K., KUMAR, A., HÄRMA, M.-A. & ANDRESSOO, J.-O. 2014. miR-1, miR-10b, miR-155, and miR-191 are novel regulators of BDNF. *Cell Mol Life Sci,* 71**,** 4443-4456.

VERHOVSHEK, T., CAI, Y., OSBORNE, M. C. & SENGELAUB, D. R. 2010. Androgen regulates brain-derived neurotrophic factor in spinal motoneurons and their target musculature. *Endocrinology,* 151**,** 253-61.

VERMA, P., CHIERZI, S., CODD, A. M., CAMPBELL, D. S., MEYER, R. L., HOLT, C. E. & FAWCETT, J. W. 2005. Axonal protein synthesis and degradation are necessary for efficient growth cone regeneration. *J Neurosci,* 25**,** 331-42.

VICARIO, A., COLLIVA, A., RATTI, A., DAVIDOVIC, L., BAJ, G., GRICMAN, Ł., COLOMBRITA, C., PALLAVICINI, A., JONES, K. R., BARDONI, B. & TONGIORGI, E. 2015. Dendritic targeting of short and long 3′ UTR BDNF mRNA is regulated by BDNF or NT-3 and distinct sets of RNA-binding proteins. *Front Mol Neurosci,* 8**,** 62.

VIVO, M., PUIGDEMASA, A., CASALS, L., ASENSIO, E., UDINA, E. & NAVARRO, X. 2008. Immediate electrical stimulation enhances regeneration and reinnervation and modulates spinal plastic changes after sciatic nerve injury and repair. *Exp Neurol,* 211**,** 180-93.

WALLER, A. 1850. Experiments on the Section of the Glossopharyngeal and Hypoglossal Nerves of the Frog, and Observations of the Alterations Produced Thereby in the Structure of Their Primitive Fibres. *Philos Trans R Soc Lond B Biol Sci,* 140**,** 423-429.

WALSH, G. S., KROL, K. M., CRUTCHER, K. A. & KAWAJA, M. D. 1999. Enhanced neurotrophin-induced axon growth in myelinated portions of the CNS in mice lacking the p75 neurotrophin receptor. *J Neurosci,* 19**,** 4155-68.

WAN, L., XIA, R. & DING, W. 2010. Short-term low-frequency electrical stimulation enhanced remyelination of injured peripheral nerves by inducing the promyelination effect of brain-derived neurotrophic factor on Schwann cell polarization. *J Neurosci Res,* 88**,** 2578-87.

WANG, H., RIVERO-MELIAN, C., ROBERTSON, B. & GRANT, G. 1994. Transganglionic transport and binding of the isolectin B4 from Griffonia simplicifolia I in rat primary sensory neurons. *Neuroscience,* 62**,** 539-51.

WANG, K. C., KIM, J. A., SIVASANKARAN, R., SEGAL, R. & HE, Z. 2002a. P75 interacts with the Nogo receptor as a co-receptor for Nogo, MAG and OMgp. *Nature,* 420**,** 74-8.

WANG, L., COPRAY, S., BROUWER, N., MEEK, M. F. & KERNELL, D. 2002b. Regional distribution of slow-twitch muscle fibers after reinnervation in adult rat hindlimb muscles. *Muscle Nerve,* 25**,** 805-15.

WARD, P. J., CLANTON, S. L. & ENGLISH, A. W. 2018. Optogenetically enhanced axon regeneration: motor versus sensory neuron-specific stimulation. *Eur J Neurosci,* 47**,** 294-304.

WARD, P. J., JONES, L. N., MULLIGAN, A., GOOLSBY, W., WILHELM, J. C. & ENGLISH, A. W. 2016. Optically-induced neuronal activity is sufficient to promote functional motor axon regeneration in vivo. *PLoS ONE,* 11**,** e0154243.

WATSON, W. E. 1968. Observations on the nucleolar and total cell body nucleic acid of injured nerve cells. *J Physiol,* 196**,** 655-676.2.

WEBBER, C. & ZOCHODNE, D. 2010. The nerve regenerative microenvironment: Early behavior and partnership of axons and Schwann cells. *Experimental Neurology,* 223**,** 51-59.

WELIN, D., NOVIKOVA, L. N., WIBERG, M., KELLERTH, J. O. & NOVIKOV, L. N. 2008. Survival and regeneration of cutaneous and muscular afferent neurons after peripheral nerve injury in adult rats. *Exp Brain Res,* 186**,** 315-23.

WEST, A. E., CHEN, W. G., DALVA, M. B., DOLMETSCH, R. E., KORNHAUSER, J. M., SHAYWITZ, A. J., TAKASU, M. A., TAO, X. & GREENBERG, M. E. 2001. Calcium regulation of neuronal gene expression. *Proc Natl Acad Sci U S A,* 98**,** 11024-31.

WHITE, D. M., WALKER, S., BRENNEMAN, D. E. & GOZES, I. 2000. CREB contributes to the increased neurite outgrowth of sensory neurons induced by vasoactive intestinal polypeptide and activity-dependent neurotrophic factor. *Brain Res,* 868**,** 31-8.

WIBERG, R., NOVIKOVA, L. N. & KINGHAM, P. J. 2018. Evaluation of apoptotic pathways in dorsal root ganglion neurons following peripheral nerve injury. *Neuroreport,* 29**,** 779-785.

WILHELM, J., XU, M., CUCORANU, D., CHMIELEWSKI, S., HOLMES, T., LAU, K., BASSELL, G. & ENGLISH, A. 2012. Cooperative roles of BDNF expression in neurons and Schwann cells are modulated by exercise to facilitate nerve regeneration. *J Neurosci,* 32**,** 5002-5009.

WILLIS, D. E., XU, M., DONNELLY, C. J., TEP, C., KENDALL, M., ERENSTHEYN, M., ENGLISH, A., SCHANEN, N. C., KIRN-SAFRAN, C. B., YOON, S. O., BASSELL, G. J. & TWISS, J. L. 2011. Axonal localization of transgene mRNA in mature PNS and CNS neurons. *J Neurosci,* 31**,** 14481-14487.

WITZEL, C., ROHDE, C. & BRUSHART, T. M. 2005. Pathway sampling by regenerating peripheral axons. *J Comp Neurol,* 485**,** 183-90.

WONG, J. N., OLSON, J. L., MORHART, M. J. & CHAN, K. M. 2015. Electrical stimulation enhances sensory recovery: A randomized controlled trial. *Ann Neurol,* 77**,** 996-1006.

WOOD, K., WILHELM, J., SABATIER, M., LIU, K., GU, J. & ENGLISH, A. 2012. Sex differences in the effectiveness of treadmill training in enhancing axon regeneration in injured peripheral nerves. *Dev Neurobiol,* 72**,** 688-698.

WYATT, S., SHOOTER, E. M. & DAVIES, A. M. 1990. Expression of the NGF receptor gene in sensory neurons and their cutaneous targets prior to and during innervation. *Neuron,* 4**,** 421-7.

YAMASHITA, T., TUCKER, K. L. & BARDE, Y. A. 1999. Neurotrophin binding to the p75 receptor modulates Rho activity and axonal outgrowth. *Neuron,* 24**,** 585-93.

YAN, Q. & JOHNSON, E. 1988. An immunohistochemical study of the nerve growth factor receptor in developing rats. *J Neurosci,* 8**,** 3481-3498.

YANG, F., JE, H. S., JI, Y., NAGAPPAN, G., HEMPSTEAD, B. & LU, B. 2009. Pro-BDNF-induced synaptic depression and retraction at developing neuromuscular synapses. *J Cell Biol,* 185**,** 727-41.

YAO, J., SASAKI, Y., WEN, Z., BASSELL, G. J. & ZHENG, J. Q. 2006. An essential role for β-actin mRNA localization and translation in Ca2+-dependent growth cone guidance. *Nat Neurosci,* 9**,** 1265.

ZHANG, F. H., HUANG, P., YANG, P. S. & ZHANG, X. 2010. [Influence of p75 neurotrophin receptor knockout on the regeneration of facial nerves after crush injury in mouse]. *Hua Xi Kou Qiang Yi Xue Za Zhi,* 28**,** 95-8.

ZHANG, J. Y., LUO, X. G., XIAN CORY, J., LIU, Z. H. & ZHOU, X. F. 2008. Endogenous BDNF is required for myelination and regeneration of injured sciatic nerve in rodents. *Eur J Neurosci,* 12**,** 4171-4180.

ZHOU, X. F., LI, W. P., ZHOU, F. H., ZHONG, J. H., MI, J. X., WU, L. L. & XIAN, C. J. 2005. Differential effects of endogenous brain-derived neurotrophic factor on the survival of axotomized sensory neurons in dorsal root ganglia: a possible role for the p75 neurotrophin receptor. *Neuroscience,* 132**,** 591-603.

ZHOU, X. F., RUSH, R. A. & MCLACHLAN, E. M. 1996. Differential expression of the p75 nerve growth factor receptor in glia and neurons of the rat dorsal root ganglia after peripheral nerve transection. *J Neurosci,* 16**,** 2901-11.

ZIGMOND, R., MOHNEY, R., SCHREIBER, R., SHADIACK, A., SUN, Y., VACCARONIELLO, Y. S. & ZHOU, Y. 1997. Changes in gene expression in adult sympathetic neurons after axonal injury. *In:* GOLDSTEIN, D. S., EISENHOFER, G. & MCCARTY, R. (eds.) *Adv Pharmacol.* Academic Press.

ZUO, J., HERNANDEZ, Y. J. & MUIR, D. 1998. Chondroitin sulfate proteoglycan with neurite-inhibiting activity is up-regulated following peripheral nerve injury. *J Neurobiol,* 34**,** 41-54.

ZUO, J., NEUBAUER, D., GRAHAM, J., KREKOSKI, C. A., FERGUSON, T. A. & MUIR, D. 2002. Regeneration of axons after nerve transection repair is enhanced by degradation of chondroitin sulfate proteoglycan. *Exp Neurol,* 176**,** 221-8.

Chapter 2:

The Val66Met BDNF polymorphism and peripheral nerve injury: Enhanced regeneration in Met-carriers that is not further improved with activity-dependent treatment.

Currently under review at Journal of Neuroscience: McGregor CE, Irwin AM, English AW (2018) The Val66Met BDNF polymorphism and peripheral nerve injury: Enhanced regeneration in Met-carriers that is not further improved with activity-dependent treatment. J Neurosci: under review

Posters presented from this work:

McGregor C, English AW (2017) "Neurite elongation is enhanced in cells heterozygous for the Val66Met polymorphism." Society for Neuroscience 2017, Washington D.C.

McGregor C, English AW (2016) The effect of electrical stimulation on muscle reinnervation and axon elongation in a mouse model of Val66Met. Society for Neuroscience, 2016, San Diego.

McGregor C, English AW (2016) The effect of electrical stimulation on muscle reinnervation and axon elongation in a mouse model of Val66Met. Symposium on Regenerative Rehabilitation, 2016, Atlanta.

McGregor C, English AW (2016) The effect of electrical stimulation on muscle reinnervation in a mouse model of Val66Met. Midwest Motoneuron Consortium, 2016, Indianapolis.

Invited talks from this work:

"The BDNF Val66Met polymorphism and peripheral nerve regeneration: a surprising benefit of a maligned SNP." September, 2018. Midwest Motoneuron Consortium, Indianapolis, Indiana.

"Less is more: enhanced axon elongation and the BDNF polymorphism Val66Met." December, 2017. Seminars in Integrative Neuroscience, Atlanta, Georgia.

"The Val66Met SNP and activity-dependent treatments to enhance nerve regeneration." March, 2017. Emory Neuroscience Candidates' Ongoing Research, Atlanta, Georgia.

Abstract

Activity-dependent treatments to enhance peripheral nerve regeneration after injury have shown great promise, and clinical trials implementing them have begun. Success of these treatments requires activity-dependent release of brain derived neurotrophic factor (BDNF). A single nucleotide polymorphism (SNP) in the *BDNF* gene known as Val66Met, which is found in nearly one third of the human population, results in defective activity-dependent BDNF secretion and could impact the effectiveness of these therapies. Here, we used a mouse model of this SNP to test the efficacy of treadmill exercise in enhancing axon regeneration in animals both heterozygous (V/M) and homozygous (M/M) for the SNP. Axon regeneration was studied four weeks after complete transection and repair of the sciatic nerve, using both electrophysiological and histological outcome measures. Regeneration was enhanced significantly without treatment in V/M mice, compared to wild type (V/V) controls. Unlike V/V mice, treatment of both V/M and M/M mice with treadmill exercise did not result in enhanced regeneration. These results were recapitulated *in vitro* using dissociated neurons containing the light-sensitive cation channel, channelrhodopsin. Three days after plating, neurites of neurons from V/M and M/M mice were longer than those of V/V neurons. In neurons from V/V mice, but not those from V/M or M/M animals, longer neurites were found after optical stimulation. Taken together, Met carriers possess an intrinsically greater capacity to regenerate axons in peripheral nerves, but this cannot be enhanced further by activity-dependent treatments.

Introduction

Every year in the US, there are more than 200,000 new cases of peripheral nerve injury (PNI). Despite the common belief that axons in peripheral nerves will spontaneously regenerate, recovery is slow, and over 90% of adults who sustain a PNI never regain full motor function (Portincasa et al., 2007, Scholz et al., 2009). Currently, there are no commonly-used non-surgical treatments for PNI (Isaacs, 2010).

Experimental treatments, such as exercise, electrical stimulation (ES), and optical stimulation, have repeatedly been shown to be effective in promoting elongation of regenerating axons, increasing recruitment of motor and sensory neurons into the regeneration process, and accelerating restoration of muscle responses to nerve stimulation (Al Majed et al., 2000b, English et al., 2007, English et al., 2009, Haastert-Talini et al., 2011, English et al., 2011b, Boeltz et al., 2013, Park et al., 2015, Ward et al., 2016, Ward et al., 2018). The success of these treatments requires increased activity of the participating neurons (Jaiswal et al., 2017), and thus they are collectively considered activity-dependent treatments (Udina et al., 2011).

Neuronal brain derived neurotrophic factor (BDNF) secretion and signaling is required for the enhancing effects of activity-based treatments (Al Majed et al., 2000a, Gordon, 2010, Wilhelm et al., 2012). Enhancement of axon regeneration is abolished in animals in which either BDNF or its receptor, tropomyosin receptor kinase B (trkB), have been selectively knocked out of neurons, indicating that neuronal BDNF as well as trkB activation are required for the enhancing effects of ES and exercise (Al Majed et al., 2000a, Gordon, 2010, Wilhelm et al., 2012, English et al., 2013). This BDNF-dependence could impose a barrier to translation of activity-dependent treatments. About 30% of Americans have a single nucleotide polymorphism (SNP) in the *BDNF* gene in which a methionine is substituted for a valine at the 66th residue (Val66Met, see Figure 1A) (Egan et al., 2003, Shimizu et al., 2004). BDNF is secreted through both a constitutive pathway and a regulated pathway (Lessmann et al., 2003). Cells expressing the Met allele have deficits in regulated, calcium-dependent release of BDNF, but not

constitutive release (Egan et al., 2003, Chen et al., 2004). This observation is mirrored by the finding that in Met-carriers, exercise-dependent increases in plasma BDNF are absent (Nascimento et al., 2015, Ieraci et al., 2016). This deficit in activity-dependent release of BDNF could inhibit the effectiveness of activity-dependent treatments for enhancing peripheral nerve regeneration.

Therefore, we tested the efficacy of two activity-based experimental therapies, treadmill training and optical stimulation, in enhancing nerve regeneration after injury in mice heterozygous and homozygous for the Val66Met polymorphism.

Methods

Animals and Surgical Treatments. All experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committee of Emory University and conformed to the Guidelines for the Use of Animals in Research of the Society for Neuroscience. Transgenic C57BL/6J mice heterozygous (V/M) and homozygous (M/M) for the met allele of the Val66Met polymorphism and their wild type littermates (V/V) were bred and maintained at the Division of Animal Resources at Emory University. Founders for these mice were a generous gift from Doctor Frances Lee (Chen et al., 2006). Mice were group housed with *ad libitum* access to food and water. Both males and females were used in control experiments. Only females were used for exercise experiments.

Mice were anesthetized with 2% isoflurane and treated with Meloxicam analgesic (2mg/kg). The right sciatic nerve was exposed in the mid-thigh, placed on a small rectangle of SILASTIC film (Dow Corning 501-1) and secured with fibrin glue: a mixture of fibrinogen and thrombin (1:2, Sigma-Aldrich, St Louis, MO) (de Vries et al., 2002, MacGillivray, 2003). The nerve was fully transected using sharp scissors three millimeters proximal to the sciatic branching. The aligned stumps on the mat were then secured with more fibrin glue. The left side of each mouse served as an unoperated control. After nerve repair, the surgical site was sutured closed, and the animal was allowed to recover from anesthesia before returning to its cage.

Treadmill Training. Female mice were exercised using an interval training protocol previously shown to be effective in enhancing axon regeneration (Wood et al., 2012). Three days after transection surgery, mice were exposed to interval training, in which the animal performed four bouts of two minutes of high intensity running (20 m/min), each separated by 5 minutes of rest. This training protocol was repeated five days per week for two weeks during their light cycle, a

regimen that has previously been shown to be effective in enhancing axon regeneration (Sabatier et al., 2008).

Motor Unit Number Estimation (MUNE). Four weeks post-injury, the extent of muscle reinnervation was investigated using motor unit number estimation (MUNE). MUNE allows for the estimation of the number of functional motor units, in this case the number of motoneurons reinnervating the lateral gastrocnemius (McComas et al., 1971, Willand et al., 2011, Gooch et al., 2014). Briefly, a stimulating cuff electrode was placed around the sciatic nerve proximal to the injury site, and three monopolar fine wire electromyography EMG electrodes (California Fine Wire Company, Grover Beach, CA) were implanted together into the belly of the lateral gastrocnemius muscle using a 25G hypodermic needle. The tips of the wires were hooked such that they were each 1mm apart from each other. The wires were attached to three separate recording amplifiers, as shown in Figure 1B, to enable detection of distinct signals within the muscle. Electrical stimulation of the nerve was applied to evoke three compound muscle action potentials (CMAPs). CMAPs were elicited with 0.1ms pulses with two second intervals and recorded using custom LabVIEW software (National Instruments, Austin, TX). We have adapted the multiple point stimulation technique used in humans, in which small electrical currents were passed through the stimulating cuff and increased in amplitude until an all-or-nothing EMG response was noted (Stein and Yang, 1990, Doherty and Brown, 1993). This lowest-threshold response on each channel was assumed to be produced by activation of a single motor unit (SMU). To be counted as an SMU, the response needed to be biphasic or triphasic and to occur more than once while stimulating voltage was held steady at just the threshold needed for activation. The average rectified voltage within an empirically determined time window for each SMU potential was calculated in LabVIEW software (Sabatier et al., 2011). The stimulating cuff was then moved to stimulate different points along the nerve until at least nine SMU potentials could be recorded. The stimulus intensity was then increased until a maximal CMAP was

evoked. The amplitude of this potential was measured as above. It was assumed that this maximum CMAP was produced by the near synchronous activation of all of the motoneurons innervating the muscle. The average maximal CMAP amplitude for each animal was divided by the average of the SMU amplitudes to estimate the number of functional motor units in the reinnervated muscle (Major et al., 2007).

Retrograde-Labeling of Motoneurons and Muscle Afferent Neurons. Immediately following electrophysiological recordings, the lateral gastrocnemius muscles of both the intact and injured sides were exposed and injected with 1µl of a 1% solution of Cholera toxin B (CTB) conjugated to a fluorescent label (Alexafluor 555; Life Technologies, Grand Island, NY, catalog number C-34776). Injections were performed using a Hamilton syringe (Hamilton, Reno, NV), and CTB was injected in 30 second intervals in two locations in the belly of the lateral gastrocnemius. This approach was used to mark sensory and motoneurons that had successfully regenerated into the muscle. After allowing three days for sufficient transport of the tracer, mice were euthanized. Animals were deeply anesthetized with pentobarbital (150mg.kg, i.p.) and perfused transcardially with cold 0.9% saline followed by 4% periodate-lysate-paraformaldehyde fixative (McLean and Nakane, 1974). Lumbar 3-5 dorsal root ganglia (DRGs) and spinal cord segments were harvested, cryoprotected in 20% sucrose, sectioned (20µm for spinal cord sections, 14µm for DRG sections), and mounted onto slides in serial sections. Using a Leica DM6000 upright microscope and low light camera, 20x images were obtained with HCImage software (Hamamatsu, Sewickley, PA). Motoneurons labeled with CTB were counted if their somata contained dense granular fluorescence extending into the primary dendrites and contained a dark region, indicating a nucleus (English, 2005). Counts of labeled neurons on the intact side of each mouse served as an uninjured control. For DRG neurons, the visibly fluorescent-labeled cells were traced using ImageJ software, and the mean gray value and area were recorded. Twenty cells that were visibly not fluorescently labeled were also recorded in order to determine

the background intensity. The fluorescently labeled cells were then counted if the mean gray value was greater than the mean + 2.5 standard deviations of the background. Only cells of the L4 segment were counted.

Motor Endplate Reinnveration. The medial and lateral gastrocnemius muscles from both the intact and injured side of mice were harvested concurrently with the spinal cord and DRGs. The muscles were cryoprotected in 20% sucrose, sectioned longitudinally (20 µm), and mounted onto slides. Sections underwent antigen retrieval in boiling 10 mM Sodium Citrate buffer (pH 8.5). Immediately following, sections were washed with 0.1M PBS, then blocked with 0.3% triton in 0.1M PBS and 10% natural goat serum for 1 hour at room temperature and incubated at 4°C overnight (14-16 hours) with antibody against the vesicular acetylcholine transporter (VAChT, 1:500, Synaptic Systems, Göttingen, Germany) to label motoneuron synaptic terminals. The following morning, sections were washed in 0.1M PBS and incubated for two hours at room temperature with secondary antibody, goat anti-guinea pig conjugated to Alexafluor 647 (1:200 Thermo Fisher Scientific, Waltham, MA) and α-bungarotoxin conjugated to Alexafluor 555 (αBT, 1:500, Sigma-Aldrich, Darmstadt, Germany) to label motor endplates. Glass coverslips were mounted using Entellan (Millipore, Darmstadt, Germany). For each muscle, 50 motor endplates were imaged, as above, and scored as reinnervated only if VAChT immunofluorescence completely filled the endplate (see Figure 4A).

Dorsal Root Ganglion Cell Culture. Previously described V/M mice were bred with transgenic mice expressing the light–sensitive cation channel, channelrhodopsin, under the control of the Thy1 promoter (Thy1ChR2, https://www.jax.org/strain/007612). To prepare cultures, adult mice of both sexes were euthanized with an overdose of isoflurane. The entire vertebral column was removed, and all of the DRGs were immediately dissected out bilaterally and placed in ice cold Hank's Balanced Salt Solution (HBSS, Corning, Corning, New York). After incubation in dispase

(2.5u/mL Sigma-Aldrich) and collagenase (200u/mL Worthington Biochemical, Lakewood, NJ) in a 37°C bead bath for 45 minutes with gentle agitation applied every 15 minutes, DRGs were then treated in 37°C DNase (Worthington Biochemical) for 2.5 minutes before addition of room temperature HBSS. Cells were triturated using a fire polished glass pipette and centrifuged for 3 minutes at 3000 rpm. The HBSS was removed and cells were resuspended in Neurobasal medium A (NB-A, Invitrogen, Carlsbad, CA) containing 2% B-27 (Invitrogen), 1% Penicillin/Streptomycin (Lonza Biowhittaker), and 1% Glutamax (Invitrogen). Cells were seeded at a density of 1000 cells per well in 48-well Opticlear plates (Axion Biosystems, Atlanta, GA) coated in laminin (Thermo Fisher Scientific, Waltham, MA) and poly-L-lysine (Sigma-Aldrich). Cells were incubated at 37°C with 5% CO_2.

Twenty-four hours after plating, the NB-A solution was replaced. Plates were removed from the incubator and placed on an Axion Biosystems plate holder on top of a 37° warming plate with 5% CO_2 piped in. Cells were then stimulated using the Lumos System (Axion Biosystems) under control of their AxIS software (Axion Biosystems). Cells were exposed to 475 nm light at an intensity of 0.59 mW/mm^2 and at a rate of 20hz for one hour for a total of 72K pulses (Park et al., 2015). Control stimulation was the same regimen, but with light of an inappropriate wavelength (655 nm). Unstimulated cells received no light input. Media was collected immediately after stimulation and stored at -80°C for analysis of BDNF protein levels. This time point was chosen based on a previous study reporting a significant increase in BDNF protein in the media from DRG explants from Thy1ChR2 animals (Park et al., 2015). Analysis was performed with help from the Emory Multiplexed Immunoassay Core. Undiluted samples were analyzed in duplicate using the BDNF U Plex plates (Meso Scale Discovery, Rockville, MD).

Seventy-two hours after plating, cells were fixed with 4°C periodate-lysate-paraformaldehyde fixative. Fixed cells were incubated with anti-tubulin β-3 antibody overnight (14-16 hours, Biolegend, San Diego, CA), followed by application of a secondary, goat anti-

mouse antibody conjugated to Alexafluor 555 (Invitrogen) and DAPI (Invitrogen). Cells were imaged with a 20x objective on an epifluorescence microscope (Ti Eclipse; Nikon) equipped with a cooled charge-coupled device (CCD) camera (HQ2; Photometrics) using Nis Elements Imaging Software (Nikon). The cell soma area and longest processes from each cell were measured from these images using the Fiji software package (ImageJ).

Experimental Design and Statistics. All results were scored while blinded to treatment and genotype. Data analyses were performed in Statistica 64, except for 95% confidence intervals (CI) and Mann-Whitney U tests, which were performed in Microsoft Excel. Power analyses were performed *a priori* (α=0.05, Power=0.8) using data from previous studies to select adequate sample sizes for all outcome measures except for MUNE, which had not previously been performed in mice. For MUNE, power analysis was performed using preliminary data. To test the assumption of homoscedasticity required for ANOVA, Levene's test was used. Unless otherwise specified, one-way ANOVA was performed with Fisher's LSD post-hoc test. For samples with unequal variance, Kruskal-Wallis ANOVA was performed.

