MECHANISMS OF MOTOR LEARNING AND BRAIN PLASTICITY POST STROKE

by

Erin E. Helm

A dissertation submitted to the Faculty of the University of Delaware in partial fulfillment of the requirements for a Doctor of Philosophy in Biomechanics and Movement Science

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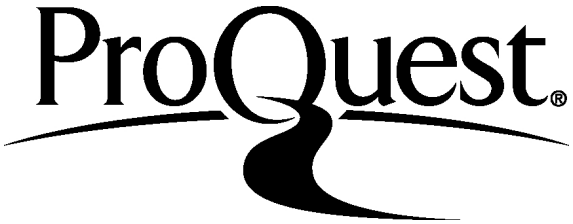

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ABSTRACT

Stroke is the leading cause of long term disability in the United States. The ability to recover motor skill function post-stroke relies largely upon the adaptive capacity of the brain following neurologic insult. The mechanisms which enable neural plasticity post-stroke are similar to those which promote neural reorganization in the healthy brain during learning. As such, parameters of neuro-rehabilitation which optimize motor learning and enhance neural plasticity are of great interest to clinicians and researchers in the field of stroke rehabilitation. The overall goal of this dissertation was to identify key molecular and behavioral requisites of motor learning post-stroke. The split belt treadmill was utilized to investigate within session learning and retention of a novel locomotor pattern in neurologically intact individuals as well as those post stroke.

To identify behavioral requisites of post-stroke learning, we examined characteristics of task practice, specifically variable and constant practice that would promote locomotor learning in those with chronic stroke. Although frequently utilized to promote motor relearning in individual's post-stroke, our results revealed that variable practice confers little benefit over constant practice in learning of a novel locomotor pattern in individuals post-stroke. The current study is the first to assess the effects of

practice characteristics on learning of a complex lower-extremity task in subjects post-stroke.

To identify mechanisms of neural plasticity that may moderate motor learning we examined the impact of a single nucleotide polymorphism on the BDNF gene (Val66Met) in learning of a novel locomotor task in subjects with chronic stroke. The results demonstrate that chronic stroke survivors, regardless of presence or absence of the polymorphism are able to adapt their walking pattern over a period of trial and error practice. The process of locomotor adaptation, however is slowed in those with the Val66Met polymorphism.

To examine behavioral parameters of rehabilitation that may promote neuroplastic processes through a BDNF related mechanism, we examined the role of high intensity exercise on locomotor learning in neurologically intact subjects. Specifically, the role of high intensity exercise in upregulation of peripheral BDNF levels as well as the role of high intensity exercise in mediation of motor skill performance and retention of a novel locomotor task was explored. In addition the impact of a single nucleotide polymorphism on the BDNF gene (Val66Met) was examined in neurologically intact adults to assess the relationship between exercise and motor learning. The results of this study demonstrate that although high intensity exercise prior to a motor learning task resulted in increased peripheral BDNF this exercise does not provide additional benefit to learning of a novel locomotor pattern in neurologically intact adults. The current results also demonstrate that presence of a single nucleotide polymorphism on the BDNF gene (Val66Met) does

not influence the magnitude of upregulation of serum BDNF with high intensity exercise, nor does it interfere with learning of a novel locomotor pattern.

Chapter 1

INTRODUCTION

1.1 Background and Significance

The adaptive capacity of the brain has been well documented in the animal model during motor skill learning and functional recovery from various neurologic insults[1–3]. Human studies have demonstrated a correlative role of motor skill learning and alterations in motor map representations in the healthy human population as well as those post-stroke[4–7]. Principles of motor learning have become a fundamental characteristic of neurological rehabilitation however empirical evidence supporting the use of specific task parameters to increase neural plasticity and thus, facilitate post stroke locomotor recovery is meager. The ability to exploit the brains capacity for learning induced reorganization requires a greater understanding of the neural substrates that enable such plasticity. Assessing the mechanisms mediating plasticity in the neurologically intact and post-stroke brain during lower extremity motor learning is fundamental to the advancement of post-stroke rehabilitation.

Stroke: ***Overview***

According to the American Heart Association (AHA), each year approximately 795,000 people experience a new or recurrent stroke [8]. Stroke is the leading cause of long term disability in the United States[8] with one in five women and one in six men experiencing a stroke within their lifetime (Seshadri et al. 2006). Specific deficits following stroke are dependent upon the area of cerebral circulation that is affected. Impairments often encompass a spectrum of cognitive, sensory and motor functions. At six-month post-stroke, 30% of stroke survivors over the age of 65 are unable to walk without an assistive device, and 26%are dependent for activities of daily living[8]. The ability to regain ambulatory function post-stroke is a common goal of stroke survivors (Bohannon RW, 1998). Moreover, improved ambulatory function is also a common goal of rehabilitation professionals as improved ambulation has been linked to increased community participation, improved cardiovascular fitness and decreased risk of stroke recurrence [8].

Post Stroke Functional Recovery: ***Brain plasticity, Motor Recovery and Rehabilitation***

Recovery post stroke can be considered a two-fold process occurring as a natural phenomenon in the weeks post stroke, and also in response to environmental demand [9]. The first appears to occur as a series of stereotyped stages, with spontaneous recover maximally expressed within the first four weeks and then gradually decreasing within the first 6 months. This spontaneous recovery is the result of various mechanisms including restitution of the ischemic penumbra, resolution of diaschisis and brain reorganization [9].