Sample Sizes and Statistical Analysis by Experiment

SMU Distributions: V/V Intact: 124 SMUs from 16 animals (6 males, 10 females); V/M Intact: 205 SMUs from 24 animals (9 males, 15 females); M/M Intact 158 SMUs from 17 animals (5 males, 12 females). Data analyzed with Mann-Whitney U test. Figure 2A.

MUNE and CMAP: Untreated (UT): V/V n= 13 (7 males, 6 females); V/M n=14 (8 males, 6 females); M/M n=11 (6 males, 5 females). Data analyzed by Kruskal-Wallis, multiple comparisons post hoc test. Figure 2B. *Fold change with Treadmill Training (TT)*: n=6 females for all genotypes. Data analyzed by Kruskal-Wallis ANOVA, multiple comparisons post-hoc test. Figure 2C.

Motoneuron Labeling Untreated: V/V n=5 (3 males, 2 females); UT V/M n=5 males; UT M/M n=5 (3 males, 2 females). Analyzed by one-way ANOVA, Fisher's LSD post-hoc test. Figure 3B. *Fold change with TT:* n=5 females for all genotypes. Data analyzed by one-way ANOVA, Fisher's LSD post-hoc test. Figure 3C.

Muscle Fiber Reinnervation: UT V/V n=7 (4 males, 3 females); UT V/M n=6 (4 males, 2 females); UT M/M n=5 (3 males, 2 females). Data analyzed by one-way ANOVA, Fisher's LSD post hoc-test Figure 4B. Fold change with TT: n=6 females for all genotypes. Data analyzed by Kruskal-Wallis ANOVA, multiple comparison post hoc test.. Figure 4C.

DRG Labeling: UT V/V n=5 (4 females, 1 male); TT V/V n=6 females; UT V/M n=6 (5 males, 1 female); TT V/M n=5 females; UT M/M n=5 (3 males, 2 females); TT M/M n=6 females. Cell counts analyzed by two-way ANOVA, Fisher's LSD post-hoc test. Figure 5C. Average median cell area after injury analyzed by two-way ANOVA, genotype by treatment. Figure 5D. *Intact Cell Size Analysis:* V/V Intact: n=5 (1 male, 4 females); V/M Intact n=5 (2 males, 3 females); M/M Intact n=6 (1 male, 5 females). Distribution of cell size in intact animals analyzed by Mann-Whitney U test. Average median cell area analyzed by one-way ANOVA. Figure 5A.

In vitro Neurite Outgrowth and Protein Analysis: For each animal/treatment, at least 30 cells were measured and averaged. Thy1ChR2 V/V n=4 (3 females, 1 male); Thy1ChR2 V/M n=5 (2 females, 3 males); Thy1ChR2 M/M n=3 males; WT n=4 (3 males, 1 female). Data were analyzed with one-way ANOVA (Figure 6B, C, F, G) or two-way ANOVA (genotype by treatment, Figure 6E), followed by Fisher's LSD where appropriate. For protein analysis, one Thy1ChR2 V/M animal was excluded as an outlier as identified in the Grubb's outlier test.

Results

Functional recovery is enhanced in untreated V/M and M/M mice.

Single motor unit potentials were recorded from intact mice four weeks after injury without treatment, and four weeks after injury in animals treated with TT. Animals in three genotypes were studied: V/V; V/M; and M/M. The distributions of the amplitudes of these potentials in intact mice, and in mice four weeks after sciatic nerve transection and repair, with or without treatment with treadmill training, are shown for the three genotypes in Figure 2A. As previously reported in both humans and rodents (Gordon et al., 2010, Gordon et al., 2011, Gordon and Tetzlaff, 2015), in untreated V/V mice, the sizes of SMU potentials four weeks after injury were greater than those found in intact animals of the same genotype (Mann-Whitney test, U= 76, p=0.009, Figure 2A, top panel). No significant difference in SMU amplitude, relative to intact mice was found in animals of this genotype treated with TT. In V/M or M/M mice no significant differences in the amplitudes of SMU potentials were found regardless of treatment (Figure 2A, bottom two panels).

To estimate the number of functional motor units, the average maximal CMAP amplitude was divided by the average SMU amplitude in each muscle studied prior to and four weeks after sciatic nerve transection and repair (Major et al., 2007). In intact animals, MUNE did not differ between the three genotypes (V/V=266.4±30.3, V/M=288.3±33.7, M/M=287.5±34.5; one-way ANOVA, $F(2,64)=0.15$, $p=0.86$). Previous studies have not reported the effect of the met allele in females as well as males. Therefore, we tested the effect of sex and genotype on MUNE four weeks after transection and repair (n=5-8). There was a significant effect of genotype ($F(2,32)=6.0$, $p=0.006$) but not sex, so data from the UT males and females were pooled based on genotype. Once pooled, the Levene's test for homogeneity of variance was significant ($p=0.0089$), indicating non-homoscedastic variance, so non-parametric Kruskal-Wallis ANOVA was used to analyze MUNE in untreated animals. A significant effect of genotype on MUNE ($H(2)=11.56$, $p=0.0031$) was found, with a mean rank of 11.00 for V/V, 23.86 for V/M, and 24.00

for M/M. Both V/M and M/M had a significantly higher mean rank than V/V (p=0.0080 and p=0.013 respectively, Figure 2B). A similar effect of genotype was found for maximal CMAP amplitude (H(2,34)=10.53, p=0.0052; mean rank of 11.15 for V/V, 23.39 for V/M, and 23.09 for M/M) (Figure 2C). Based on post-hoc testing, the maximal CMAP in V/V animals (0.10±0.014) was significantly smaller than V/M animals (0.30±0.070, p=0.012) and M/M animals (0.23±0.042, p=0.021).

The effect of treadmill training on motor unit reinnervation differed across genotypes. In treadmill trained V/V animals, a nearly four-fold increase in MUNE was found relative to untreated controls (95% CI [2.72, 5.11] Figure 2C). In contrast, the fold change in treadmill trained V/M animals was not different from untreated (1.31-fold change, 95% CI [0.59, 2.04]). Treadmill training may actually result in decreased functional recovery in M/M animals (95% CI [0.32, 0.90]). A significant difference in response to treadmill training was found between genotypes (Kruskal-Wallis ANOVA, H(2)=11.09, p=0.0039), with a mean rank of 15.17 for V/V, 8.17 for V/M, and 5.17 for M/M. Ranks for V/V and M/M were significantly different from each other (p=0.0035).

Motor axon regeneration is enhanced in untreated but not treadmill trained mice heterozygous for $BDNF_{met}$

To assay the extent of successful motor axon regeneration, we injected a retrograde tracer bilaterally into the lateral gastrocnemius muscles and counted the number of labeled motoneurons (Figure 3A). The ratio of counts on the injured side to the intact side of each mouse was used to evaluate the proportion of the entire pool of motoneurons reinnervating the muscle. Four weeks after peripheral nerve injury, fewer than half of injured motoneurons had regenerated into the lateral gastrocnemius muscle in untreated V/V mice (0.45± 0.077, Figure 3B), as has been shown previously (Ward et al, 2018). Regeneration in untreated V/M mice was almost twice that of V/V (0.81±0.068). Motoneuron regeneration in M/M mice was similar to V/V

mice at 0.50±0.16. The omnibus result of a one-way ANOVA comparing the three genotypes was significant ($F(2,12)=6.31$, $p=0.01$). Post-hoc paired testing revealed that the injured/intact ratio in untreated V/M mice was significantly larger than that in either untreated V/V mice or M/M mice ($p=0.009$ and $p=0.01$ respectively, Figure 3B). There was no significant difference between untreated V/V and M/M mice (p=ns).

To analyze the effect of genotype on response to treadmill training, the fold change (TT/UT) in number of retrogradely labeled motoneurons was calculated. Treadmill training increased the number of labeled motoneurons in V/V mice to 1.72-fold (95% CI [1.31, 2.13]). In contrast, treadmill training may have been detrimental to motoneuron regeneration in V/M mice (0.62-fold change, 95% CI [0.47, 0.76]). Treadmill training had no effect on motoneuron regeneration in M/M mice (0.93-fold change, 95% CI [0.20, 1.66]). The omnibus result of a one-way ANOVA comparing the three genotypes was significant ($F(2,13)=7.7$, $p= 0.006$, Figure 3C). Using post-hoc paired testing, significant differences in the fold-change of the proportion of labeled motoneurons were found between V/V and V/M mice ($p=0.002$) and V/V and M/M mice ($p=0.01$). No difference was found between treadmill trained V/M and M/M mice (p=ns).

Muscle fiber reinnervation is enhanced in untreated but not treadmill trained mice heterozygous for $BDNF_{met}$

Muscle fiber reinnervation was analyzed by determining the proportion of alpha bungarotoxin-positive motor endplates where VAChT, a marker of cholinergic motoneuron terminals, was expressed. Only motor endplates with complete coverage by VAChT immunofluorescent structures were counted (Figure 4A). With no treatment, 18.6±8.1% of motor endplates in V/V animals were occupied by motoneuron terminals four weeks after sciatic nerve transection and repair (Figure 4B). In untreated V/M mice, 46.3±9.84% of motor endplates were occupied by motoneuron terminals, and in M/M mice, 22±2.37% of motor endplates were occupied. The results of a one way ANOVA of genotype were significant ($F(2,15)=4.11$,

p=0.038). Post hoc testing revealed that in muscles collected from untreated V/M mice, a significantly greater percentage of motor endplates were covered by VAChT positive motor nerve terminals than in untreated V/V mice (p=0.016) and M/M mice (p=0.046) (Fig. 4B). In V/V animals, treadmill training resulted in a 2.6-fold change in percent of VAChT positive motor endplates (95% CI [1.71, 3.57], Fig. 4C). In contrast, treadmill training had no effect on percentage of VAChT positive motor endplates in V/M mice (95% CI [-0.40, 1.45]) or M/M mice (95% CI [0.53, 2.39]). A Levene's test of variance was significant (p=0.0077). There was a significant difference in response to treadmill training between genotypes (H(2)=7.04, p=0.030) with a mean rank of 13.50 for V/V, 5.33 for V/M, and 9.67 for M/M. Post hoc testing revealed a significant difference between V/V and V/M mice (p=0.024). The difference between V/M and M/M mice was not significant (p=ns).

Treadmill training does not enhance DRG regeneration

To count the number of sensory neurons that successfully reinnervated the lateral gastrocnemius, a retrograde tracer was injected into the muscle four weeks after injury, and the number of labeled L4 DRG neurons was counted (Figure 5). Two way factorial ANOVA of genotype and treatments revealed a significant effect of genotype on the number of labeled DRG neurons (F(2,27)=6.53, p=0.0049, Figure 5C). Treadmill training did not increase the number of DRG neurons innervating the lateral gastrocnemius in any genotype (p=ns), and in fact may have decreased the number of DRG neurons innervating the muscle in V/V mice, though the results were not significant (p=0.18). Unlike the previous measures, there was no significant difference between untreated V/V and V/M mice (p=0.11), though *post hoc* testing revealed a significant difference between untreated V/M and M/M mice (p=0.013).

DRG neurons are often categorized by size: nociceptive cells are generally small (areas between 100 and 500μm^2), and proprioceptive and low threshold mechanoreceptive cells are generally larger (areas between 500 and 1500μm^2) (Lawson, 1979, Lawson and Waddell,

1991). In intact animals, the distribution of cell areas of DRG neurons in M/M animals was shifted significantly to the left of that of V/V animals (Figure 5A, Mann-Whitney U test, U=179, p=0.026), meaning that relatively more small DRG neurons and fewer large DRG neurons were found in the M/M mice. A leftward shift of the size distribution of V/M DRG neurons was not significantly from that of either V/V (U=216, p=0.14) or V/M (U=222, p=0.18) mice. The omnibus result of a one-way ANOVA of the average median cell sizes of the three genotypes was significant (Figure 5A inset, $F(2,12)=5.77$, $p=0.018$). The median cell area for V/V DRG neurons was significantly larger than that found in M/M mice ($p=0.0055$) but not V/M mice ($p=0.12$). Median cell areas of V/M and M/M mice were not significantly different ($p=0.10$). After nerve transection, regardless of treatment, no differences in the sizes of sensory neurons whose axons had regenerated successfully were noted (Figure 5D). Median cell area did not differ significantly across genotypes (two-way ANOVA, no effect of genotype (Figure 5D, $F(2,27)=0.53$, $p=0.60$); treatment ($F(1,27)=0.27$, $p=0.61$); or interaction of genotype by treatment ($F(2,27)=0.36$, $p=0.70$).

Neurite outgrowth is enhanced in V/M and M/M neurons

Adult DRG neurons derived from mice expressing the light-sensitive cation channel, channelrhodopsin, were cultured for 72 hours. Wild type (WT) neurons were also cultured as light-insensitive controls. To evaluate the effects of activity and genotype on neurite outgrowth, the longest neurite length for each neuron studied was measured. Figure 6A is a representative image of Thy1ChR2 V/V DRG neurons. Seventy-two hours after plating, the average longest neurite length for untreated Thy1ChR2 V/V neurons was 235.22±4.6µm (Figure 6B). Thy1ChR2 V/M and Thy1ChR2 M/M neurons grew substantially longer neurites, with averages of 325.10±8.8µm and 357.22±22.1µm respectively. The omnibus result of one-way ANOVA was significant for an effect of genotype ($F(3,12)=15.84$, $p=0.00018$). Average longest neurite length from Thy1ChR2 V/V neurons was significantly shorter than average neurite length from

Thy1ChR2 V/M neurons (p=0.013) and Thy1ChR2 M/M neurons (p=0.00016), but not WT neurons (p=0.95). Thy1ChR2 V/M and Thy1ChR2 M/M neurons did not differ in longest neurite length (p=0.91).

Optical stimulation enhances neurite elongation in V/V neurons *in vitro*

To measure neurite outgrowth in response to activity-dependent treatment, cells were exposed to one hour of 20 Hz light stimulation at 24 hours in culture, and were fixed and stained 48 hours later. Longest neurite length was measured, and fold-change (optically stimulated/untreated) was calculated. A 1.2-fold change in neurite length was recorded in Thy1ChR2 V/V cells stimulated with light (95% CI [1.12, 1.36] Figure 6C). In contrast, no response to light stimulation was found in the other three genotypes studied (Thy1ChR2 V/M 0.92-fold change, 95% CI [0.82, 1.03]; Thy1ChR2 M/M 0.87-fold change, 95% CI [0.61, 1.13]; WT 0.96-fold change, 95% CI [0.78, 1.15]). The omnibus result of a one-way ANOVA was significant ($F(3,12)=4.03$, $p=0.034$). Post-hoc testing revealed Thy1ChR2 V/V fold change was significantly different from Thy1ChR2 V/M fold change (p=0.013), ThyChR2 M/M fold change (p=0.011), and WT fold change (p=0.034). As a control, cells were also stimulated with an inappropriate wavelength of light (655nm). Neurite length did not differ with this control stimulation in any genotype, and there was no difference between any of the genotypes (p=ns).

These differences in longest neurite length could be due to neurons growing longer neurites or differences in the proportion of cells that grew any neurites. To test this, we analyzed the proportion of cells with neurites for each genotype and treatment group, and found no effect of genotype ($F(2,18)=1.79$, $p=0.20$), treatment ($F(1,18)=1.27$, $p=0.28$), or an interaction of genotype*treatment ($F(2,18)=1.20$, $p=0.32$, Figure 6E). There also was no significant difference between genotypes or treatments in the soma sizes of the cultured neurons studied (see Figure 6D).

There is no difference in basal release of BDNF between genotypes

Immediately after light stimulation, media was collected from the DRG culture for analysis of secreted BDNF protein (Figure 6F). The omnibus result of a one-way ANOVA revealed no significant effect of genotype ($F(3,11)=1.10$, $p=0.39$). Optical stimulation increased BDNF concentration two-fold in media collected from Thy1ChR2 V/V cells (Figure 6G, 2.21, 95% CI [2.059, 2.306]). In contrast, there was no difference in BDNF concentration in media collected from stimulated Thy1ChR2 V/M neurons (1.40, 95% CI [0.50, 2.31]), Thy1ChR2 M/M neurons (1.17, 95% CI [0.216, 2.13]), or WT neurons (1.42, 95% CI [0.36, 2.49]). Due to high within group variability, one-way ANOVA revealed no significant effect of genotype on fold-change of BDNF concentration ($F(3,10)=1.30$, $p=0.33$).

Discussion

Activity-dependent treatments have proven useful in enhancing axon regeneration after peripheral nerve injury. Their effectiveness requires BDNF-trkB signaling in the neurons whose axons are regenerating. We used neuroanatomical and electrophysiological methods to analyze the ability of one such treatment, treadmill training, to enhance axon regeneration in mice carrying the Met allele of the Val66Met SNP, which have deficient activity-dependent release of BDNF. Here, we present two main findings. First, without treatment, axon regeneration in heterozygous V/M mice is enhanced *in vivo* and neurite outgrowth of cultured DRG neurons from both V/M and M/M mice is longer *in vitro* when compared to cells from V/V mice. Second, treadmill training, which is a powerful activity-dependent promoter of axon regeneration, did not enhance regeneration in Met-carriers *in vivo,* and optical stimulation did not enhance neurite outgrowth *in vitro.*

Without any treatment, motor axon regeneration in V/M mice was enhanced significantly compared to V/V mice. Our results from MUNE, retrograde labeling of motoneurons and DRG neurons, and analysis of motor endplate reinnervation support the conclusion that axons of more motoneurons have effectively regenerated and reinnervated a muscle target in untreated V/M mice. The simplest interpretation of these findings is that the process of regeneration/reinnervation is accelerated in these animals. As motor axons regenerate into muscles, the first axons to innervate the muscle capture a large number of muscle fibers, skewing this innervation ratio higher (Rafuse and Gordon, 1998). Over time, more axons reach the muscle, and innervation ratios return to those found pre-injury. Our finding of larger SMU amplitudes four weeks after injury in untreated V/V mice would be consistent with such an increase in innervation ratio. That SMU amplitudes were not larger in untreated Met-carriers at the same post-injury time could indicate that at four weeks, regeneration is more advanced than in V/V mice. Our finding that neurites from cultured DRG neurons expressing the Met allele

were significantly longer 72 hours after plating than those derived from V/V mice also is interpreted as more rapid regeneration.

The enhanced regeneration of axons in the Met-carriers was an unexpected result. The valine to methionine replacement in this SNP occurs in the pro-domain of the BDNF molecule, which is cleaved to produce mature BDNF. The presence of the Met allele results in inefficient packaging of BDNF into calcium-sensitive vesicles, making activity-dependent release of BDNF deficient in V/M and M/M mice (Egan et al., 2003, Chen et al., 2004). These vesicles release BDNF in response to calcium influx, such as might occur in motoneurons during exercise (Lessmann et al., 2003). In contrast, no difference in basal release of BDNF has been reported (Chen et al., 2006, Notaras et al., 2017). In addition, the Met-containing prodomain of *bdnf* has been shown to selectively activate the common neurotrophin receptor, $p75^{NTR}$, which has been associated with growth cone retraction and dendritic spine disassembly (Anastasia et al., 2013, Giza et al., 2018). Both motoneurons and DRG neurons express $p75^{NTR}$, but whether endogenous levels of *secreted* Met-containing prodomain are sufficient to impact axon regeneration in these cell types remains an open question (Zhou et al., 1996, Ibáñez and Simi, 2012). For these reasons, we did not anticipate improved regeneration in the untreated V/M and M/M mice. If anything, we anticipated worse regeneration among the Met-carriers.

The mechanism behind the observed enhanced regeneration/neurite outgrowth in Met-carriers is currently unknown. One possibility could be greater constitutive release of BDNF resulting from the deficiency in regulated release described above. However, we found no difference in basal levels of BDNF secretion in our culture experiments, consistent with previous studies (Chen et al., 2006, Notaras et al., 2017). Another possible explanation could be increased trkB expression in the regenerating the axons, resulting in more effective ligand-binding despite less available ligand. Higher levels of full length trkB and lower levels of the dominant-negative truncated form of trkB have been found in the dorsal hippocampus in M/M mice (Notaras et al., 2017). A similar elevation of trkB expression in DRG neurons and

motoneurons in mice with the Met allele could be a part of a mechanism explaining the enhanced axon growth observed. Additionally, compensatory changes in other ligands for trkB, such as neurotrophin 4/5 (NT4/5), could also account for differences in axon regeneration. Neuronal release of NT4/5 contributes to axon regeneration after injury (English et al., 2005, English et al., 2011a), though basal levels of neuronal NT 4/5 secretion have not been studied, nor has activity-dependent secretion (Lessmann et al., 2003). These possible mechanisms should be explored further in future studies.

Carrying the Met allele of the BDNF Val66Met polymorphism has been reported as a risk factor for numerous diseases and disorders, including Alzheimer's disease, obsessive compulsive disorder, anorexia nervosa, and bipolar disorder (Sklar et al., 2002, Hall et al., 2003, Ribases et al., 2003, Neves-Pereira et al., 2002, Notaras et al., 2015). Here, we find a possible beneficial result of this allele—better axon regeneration after injury. We are not the first to report a surprising benefit of carrying the Met allele. Met-carriers more effectively recover executive functioning after traumatic brain injury, although no effect is seen on recovery of general intelligence (Krueger et al., 2011, Rostami et al., 2011). Additionally, Met-carriers have a lower risk of mortality following severe TBI (Failla et al., 2015). In stroke models, the Met allele was associated with enhanced motor performance after a transient middle cerebral artery occlusion (Qin et al., 2014). These findings taken together could indicate that having this allele is not simply bad—indeed, it would be hard to explain the high prevalence of the allele in East Asian populations if the results were unequivocally negative (Shimizu et al., 2004).

Neither treadmill training nor optical stimulation further enhanced regeneration in Met-carriers. This result was expected due to the BDNF-dependence of these treatments and the deficient activity-dependent BDNF-release found in Met-carriers (Egan et al., 2003, Chen et al., 2006, Wilhelm et al., 2012). As expected, treadmill training markedly improved MUNE, motoneuron labeling, and motor endplate occupation in V/V mice.

An unexpected result was the difference in DRG cell size found in intact animals. The L4 DRGs from M/M animals contained smaller neurons than those from V/V animals. BDNF is a trophic signal in the DRG, but trkB is not expressed by all DRG neurons. In intact animals, 20-50% of DRG neurons express trkB, and those neurons tend to be large diameter neurons as well as medium diameter low threshold mechanoreceptors (McMahon et al., 1994, Wright and Snider, 1995, Li et al., 2011). The lack of large neurons in M/M DRGs could be developmental—insufficient BDNF secretion could have led to the death of large neurons. Further investigation of genotypic differences in DRG neuron type through probing molecular expression is warranted.

We hypothesized that treadmill training would increase the number L4 DRG neurons innervating the lateral gastrocnemius in V/V mice. Previous studies had found that activity-dependent treatments such as electrical stimulation and voluntary exercise enhanced DRG regeneration *in vivo* and neurite outgrowth *in vitro* (Molteni et al., 2004, Geremia et al., 2007). However, another study found electrical stimulation markedly enhanced regeneration in *cutaneous* DRG neurons but *decreased* the number of successfully regenerating muscle afferents (Brushart et al., 2005). We found no effect of treadmill training on DRG axon regeneration in any of our genotypes. In contrast, in our *in vitro* studies, optogenetic stimulation increased neurite length in V/V cells. This could be because our cultures primarily favored large DRG neurons, which may respond favorably to increased neural activity (Modol et al., 2015). The role for activity-dependent treatments in enhancing axon regeneration in DRG neurons could therefore depend entirely on cell-type, and further exploration into cell type could elucidate differences in the effects of neuronal activity. Our *in vitro* results are also in contrast to (Enes et al., 2010), who found that continuous depolarization through high levels of KCl or electrical stimulation decreased neurite outgrowth in dissociated DRG neurons. However, Park et al., demonstrated that limited depolarization increases DRG growth, whereas stimulating for longer

than 3 hours abolishes this enhancement. Thus, in addition to cell-type specific reactions to activity-dependent treatments, there may also be a dose-dependence in how cells respond.

Conclusion

Clinical trials testing the efficacy of activity-dependent treatments for nerve damage have already begun (Gordon et al., 2007, Gordon et al., 2010). In these studies, brief electrical stimulation is applied after carpal tunnel release surgery, resulting in increased MUNE 6-8 months after surgery. While promising, the enhancing effects of electrical stimulation could be diminished by inclusion of Met-carriers, who, based on the results presented above, might be expected to have naturally better outcomes under control conditions and not to respond to this activity-dependent treatment. Without genotyping of patients, benefits of activity-dependent treatments for non-met-carriers could be masked. More importantly, as activity-based treatments increase in popularity to treat peripheral neuropathies, health care professionals need to account for patient genotype. For the substantial portion of the population carrying the Met allele, personalized medicine that does not rely on endogenous BDNF secretion may be necessary.

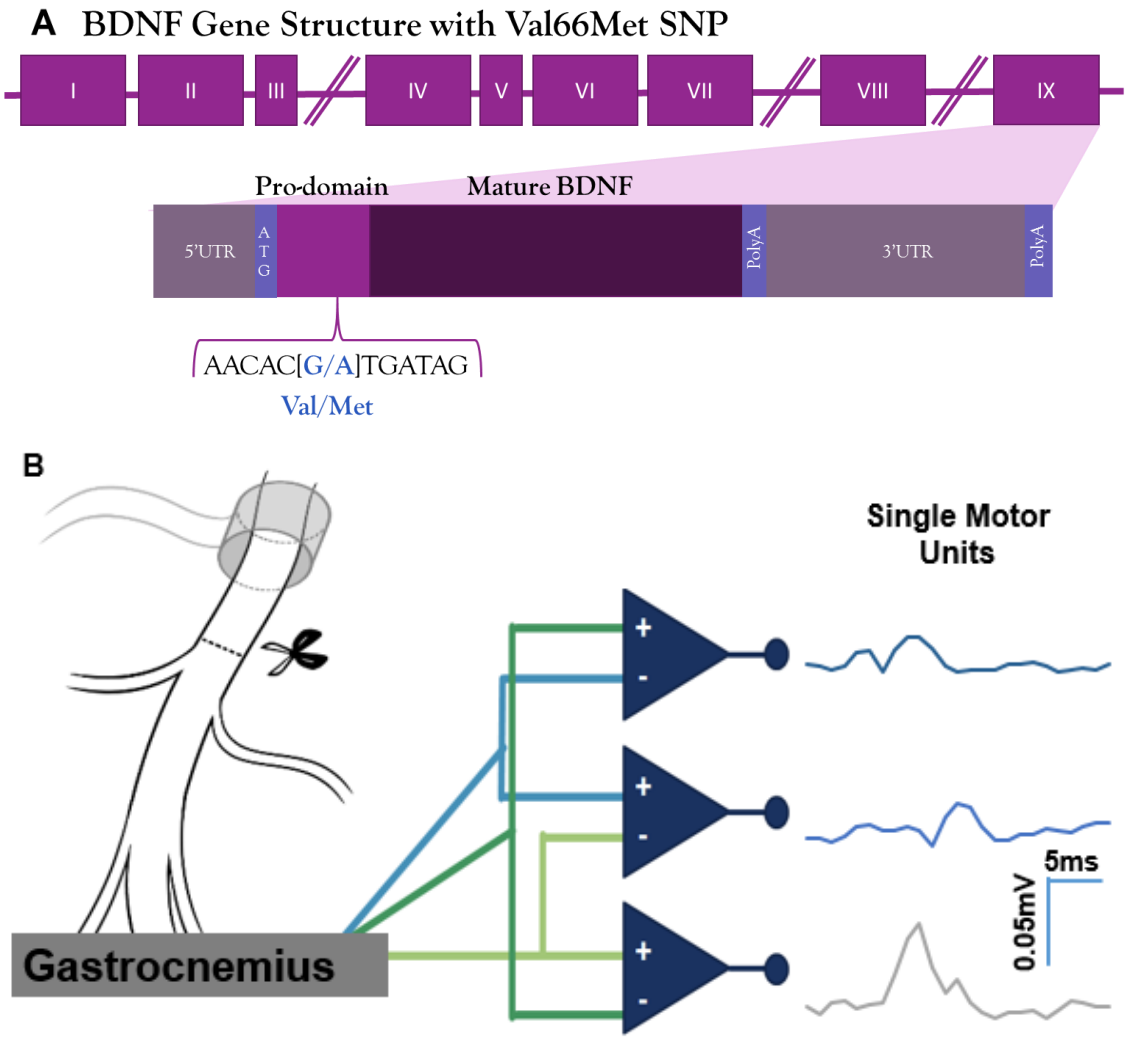


Figure 1. A. Structure of BDNF gene and location of Val66Met SNP in the coding exon IX. The G to A substitution in the prodomain results in a valine to methionine substitution and a decreased Ca^{2+}- dependent release of BDNF. **B.** Recording single motor unit (SMU) potentials using a tripolar electrode. A stimulating cuff is placed around the sciatic nerve. A tripolar electrode is inserted into the lateral gastrocnemius muscle, and amplitudes of SMU potentials are recorded in three channels.

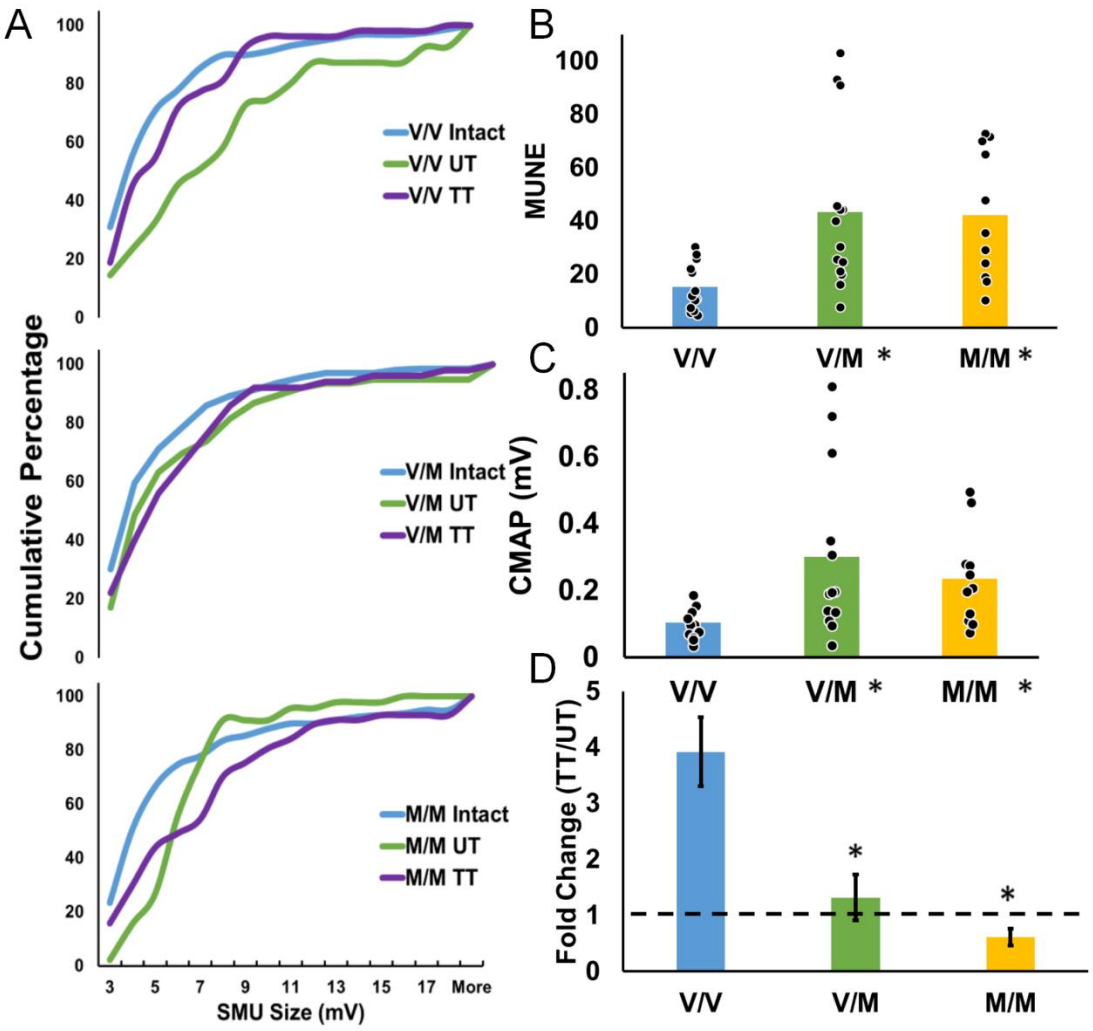

A
Cumulative Percentage
V/V Intact
V/V UT
V/V TT
V/M Intact
V/M UT
V/M TT
M/M Intact
M/M UT
M/M TT
SMU Size (mV)
B
MUNE
C
CMAP (mV)
D
Fold Change (TT/UT)
V/V
V/M
M/M

Figure 2. **Treadmill training enhances functional recovery in V/V but not Met-carriers. A.** Cumulative histograms of single motor unit potential (SMU) amplitudes. In V/V mice, sciatic nerve transection resulted in larger amplitude potentials four weeks after injury (top panel), and TT did not. In Met-carriers, SMU potential amplitude was not significantly different, regardless of injury or treatment (bottom two panels). **B**. Motor unit number estimation (MUNE) is shown four weeks after injury for the three genotypes. Data from individual animals are solid symbols overlaid on the bars. Bars indicate mean value. Asterisks denote $p<0.05$ with respect to V/V mice. **C**. Maximum CMAP potential is shown four weeks after injury in the three genotypes studied. Data from individual animals are solid symbols overlaid on the bars. Bars indicate mean value. Asterisks denote $p<0.05$ with respect to V/V mice. **D.** Fold change in MUNE with treadmill training (TT) is represented for the three genotypes. Data represented as mean (+SEM). Asterisks denote $p<0.05$ with respect to V/V mice.

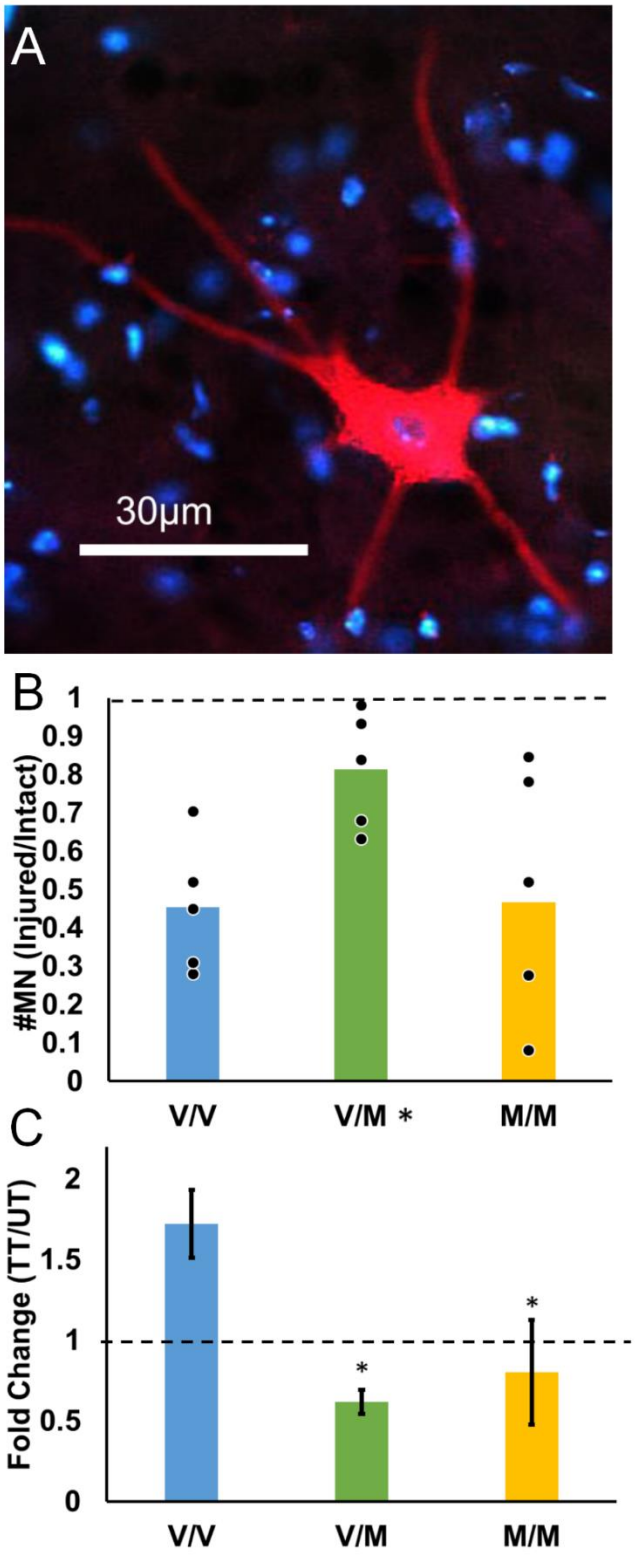

A
30µm
B
#MN (Injured/Intact)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
V/V
V/M *
M/M
C
Fold Change (TT/UT)
2
1.5
1
0.5
0
*
*
V/V
V/M
M/M

Figure 3. **Motor axon regeneration is enhanced in untreated but not treadmill trained mice heterozygous for $BDNF_{met}$ A.** A single motoneuron labeled by a retrograde tracer that had been injected into the lateral gastrocnemius muscle four weeks after sciatic nerve transection and repair. Motoneurons like this one were counted as labeled if the soma was labeled with dense granular fluorescence which extends into the primary dendrites and contains a dark region, indicating a nucleus. Red is CTB-555, Blue is DAPI. **B.** The ratio of the number of labeled motoneurons (injured/intact) is shown. Data from individual animals are solid symbols overlaid on the bars. Bars indicate mean value. Asterisks denote $p<0.05$ with respect to V/V mice. **C.** Fold-change in the ratio of retrogradely labeled motoneurons is represented for the three genotypes. Data represented as mean (+SEM). Asterisks denote $p<0.05$ with respect to V/V animals.

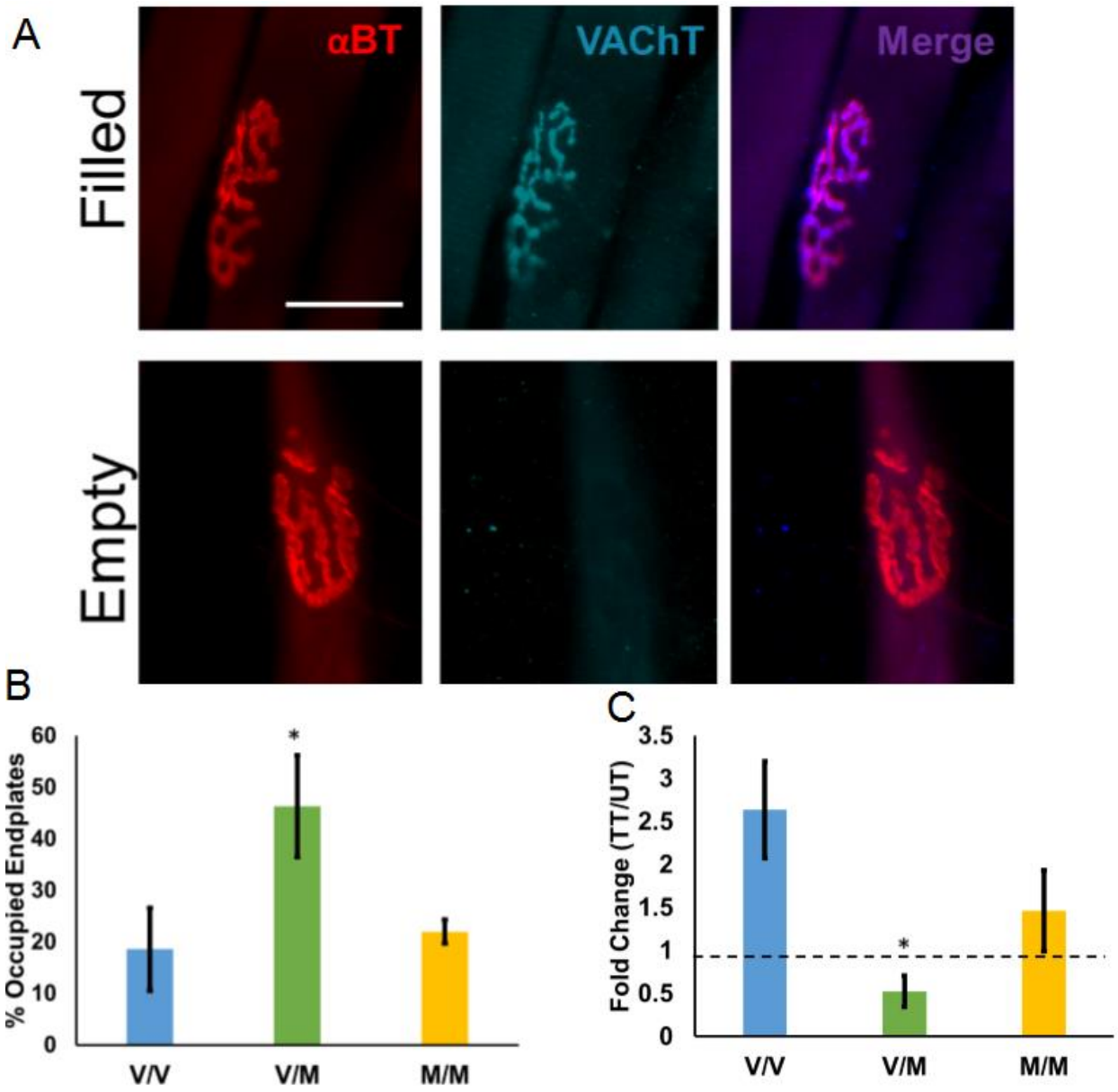


Figure 4. Muscle fiber reinnervation is enhanced in untreated but not treadmill trained mice heterozygous for $BDNF_{met}$ A. Using the binding of fluorescent alpha bungarotoxin (αBT), two motor endplates are shown (top and bottom left panels). Immunoreactivity to VAChT marks motoneuron terminals (top and bottom middle panels). Motor endplates were counted as filled if the VAChT completely covered the motor endplate (top panels). Fifty motor endplates were scored for each animal. Scale bar=25 µm. **B.** A higher percentage of motor endplates were scored as VAChT+ in muscles harvested from untreated V/M mice than V/V or M/M mice. Asterisks denote $p<0.05$ with respect to V/V mice. **C**. Effect of treadmill training (TT) on motor

endplate reoccupation. Mean (± SEM) fold change in TT animals relative to untrained controls (UT) are shown for the three genotypes studied. Asterisks denote $p<0.05$ with respect to V/V mice.

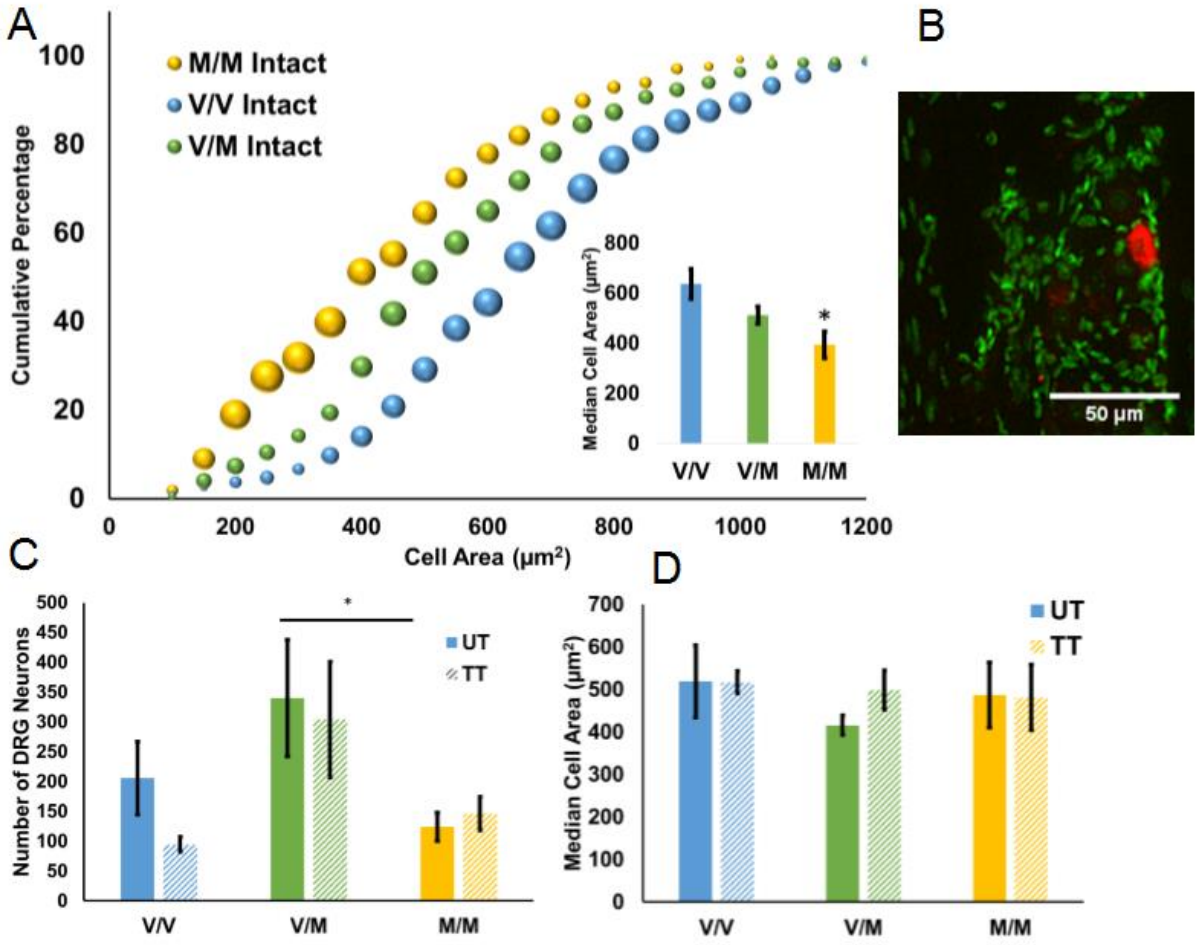


Figure 5. Treadmill training does not enhance DRG regeneration. A. In intact animals, the DRG cell size distribution was significantly smaller in M/M mice compared to V/V mice. The median cell size was significantly smaller in M/M mice compared to V/V mice. Asterisk denotes $p<0.05$ with respect to V/V mice. **B.** Representative image of labeled DRG neuron: CTB 555 in red; DAPI in green. **C.** Treadmill training did not increase the number of labeled DRG neurons in any genotype. Significantly more labeled DRG neurons were found in V/M mice than M/M mice. Asterisk denotes $p<.05$. **D.** The median cell area for DRG neurons that successfully innervated the lateral gastrocnemius did not differ with treatment or genotype.

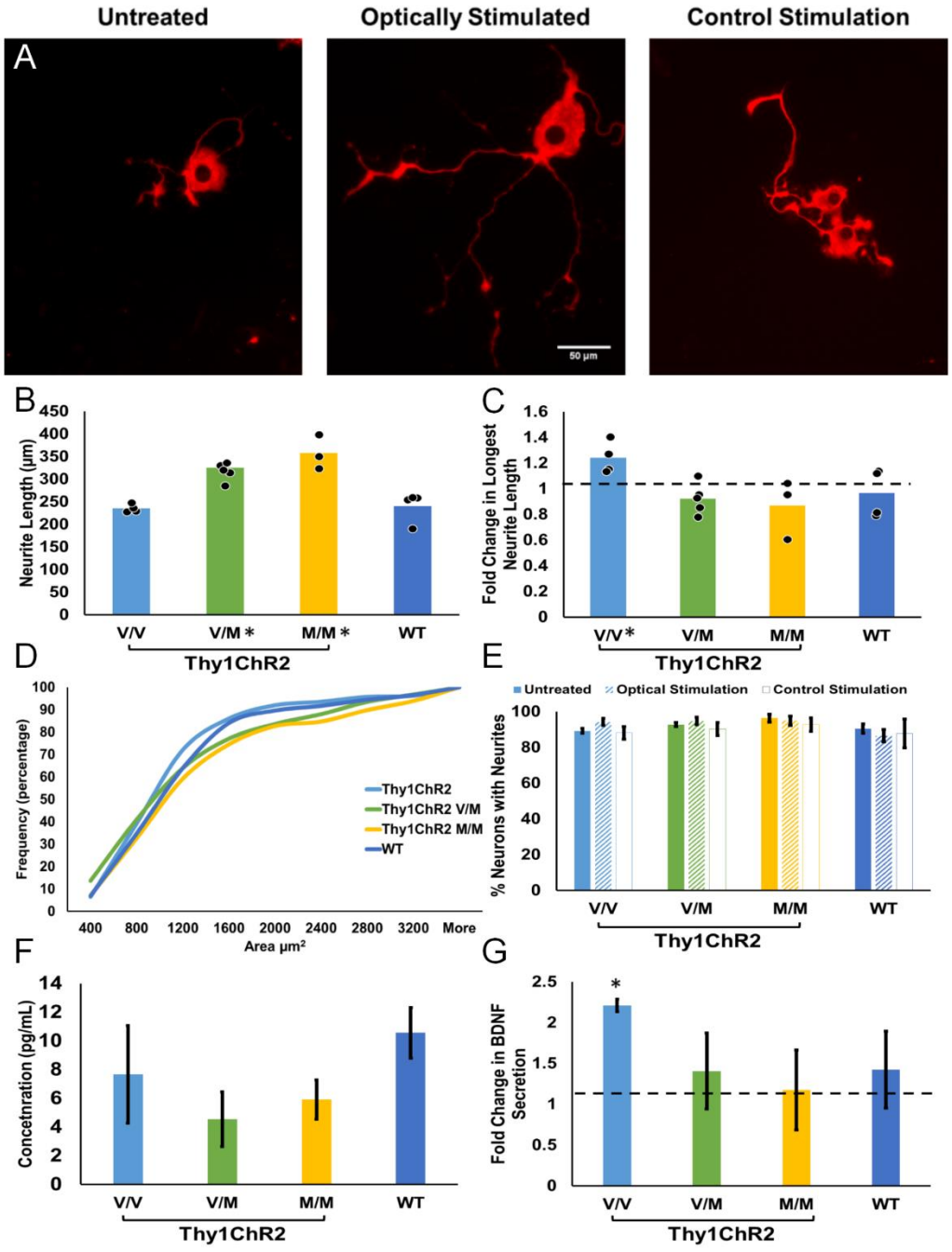

Untreated
Optically Stimulated
Control Stimulation
A
50 µm
B
Neurite Length (µm)
V/V
V/M *
M/M *
WT
Thy1ChR2
C
Fold Change in Longest Neurite Length
V/V*
V/M
M/M
WT
Thy1ChR2
D
Frequency (percentage)
Area µm²
Thy1ChR2
Thy1ChR2 V/M
Thy1ChR2 M/M
WT
E
Untreated
Optical Stimulation
Control Stimulation
% Neurons with Neurites
V/V
V/M
M/M
WT
Thy1ChR2
F
Concetnration (pg/mL)
V/V
V/M
M/M
WT
Thy1ChR2
G
Fold Change in BDNF Secretion
*
V/V
V/M
M/M
WT
Thy1ChR2

Figure 6. A. Representative images of cultured V/V DRG neurons immunofluorescent for beta tubulin III. **B**. The mean longest neurite length is shown for untreated cultures of the four genotypes studied. Data from individual cultures are shown as solid symbols overlaid on the bars. Asterisks denote $p<0.05$ with respect to Thy1ChR2 V/V mice. **C.** Fold-change in longest neurite length after 1hr light stimulation is shown for the four genotypes studied. Data from individual cultures are shown as solid symbols overlaid on the bars. Asterisk denotes significant difference from 1. **D**. A cumulative histogram of the areas of cultured DRG neurons for the four genotypes. **E.** The percent of neurons that grew neurites is shown for the four genotypes. Cells were untreated, stimulated optically with 472nm light, or stimulated with control stimulation of light of an inappropriate wavelength. Data are represented as mean (± SEM). **F**. Media was collected for protein analysis of basal levels of secreted BDNF protein. Data are represented as mean (± SEM). **G.** Immediately after light stimulation, media was collected for protein analysis of BDNF secretion. Fold-change of BDNF secretion is represented. Data expressed as mean (± SEM). Asterisks denote $p<0.05$ with respect to 1.

References

AL MAJED, A., BRUSHART, T. & GORDON, T. 2000a. Electrical stimulation accelerates and increases expression of BDNF and trkB mRNA in regenerating rat femoral motoneurons. *Eur J Neurosci,* 12**,** 4381-4390.

AL MAJED, A., NEUMANN, C., BRUSHART, T. & GORDON, T. 2000b. Brief electrical stimulation promotes the speed and accuracy of motor axonal regeneration. *J Neurosci,* 20**,** 2602-2608.

ANASTASIA, A., DEINHARDT, K., CHAO, M., WILL, N., IRMADY, K., LEE, F., HEMPSTEAD, B. & BRACKEN, C. 2013. Val66Met polymorphism of BDNF alters prodomain structure to induce neuronal growth cone retraction. *Nat Commun,* 4**,** 2490.

BOELTZ, T., IRELAND, M., MATHIS, K., NICOLINI, J., POPLAVSKI, K., ROSE, S. & ENGLISH, A. 2013. Effects of treadmill training on functional recovery following peripheral nerve injury in rats. *J Neurophysiol,* 109**,** 2645-2657.

BRUSHART, T. M., JARI, R., VERGE, V., ROHDE, C. & GORDON, T. 2005. Electrical stimulation restores the specificity of sensory axon regeneration. *Exp Neurol,* 194**,** 221-229.

CHEN, Z., JING, D., BATH, K., IERACI, A., KHAN, T., SIAO, C., HERRERA, D., TOTH, M., MCEWEN, B., HEMPSTEAD, B. & LEE, F. 2006. Genetic variant BDNF (Val66Met) polymorphism alters anxiety-related behavior. *Science,* 314**,** 140-143.

CHEN, Z., PATEL, P., SANT, G., MENG, C., TENG, K., HEMPSTEAD, B. & LEE, F. 2004. Variant brain-derived neurotrophic factor (BDNF) (Met66) alters the intracellular trafficking and activity-dependent secretion of wild-type BDNF in neurosecretory cells and cortical neurons. *J Neurosci,* 24**,** 4401-4411.

DE VRIES, J., MENOVSKY, T., VAN GULIK, S. & WESSELING, P. 2002. Histological effects of fibrin glue on nervous tissue: a safety study in rats. *Surg Neurol,* 57**,** 415-22; discussion 422.

DOHERTY, T. J. & BROWN, W. F. 1993. The estimated numbers and relative sizes of thenar motor units as selected by multiple point stimulation in young and older adults. *Muscle Nerve,* 16**,** 355-66.

EGAN, M., KOJIMA, M., CALLICOTT, J., GOLDBERG, T., KOLACHANA, B., BERTOLINO, A., ZAITSEV, E., GOLD, B., GOLDMAN, D., DEAN, M., LU, B. & WEINBERGER, D. 2003. The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. *Cell,* 112**,** 257-269.

ENES, J., LANGWIESER, N., RUSCHEL, J., CARBALLOSA-GONZALEZ, M. M., KLUG, A., TRAUT, M. H., YLERA, B., TAHIROVIC, S., HOFMANN, F., STEIN, V., MOOSMANG, S., HENTALL, I. D. & BRADKE, F. 2010. Electrical activity suppresses axon growth through Cav1.2 channels in adult primary sensory neurons. *Curr Biol,* 20**,** 1154-1164.

ENGLISH, A. 2005. Enhancing axon regeneration in peripheral nerves also increases functionally inappropriate reinnervation of targets. *J Comp Neurol,* 490**,** 427-441.

ENGLISH, A., CUCORANU, D., MULLIGAN, A., RODRIGUEZ, J. & SABATIER, M. 2011a. Neurotrophin-4/5 is implicated in the enhancement of axon regeneration produced by treadmill training following peripheral nerve injury. *Eur J Neurosci,* 33**,** 2265-2271.

ENGLISH, A., CUCORANU, D., MULLIGAN, A. & SABATIER, M. 2009. Treadmill training enhances axon regeneration in injured mouse peripheral nerves without increased loss of topographic specificity. *J Comp Neurol,* 517**,** 245-255.

ENGLISH, A., LIU, K., NICOLINI, J., MULLIGAN, A. & YE, K. 2013. Small-molecule trkB agonists promote axon regeneration in cut peripheral nerves. *Proc Natl Acad Sci U S A,* 110**,** 16217-16222.

ENGLISH, A., SCHARTZ, G., W, M., SABATIER, M. & MULLIGAN, A. 2007. Electrical stimulation promotes peripheral axon regeneration by enhanced neuronal neurotrophin signaling. *Dev Neurobiol,* 67**,** 158-172.

ENGLISH, A., W, M. & CARRASCO, D. 2005. Neurotrophin-4/5 is required for the early growth of regenerating axons in peripheral nerves. *Eur J Neurosci,* 21**,** 2624-2634.

ENGLISH, A., WILHELM, J. & SABATIER, M. 2011b. Enhancing recovery from peripheral nerve injury using treadmill training. *Ann Anat,* 193**,** 354-361.

FAILLA, M. D., KUMAR, R., PEITZMAN, A., CONLEY, Y. P., FERRELL, R. E. & WAGNER, A. K. 2015. Variation in the BDNF gene interacts with age to predict mortality in a prospective, longitudinal cohort with severe TBI. *Neurorehabil Neural Repair,* 29**,** 234-246.

GEREMIA, N., GORDON, T., BRUSHART, T., AL MAJED, A. & VERGE, V. 2007. Electrical stimulation promotes sensory neuron regeneration and growth-associated gene expression. *Exp Neurol,* 205**,** 347-359.

GIZA, J. I., KIM, J., MEYER, H. C., ANASTASIA, A., DINCHEVA, I., ZHENG, C. I., LOPEZ, K., BAINS, H., YANG, J., BRACKEN, C., LISTON, C., JING, D., HEMPSTEAD, B. L. & LEE, F. S. 2018. The BDNF Val66Met Prodomain Disassembles Dendritic Spines Altering Fear Extinction Circuitry and Behavior. *Neuron,* 99**,** 163-178.e6.

GOOCH, C., DOHERTY, T., CHAN, K., BROMBERG, M., LEWIS, R., STASHUK, D., BERGER, M., ANDARY, M. & DAUBE, J. 2014. Motor unit number estimation: a technology and literature review. *Muscle Nerve,* 50**,** 884-893.

GORDON, T. 2010. The physiology of neural injury and regeneration: the role of neurotrophic factors. *J Commun Disord,* 43**,** 265-273.

GORDON, T., AMIRJANI, N., EDWARDS, D. C. & CHAN, K. M. 2010. Brief post-surgical electrical stimulation accelerates axon regeneration and muscle reinnervation without affecting the functional measures in carpal tunnel syndrome patients. *Exp Neurol,* 223**,** 192-202.

GORDON, T., BRUSHART, T. M., AMIRJANI, N. & CHAN, K. M. 2007. The potential of electrical stimulation to promote functional recovery after peripheral nerve injury--comparisons between rats and humans. *Acta Neurochir Suppl,* 100**,** 3-11.

GORDON, T. & TETZLAFF, W. 2015. Regeneration-associated genes decline in chronically injured rat sciatic motoneurons. *Eur J Neurosci,* 42**,** 2783-91.

GORDON, T., TYREMAN, N. & RAJI, M. A. 2011. The basis for diminished functional recovery after delayed peripheral nerve repair. *J Neurosci,* 31**,** 5325-34.

HAASTERT-TALINI, K., SCHMITTE, R., KORTE, N., KLODE, D., RATZKA, A. & GROTHE, C. 2011. Electrical stimulation accelerates axonal and functional peripheral nerve regeneration across long gaps. *J Neurotrauma,* 28**,** 661-74.

HALL, D., DHILLA, A., CHARALAMBOUS, A., GOGOS, J. A. & KARAYIORGOU, M. 2003. Sequence variants of the brain-derived neurotrophic factor (BDNF) gene are strongly associated with obsessive-compulsive disorder. *Am J Hum Genet,* 73**,** 370-376.

IBÁÑEZ, C. & SIMI, A. 2012. p75 neurotrophin receptor signaling in nervous system injury and degeneration: paradox and opportunity. *Trends Neurosci,* 35**,** 431-440.

IERACI, A., MADAIO, A. I., MALLEI, A., LEE, F. S. & POPOLI, M. 2016. Brain-derived neurotrophic factor Val66Met human polymorphism impairs the beneficial exercise-induced neurobiological changes in mice. *Neuropsychopharmacology*.

ISAACS, J. 2010. Treatment of acute peripheral nerve injuries: current concepts. *J Hand Surg Am,* 35**,** 491-7; quiz 498.

JAISWAL, P. B., TUNG, J. K., GROSS, R. E. & ENGLISH, A. W. 2017. Motoneuron activity is required for enhancements in functional recovery after peripheral nerve injury in exercised female mice. *J Neurosci Res*.

KRUEGER, F., PARDINI, M., HUEY, E. D., RAYMONT, V., SOLOMON, J., LIPSKY, R. H., HODGKINSON, C. A., GOLDMAN, D. & GRAFMAN, J. 2011. The role of the Met66 brain-derived neurotrophic factor allele in the recovery of executive functioning after combat-related traumatic brain injury. *J Neurosci,* 31**,** 598-606.

LAWSON, S. N. 1979. The postnatal development of large light and small dark neurons in mouse dorsal root ganglia: a statistical analysis of cell numbers and size. *J Neurocytol,* 8**,** 275-294.

LAWSON, S. N. & WADDELL, P. J. 1991. Soma neurofilament immunoreactivity is related to cell size and fibre conduction velocity in rat primary sensory neurons. *J Physiol,* 435**,** 41-63.

LESSMANN, V., GOTTMANN, K. & MALCANGIO, M. 2003. Neurotrophin secretion: current facts and future prospects. *Prog Neurobiol,* 69**,** 341-374.

LI, L., RUTLIN, M., ABRAIRA, VICTORIA E., CASSIDY, C., KUS, L., GONG, S., JANKOWSKI, MICHAEL P., LUO, W., HEINTZ, N., KOERBER, H. R., WOODBURY, C. J. & GINTY, DAVID D. 2011. The functional organization of cutaneous low-threshold mechanosensory neurons. *Cell,* 147**,** 1615-1627.

MACGILLIVRAY, T. E. 2003. Fibrin sealants and glues. *J Card Surg,* 18**,** 480-5.

MAJOR, L., HEGEDUS, J., WEBER, D., GORDON, T. & JONES, K. 2007. Method of counting motor units in mice and validation using a mathematical model. *J Neurophysiol,* 97**,** 1846-1856.

MCCOMAS, A. J., FAWCETT, P. R., CAMPBELL, M. J. & SICA, R. E. 1971. Electrophysiological estimation of the number of motor units within a human muscle. *J Neurol Neurosurg Psychiatry,* 34**,** 121-31.

MCLEAN, I. W. & NAKANE, P. K. 1974. Periodate-lysine-paraformaldehyde fixative. A new fixation for immunoelectron microscopy. *J Histochem Cytochem,* 22**,** 1077-83.

MCMAHON, S. B., ARMANINI, M. P., LING, L. H. & PHILLIPS, H. S. 1994. Expression and coexpression of Trk receptors in subpopulations of adult primary sensory neurons projecting to identified peripheral targets. *Neuron,* 12**,** 1161-1171.

MODOL, L., SANTOS, D., COBIANCHI, S., GONZALEZ-PEREZ, F., LOPEZ-ALVAREZ, V. & NAVARRO, X. 2015. NKCC1 activation is required for myelinated sensory neurons regeneration through JNK-dependent pathway. *J Neurosci,* 35**,** 7414-27.

MOLTENI, R., ZHENG, J.-Q., YING, Z., GÓMEZ-PINILLA, F. & TWISS, J. L. 2004. Voluntary exercise increases axonal regeneration from sensory neurons. *Proc Natl Acad Sci U S A,* 101**,** 8473.

NASCIMENTO, C., PEREIRA, J., PIRES DE ANDRADE, L., GARUFFI, M., AYAN, C., KERR, D., TALIB, L., COMINETTI, M. & STELLA, F. 2015. Physical exercise improves peripheral BDNF levels and cognitive functions in elderly mild cognitive impairment individuals with different BDNF Val66Met genotypes. *J Alzheimers Dis,* 43**,** 81-91.

NEVES-PEREIRA, M., MUNDO, E., MUGLIA, P., KING, N., MACCIARDI, F. & KENNEDY, J. L. 2002. The brain-derived neurotrophic factor gene confers susceptibility to bipolar disorder: evidence from a family-based association study. *Am J Hum Genet,* 71**,** 651-5.

NOTARAS, M., DU, X., GOGOS, J., VAN DEN BUUSE, M. & HILL, R. A. 2017. The BDNF Val66Met polymorphism regulates glucocorticoid-induced corticohippocampal remodeling and behavioral despair. *Transl Psychiatry,* 7**,** e1233.

NOTARAS, M., HILL, R. & VAN DEN BUUSE, M. 2015. The BDNF gene Val66Met polymorphism as a modifier of psychiatric disorder susceptibility: progress and controversy. *Mol Psychiatry,* 20**,** 916-30.

PARK, S., KOPPES, R. A., FRORIEP, U. P., JIA, X., ACHYUTA, A. K. H., MCLAUGHLIN, B. L. & ANIKEEVA, P. 2015. Optogenetic control of nerve growth. *Sci Rep,* 5**,** 9669.

PORTINCASA, A., GOZZO, G., PARISI, D., ANNACONTINI, L., CAMPANALE, A., BASSO, G. & MAIORELLA, A. 2007. Microsurgical treatment of injury to peripheral nerves in upper and lower limbs: a critical review of the last 8 years. *Microsurgery,* 27**,** 455-462.

QIN, L., JING, D., PARAUDA, S., CARMEL, J., RATAN, R. R., LEE, F. S. & CHO, S. 2014. An Adaptive Role for BDNF Val66Met Polymorphism in Motor Recovery in Chronic Stroke. *J Neurosci,* 34**,** 2493-2502.

RAFUSE, V. F. & GORDON, T. 1998. Incomplete rematching of nerve and muscle properties in motor units after extensive nerve injuries in cat hindlimb muscle. *J Physiol,* 509**,** 909-926.

RIBASES, M., GRATACOS, M., ARMENGOL, L., DE CID, R., BADIA, A., JIMENEZ, L., SOLANO, R., VALLEJO, J., FERNANDEZ, F. & ESTIVILL, X. 2003. Met66 in the brain-derived neurotrophic factor (BDNF) precursor is associated with anorexia nervosa restrictive type. *Mol Psychiatry,* 8**,** 745-51.

ROSTAMI, E., KRUEGER, F., ZOUBAK, S., DAL MONTE, O., RAYMONT, V., PARDINI, M., HODGKINSON, C. A., GOLDMAN, D., RISLING, M. & GRAFMAN, J. 2011. BDNF Polymorphism Predicts General Intelligence after Penetrating Traumatic Brain Injury. *PLoS ONE,* 6**,** e27389.

SABATIER, M. J., REDMON, N., SCHWARTZ, G. & ENGLISH, A. W. 2008. Treadmill training promotes axon regeneration in injured peripheral nerves. *Exp Neurol,* 211**,** 489-493.

SABATIER, M. J., TO, B. N., NICOLINI, J. & ENGLISH, A. W. 2011. Effect of slope and sciatic nerve injury on ankle muscle recruitment and hindlimb kinematics during walking in the rat. *J Exp Biol,* 214**,** 1007-16.

SCHOLZ, T., KRICHEVSKY, A., SUMARTO, A., JAFFURS, D., WIRTH, G. A., PAYDAR, K. & EVANS, G. R. 2009. Peripheral nerve injuries: an international survey of current treatments and future perspectives. *J Reconstr Microsurg,* 25**,** 339-44.

SHIMIZU, E., HASHIMOTO, K. & IYO, M. 2004. Ethnic difference of the BDNF 196G/A (val66met) polymorphism frequencies: the possibility to explain ethnic mental traits. *Am J Med Genet B Neuropsychiatr Genet,* 126b**,** 122-3.

SKLAR, P., GABRIEL, S. B., MCINNIS, M. G., BENNETT, P., LIM, Y., TSAN, G., SCHAFFNER, S., KIROV, G., JONES, I., OWEN, M., CRADDOCK, N., DEPAULO, J. R. & LANDER, E. S. 2002. Family-based association study of 76 candidate genes in bipolar disorder: BDNF is a potential risk locus. Brain-derived neutrophic factor. *Mol Psychiatry,* 7**,** 579-93.

STEIN, R. B. & YANG, J. F. 1990. Methods for estimating the number of motor units in human muscles. *Ann Neurol,* 28**,** 487-95.

UDINA, E., COBIANCHI, S., ALLODI, I. & NAVARRO, X. 2011. Effects of activity-dependent strategies on regeneration and plasticity after peripheral nerve injuries. *Ann Anat,* 193**,** 347-353.

WARD, P. J., CLANTON, S. L. & ENGLISH, A. W. 2018. Optogenetically enhanced axon regeneration: motor versus sensory neuron-specific stimulation. *Eur J Neurosci,* 47**,** 294-304.

WARD, P. J., JONES, L. N., MULLIGAN, A., GOOLSBY, W., WILHELM, J. C. & ENGLISH, A. W. 2016. Optically-induced neuronal activity is sufficient to promote functional motor axon regeneration in vivo. *PLoS ONE,* 11**,** e0154243.

WILHELM, J., XU, M., CUCORANU, D., CHMIELEWSKI, S., HOLMES, T., LAU, K., BASSELL, G. & ENGLISH, A. 2012. Cooperative roles of BDNF expression in neurons and Schwann cells are modulated by exercise to facilitate nerve regeneration. *J Neurosci,* 32**,** 5002-5009.

WILLAND, M., HOLMES, M., BAIN, J., FAHNESTOCK, M. & DE BRUIN, H. 2011. Determining the effects of electrical stimulation on functional recovery of denervated rat

gastrocnemius muscle using motor unit number estimation. *Conf Proc IEEE Eng Med Biol Soc*.

WOOD, K., WILHELM, J., SABATIER, M., LIU, K., GU, J. & ENGLISH, A. 2012. Sex differences in the effectiveness of treadmill training in enhancing axon regeneration in injured peripheral nerves. *Dev Neurobiol,* 72**,** 688-698.

WRIGHT, D. E. & SNIDER, W. D. 1995. Neurotrophin receptor mRNA expression defines distinct populations of neurons in rat dorsal root ganglia. *J Comp Neurol,* 351**,** 329-38.

ZHOU, X. F., RUSH, R. A. & MCLACHLAN, E. M. 1996. Differential expression of the p75 nerve growth factor receptor in glia and neurons of the rat dorsal root ganglia after peripheral nerve transection. *J Neurosci,* 16**,** 2901-11.

Chapter 3:

Enhanced regeneration in Met-carriers of BDNF Val66Met polymorphism is trkB-dependent

Posters presented from this work:

McGregor C, English AW (2017) “Neurite elongation is enhanced in cells heterozygous for the Val66Met polymorphism.” Society for Neuroscience 2017, Washington D.C.

Invited talks from this work:

“The BDNF Val66Met polymorphism and peripheral nerve regeneration: a surprising benefit of a maligned SNP.” September, 2018. Midwest Motoneuron Consortium, Indianapolis, Indiana.

Abstract

The BDNF Val66Met polymorphism results in deficient activity-dependent secretion of BDNF. We previously reported that transgenic mice expressing the Met allele of this polymorphism have enhanced axon regeneration after peripheral nerve injury *in vivo* and enhanced neurite outgrowth *in vivo*. Here we treated animals with antagonists to the two receptors for BDNF, trkB and $p75^{NTR}$, at the time of injury. We also applied these drugs to cultured adult DRG neurons as well as a fusion protein, trkB-Fc, which sequesters trkB ligands. Antagonizing trkB resulted in decreased motoneuron regeneration in the heterozygous Met-carriers and decreased neurite outgrowth *in vitro* in both heterozygous and homozygous Met-carriers. Additionally, trkB-Fc abolished the enhanced neurite outgrowth in the Met-carrying cells. The $p75^{NTR}$ inhibitor, LM11A-31, had a modest enhancing effect in axon regeneration in wild type mice, but no effect in the Met-carriers.

Introduction

Peripheral nerves have the capacity to regenerate after injury, but the process is slow and often incomplete (Portincasa et al., 2007). Treatments that enhance the cell-intrinsic mechanisms for regeneration, such as electrical stimulation, have been gaining attention in past years, and have even garnered clinical trials (Gordon et al., 2010, Wong et al., 2015). These treatments depend on the injured neuron's ability to secrete brain derived neurotrophic factor (BDNF) (English et al., 2014, Wilhelm et al., 2012).

Genetic differences in the human population can lead to high variance in response to treatment and difficulty in translating treatments from bench to bedside. Of particular interest is the single nucleotide polymorphism (SNP) in the *BDNF* gene Val66Met, which results in a valine to methionine substitution at the 66th codon (Egan et al., 2003). This polymorphism is very common—a third of Americans carry the Met allele, as do up to 50% of East Asians (Egan et al., 2003, Shimizu et al., 2004). Expressing the met form of BDNF ($BDNF_{Met}$) results in decreased activity-dependent secretion of BDNF as well as possible increased activation of the common neurotrophin receptor ($p75^{NTR}$) (Egan et al., 2003, Chen et al., 2004, Chen et al., 2006, Anastasia et al., 2013). Both the deficiency of secreted BDNF and increased $p75^{NTR}$ activation could lead to a decreased response to activity-dependent treatments, and indeed, we have shown this to be true in an animal model of the SNP (McGregor et al., 2018).

BDNF has two receptors—tropomyosin receptor kinase B (trkB) and the common neurotrophin receptor, $p75^{NTR}$. TrkB activation enhances axon growth, whereas $p75^{NTR}$ is involved in anti-growth signaling and growth cone retraction (Boyd and Gordon, 2003, Anastasia et al., 2013). Mature BDNF has a higher affinity for trkB than $p75^{NTR}$, but when a high concentration of exogenously BDNF is applied, growth is inhibited as $p75^{NTR}$ is activated (Boyd and Gordon, 2002). Unlike $BDNF_{Val}$, the prodomain of $BDNF_{Met}$ is bioactive and able to activate $p75^{NTR}$ once cleaved from mature BDNF, resulting in dendrite collapse and growth cone retraction (Anastasia et al., 2013, Giza et al., 2018). Surprisingly, we discovered that in mice

either heterozygous or homozygous for the Met allele, enhanced axon regeneration compared to wild-type valine/valine mice was found after sciatic nerve transection and repair with no treatment. Understanding the mechanism behind this enhanced regeneration as well as finding treatments that can enhance axon regeneration in Met-carriers is of the utmost importance for furthering the field of peripheral nerve regeneration.

We hypothesized that blocking $p75^{NTR}$ activation with an antagonist, LM11A-31 (Massa et al., 2006), would enhance regeneration in mice heterozygous, homozygous, or lacking the Met allele. We further hypothesized that antagonizing trkB with a competitive antagonist, ANA-12, (Cazorla et al., 2011) would inhibit the enhanced regeneration found in mice carrying the met allele of BDNF. We tested these pharmacological agents on the effect of axon regeneration *in vivo* and neurite outgrowth *in vitro*. We found that LM11A-31 was ineffective in enhancing regeneration in any of the mice, but that blocking the trkB receptor with ANA-12 resulted in abolishment of the enhanced regeneration in the Met-carriers.

Methods

Animals and Surgical Treatments. All experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committee of Emory University and conformed to the Guidelines for the Use of Animals in Research of the Society for Neuroscience. A transgenic C57BL/6J mouse homozygous for the met allele (M/M) was received as a generous gift from Dr. Frances Lee (Chen et al., 2006). This founder was used to breed heterozygous (V/M) and wild type (V/V) offspring. Mice were bred and maintained at the Division of Animal Resources at Emory University. Mice were group-housed on a 12/12 light dark schedule with *ad libitum* access to food and water. Both males and females were used for all experiments.

Mice were anesthetized with 2% isoflurane and treated with Meloxicam analgesic (2mg/kg). The right sciatic nerve was exposed in the mid-thigh, placed on a small rectangle of SILASTIC film (Dow Corning 501-1) and secured with fibrin glue (de Vries et al., 2002, MacGillivray, 2003). Fibrin glue was used to deliver the antagonists LM11A-31 (791mg/mL in sterile water, Tocris, Minneapolis, MN) and ANA-12 (2mg/mL in 40% PEG300, 40% DMSO, and 20% sterile water, Tocris). This concentration of ANA-12 was chosen because it was the maximum drug we could get into solution. Mixing the drug with the fibrin glue has been shown to release the drug over the course of three days (Spicer and Mikos, 2010). Fibrin glue was mixed 1:1:2 (fibrinogen: drug: thrombin). The nerve was fully transected using sharp scissors three millimeters proximal to the sciatic branching. The left side of each mouse served as an unoperated control. After nerve repair, the surgical site was sutured closed, and the animal was allowed to recover from anesthesia before returning to its cage.

Motor Unit Number Estimation (MUNE). Four weeks post-injury, functional muscle reinnervation was investigated using motor unit number estimation (MUNE) (McComas et al., 1971). MUNE was performed as previously described (McGregor et al., 2018). Briefly, a bipolar stimulating cuff, constructed from silastic tubing and fine wire (Cooner Wire, Chastworth, CA;

part number AS631) was placed around the injured nerve. Three monopolar fine wire electromyography (EMG) electrodes (California Fine Wire Company, Grovery Beach, CA) were implanted into the lateral gastrocnemius using a 25G hypodermic needle. One mm of insulation was removed from the tips of the wires and hooked such that they were each at least 1mm apart from each other when placed in the muscle. The three wires were attached to three differential amplifiers, which allowed for simultaneous recording from three channels. Stimulations consisted of 0.1ms pulses applied at two second intervals and muscle EMG activity was recorded using custom LabVIEW software (National Instruments, Austin, TX). Stimulus intensity was graded until a single all-or-none EMG response was observed. This lowest-threshold response on each channel was assumed to be produced by activation of a single motor unit (SMU). To be counted as an SMU, the response needed to be biphasic or triphasic and to occur more than once while stimulating voltage was held steady at just the threshold needed for activation. The average rectified voltage within an empirically determined time window for each SMU potential was calculated in LabVIEW software (Sabatier et al., 2011). Multi-point stimulation was used by moving the cuff electrode to different points along the nerve to increase the number of SMUs recorded (5-12 per animal) (Doherty and Brown, 1993, Stein and Yang, 1990). Stimulus intensity was then increased to reach the maximal compound muscle action potential (CMAP). The average maximal CMAP amplitude for each animal was divided by the average of the SMU amplitudes to estimate the number of functional motor units in the reinnervated muscle.

Retrograde Labeling of Motoneurons. Immediately following electrophysiological recordings, the lateral gastrocnemius of both the injured and intact sides of each mouse were exposed and using a Hamilton syringe (Hamilton, Reno, NV), 1µL of a 1% solution of Cholera toxin B (CTB) conjugated to a fluorescent label (Alexafluor 555; life Technologies, Grand Island, NY, catalog number C-34776) was slowly injected into the muscle over 30 seconds. Motoneurons which had successfully regenerated into the muscle took up the tracer. After

allowing three days for sufficient transport of the tracer into the cell body, mice were euthanized with an overdose of pentobarbital (150mg/kg, i.p.) and perfused transcardially with cold 0.9% saline followed by 4% periodate-lysate-paraformaldehyde fixative (McLean and Nakane, 1974). Lumbar 3-5 spinal cord segments were harvested and cryoprotected in 20% sucrose, sectioned on a cryostat (20 µm sections), and mounted onto slides in serial sections. Images were obtained using a fluorescence microscope (Leica DM6000) with a lowlight camera (Hamamatsu, Sewickley, PA) using HCImage software (Hamamatsu, Sewickley, PA). Motoneurons labeled with CTB were counted if their somata contained dense granular fluorescence extending into the primary dendrites and contained a dark region indicating a nucleus (Figure 2A (English, 2005)).

Motor Endplate Reinnervation. Concurrent to spinal cord harvest, both the intact and injured gastrocnemius muscles were harvested. The muscles were cryoprotected in 20% sucrose, sectioned longitudinally (20 µm), and mounted onto slides in non-serial sections. Antigen retrieval was performed using boiling 10mM Sodium Citrate buffer (pH 8.5). After 20 minutes, sections were washed three times with 0.1M PBS, then blocked with 0.3% triton in 0.1M PBS and 10% natural goat serum for 1 hour at room temperature. Sections were incubated overnight (14-16 hours) with antibody against the vesicular acetylcholine transporter (VAChT, 1:500, Synaptic Systems, Göttingen, Germany) to label motoneuron synaptic terminals. The following morning, sections were washed three times in 0.1M PBS and incubated for two hours at room temperature with secondary antibody (goat anti-guinea pig conjugated to Alexafluor 647, 1:200, Thermo Fisher Scientific, Waltham, MA) and α-bungarotoxin conjugated to Alexafluor 555 (αBT, 1:500, Sigma-Aldrich, Darmstadt, Germany) to label motor end plates. Once sections were dry, glass coverslips were mounted using Entellan (Millipore, Darmstadt, Germany). For each muscle, 50 motor endplates were imaged as above. Endplates were scores as reinnervated only if VAChT immunofluorescence completely filled the endplate (Figure 3A).

Dorsal Root Ganglion Cell Culture. Adult mice (>2 months of age) were deeply anesthetized with an overdose of isoflurane. The vertebral column was removed and washed with cold, sterile Hank's basal salt solution (HBSS, Corning, Corning, New York). All dorsal root ganglia (DRG) were dissected out bilaterally and placed in fresh cold HBSS. Once the dissection was complete, DRGs were incubated in dispase (2.5u/mL Sigma-Aldrich) and collagenase (200u/mL Worthington Biochemical, Lakewood, NJ) in a 37°C bead bath for 45 minutes with gentle agitation applied every 15 minutes. Dispase was then removed, and DRGs were incubated with 37°C DNase (Worthington Biochemical) for 2.5 minutes. Room temperature HBSS was added, and cells were triturated using a fire polished glass pipette and centrifuged for 3 minutes at 3000 rpm. The HBSS was removed and cells were resuspended in Neurobasal medium A (NB-A, Invitrogen, Carlsbad, CA) containing 2% B-27 (Invitrogen), 1% Penicillin/Streptomycin (Lonza Biowhittaker), and 1% Glutamax (Invitrogen). Cells were plated on glass coverslips coated in laminin (Thermo Fisher Scientific, Waltham, MA) and poly-L-lysine (Sigma-Aldrich). Twenty-four hours after plating, the NB-A solution was replaced. For drug groups, LM11A-31 (100nM), trkB-FC (5.63nM), or ANA-12 (100nM) was included in the NB-A. Media was collected at 24 hours for future analysis of protein levels.

Seventy-two hours after plating, cells were fixed with cold PLP for fifteen minutes. After three washes and blocking with natural goat serum and triton, cells were incubated overnight with anti-tubulin β-3 antibody overnight (14-16 hours, Biolegend, San Diego, CA). The following morning, cells were washed with 0.1M PBS and incubated with a secondary antibody, goat anti-mouse antibody conjugated to Alexafluor 555 (Invitrogen) and DAPI (Invitrogen). Coverslips were mounted onto slides using Entellan. The cell soma area and longest processes from each cell were measured from these images using the Fiji software package (ImageJ).

Experimental Design and Statistics. All results were scored while blinded to treatment and genotype. Data analyses were performed in Statistica 64. Two-way ANOVA of genotype by treatment were performed for the different experiments. Post-hoc testing was performed using

Fishers LSD. Planned comparisons were performed using student's t-test. Untreated control values for MUNE were previously reported in McGregor et al., 2018 (n=11-14). For *in vivo* ANA-12 and LM11A-31 experiments, n=4-6. For *in vitro* experiments, at least 30 cells per well were measured, and they were averaged. Averaged values from 2-4 animals were used.

Results

trkB blockade inhibited functional recovery in heterozygous Met-carriers. Four weeks after injury, the number of successfully reinnervated motor units was investigated through EMG recording of the lateral gastrocnemius. Individual single motor unit amplitudes were recorded as well as the maximum compound muscle action potentials. By dividing, we calculated the estimated number of motor units innervating the lateral gastrocnemius. Control values for the three genotypes were previously reported in McGregor et al., 2018.

A trkB antagonist (ANA-12) was applied to the nerve at the time of injury, and MUNE was recorded four weeks later. This method of drug delivery results in drug release over three days (Spicer and Mikos, 2010). Two-way ANOVA revealed a significant effect of genotype*treatment on MUNE ($F(2,45)=3.72$, $p=0.032$). As previously reported in McGregor et al., the untreated V/V MUNE value is significantly smaller than V/M and M/M ($p=0.0034$ and $p=0.0079$ respectively). ANA-12 application resulted in significantly decreased MUNE in V/M only from 43.37±8.19 to 12.23±4.19 ($p=0.011$, Figure 1A). This value was not significantly different from untreated V/V ($p=0.76$). In homozygous M/M animals, ANA-12 application resulted in a moderately decreased MUNE (42.11±7.29 to 26.68±7.96), but this did not reach significance ($p=0.25$). Treatment with ANA-12 did result in decreasing M/M MUNE such that it was no longer significantly different from untreated V/V ($p=0.40$). ANA-12 treatment resulted in a slight increase in MUNE in wild type V/V animals (15.21±2.26 to 29.80±10.92), but this also did not reach significance ($p=0.20$).

The application of a $p75^{NTR}$ antagonist (LM11A-31) to the injured nerve at the time of injury did not result in a significant overall change in MUNE (two-way ANOVA, Figure 1B). A planned comparison between untreated V/V animals (15.21±2.26) and V/V animals treated with LM11A-31 (43.08±16.29) revealed a significant effect of the drug on MUNE ($p=0.017$). There was no significant effect of LM11A-31 on V/M or M/M MUNE (p=ns).

trkB blockade inhibited motor axon regeneration in V/M mice. To assay successful motor axon regeneration into the lateral gastrocnemius, we injected a retrograde tracer into the muscle and counted the number of labeled motoneurons. The uninjured side was used as a control, and values are given as a ratio of injured/intact.

Application of ANA-12 to the injured nerve resulted in decreased motor axon regeneration in all three genotypes (two-way ANOVA, significant effect of treatment ($F(1,27)=4.68$, $p=0.039$). trkB blockade had no effect on fold-change on the ratio of injured/intact motoneurons in V/V mice (0.54, 95% CI [-0.15, 1.2]) or M/M mice (0.73, 95% CI [0.14, 1.59] Figure 2B). Fold-change in the ratio of injured/intact labeled motoneurons was significantly inhibited in V/M mice (0.46, 95% CI [0.17, 0.76]). The omnibus result of a one-way ANOVA revealed no significant effect of genotype on fold-change with ANA-12 treatment ($F(2,12)=1.07$, $p=0.37$).

The omnibus result of a one-way ANOVA revealed a significant effect of genotype on fold-change in motoneuron proportion in response to treatment with LM11A-31 ($F(2,11)=5.65$, $p=0.020$, Figure 2C). V/V had almost 2-fold change in proportion of retrogradely labeled motoneurons with LM11A-31 treatment (95% CI [1.11, 2.68]). This was significantly different from both V/M ($p=0.010$) and M/M ($p=0.018$). V/M and M/M were not different from each other ($p=0.96$).

trkB blockade increased motor endplate reoccupation. To assay successful reinnervation of the muscle fibers, we determined the proportion of motor endplates covered by motoneuron terminals. Endplates were labeled with α-bungarotoxin, a marker of acetylcholine receptors, and motor terminals were labeled with VAChT, a marker of cholinergic motor terminals (Figure 3A). Two-way ANOVA revealed a significant effect of genotype ($F(2,26)=4.11$, $p=0.028$), treatment ($F(1,26)=6.93$, $p=0.014$), and genotype*treatment interaction ($F(2,26)=7.55$, $p=0.0026$) on motor endplate reinnervation. Application of ANA-12 at time of injury significantly increased the percentage of filled motor endplates in M/M mice from 23.67±5.87% to 51.6±7.96% ($p=0.0026$,

Figure 3B). The percentage of VAChT-positive motor endplates in V/V mice also increased from 8.4±2.71% to 32.5±6.29% (p=0.015). The decrease in proportions in V/M mice from 32.33±6.21 to 19.33±5.46% was not statistically significant (p=0.12). Similar to previous reports, muscles collected from untreated V/V mice had a significantly smaller percentage of VAChT-positive motor endplates compared to muscles from untrained V/M mice (p=0.0083, McGregor et al., submitted).

Applying the $p75^{NTR}$ antagonist LM11A-31 to the nerve at the time of injury had no significant effect on motor endplate coverage (two-way ANOVA, genotype ($F(2,26)=2.37$, $p=0.11$); treatment ($F(1,26)=0.032$, $p=0.86$); or genotype*treatment ($F(2,26)=1.63$, $p=0.22$), Figure 3C). A planned comparison between LM11A-31 treated V/V animals and untreated V/V animals did not reveal a significant difference in VAChT covered motor endplates (p=0.078).

trkB inhibition results in decreased longest neurite length in DRG neurons from M/M mice. Adult DRG neurons were cultured for three days. For 48 hours, cultures were incubated with media containing trkB-Fc, ANA-12, or LM11A-31. After three days, cells were fixed and labeled for beta tubulin so that longest neurite lengths could be analyzed (Figure 4A).

As previously reported, there was a significant effect of genotype ($F(2,13)=16.51$, $p=0.00027$) on longest neurite length (Figure 4B). Untreated M/M neurons grew the longest neurites with an average of 218 ± 12.2 µm. This was significantly longer than both V/M neurites (127.7±27.7 µm, p=0.0028) and V/V neurites (101.68 ± 21.5 µm, p=0.00016). V/M neurites were not significantly longer than V/V neurites (p=0.16).

Two-way ANOVA of genotype by treatment revealed a significant effect of ANA-12 treatment on longest neurite outgrowth ($F(1,13)=7.25$, $p=0.018$, Figure 4B). In DRG neurons from M/M mice, the endogenous increased neurite outgrowth was blocked by treatment of 100nM ANA-12 for 48 hours (133.6 ± 13.0 µm, p=0.0026, Figure 4B). In cells from M/M mice treated with ANA-12, longest neurite lengths were not significantly different than untreated V/V

cells (p=0.093). ANA-12 had no significant effect on neurite length in V/M or V/V neurons (p=ns).

Two-way ANOVA of genotype by treatment revealed a significant effect of treatment on longest neurite length in DRG neurons treated with trkB-Fc ($F(1,15)=4.72$, $p=0.046$, Figure 4C). Treatment with the trkB fusion protein that sequesters all trkB ligands blocked the endogenous enhanced neurite outgrowth in M/M cells (153.3±9.38, $p=0.034$). Longest neurite length in trkB-Fc treated M/M cells were not significantly different from untreated V/V cells ($p=0.086$).

Two-way ANOVA of genotype by treatment revealed a significant effect of genotype on longest neurite length ($F(1,7)=8.08$, $p=0.25$, Figure 4D). Treatment of DRG neurons from M/M mice with LM11A-31 resulted in decreased longest neurite length (132.4±8.9, $p=0.042$).

Discussion

We have previously reported that axon regeneration after peripheral nerve injury is enhanced in mice carrying the met allele of the BDNF Val66Met polymorphism (McGregor et al., 2018). Here, we tested the effect of antagonizing two different BDNF receptors—the trkB receptor, which is involved in progrowth signaling, and $p75^{NTR}$, which is involved in anti-growth signaling—in order to elucidate how this allele might be conferring enhanced regeneration. Application of trkB antagonist ANA-12 to the nerve at the time of injury attenuated the enhanced regeneration in V/M mice and abolished enhanced neurite outgrowth in cultured DRG neurons both heterozygous and homozygous for the met allele, indicating that this enhancement is trkB-dependent. Furthermore, application of trkB-Fc, a soluble fusion protein which sequesters BDNF ligands, also attenuated enhanced neurite outgrowth in Met-carriers. Application of the $p75^{NTR}$ antagonist, LM11A-31, produced a enhancement on regeneration in V/V mice, but no effect on regeneration in the Met-carriers.

The trkB-dependence of the enhanced regeneration seen in Met-carriers has led us to a number of hypotheses. The first is that trkB expression is increased in the injured sensory and motor neurons of mice with the met allele. With increased expression of trkB, limited ligand secretion could result in more efficient signaling. Higher levels of trkB protein and lower levels of the dominant-negative form of trkB have been found in the hippocampus of M/M animals (Notaras et al., 2017). If this were the mechanism of basal levels of enhanced peripheral axon regeneration in Met-carriers, application of a trkB inhibitor to the injured nerve would result in worse regeneration, and application of LM11A-31 would have no effect, as all available ligand already preferentially binds trkB. This prediction of the hypothesis is consistent with our results. Further evidence through trkB protein analysis of motoneurons, DRG neurons, and Schwann cells is warranted.

Another possible mechanism to explain the enhanced axon regeneration found in met allele carrier mice is increased secretion of ligands for trkB. Basal levels of BDNF secretion

have not been found to be different in Met-carriers *in vitro,* in tissue samples, nor in human serum (Chen et al., 2006, Egan et al., 2003, Lemos et al., 2015, Notaras et al., 2017, McGregor et al., 2018). Increased secretion of neurotrophin 4/5 (NT4/5), which binds trkB and is not released in an activity-dependent manner, could account for higher levels of ligand (Lessmann et al., 2003). Application of ANA-12 would block the effects of increased ligand, as would the soluble antibody, trkB-FC. While this is consistent with our results, application of a $p75^{NTR}$ antagonist would likely enhance regeneration in this scenario, as high levels of ligand generally result in $p75^{NTR}$ activation and anti-growth signaling (Boyd and Gordon, 2002, Boyd and Gordon, 2001). This is inconsistent with our findings, in which LM11A-31 applied to the fibrin glue had no effect on regeneration *in vivo* nor did LM11A-31 in the media have any effect *in vitro* in the Met-carriers.

We also considered the hypothesis that in Met-carriers, trkB could be ligand-independently transactivated to compensate for decreased BDNF availability. It is known that trkB can be transactivated downstream for the activation of a number of receptors, as well as by zinc ions (Lee and Chao, 2001, Lee et al., 2002, Iwakura et al., 2008, Hwang et al., 2005). Our data do not support this hypothesis, as trkB-FC, which sequesters all ligands for trkB, blocked enhanced neurite outgrowth when added to the media of the DRG cultures. Therefore, the actions of trkB in the met-carrier mice must be ligand-dependent.

The application of LM11A-31 to the injured nerve at the time of injury did not enhance regeneration in the Met-carriers, although in MUNE, retrograde labeling, and VAChT coverage there was modest improvement in the V/V mice with LM11A-31 that did not reach statistical significance with VAChT coverage. This could be a dosing issue—fibrin glue drug delivery lasts for three days, and any effect could have been masked by the four week period before analysis of axon regeneration (Spicer and Mikos, 2010). Others using oral administration of LM11A-31 have found increased neurogenesis and decreased activation of downstream effectors of

$p75^{NTR}$ (Shi et al., 2013). Surprisingly, there was no effect of LM11A-31 on V/V DRG neurite outgrowth *in vitro.*

The effect of blocking $p75^{NTR}$ on peripheral nerve regeneration is anything but clear. In knockout studies, deleting $p75^{NTR}$ has been found to be detrimental to regeneration, particularly with regard to Schwann cell remyelination (Song et al., 2009, Cosgaya et al., 2002). Others have found deletion of $p75^{NTR}$ enhances regeneration of DRG neurons and motoneurons (Scott and Ramer, 2010, Boyd and Gordon, 2001). Both drug antagonism and genetic knockout affect all cells—although by adding the antagonist to glue, we limited the site of action to cells at site of injury—mainly the Schwann cells and growth cones of the injured neurons. Further investigation using cell-type specific knock out of $p75^{NTR}$ could elucidate the role of this complicated protein in peripheral axon regeneration.

Met-carriers, both human and rodent, have been found to have smaller hippocampi, perhaps resulting from decreased dendritic arborization (Egan et al., 2003, Chen et al., 2006). Indeed, Chen et al., used Golgi staining to visualize the dendritic arbors of M/M hippocampal neurons, and found them to be smaller than their wild type counterparts (Chen et al., 2006). Previous reports have indicated that the prodomain of $BDNF_{Met}$ is bioactive, and could play a role in inhibiting dendritic and axonal growth through $p75^{NTR}$, providing a mechanism for this deficient growth (Anastasia et al., 2013, Giza et al., 2018). Here, we applied a $p75^{NTR}$ antagonist, but did not find further enhancement of V/M or M/M regeneration or neurite outgrowth. In fact, $p75^{NTR}$ antagonism resulted in shorter neurites in DRG neurons cultured from M/M mice. This could be because $p75^{NTR}$ is required for axonal transport of BDNF/trkB signaling endosomes, which travel to the cell body to influence somal signal transduction (Curtis et al., 1995, von Bartheld et al., 1996).

Unlike motoneuron regeneration and DRG neuron neurite outgrowth, application of ANA-12 to the injured nerve stump enhanced motor endplate reoccupation both in V/V mice and M/M mice. ANA-12 decreased the proportion of motoneurons regenerating into the muscle by over

half in the V/V mice, but significantly increased the percentage of VAChT-positive endplates by almost four times. BDNF has been shown to inhibit the maturation of neuromuscular synapses (Song and Jin, 2015), and our results are consistent with this conclusion.

Conclusion

The enhanced regeneration is found in Met-carriers is trkB-dependent. Further protein analysis of both trkB and its ligands would provide further insight into how trkB is enhancing regeneration. In peripheral nerve regeneration in Met-carriers, $p75^{NTR}$ has little effect on basal regeneration.

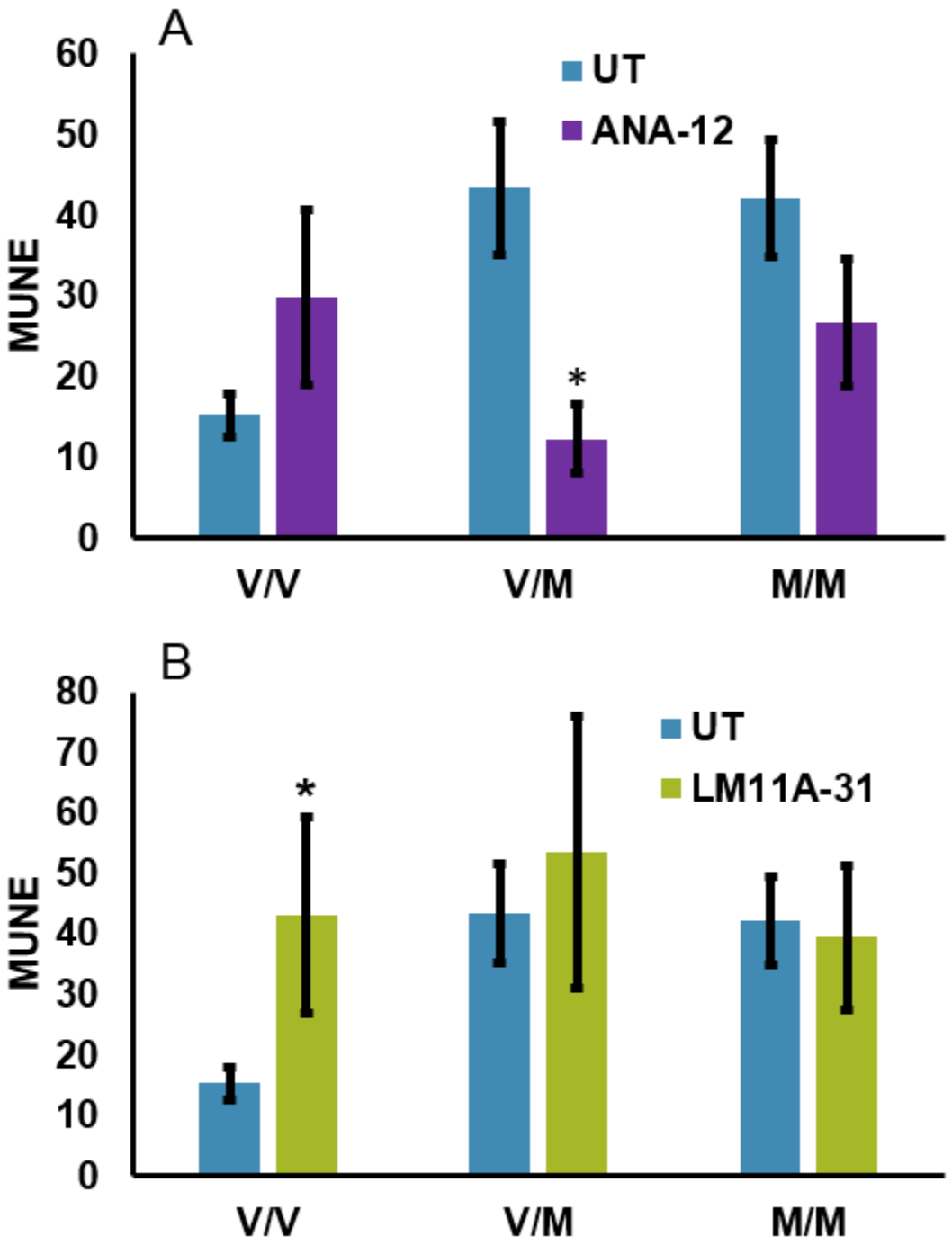


Figure 1. **Motor unit number estimation four weeks after injury with treatment with trkB antagonist ANA-12 and p75^{NTR} antagonist LM11A-31.** A. As previously reported, V/M and M/M mice had increased MUNE four weeks after injury with no treatment. Inhibiting trkB blocked basal levels of enhanced regeneration in V/M mice. There was no significant effect in V/V or

M/M mice. Asterisk denotes $p<0.05$ with respect to untreated V/M. B. Treatment with LM11A-31 resulted in enhancement of V/V MUNE four weeks after injury. No effect was seen in V/M or M/M mice. Asterisk denotes $p<0.05$ with respect to UT V/V.

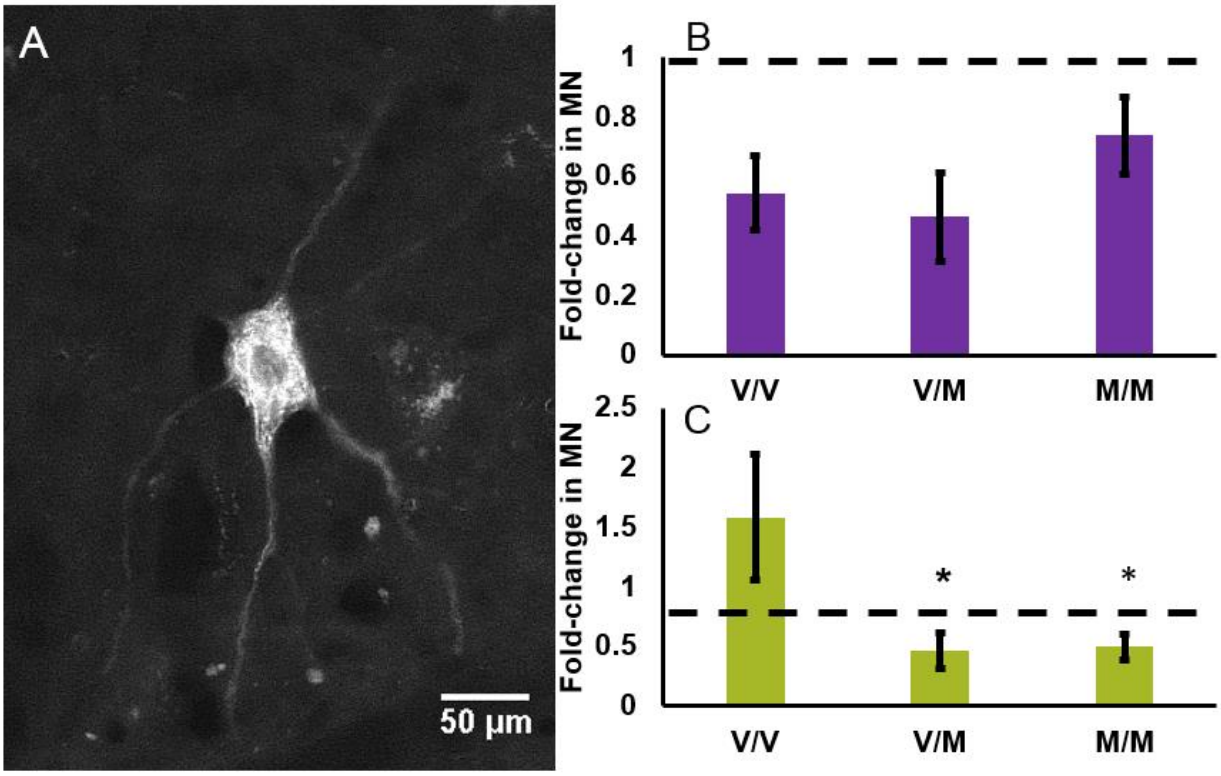


Figure 2. **Motor axon regeneration into the lateral gastrocnemius.** A. CTB conjugated to Alexafluor 555 was injected into the lateral gastrocnemius. Labeled motoneurons were counted on both the injured and intact sides. B. Treatment with ANA-12 resulted in decreased motor axon regeneration in all three genotypes. Values are given as injured over intact. C. LM11A-31 resulted in a modest enhancement in motor axon regeneration in V/V mice, but not V/M or M/M mice. Asterisks denote $p<0.05$. Error bars are SEM.

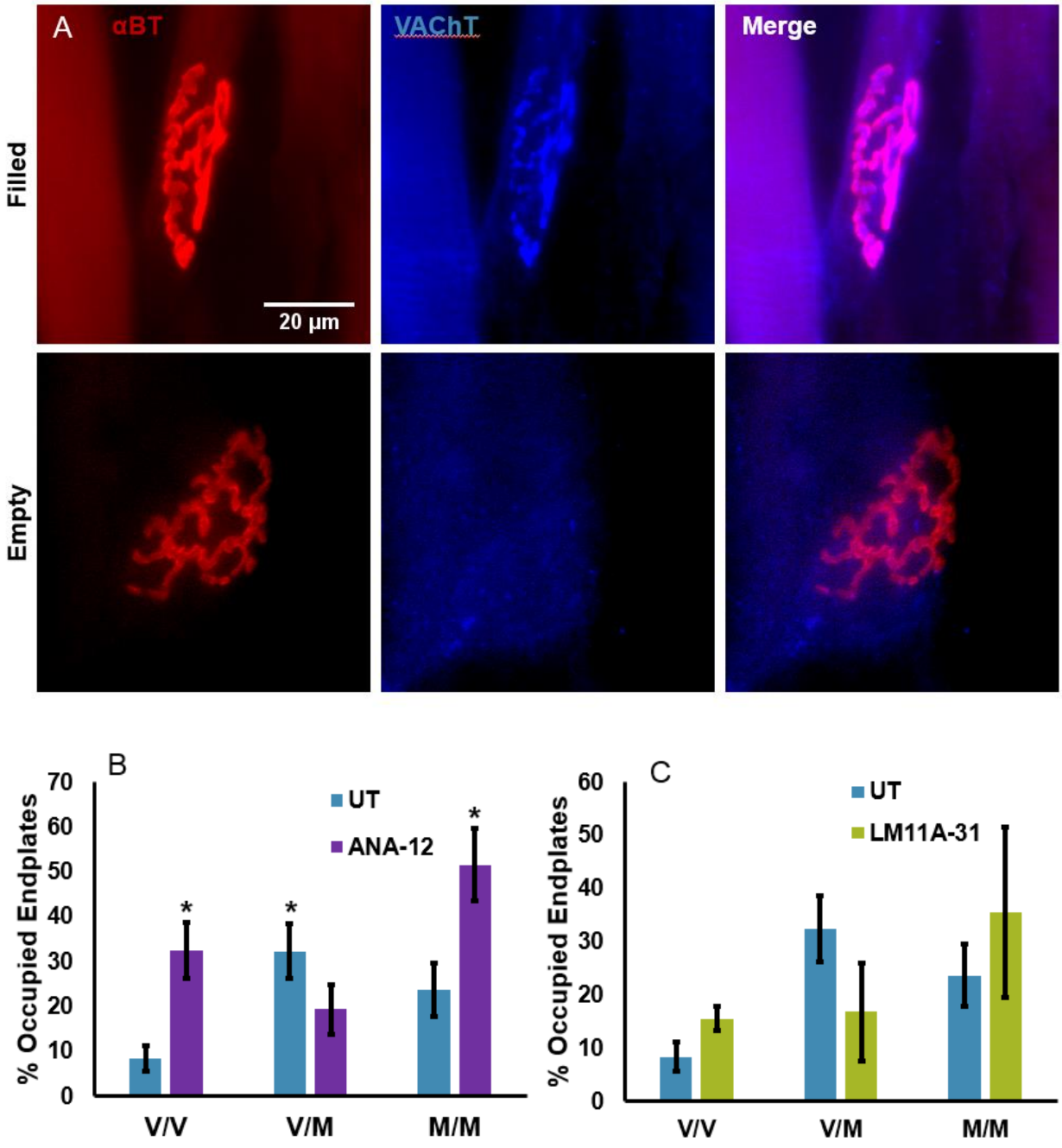


Figure 3. **VAChT labeling of motor endplates**. A. Motor endplates were labeled with α-bungarotoxin (left panel). Motor axon terminals were labeled with an antibody against VAChT (middle panel). Motor endplates were scored as occupied when completely covered with VAChT (top panel). B. Treatment with ANA-12 resulted in improved motor endplate occupation in V/V

and M/M mice. V/M mice had increased motor endplate occupation with no treatment compared to V/V mice. ANA-12 treated M/M mice had increased occupied motor endplates compared to all other groups. Asterisks denote $p<0.05$ compared to untreated V/V. B. Treatment with LM11A-31 had no effect on motor endplate occupation 4 weeks after peripheral nerve transection.

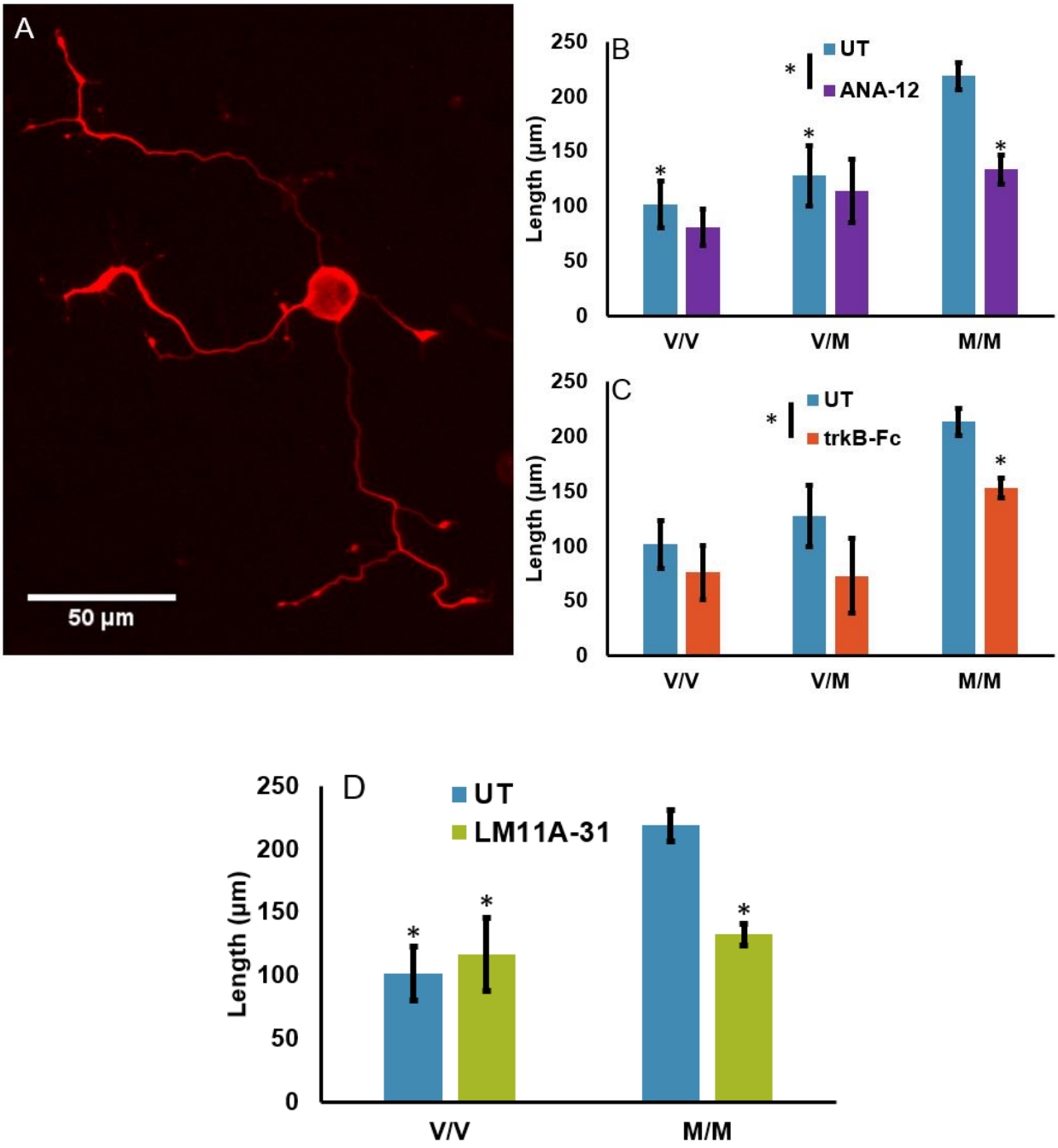


Figure 4. **trkB inhibition blocked enhanced neurite outgrowth in cells from M/M mice**. A. Cells were labeled with anti-β tubulin III seventy-two hours after plating and longest neurite lengths were analyzed. B. Enhanced basal levels of neurite outgrowth were found from adult

DRG neurons cultured from M/M mice. Treatment with 100nM ANA-12 blocked this enhanced outgrowth. Asterisks denote $p<0.05$ with respect to UT M/M. C. Treatment with 5.63nM trkB-Fc had a significant effect on longest neurite length. Enhanced neurite outgrowth from M/M neurons was blocked by trkB-Fc treatment. Asterisk denotes $p<0.05$ with respect to UT M/M. D. Treatment with 100nM of LM11A-31 had a significant effect on longest neurite length. Asterisks denote $p<0.05$ with respect to UT M/M. Error bars are for SEM.

References

ANASTASIA, A., DEINHARDT, K., CHAO, M., WILL, N., IRMADY, K., LEE, F., HEMPSTEAD, B. & BRACKEN, C. 2013. Val66Met polymorphism of BDNF alters prodomain structure to induce neuronal growth cone retraction. *Nat Commun,* 4**,** 2490.

BOYD, J. G. & GORDON, T. 2001. The neurotrophin receptors, trkB and p75, differentially regulate motor axonal regeneration. *J Neurobiol,* 49**,** 314-25.

BOYD, J. G. & GORDON, T. 2002. A dose-dependent facilitation and inhibition of peripheral nerve regeneration by brain-derived neurotrophic factor. *Eur J Neurosci,* 15**,** 613-26.

BOYD, J. G. & GORDON, T. 2003. Neurotrophic factors and their receptors in axonal regeneration and functional recovery after peripheral nerve injury. *Mol Neurobiol,* 27**,** 277-324.

CAZORLA, M., PRÉMONT, J., MANN, A., GIRARD, N., KELLENDONK, C. & ROGNAN, D. 2011. Identification of a low–molecular weight TrkB antagonist with anxiolytic and antidepressant activity in mice. *J Clin Invest,* 121**,** 1846-1857.

CHEN, Z., JING, D., BATH, K., IERACI, A., KHAN, T., SIAO, C., HERRERA, D., TOTH, M., MCEWEN, B., HEMPSTEAD, B. & LEE, F. 2006. Genetic variant BDNF (Val66Met) polymorphism alters anxiety-related behavior. *Science,* 314**,** 140-143.

CHEN, Z., PATEL, P., SANT, G., MENG, C., TENG, K., HEMPSTEAD, B. & LEE, F. 2004. Variant brain-derived neurotrophic factor (BDNF) (Met66) alters the intracellular trafficking and activity-dependent secretion of wild-type BDNF in neurosecretory cells and cortical neurons. *J Neurosci,* 24**,** 4401-4411.

COSGAYA, J. M., CHAN, J. R. & SHOOTER, E. M. 2002. The neurotrophin receptor p75NTR as a positive modulator of myelination. *Science,* 298**,** 1245-8.

CURTIS, R., ADRYAN, K. M., STARK, J. L., PARK, J. S., COMPTON, D. L., WESKAMP, G., HUBER, L. J., CHAO, M. V., JAENISCH, R., LEE, K. F. & ET AL. 1995. Differential role of the low affinity neurotrophin receptor (p75) in retrograde axonal transport of the neurotrophins. *Neuron,* 14**,** 1201-11.

DE VRIES, J., MENOVSKY, T., VAN GULIK, S. & WESSELING, P. 2002. Histological effects of fibrin glue on nervous tissue: a safety study in rats. *Surg Neurol,* 57**,** 415-22; discussion 422.

DOHERTY, T. J. & BROWN, W. F. 1993. The estimated numbers and relative sizes of thenar motor units as selected by multiple point stimulation in young and older adults. *Muscle Nerve,* 16**,** 355-66.

EGAN, M., KOJIMA, M., CALLICOTT, J., GOLDBERG, T., KOLACHANA, B., BERTOLINO, A., ZAITSEV, E., GOLD, B., GOLDMAN, D., DEAN, M., LU, B. & WEINBERGER, D. 2003. The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. *Cell,* 112**,** 257-269.

ENGLISH, A. 2005. Enhancing axon regeneration in peripheral nerves also increases functionally inappropriate reinnervation of targets. *J Comp Neurol,* 490**,** 427-441.

ENGLISH, A. W., WILHELM, J. C. & WARD, P. J. 2014. Exercise, neurotrophins, and axon regeneration in the PNS. *Physiology (Bethesda),* 29**,** 437-45.

GIZA, J. I., KIM, J., MEYER, H. C., ANASTASIA, A., DINCHEVA, I., ZHENG, C. I., LOPEZ, K., BAINS, H., YANG, J., BRACKEN, C., LISTON, C., JING, D., HEMPSTEAD, B. L. & LEE, F. S. 2018. The BDNF Val66Met Prodomain Disassembles Dendritic Spines Altering Fear Extinction Circuitry and Behavior. *Neuron,* 99**,** 163-178.e6.

GORDON, T., AMIRJANI, N., EDWARDS, D. C. & CHAN, K. M. 2010. Brief post-surgical electrical stimulation accelerates axon regeneration and muscle reinnervation without affecting the functional measures in carpal tunnel syndrome patients. *Exp Neurol,* 223**,** 192-202.

HWANG, J. J., PARK, M. H., CHOI, S. Y. & KOH, J. Y. 2005. Activation of the Trk signaling pathway by extracellular zinc. Role of metalloproteinases. *J Biol Chem,* 280**,** 11995-2001.

IWAKURA, Y., NAWA, H., SORA, I. & CHAO, M. V. 2008. Dopamine D1 Receptor-induced Signaling through TrkB Receptors in Striatal Neurons. *J Biol Chem,* 283**,** 15799-15806.

LEE, F. S. & CHAO, M. V. 2001. Activation of Trk neurotrophin receptors in the absence of neurotrophins. *Proc Natl Acad Sci U S A,* 98**,** 3555-3560.

LEE, F. S., RAJAGOPAL, R., KIM, A. H., CHANG, P. C. & CHAO, M. V. 2002. Activation of trk neurotrophin receptor signaling by pituitary adenylate cyclase-activating polypeptides. *J Biol Chem,* 277**,** 9096-9102.

LEMOS, J. R., ALVES, C. R., DE SOUZA, S. B. C., MARSIGLIA, J. D. C., SILVA, M. S. M., PEREIRA, A. C., TEIXEIRA, A. L., VIEIRA, E. L. M., KRIEGER, J. E., NEGRÃO, C. E., ALVES, G. B., DE OLIVEIRA, E. M., BOLANI, W., DIAS, R. G. & TROMBETTA, I. C. 2015. Peripheral vascular reactivity and serum BDNF responses to aerobic training are impaired by the BDNF Val66Met polymorphism. *Physiol Genomics,* 48**,** 116-123.

LESSMANN, V., GOTTMANN, K. & MALCANGIO, M. 2003. Neurotrophin secretion: current facts and future prospects. *Prog Neurobiol,* 69**,** 341-374.

MACGILLIVRAY, T. E. 2003. Fibrin sealants and glues. *J Card Surg,* 18**,** 480-5.

MASSA, S. M., XIE, Y., YANG, T., HARRINGTON, A. W., KIM, M. L., YOON, S. O., KRAEMER, R., MOORE, L. A., HEMPSTEAD, B. L. & LONGO, F. M. 2006. Small, nonpeptide p75NTR ligands induce survival signaling and inhibit proNGF-induced death. *J Neurosci,* 26**,** 5288-300.

MCCOMAS, A. J., FAWCETT, P. R., CAMPBELL, M. J. & SICA, R. E. 1971. Electrophysiological estimation of the number of motor units within a human muscle. *J Neurol Neurosurg Psychiatry,* 34**,** 121-31.

MCGREGOR, C. E., IRWIN, A. M. & ENGLISH, A. W. 2018. The Val66Met BDNF polymorphism and peripheral nerve injury: Enhanced regeneration in Met-carriers that is not further improved with activity-dependent treatment. *J Neurosci,* submitted.

MCLEAN, I. W. & NAKANE, P. K. 1974. Periodate-lysine-paraformaldehyde fixative. A new fixation for immunoelectron microscopy. *J Histochem Cytochem,* 22**,** 1077-83.

NOTARAS, M., DU, X., GOGOS, J., VAN DEN BUUSE, M. & HILL, R. A. 2017. The BDNF Val66Met polymorphism regulates glucocorticoid-induced corticohippocampal remodeling and behavioral despair. *Transl Psychiatry,* 7**,** e1233.

PORTINCASA, A., GOZZO, G., PARISI, D., ANNACONTINI, L., CAMPANALE, A., BASSO, G. & MAIORELLA, A. 2007. Microsurgical treatment of injury to peripheral nerves in upper and lower limbs: a critical review of the last 8 years. *Microsurgery,* 27**,** 455-462.

SABATIER, M. J., TO, B. N., NICOLINI, J. & ENGLISH, A. W. 2011. Effect of slope and sciatic nerve injury on ankle muscle recruitment and hindlimb kinematics during walking in the rat. *J Exp Biol,* 214**,** 1007-16.

SCOTT, A. L. M. & RAMER, M. S. 2010. Schwann cell p75NTR prevents spontaneous sensory reinnervation of the adult spinal cord. *Brain,* 133**,** 421-432.

SHI, J., LONGO, F. M. & MASSA, S. M. 2013. A small molecule p75(NTR) ligand protects neurogenesis after traumatic brain injury. *Stem Cells,* 31**,** 2561-74.

SHIMIZU, E., HASHIMOTO, K. & IYO, M. 2004. Ethnic difference of the BDNF 196G/A (val66met) polymorphism frequencies: the possibility to explain ethnic mental traits. *Am J Med Genet B Neuropsychiatr Genet,* 126b**,** 122-3.

SONG, W. & JIN, X. A. 2015. Brain-derived neurotrophic factor inhibits neuromuscular junction maturation in a cAMP-PKA-dependent way. *Neurosci Lett,* 591**,** 8-12.

SONG, X. Y., ZHANG, F. H., ZHOU, F. H., ZHONG, J. & ZHOU, X. F. 2009. Deletion of p75NTR impairs regeneration of peripheral nerves in mice. *Life Sci,* 84**,** 61-8.

SPICER, P. P. & MIKOS, A. G. 2010. Fibrin glue as a drug delivery system. *J Control Release,* 148**,** 49-55.

STEIN, R. B. & YANG, J. F. 1990. Methods for estimating the number of motor units in human muscles. *Ann Neurol,* 28**,** 487-95.

VON BARTHELD, C. S., WILLIAMS, R., LEFCORT, F., CLARY, D. O., REICHARDT, L. F. & BOTHWELL, M. 1996. Retrograde transport of neurotrophins from the eye to the brain in chick embryos: roles of the p75NTR and trkB receptors. *J Neurosci,* 16**,** 2995-3008.

WILHELM, J., XU, M., CUCORANU, D., CHMIELEWSKI, S., HOLMES, T., LAU, K., BASSELL, G. & ENGLISH, A. 2012. Cooperative roles of BDNF expression in neurons and Schwann cells are modulated by exercise to facilitate nerve regeneration. *J Neurosci,* 32**,** 5002-5009.

WONG, J. N., OLSON, J. L., MORHART, M. J. & CHAN, K. M. 2015. Electrical stimulation enhances sensory recovery: A randomized controlled trial. *Ann Neurol,* 77**,** 996-1006.

Chapter 4:

General Discussion and Future Directions

Discussion

The underlying hypothesis of this thesis was that activity-dependent treatments to enhance peripheral nerve regeneration would be ineffective in rodents both heterozygous and homozygous for the met allele of the BDNF Val66Met polymorphism. This was tested both *in vivo* using treadmill training as well as *in vitro* using optogenetic stimulation of cultured adult DRG neurons expressing the light sensitive cation channel, channelrhodopsin (ChR2). The original hypothesis was supported, and Met-carriers did not respond to these activity-dependent treatments; however, enhanced axon regeneration was found in Met-carriers *in vivo,* which was then recapitulated with the finding of enhanced neurite outgrowth *in vitro*. These results led to two important questions—could axon regeneration in Met-carriers be further enhanced, and what was mechanism behind their enhanced regeneration?

The enhanced regeneration was originally found *in vivo*. The first question regarding mechanism was whether systemic changes within the animal led to the enhanced regeneration, or whether there were intrinsic neuronal differences that caused enhanced axonal growth. Met-carrying mice have some phenotypic differences from wild type—they are heavier, particularly the males, more aggressive, and have increased anxiety-like behavior (Chen et al., 2006). Like humans with the met allele, the mice have smaller hippocampi (Egan et al., 2003, Chen et al., 2006). Importantly, M/M mice do not differ from their V/V counterparts in basal levels of activity recorded in in open field tests (Chen et al., 2006, Lee et al., 2015, Yu et al., 2012, Li et al., 2010) or in voluntary wheel running (Chen et al., 2017, Ieraci et al., 2016). Thus, to test whether enhanced regeneration was a result of intrinsic neuronal properties, we cultured adult DRG neurons. Neurite outgrowth was enhanced in both V/M and M/M cultures, indicating that the enhancement in axonal outgrowth is a neuronal property and not a result of other phenotypic changes. In fact, enhanced neurite outgrowth in the homozygous M/M cultures was more robust than the enhanced axon regeneration seen *in vivo*, indicating that phenotypic changes might actually inhibit natural regenerative capabilities in these animals.

The prodomain of $BDNF_{Met}$ has been found to activate the pan-neurotrophin receptor $p75^{NTR}$, resulting in growth cone retraction and dendrite retraction (Anastasia et al., 2013, Giza et al., 2018). This differs from the cleaved prodomain of $BDNF_{Val}$, which is unable to activate the receptor. Thus, to test whether axon regeneration could be further enhanced in Met-carriers, the first target was manipulation of $p75^{NTR}$ signaling. Therefore, LM11A-31, a $p75^{NTR}$ inhibitor, was applied to the site of injury in V/V, V/M and M/M mice (Shi et al., 2013). The drug was mixed with the fibrin glue used to secure the two nerve stumps together during repair. This method results in drug dispersal over the course of three days (Spicer and Mikos, 2010). Although there was a trend toward modest improvement in the V/V mice, this treatment did not enhance axon regeneration *in vivo* in the V/M and M/M mice, nor did it have an effect *in vitro* on neurite outgrowth, indicating that $p75^{NTR}$ activation after peripheral nerve injury has no significant effect on basal levels of regeneration in Met-carriers, and blocking it could not further enhance their regeneration.

To determine the mechanism behind the enhanced basal levels of regeneration in the Met-carriers, the trkB antagonist ANA-12 was applied to the cultures of adult DRG neurons. Addition of ANA-12 to the media resulted in abolishment of enhanced neurite outgrowth in both V/M and M/M cultures. This was a surprise; it indicated that the basal levels of increased neurite outgrowth were trkB-dependent, despite no differences in basal levels of BDNF secretion between genotypes (Chen et al., 2006, Notaras et al., 2017, McGregor et al., 2018). Thus, several possible trkB-dependent mechanisms for enhanced regeneration in Met-carriers were hypothesized and will be explored below (Figure 1):

1. There is more trkB expression in Met-carriers.
2. There are higher levels of trkB ligand secretion in Met-carriers.
3. trkB is activated in a ligand-independent mechanism in Met-carriers4

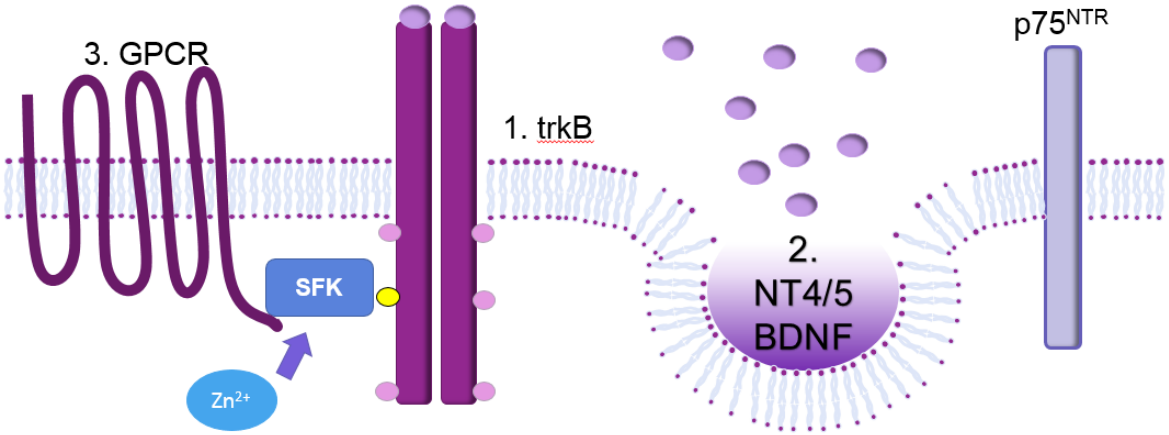


Figure 1. Possible mechanisms of enhanced regeneration in Met-Carriers. 1. Increased expression of trkB receptor. With increased trkB receptor, secreted ligands are more likely to bind trkB and less likely to bind $p75^{NTR}$. 2. Increased constitutive secretion of trkB ligands. NT-4/5 is a ligand for trkB, and a compensatory increase in secretion could enhance regeneration. 3. Ligand-independent transactivation by other molecules which activate SFKs to phosphorylate trkB. Numerous receptors including GPCRs, RTKs, as well as zinc ions have been shown to transactivate trkB.

There is more trkB expression in Met-carriers

BDNF can bind both trkB and $p75^{NTR}$. Both of these receptors are expressed by motoneurons, DRG neurons, and Schwann cells, though in the case of the latter, only the non-signaling, truncated form of trkB is expressed. It has been proposed that through these two receptors, BDNF has a biphasic regulation of axon regeneration (Boyd and Gordon, 2003). Mature BDNF has a higher affinity for trkB than $p75^{NTR}$, and at lower levels of exogenous BDNF application, trkB is activated, and axon regeneration is enhanced (Boyd and Gordon, 2002). As levels of BDNF applied are increased, trkB receptors become saturated with ligand, and $p75^{NTR}$ becomes activated and inhibits growth (Boyd and Gordon, 2002). Activation of $p75^{NTR}$ could happen within the injured neurons or in nearby Schwann cells, but both result in growth inhibition (Scott and Ramer, 2010). This balance of opposing actions of $p75^{NTR}$ and trkB could be shifted by altered expression of the receptors. With higher trkB expression, similar ligand

secretion would result in less activation of $p75^{NTR}$. Similarly, lower $p75^{NTR}$ expression would result in less anti-growth signaling. Thus, even with deficient activity-dependent BDNF secretion, Met-carriers could have enhanced trkB signaling with basal levels of constitutive secretion.

There is evidence for higher trkB expression in Met-carriers. Notaras et al., used Western blotting to probe trkB expression in the dorsal hippocampus and cortex of mice homozygous for the met allele and found increased full length trkB and decreased truncated trkB protein in the Met-carriers (Notaras et al., 2017). Ieraci et al. similarly found increased basal levels of trkB expression in the hippocampus of homozygous M/M mice (Ieraci et al., 2016). To date, trkB expression in Met-carriers has not been studied in other neuronal areas. Protein analysis on DRGs, motoneurons, and growth cones of regenerating axons could reveal whether increased trkB expression is a mechanism behind enhanced regeneration in Met-carriers.

There are higher levels of trkB ligand secretion in Met-carriers

BDNF is unique to the other neurotrophins in that it is secreted through two separate pathways. Like the other neurotrophins, BDNF is released constitutively in small vesicles that exocytose near the cell body. The second pathway is unique to BDNF—activity-dependent secretion through calcium-sensitive vesicles that are released in the distal dendrites and axon (Lessmann et al., 2003, Mowla et al., 1999, Kuczewski et al., 2009, Brigadski et al., 2005). The met allele of the Val66Met SNP results specifically in deficient packaging of BDNF into calcium-sensitive secretory vesicles (Chen et al., 2004). Because $BDNF_{Met}$ is still able to be packaged into constitutive secretory vesicles, one possible outcome of deficient activity-dependent secretion is increased constitutive secretion.

Egan et al. originally explored this hypothesis in their paper first describing the Val66Met polymorphism. In hippocampal cells transfected with constructs for GFP-bound $BDNF_{Val}$ or $BDNF_{Met}$, basal levels of mRNA expression and protein secretion were the same, but regulated secretion was deficient only for $BDNF_{Met}$ (Egan et al., 2003). Chen et al. tested secretion of

BDNF from hippocampal neurons cultured from their transgenic mouse that expressed the met allele of the SNP. Once again, basal levels of BDNF secretion were not different between the genotypes, though a marked deficiency was seen in activity-dependent secretion (Chen et al., 2006). Additionally, $BDNF_{Met}$ was found to dimerize with $BDNF_{Val}$, resulting in decreased packaging of both forms of BDNF into the regulated pathway and higher accumulation of BDNF near the cell soma (Chen et al., 2004). Using the mice developed by Chen et al., others assayed protein levels using brain lysates from the hippocampus and prefrontal cortex. This technique allows for information of what is happening *in vivo* but is not specific to *secreted* BDNF. Regardless, Bath et al. and Ieraci et al. saw significantly less BDNF protein in M/M hippocampal lysates than V/V (Bath et al., 2012, Ieraci et al., 2016). Ieraci et al. also analyzed BDNF mRNA levels in the hippocampus through PCR and found significantly decreased BDNF mRNA transcripts in M/M hippocampal lysates (Ieraci et al., 2016). Conversely, Notaras et al. found no differences in basal levels of BDNF protein in either the hippocampus or cortex (Notaras et al., 2017). These four experiments utilized hippocampal neurons, so we tested BDNF secretion from cultured adult DRG neurons expressing ChR2 stimulated with 1 hour of 20Hz light. Like the previous groups, we found no difference in basal levels of BDNF secretion (McGregor et al., 2018).

BDNF is not the only ligand for trkB. NT-4/5 also activates trkB and has also been found to enhance peripheral nerve regeneration (English et al., 2011, English et al., 2005). Unlike BDNF, NT-4/5 is released only through constitutive secretion (Lessmann et al., 2003). Interestingly, NT-4/5 activation of trkB results in a slower internalization and degradation of the receptor than BDNF activation, resulting in longer periods of signaling after ligand-binding (Proenca et al., 2016). NT-4/5 could therefore be a more potent ligand for trkB than BDNF, and increased constitutive release of NT-4/5 could explain the enhanced regeneration found in Met-carriers.

By treating cultures of adult DRG neurons with trkB-Fc, an antibody that sequesters all ligands for trkB, the enhanced growth in the V/M and M/M cultures was blocked (Meyer-Franke et al., 1998). This could be evidence for higher ligand secretion from cells carrying the met allele. However, as mentioned above, as ligand increases, so does potential activation of $p75^{NTR}$. NT-4/5, like BDNF, can activate $p75^{NTR}$. Consequently, if Met-carriers have higher endogenous secretion of trkB ligands, one might expect higher $p75^{NTR}$ activation. Treatment with a $p75^{NTR}$ antagonist did not enhance either neurite outgrowth *in vitro* or peripheral nerve regeneration *in vivo* in Met-carriers. Therefore this hypothesis is less likely than the increased trkB expression hypothesis discussed above. More detailed protein analysis of culture media would illuminate if there are differences in trkB ligand secretion.

Ligand-independent activation of trkB

In addition to ligand-dependent activation of trkB, trkB can also be transactivated through ligand-independent mechanisms. trkB is a receptor tyrosine kinase (RTK). Transactivation of RTKs by G protein-coupled receptors (GPCR) was first documented in epidermal growth factor receptors (Daub et al., 1997, Luttrell and Luttrell, 2004). These interactions are mediated through GPCR activation of Src family kinases (SFK) (Luttrell and Luttrell, 2004). SFKs are intracellular tyrosine kinases that interact with activated phosphorylated trks through the Src homology domain (SH2) in a similar mechanism to PLC_{γ} (Iwasaki et al., 1998). As tyrosine kinases, SFKs are able to phosphorylate tyrosine residues, and they may have a role in canonical ligand-dependent activation of trkB. Ligand activation of trkB results in higher activation of SFKs, and inhibiting SFK activity can block phosphorylation of residue Y705/706 on trkB (Huang and McNamara, 2010). While this residue is not associated with specific downstream signaling molecules, it is the first residue to be phosphorylated upon trkB activation, and phosphorylation of this residue may result in a conformational change that allows for the phosphorylation of other residues (Segal et al., 1996). Therefore, SFKs may play

an important role in ligand-dependent trkB activation as well as ligand-independent transactivation.

Given the ability of SFKs and GPCRs to transactivate other RTKs, Lee and Chao searched for GPCRs that might transactivate trkB. They found that adenosine activation of adenosine 2A receptors resulted in trk phosphorylation (Lee and Chao, 2001). This trkB activation was slower than that seen with neurotrophins (Lee and Chao, 2001). Ligand-independent activation resulted in activation of downstream signaling events, such as phosphorylation of Akt and increased cell survival (Lee and Chao, 2001, Wiese et al., 2007). Like previously discovered RTK transactivation, adenosine transactivation of trkB is dependent upon SFKs (Rajagopal and Chao, 2006). The adenosine receptor 2A has been studied in a peripheral nerve injury model. Motoneurons and DRG neurons contain the adenosine 2A receptor, and activation or this receptor results in increased motoneuron survival after neonatal facial nerve transection (Wiese et al., 2007, Kaelin-Lang et al., 1998). Activation of this receptor was insufficient to increase motoneuron survival in embryonic motoneurons lacking the trkB receptor (Wiese et al., 2007).

Soon after the discovery of trkB transactivation by adenosine, other ligands/receptors were investigated for their ability to activate the receptor. Pituitary adenylate cyclase-activating polypeptides (PACAP) were found to transactivate trkB in dissociated hippocampal neurons (Lee et al., 2002). This transactivation occurred mainly on intracellular membranes and not on trkB receptors on the extracellular membrane (Rajagopal et al., 2004). PACAP is upregulated in DRG neurons after peripheral nerve injury and motoneurons after facial nerve injury (Pettersson et al., 2014, Zhang et al., 1996, Zhou et al., 1999). In a facial nerve injury model, application of exogenous PACAP to the site of injury enhanced functional recovery (Kimura et al., 2004, Suarez et al., 2006). PACAP also enhances DRG neurite outgrowth *in vitro* (Suarez et al., 2006). Whether these effects were mediated by transactivation of trkB was not probed.

In DRGs, low density lipoprotein receptor-related protein 1 (LRP1) is expressed in both large and small DRG cells, and injection of the LRP1 ligand a2 macroglobulin (a2M) results in trk phosphorylation (Shi et al., 2009). Unlike the previous two mentioned transactivators of trkB, LRP1 is not a GPCR. Instead, it is a low-density lipoprotein receptor (LDLR) which has numerous ligands and through interactions with other cell-surface receptors is involved in numerous cell signaling events, such as activating protein kinase A (PKA) and the MAPK/ERK pathway (Herz et al., 1988, Lillis et al., 2005). Cell signaling through LRP1 is mediated by tyrosine phosphorylation of the receptor (Su et al., 2002). In cultured developing cerebellar neurons and adult DRG neurons, addition of LRP1 ligand results in increased neurite outgrowth (Shi et al., 2009, Yoon et al., 2013). Inhibiting SFKs blocked trk activity in response to LRP1 ligands in the adult DRG neurons (Yoon et al., 2013). In a model of sciatic nerve crush, addition of a2M increased the number of axons in the distal nerve stump five days after injury (Arandjelovic et al., 2007). The effects of a2M were LRP1-dependent, though in this study, transactivation of trkB was not probed.

Other ligand/receptor transactivators of trkB include the dopamine D1 receptor (Iwakura et al., 2008), which is a GPCR expressed in both DRG and motoneurons (Dubois et al., 1986, Zhu et al., 2007, Galbavy et al., 2013), and pineal *N*-acetylserotonin (NAS), a metabolite of serotonin and precursor to melatonin (Jang et al., 2010). Epidermal growth factor receptor (ErbB), an RTK, has also been found to transactivate trkB (Puehringer et al., 2013). To date, dopamine D1 receptors and NAS have not been tested in models of peripheral nerve regeneration, but ErbB, which is expressed in both motor and sensory neurons (Pearson and Carroll, 2004), enhances regeneration after sciatic nerve injury (Joung et al., 2010). Whether this enhancement was trkB-mediated was not probed.

Zinc can also transactivate trkB (Hwang et al., 2005, Huang et al., 2008). Zinc has numerous functions in the nervous system, reviewed by (Frederickson et al., 2005), but one interesting property of zinc in the nervous system is that it is released from synaptic vesicles. In

the spinal cord, zinc is localized to synaptic terminals on motoneuron cell bodies (Jo et al., 2000). As a cation, zinc can enter the cell through cation channels (such as NMDARs) to act intracellularly (Frederickson et al., 2005). Addition of zinc to the media of cultured neurons increases trkB phosphorylation, as does cellular depolarization, resulting in the opening of cation channels through which zinc can enter the cell (Huang et al., 2008). Like GPCRs, zinc activates trkB through SFKs (Huang and McNamara, 2010, Huang et al., 2008, Xu et al., 2011). Neonatal zinc deficiency leads to SFK- and trkB-mediated hippocampal cell death (Xu et al., 2011). Interestingly, in this model, BDNF levels increased as in apparent compensation for decreased zinc (Xu et al., 2011). To date, no studies have analyzed the effect of zinc on peripheral nerve regeneration after injury, but it is possible that zinc activation of trkB would act as a proregenerative signal in motoneurons and DRG neurons, and therefore increased zinc secretion is a possible mechanism behind enhanced regeneration in Met-carriers.

This plethora of non-canonical activators of trkB provide numerous possibilities for compensatory trkB activation in Met-carriers. Many pharmacological agents are available to specifically investigate these activators and/or their receptors, and using our DRG culture system, it would be possible to probe which, if any, of these ligands/receptors is responsible for the enhanced regeneration in Met-carriers. However, first whether the enhanced neurite outgrowth is ligand-independent was tested by treating cultures with TrkB-Fc. TrkB-Fc is a soluble protein containing the ligand-binding domain of trkB, allowing for sequestration of endogenous trkB ligands (Meyer-Franke et al., 1998). If trkB was enhancing neurite outgrowth through transactivation, addition of trkB-Fc to the media would have no effect on neurite outgrowth. In reality, there was a dramatic inhibition of neurite outgrowth with trkB-Fc treatment, indicating that transactivation is not the mechanism by which Met-carriers have enhanced regeneration after injury.

Three hypotheses for how Met-carriers could have trkB-dependent enhanced axon regeneration after injury have been presented: modulation of receptor expression, modulation of

ligand expression, and modulation of mechanism of receptor activation. The evidence from cultured DRG neurons using a trkB antagonist and trkB ligand sequestration indicates the first hypothesis, changes in trkB expression on the injured neurons, is the likeliest. Further protein analysis could confirm this hypothesis.

Personalized medicine

The finding that Met-carriers do not respond to activity-dependent treatment means that in the future, to appropriately treat peripheral nerve injuries using activity-dependent therapies, patients will need to be genotyped. The strategy of genotyping patients in order to best treat their ailments is a hallmark of the growing field of personalized medicine.

The field of personalized medicine is still new, but applications are diverse. Gene expression profiling can identify asymptomatic carriers of various viruses, children with undiagnosed diseases, and adults with difficult to diagnose ailments (Bryce and McWilliams, 2013). GWAS has also been implemented to identify gene variants that are risk factors for common diseases (Li and Meyre, 2014). Genome studies are being used to anticipate adverse reactions to certain drugs, such as Warfarin (Johnson et al., 2011). With regard to the Val66Met SNP, certain drugs have been found less effective in the Met-carrying population. For example, olanzapine, a schizophrenia drug, is less therapeutic for Met-carriers, whereas other commonly prescribed drugs have no difference in efficacy (Nikolac Perkovic et al., 2014). Met-carriers, particularly heterozygotes, respond better to selective serotonin reuptake inhibitors prescribed for depression (Yan et al., 2014). In this study, we have identified a new application of personalized medicine—genotyping patients in order to determine whether non-pharmacological treatments will be effective.

Val66Met and neuronal insult

Carrying the Met allele of the BDNF Val66Met polymorphism has been reported as a risk factor for increasing susceptibility and symptom severity in numerous diseases and disorders, including Alzheimer's disease, obsessive compulsive disorder, anorexia nervosa, and bipolar

disorder (Sklar et al., 2002, Hall et al., 2003, Ribases et al., 2003, Neves-Pereira et al., 2002, Notaras et al., 2015). Here, we find a possible beneficial result of the met allele—better axon regeneration after peripheral nerve injury. The effect of the Val66Met polymorphism has been researched with respect to other neuronal injuries, including stroke, traumatic brain injury (TBI), and spinal cord injury, with some conflicting yet promising results.

Only two studies regarding the Val66Met polymorphism and spinal cord injury have been performed. A survey of 11 Caucasian patients and one Native Hawaiian with cervical myelopathy revealed a higher than average occurrence of the Met allele (60% of patients vs an expected 30%) (Abode-Iyamah et al., 2016). There was no difference found in gait performance, although worse physical performance was self-reported by patients (Abode-Iyamah et al., 2016). Eleven patients with incomplete spinal cord injury were treated with low, medium, and high intensity exercise, and their blood serum was collected and analyzed for BDNF protein. Four of these patients were heterozygous for the met allele. Only high intensity exercise increased serum BDNF levels, and as mentioned previously, exercise did not increase serum levels of BDNF in the Met-carriers (Leech and Hornby, 2017). No animal models of spinal cord injury have been used to examine the effect of the met allele. Further exploration of how the met allele affects severity of spinal cord injuries and functional recovery would be illuminating.

The effect of the met allele of the Val66Met polymorphism has been far more extensively researched with respect to stroke. Animal models of stroke have been limited, but Met-carriers have been found to have worse earlier recovery from stroke followed by enhanced motor recovery later (Qin et al., 2014, Qin et al., 2011). Studies of human patients have been more equivocal, but there is some agreement that early outcomes are poorer in Met-carriers with no difference in long term outcome (Balkaya and Cho, 2018). The contradicting outcomes in stroke research may be a result of the effect of the met allele on hippocampal-based cognitive tasks. As mentioned previously, Met-carriers have decreased hippocampal volume (Egan et al., 2003). The met allele also impairs synaptic plasticity in the hippocampus, which could hamper

performance on certain cognitive tasks (Ninan et al., 2010). Indeed, memory is impaired in Met-carriers without stroke (Egan et al., 2003, Chen et al., 2006, Hariri et al., 2003). Thus, in outcomes that require learning, it would not be surprising to find poorer outcomes among Met-carriers, and that is precisely what is reported (Charalambous et al., 2018, van der Vliet et al., 2017). In measures of functional recovery, Met-carriers are generally found to be at no disadvantage compared to Val homozygotes (Balkaya and Cho, 2018).

The effect of the Val66Met allele has also been studied with regard to TBI. Analysis of cognition 30 years after injury revealed Met-carriers are protected from executive dysfunction impairment and had higher scores on intelligence, working memory, and processing speed (Krueger et al., 2011, Barbey et al., 2014). These differences are not seen in the uninjured population. Met-carriers more effectively recover executive functioning after traumatic brain injury, although no effect is seen on recovery of general intelligence (Krueger et al., 2011, Rostami et al., 2011). Additionally, Met-carriers over 45 have a lower risk of mortality following severe TBI (Failla et al., 2015). Concussed Met-carriers have improved olfactory performance (Larson-Dupuis et al., 2015). The protectiveness of the met allele in TBI may be gender specific—women with the met allele tend to fare better than male Met-carriers (Finan et al., 2018). The mouse model of the Val66Met SNP has not been utilized in TBI studies, so the mechanism of this protection is not currently understood.

Although the met allele may protect against certain aspects of neuronal insult, it can also result in worse emotional outcome. Met-carriers are at higher risk for post-stroke depression (Kim et al., 2008). Met-carriers, particularly males, are at higher risk for anxiety and depression after TBI (Wang et al., 2018). In the study of cervical myelopathy, Met-carriers had worse self-reported mental health (Abode-Iyamah et al., 2016). This could confound analysis of functional recovery—depression after injury impairs recovery (Bilge et al., 2008, van de Weg et al., 1999). Moreover, emotional distress lowers quality of life, and thus the met allele of the Val66Met SNP should be considered a risk factor for mental health complications after neuronal insult.

These findings taken together could indicate that carrying the Met allele could be protective from various forms of neuronal insult. Indeed, it would be difficult to explain the high prevalence of the allele in East Asian populations if the results were unequivocally negative (Shimizu et al., 2004, Petryshen et al., 2009). Further investigation into possible beneficial effects of the met allele could inform health care providers on how best to care for their patients.

Future Directions

We were unable to further enhance regeneration in Met-carriers with either activity-dependent treatments or with application of LM11A-31 to the injured nerve stump. Other experimental therapies may prove useful, however, such as treatment with small-molecule trkB agonists, which have proven to be effective in wild type mice (English et al., 2013). Even more beneficial would be finding trkB-independent therapies to enhance peripheral nerve regeneration. This could be accomplished through targeting downstream effectors of trkB, such as transcription factors ATF-3, CREB, and STAT-3, which have all been associated with enhanced peripheral nerve regeneration (Seijffers et al., 2007, White et al., 2000, Makwana and Raivich, 2005).

As I transition to my postdoctoral work, one possibility for continuing research on the Val66Met polymorphism is to use human induced pluripotent stem cells (iPSCs). These cells can be differentiated into motoneurons, allowing for an *in vitro* model of adult motoneuron axon regeneration (Shimojo et al., 2015). Furthermore, using gene editing, Met-carrying cells can be changed to a V/V genotype, and V/V cells can be made to express the Met allele (Barrangou and Doudna, 2016). Changes in axon regeneration can then be further probed.

Conclusion

As activity-dependent treatments begin to be explored for human patients of peripheral nerve injury, genotype will need to be considered. The prevalence of the Val66Met polymorphism will undoubtedly affect treatments in realms beyond peripheral nerve injury. However, despite the challenges identified here of enhancing peripheral nerve regeneration in

Met-carriers, this body of work presents one of the few benefits associated with the met allele of the Val66Met BDNF polymorphism.

References

ABODE-IYAMAH, K. O., STONER, K. E., GROSSBACH, A. J., VILJOEN, S. V., MCHENRY, C. L., PETRIE, M. A., DAHDALEH, N. S., GROSLAND, N. M., SHIELDS, R. K. & HOWARD, M. A. 2016. Effects of brain derived neurotrophic factor Val66Met polymorphism in patients with cervical spondylotic myelopathy. *Journal of Clinical Neuroscience,* 24**,** 117-121.

ANASTASIA, A., DEINHARDT, K., CHAO, M., WILL, N., IRMADY, K., LEE, F., HEMPSTEAD, B. & BRACKEN, C. 2013. Val66Met polymorphism of BDNF alters prodomain structure to induce neuronal growth cone retraction. *Nat Commun,* 4**,** 2490.

ARANDJELOVIC, S., DRAGOJLOVIC, N., LI, X., MYERS, R. R., CAMPANA, W. M. & GONIAS, S. L. 2007. A derivative of the plasma protease inhibitor α2-macroglobulin regulates the response to peripheral nerve injury. *J Neurochem,* 103**,** 694-705.

BALKAYA, M. & CHO, S. 2018. Genetics of stroke recovery: BDNF val66met polymorphism in stroke recovery and its interaction with aging. *Neurobiology of Disease*.

BARBEY, A. K., COLOM, R., PAUL, E., FORBES, C., KRUEGER, F., GOLDMAN, D. & GRAFMAN, J. 2014. Preservation of general intelligence following traumatic brain injury: contributions of the Met66 brain-derived neurotrophic factor. *PLOS ONE,* 9**,** e88733.

BARRANGOU, R. & DOUDNA, J. A. 2016. Applications of CRISPR technologies in research and beyond. *Nat Biotechnol,* 34**,** 933-941.

BATH, K. G., JING, D. Q., DINCHEVA, I., NEEB, C. C., PATTWELL, S. S., CHAO, M. V., LEE, F. S. & NINAN, I. 2012. BDNF Val66Met impairs fluoxetine-induced enhancement of adult hippocampus plasticity. *Neuropsychopharmacology,* 37**,** 1297-1304.

BILGE, C., KOCER, E., KOCER, A. & TURK BORU, U. 2008. Depression and functional outcome after stroke: the effect of antidepressant therapy on functional recovery. *Eur J Phys Rehabil Med,* 44**,** 13-8.

BOYD, J. G. & GORDON, T. 2002. A dose-dependent facilitation and inhibition of peripheral nerve regeneration by brain-derived neurotrophic factor. *Eur J Neurosci,* 15**,** 613-26.

BOYD, J. G. & GORDON, T. 2003. Neurotrophic factors and their receptors in axonal regeneration and functional recovery after peripheral nerve injury. *Mol Neurobiol,* 27**,** 277-324.

BRIGADSKI, T., HARTMANN, M. & LESSMANN, V. 2005. Differential vesicular targeting and time course of synaptic secretion of the mammalian neurotrophins. *J Neurosci,* 25**,** 7601-14.

BRYCE, A. H. & MCWILLIAMS, R. 2013. Current status and future directions of personalized medicine. *Genome Medicine,* 5**,** 62-62.

CHARALAMBOUS, C. C., ALCANTARA, C. C., FRENCH, M. A., LI, X., MATT, K. S., KIM, H. E., MORTON, S. M. & REISMAN, D. S. 2018. A single exercise bout and locomotor learning after stroke: physiological, behavioural, and computational outcomes. *J Physiol,* 596**,** 1999-2016.

CHEN, Y.-W., SURGENT, O., RANA, B. S., LEE, F. & AOKI, C. 2017. Variant BDNF-Val66Met polymorphism is associated with layer-specific alterations in GABAergic innervation of pyramidal neurons, elevated anxiety and reduced vulnerability of adolescent male mice to activity-based anorexia. *Cerebral Cortex,* 27**,** 3980-3993.

CHEN, Z., JING, D., BATH, K., IERACI, A., KHAN, T., SIAO, C., HERRERA, D., TOTH, M., MCEWEN, B., HEMPSTEAD, B. & LEE, F. 2006. Genetic variant BDNF (Val66Met) polymorphism alters anxiety-related behavior. *Science,* 314**,** 140-143.

CHEN, Z., PATEL, P., SANT, G., MENG, C., TENG, K., HEMPSTEAD, B. & LEE, F. 2004. Variant brain-derived neurotrophic factor (BDNF) (Met66) alters the intracellular trafficking and activity-dependent secretion of wild-type BDNF in neurosecretory cells and cortical neurons. *J Neurosci,* 24**,** 4401-4411.

DAUB, H., WALLASCH, C., LANKENAU, A., HERRLICH, A. & ULLRICH, A. 1997. Signal characteristics of G protein-transactivated EGF receptor. *EMBO J,* 16**,** 7032-44.

DUBOIS, A., SAVASTA, M., CURET, O. & SCATTON, B. 1986. Autoradiographic distribution of the D1 agonist [3H]SKF 38393, in the rat brain and spinal cord. Comparison with the distribution of D2 dopamine receptors. *Neuroscience,* 19**,** 125-137.

EGAN, M., KOJIMA, M., CALLICOTT, J., GOLDBERG, T., KOLACHANA, B., BERTOLINO, A., ZAITSEV, E., GOLD, B., GOLDMAN, D., DEAN, M., LU, B. & WEINBERGER, D. 2003. The BDNF val66met polymorphism affects activity-dependent secretion of BDNF and human memory and hippocampal function. *Cell,* 112**,** 257-269.

ENGLISH, A., CUCORANU, D., MULLIGAN, A., RODRIGUEZ, J. & SABATIER, M. 2011. Neurotrophin-4/5 is implicated in the enhancement of axon regeneration produced by treadmill training following peripheral nerve injury. *Eur J Neurosci,* 33**,** 2265-2271.

ENGLISH, A., LIU, K., NICOLINI, J., MULLIGAN, A. & YE, K. 2013. Small-molecule trkB agonists promote axon regeneration in cut peripheral nerves. *Proc Natl Acad Sci U S A,* 110**,** 16217-16222.

ENGLISH, A., W, M. & CARRASCO, D. 2005. Neurotrophin-4/5 is required for the early growth of regenerating axons in peripheral nerves. *Eur J Neurosci,* 21**,** 2624-2634.

FAILLA, M. D., KUMAR, R., PEITZMAN, A., CONLEY, Y. P., FERRELL, R. E. & WAGNER, A. K. 2015. Variation in the BDNF gene interacts with age to predict mortality in a prospective, longitudinal cohort with severe TBI. *Neurorehabil Neural Repair,* 29**,** 234-246.

FINAN, J. D., UDANI, S. V., PATEL, V. & BAILES, J. E. 2018. The Influence of the Val66Met Polymorphism of Brain-Derived Neurotrophic Factor on Neurological Function after Traumatic Brain Injury. *J Alzheimers Dis*.

FREDERICKSON, C. J., KOH, J. Y. & BUSH, A. I. 2005. The neurobiology of zinc in health and disease. *Nat Rev Neurosci,* 6**,** 449-62.

GALBAVY, W., SAFAIE, E., REBECCHI, M. J. & PUOPOLO, M. 2013. Inhibition of tetrodotoxin-resistant sodium current in dorsal root ganglia neurons mediated by D1/D5 dopamine receptors. *Mol Pain,* 9**,** 60.

GIZA, J. I., KIM, J., MEYER, H. C., ANASTASIA, A., DINCHEVA, I., ZHENG, C. I., LOPEZ, K., BAINS, H., YANG, J., BRACKEN, C., LISTON, C., JING, D., HEMPSTEAD, B. L. & LEE, F. S. 2018. The BDNF Val66Met Prodomain Disassembles Dendritic Spines Altering Fear Extinction Circuitry and Behavior. *Neuron,* 99**,** 163-178.e6.

HALL, D., DHILLA, A., CHARALAMBOUS, A., GOGOS, J. A. & KARAYIORGOU, M. 2003. Sequence variants of the brain-derived neurotrophic factor (BDNF) gene are strongly associated with obsessive-compulsive disorder. *Am J Hum Genet,* 73**,** 370-376.

HARIRI, A. R., GOLDBERG, T. E., MATTAY, V. S., KOLACHANA, B. S., CALLICOTT, J. H., EGAN, M. F. & WEINBERGER, D. R. 2003. Brain-derived neurotrophic factor val66met polymorphism affects human memory-related hippocampal activity and predicts memory performance. *J Neurosci,* 23**,** 6690-4.

HERZ, J., HAMANN, U., ROGNE, S., MYKLEBOST, O., GAUSEPOHL, H. & STANLEY, K. K. 1988. Surface location and high affinity for calcium of a 500-kd liver membrane protein closely related to the LDL-receptor suggest a physiological role as lipoprotein receptor. *The EMBO Journal,* 7**,** 4119-4127.

HUANG, Y. Z. & MCNAMARA, J. O. 2010. Mutual regulation of Src family kinases and the neurotrophin receptor trkB. *J Biol Chem,* 285**,** 8207-8217.

HUANG, Y. Z., PAN, E., XIONG, Z.-Q. & MCNAMARA, J. O. 2008. Zinc-mediated transactivation of trkB potentiates the hippocampal mossy fiber-CA3 pyramid synapse. *Neuron,* 57**,** 546-558.

HWANG, J. J., PARK, M. H., CHOI, S. Y. & KOH, J. Y. 2005. Activation of the Trk signaling pathway by extracellular zinc. Role of metalloproteinases. *J Biol Chem,* 280**,** 11995-2001.

IERACI, A., MADAIO, A. I., MALLEI, A., LEE, F. S. & POPOLI, M. 2016. Brain-derived neurotrophic factor Val66Met human polymorphism impairs the beneficial exercise-induced neurobiological changes in mice. *Neuropsychopharmacology*.

IWAKURA, Y., NAWA, H., SORA, I. & CHAO, M. V. 2008. Dopamine D1 Receptor-induced Signaling through TrkB Receptors in Striatal Neurons. *J Biol Chem,* 283**,** 15799-15806.

IWASAKI, Y., GAY, B., WADA, K. & KOIZUMI, S. 1998. Association of the Src family tyrosine kinase Fyn with TrkB. *J Neurochem,* 71**,** 106-11.

JANG, S.-W., LIU, X., PRADOLDEJ, S., TOSINI, G., CHANG, Q., IUVONE, P. M. & YE, K. 2010. N-acetylserotonin activates TrkB receptor in a circadian rhythm. *Proc Natl Acad Sci U S A,* 107**,** 3876.

JO, S. M., DANSCHER, G., SCHRODER, H. D., WON, M. H. & COLE, T. B. 2000. Zinc-enriched (ZEN) terminals in mouse spinal cord: immunohistochemistry and autometallography. *Brain Res,* 870**,** 163-9.

JOHNSON, J. A., GONG, L., WHIRL-CARRILLO, M., GAGE, B. F., SCOTT, S. A., STEIN, C. M., ANDERSON, J. L., KIMMEL, S. E., LEE, M. T., PIRMOHAMED, M., WADELIUS, M., KLEIN, T. E. & ALTMAN, R. B. 2011. Clinical Pharmacogenetics Implementation Consortium Guidelines for CYP2C9 and VKORC1 genotypes and warfarin dosing. *Clin Pharmacol Ther,* 90**,** 625-9.

JOUNG, I., YOO, M., WOO, J. H., CHANG, C. Y., HEO, H. & KWON, Y. K. 2010. Secretion of EGF-like domain of heregulinbeta promotes axonal growth and functional recovery of injured sciatic nerve. *Mol Cells,* 30**,** 477-84.

KAELIN-LANG, A., LAUTERBURG, T. & BURGUNDER, J.-M. 1998. Expression of adenosine A2a receptor gene in rat dorsal root and autonomic ganglia. *Neurosci Lett,* 246**,** 21-24.

KIM, J.-M., STEWART, R., KIM, S.-W., YANG, S.-J., SHIN, I.-S., KIM, Y.-H. & YOON, J.-S. 2008. BDNF genotype potentially modifying the association between incident stroke and depression. *Neurobiology of Aging,* 29**,** 789-792.

KIMURA, H., KAWATANI, M., ITO, E. & ISHIKAWA, K. 2004. PACAP facilitate the nerve regeneration factors in the facial nerve injury. *Regul Pept,* 123**,** 135-8.

KRUEGER, F., PARDINI, M., HUEY, E. D., RAYMONT, V., SOLOMON, J., LIPSKY, R. H., HODGKINSON, C. A., GOLDMAN, D. & GRAFMAN, J. 2011. The role of the Met66 brain-derived neurotrophic factor allele in the recovery of executive functioning after combat-related traumatic brain injury. *J Neurosci,* 31**,** 598-606.

KUCZEWSKI, N., PORCHER, C., LESSMANN, V., MEDINA, I. & GAIARSA, J. L. 2009. Activity-dependent dendritic release of BDNF and biological consequences. *Mol Neurobiol,* 39**,** 37-49.

LARSON-DUPUIS, C., CHAMARD, E., FALARDEAU, V., FRASNELLI, J., BEAULIEU, C., POIRIER, J., CARRIER, J., LASSONDE, M., THEORET, H., BACON, B. A. & DE BEAUMONT, L. 2015. Impact of BDNF Val66Met polymorphism on olfactory functions of female concussed athletes. *Brain Inj,* 29**,** 963-70.

LEE, B. G., ANASTASIA, A., HEMPSTEAD, B. L., LEE, F. S. & BLENDY, J. A. 2015. Effects of the BDNF Val66Met polymorphism on anxiety-like behavior following nicotine withdrawal in mice. *Nicotine Tob Res,* 17**,** 1428-1435.

LEE, F. S. & CHAO, M. V. 2001. Activation of Trk neurotrophin receptors in the absence of neurotrophins. *Proc Natl Acad Sci U S A,* 98**,** 3555-3560.

LEE, F. S., RAJAGOPAL, R., KIM, A. H., CHANG, P. C. & CHAO, M. V. 2002. Activation of trk neurotrophin receptor signaling by pituitary adenylate cyclase-activating polypeptides. *J Biol Chem,* 277**,** 9096-9102.

LEECH, K. A. & HORNBY, T. G. 2017. High-intensity locomotor exercise increases brain-derived neurotrophic factor in individuals with incomplete spinal cord injury. *J Neurotrauma,* 34**,** 1240-1248.

LESSMANN, V., GOTTMANN, K. & MALCANGIO, M. 2003. Neurotrophin secretion: current facts and future prospects. *Prog Neurobiol,* 69**,** 341-374.

LI, A. & MEYRE, D. 2014. Jumping on the Train of Personalized Medicine: A Primer for Non-Geneticist Clinicians: Part 3. Clinical Applications in the Personalized Medicine Area. *Current Psychiatry Reviews,* 10**,** 118-132.

LI, W. J., YU, H., YANG, J. M., GAO, J., JIANG, H., FENG, M., ZHAO, Y. X. & CHEN, Z. Y. 2010. Anxiolytic effect of music exposure on BDNFMet/Met transgenic mice. *Brain Res,* 1347**,** 71-9.

LILLIS, A. P., MIKHAILENKO, I. & STRICKLAND, D. K. 2005. Beyond endocytosis: LRP function in cell migration, proliferation and vascular permeability. *J Thromb Haemost,* 3**,** 1884-93.

LUTTRELL, D. K. & LUTTRELL, L. M. 2004. Not so strange bedfellows: G-protein-coupled receptors and Src family kinases. *Oncogene,* 23**,** 7969-78.

MAKWANA, M. & RAIVICH, G. 2005. Molecular mechanisms in successful peripheral regeneration. *Febs j,* 272**,** 2628-38.

MCGREGOR, C. E., IRWIN, A. M. & ENGLISH, A. W. 2018. The Val66Met BDNF polymorphism and peripheral nerve injury: Enhanced regeneration in Met-carriers that is not further improved with activity-dependent treatment. *J Neurosci,* submitted.

MEYER-FRANKE, A., WILKINSON, G. A., KRUTTGEN, A., HU, M., MUNRO, E., HANSON, M. G., JR., REICHARDT, L. F. & BARRES, B. A. 1998. Depolarization and cAMP elevation rapidly recruit TrkB to the plasma membrane of CNS neurons. *Neuron,* 21**,** 681-93.

MOWLA, S. J., PAREEK, S., FARHADI, H. F., PETRECCA, K., FAWCETT, J. P., SEIDAH, N. G., MORRIS, S. J., SOSSIN, W. S. & MURPHY, R. A. 1999. Differential sorting of nerve growth factor and brain-derived neurotrophic factor in hippocampal neurons. *J Neurosci,* 19**,** 2069-80.

NEVES-PEREIRA, M., MUNDO, E., MUGLIA, P., KING, N., MACCIARDI, F. & KENNEDY, J. L. 2002. The brain-derived neurotrophic factor gene confers susceptibility to bipolar disorder: evidence from a family-based association study. *Am J Hum Genet,* 71**,** 651-5.

NIKOLAC PERKOVIC, M., NEDIC ERJAVEC, G., ZIVKOVIC, M., SAGUD, M., UZUN, S., MIHALJEVIC-PELES, A., KOZUMPLIK, O., MUCK-SELER, D. & PIVAC, N. 2014. Association between the brain-derived neurotrophic factor Val66Met polymorphism and therapeutic response to olanzapine in schizophrenia patients. *Psychopharmacology (Berl),* 231**,** 3757-64.

NINAN, I., BATH, K. G., DAGAR, K., PEREZ-CASTRO, R., PLUMMER, M. R., LEE, F. S. & CHAO, M. V. 2010. The BDNF Val66Met polymorphism impairs NMDA receptor-dependent synaptic plasticity in the hippocampus. *The Journal of neuroscience : the official journal of the Society for Neuroscience,* 30**,** 8866-8870.

NOTARAS, M., DU, X., GOGOS, J., VAN DEN BUUSE, M. & HILL, R. A. 2017. The BDNF Val66Met polymorphism regulates glucocorticoid-induced corticohippocampal remodeling and behavioral despair. *Transl Psychiatry,* 7**,** e1233.

NOTARAS, M., HILL, R. & VAN DEN BUUSE, M. 2015. The BDNF gene Val66Met polymorphism as a modifier of psychiatric disorder susceptibility: progress and controversy. *Mol Psychiatry,* 20**,** 916-30.

PEARSON, R. J., JR. & CARROLL, S. L. 2004. ErbB transmembrane tyrosine kinase receptors are expressed by sensory and motor neurons projecting into sciatic nerve. *J Histochem Cytochem,* 52**,** 1299-311.

PETRYSHEN, T. L., SABETI, P. C., ALDINGER, K. A., FRY, B., FAN, J. B., SCHAFFNER, S. F., WAGGONER, S. G., TAHL, A. R. & SKLAR, P. 2009. Population genetic study of the brain-derived neurotrophic factor (BDNF) gene. *Molecular Psychiatry,* 15**,** 810.

PETTERSSON, L. M., GEREMIA, N. M., YING, Z. & VERGE, V. M. 2014. Injury-associated PACAP expression in rat sensory and motor neurons is induced by endogenous BDNF. *PLoS One,* 9**,** e100730.

PROENCA, C. C., SONG, M. & LEE, F. S. 2016. Differential effects of BDNF and neurotrophin 4 (NT4) on endocytic sorting of TrkB receptors. *J Neurochem,* 138**,** 397-406.

PUEHRINGER, D., OREL, N., LÜNINGSCHRÖR, P., SUBRAMANIAN, N., HERRMANN, T., CHAO, M. V. & SENDTNER, M. 2013. EGF transactivation of Trk receptors regulates the migration of newborn cortical neurons. *Nature Neurosci,* 16**,** 407-415.

QIN, L., JING, D., PARAUDA, S., CARMEL, J., RATAN, R. R., LEE, F. S. & CHO, S. 2014. An Adaptive Role for BDNF Val66Met Polymorphism in Motor Recovery in Chronic Stroke. *J Neurosci,* 34**,** 2493-2502.

QIN, L., KIM, E., RATAN, R., LEE, F. S. & CHO, S. 2011. Genetic variant of BDNF (Val66Met) polymorphism attenuates stroke-induced angiogenic responses by enhancing anti-angiogenic mediator CD36 expression. *J Neurosci,* 31**,** 775-783.

RAJAGOPAL, R. & CHAO, M. V. 2006. A role for Fyn in Trk receptor transactivation by G-protein-coupled receptor signaling. *Mol Cell Neurosci,* 33**,** 36-46.

RAJAGOPAL, R., CHEN, Z.-Y., LEE, F. S. & CHAO, M. V. 2004. Transactivation of trk neurotrophin receptors by G-protein-coupled receptor ligands occurs on intracellular membranes. *J Neurosci,* 24**,** 6650.

RIBASES, M., GRATACOS, M., ARMENGOL, L., DE CID, R., BADIA, A., JIMENEZ, L., SOLANO, R., VALLEJO, J., FERNANDEZ, F. & ESTIVILL, X. 2003. Met66 in the brain-derived neurotrophic factor (BDNF) precursor is associated with anorexia nervosa restrictive type. *Mol Psychiatry,* 8**,** 745-51.

ROSTAMI, E., KRUEGER, F., ZOUBAK, S., DAL MONTE, O., RAYMONT, V., PARDINI, M., HODGKINSON, C. A., GOLDMAN, D., RISLING, M. & GRAFMAN, J. 2011. BDNF Polymorphism Predicts General Intelligence after Penetrating Traumatic Brain Injury. *PLoS ONE,* 6**,** e27389.

SCOTT, A. L. M. & RAMER, M. S. 2010. Schwann cell p75NTR prevents spontaneous sensory reinnervation of the adult spinal cord. *Brain,* 133**,** 421-432.

SEGAL, R. A., BHATTACHARYYA, A., RUA, L. A., ALBERTA, J. A., STEPHENS, R. M., KAPLAN, D. R. & STILES, C. D. 1996. Differential utilization of Trk autophosphorylation sites. *J Biol Chem,* 271**,** 20175-81.

SEIJFFERS, R., MILLS, C. D. & WOOLF, C. J. 2007. ATF3 increases the intrinsic growth state of DRG neurons to enhance peripheral nerve regeneration. *J Neurosci,* 27**,** 7911.

SHI, J., LONGO, F. M. & MASSA, S. M. 2013. A small molecule p75(NTR) ligand protects neurogenesis after traumatic brain injury. *Stem Cells,* 31**,** 2561-74.

SHI, Y., MANTUANO, E., INOUE, G., CAMPANA, W. M. & GONIAS, S. L. 2009. Ligand binding to LRP1 transactivates trk receptors by a Src family kinase–dependent pathway. *Science Signaling,* 2**,** ra18-ra18.

SHIMIZU, E., HASHIMOTO, K. & IYO, M. 2004. Ethnic difference of the BDNF 196G/A (val66met) polymorphism frequencies: the possibility to explain ethnic mental traits. *Am J Med Genet B Neuropsychiatr Genet,* 126b**,** 122-3.

SHIMOJO, D., ONODERA, K., DOI-TORII, Y., ISHIHARA, Y., HATTORI, C., MIWA, Y., TANAKA, S., OKADA, R., OHYAMA, M., SHOJI, M., NAKANISHI, A., DOYU, M., OKANO, H. & OKADA, Y. 2015. Rapid, efficient, and simple motor neuron differentiation from human pluripotent stem cells. *Molecular Brain,* 8**,** 79.

SKLAR, P., GABRIEL, S. B., MCINNIS, M. G., BENNETT, P., LIM, Y., TSAN, G., SCHAFFNER, S., KIROV, G., JONES, I., OWEN, M., CRADDOCK, N., DEPAULO, J. R. & LANDER, E.

S. 2002. Family-based association study of 76 candidate genes in bipolar disorder: BDNF is a potential risk locus. Brain-derived neutrophic factor. *Mol Psychiatry,* 7**,** 579-93.

SPICER, P. P. & MIKOS, A. G. 2010. Fibrin glue as a drug delivery system. *J Control Release,* 148**,** 49-55.

SU, H. P., NAKADA-TSUKUI, K., TOSELLO-TRAMPONT, A. C., LI, Y., BU, G., HENSON, P. M. & RAVICHANDRAN, K. S. 2002. Interaction of CED-6/GULP, an adapter protein involved in engulfment of apoptotic cells with CED-1 and CD91/low density lipoprotein receptor-related protein (LRP). *J Biol Chem,* 277**,** 11772-9.

SUAREZ, V., GUNTINAS-LICHIUS, O., STREPPEL, M., INGOROKVA, S., GROSHEVA, M., NEISS, W. F., ANGELOV, D. N. & KLIMASCHEWSKI, L. 2006. The axotomy-induced neuropeptides galanin and pituitary adenylate cyclase-activating peptide promote axonal sprouting of primary afferent and cranial motor neurones. *Eur J Neurosci,* 24**,** 1555-64.

VAN DE WEG, F. B., KUIK, D. J. & LANKHORST, G. J. 1999. Post-stroke depression and functional outcome: a cohort study investigating the influence of depression on functional recovery from stroke. *Clin Rehabil,* 13**,** 268-72.

VAN DER VLIET, R., RIBBERS, G. M., VANDERMEEREN, Y., FRENS, M. A. & SELLES, R. W. 2017. BDNF Val66Met but not transcranial direct current stimulation affects motor learning after stroke. *Brain Stimul,* 10**,** 882-892.

WANG, Y.-J., CHEN, K.-Y., KUO, L.-N., WANG, W.-C., HSU, Y.-W., WONG, H. S.-C., LIN, C.-M., LIAO, K.-H., ZHANG, Y.-F., CHIANG, Y.-H. & CHANG, W.-C. 2018. The association between BDNF Val66Met polymorphism and emotional symptoms after mild traumatic brain injury. *BMC Medical Genetics,* 19**,** 13.

WHITE, D. M., WALKER, S., BRENNEMAN, D. E. & GOZES, I. 2000. CREB contributes to the increased neurite outgrowth of sensory neurons induced by vasoactive intestinal polypeptide and activity-dependent neurotrophic factor. *Brain Res,* 868**,** 31-8.

WIESE, S., JABLONKA, S., HOLTMANN, B., OREL, N., RAJAGOPAL, R., CHAO, M. V. & SENDTNER, M. 2007. Adenosine receptor A2A-R contributes to motoneuron survival by transactivating the tyrosine kinase receptor TrkB. *Proc Natl Acad Sci U S A,* 104**,** 17210-5.

XU, H., GAO, H.-L., ZHENG, W., XIN, N., CHI, Z.-H., BAI, S.-L. & WANG, Z.-Y. 2011. Lactational zinc deficiency-induced hippocampal neuronal apoptosis by a BDNF-independent TrkB signaling pathway. *Hippocampus,* 21**,** 495-501.

YAN, T., WANG, L., KUANG, W., XU, J., LI, S., CHEN, J. & YANG, Y. 2014. Brain-derived neurotrophic factor Val66Met polymorphism association with antidepressant efficacy: a systematic review and meta-analysis. *Asia Pac Psychiatry,* 6**,** 241-51.

YOON, C., VAN NIEKERK, E. A., HENRY, K., ISHIKAWA, T., ORITA, S., TUSZYNSKI, M. H. & CAMPANA, W. M. 2013. Low-density lipoprotein receptor-related protein 1 (LRP1)-dependent cell signaling promotes axonal regeneration. *The Journal of Biological Chemistry,* 288**,** 26557-26568.

YU, H., WANG, D.-D., WANG, Y., LIU, T., LEE, F. S. & CHEN, Z.-Y. 2012. Variant brain-derived neurotrophic factor Val66Met polymorphism alters vulnerability to stress and response to antidepressants. *J Neurosci,* 32**,** 4092-4101.

ZHANG, Y. Z., HANNIBAL, J., ZHAO, Q., MOLLER, K., DANIELSEN, N., FAHRENKRUG, J. & SUNDLER, F. 1996. Pituitary adenylate cyclase activating peptide expression in the rat dorsal root ganglia: Up-regulation after peripheral nerve injury. *Neuroscience,* 74**,** 1099-1110.

ZHOU, X., RODRIGUEZ, W. I., CASILLAS, R. A., MA, V., TAM, J., HU, Z., LELIEVRE, V., CHAO, A. & WASCHEK, J. A. 1999. Axotomy-induced changes in pituitary adenylate cyclase activating polypeptide (PACAP) and PACAP receptor gene expression in the adult rat facial motor nucleus. *J Neurosci Res,* 57**,** 953-61.

ZHU, H., CLEMENS, S., SAWCHUK, M. & HOCHMAN, S. 2007. Expression and distribution of all dopamine receptor subtypes (D(1) – D(5)) in the mouse lumbar spinal cord: A real-time PCR and non-autoradiographic in situ hybridization study. *Neuroscience,* 149**,** 885-897.