Neural reorganization within the spared motor region of the injured as well as uninjured hemisphere is thought to be due to necessity for recovery of motor function. This reorganization is activity dependent and can be broadly defined as neural plasticity. Murphy and Corbett (2009) cite two related factors that enable plasticity in the brain post-stroke; diffuse and redundant connectivity within the central nervous system (CNS), and creation of structural and functional circuits that form through reorganization of the functional somatotopic maps[10].

Restoration of movement function post-stroke is thought to be a function of motor learning or relearning whereby lost action patterns may be restored and new compensatory patterns are acquired due to reorganization of neural connections within the brain[11]. Post stroke rehabilitation interventions attempt to capitalize on the ability of the brain to reorganize in response to functional demand in order to minimize functional impairments[12]. Certain rehabilitation interventions have been found to have a greater capacity to improve function and drive restorative neural plasticity post stroke than others.

Animal models indicate that certain activities induce greater neuroplastic changes in the brain post-stroke and as such promote greater functional recovery. Task specificity, repetition and intensity of practice with the affected limb are all requisites to induce neuroplasticity [12]. Furthermore, practice of movement with the affected limb is necessary but not sufficient to induce changes to the ipsilesional side of the brain and provide subsequent functional improvements. One of the first neurophysiological studies demonstrating the ability of the motor cortex to reorganize in response to post-injury

training was performed by Nudo and colleagues in 1996[2]. The group found that animals trained to receive food pellets with the impaired hand had significantly altered connectivity within the area surrounding the lesion within the M1 cortex, providing one of the first demonstrations of the neurophysiological basis of post-stroke skill training. Plautz and colleagues (2000) took this one step further, demonstrating that simple repetition is not enough to elicit cortical reorganization, rather a learning component is required[13]. Plautz and colleagues (2000) found that cortical maps remained stable from one training session to the next when the primate completed unskilled motor tasks, however modification in the neural networks were induced when they were required to learn a new skill.

Given motor learning is a requisite for the neural reorganization that drives recovery of function, there is a need to identify mechanisms of post-stroke rehabilitation and motor learning that promote the greatest amount of brain plasticity to enable recovery.

Motor Learning Post Stroke*: Definitions and Distinctions, Current Evidence, and Practice Paradigms.*

Definitions and Distinctions

Motor learning can be defined as a set of processes associated with practice or experience leading to relatively permanent changes in skilled behavior (Schmidt, 1988). To learn a motor skill requires increased practice over much longer time periods and may

be influenced by offline-learning, consolidation and long term storage processes along with various cognitive processes including attention and decision making[14]. In order to adequately evaluate motor skill learning in post stroke therapeutic interventions, we must distinguish between acquisition and retention as distinct components of the motor learning process. Within the current proposal within session trial and error performance will be known as acquisition [15]. The ability to perform the skill at a period of time removed from the initial intervention without degradation of performance will be known as retention of the motor skill [15]. Within the literature, to effectively argue that the subject has "learned" something one must be able to demonstrate either retention of the skill at time removed from the initial practice, or generalization of the skill to other motor tasks. Retention of the skill or recall of the "motor memory" [16] is often demonstrated by "savings" in error based learning tasks. This savings refers to a more rapid rate of relearning compared with the rate of original learning [17,18].

Consolidation, or "off-line learning," results in improvements in a motor skill without practice and is considered the process of motor learning occurring between skill acquisition and retention without physical practice [15]. Krakauer and Shadmehr (2006) define this consolidation as "a set of processes whereby a long-term motor memory becomes more stable with the passage of time"[16]. This "off-line" process strengthens the memory representation resulting in an improvement in performance between practice sessions or increased resistance to interference from a secondary task [15].

Adaptation may be defined as the process of modifying or adjusting an already well-learned movement or motor skill that occurs over a period of trial-and-error practice

when exposed to a novel, perturbing environment [19]. Given this definition, motor adaptation can be considered as one specific component of motor skill learning. Adaptation occurs with trial by trial error modification in response to an environmental perturbation that alters the baseline movement pattern [20]. The perturbation provides a sensory prediction error in which the predicted outcome of the movement does not match the observed outcome [20,21]. In order for a subject to successfully adapt to the error, a process of CNS recalibration must take place to optimize the feed forward motor plan and minimize the costs of the task [21]. In a true adaptation paradigm, storage of the new motor pattern within the CNS will be reflected through 'after- effects' in which, upon removal of the stimulus, the subject cannot retrieve the previous motor behavior [22,23]. The subject must de-adapt, during a period of continued practice without the perturbation, in order to return to their previous baseline motor performance [23,24]. With continual adaptation and de-adaptation to an environmental perturbation the subject may develop the ability to switch between patterns of movement without a need for practice [19]. The ability to learn this new motor pattern may be demonstrated through "savings" [17,18].

Adaptation is typically thought to play a prominent role in error based learning through updating of an internal model, however, a role for operant reinforcement and use dependent plasticity cannot be discounted [17,25]. As such, within the current proposal we choose to discuss adaptation as a category of skill acquisition, with recognition that acquisition during error based learning paradigms requires various active learning processes that cannot be completely divorced from one another[25]. However, it must be noted that adaptation refers to refinement or modification of an already well learned

motor skill[19], rather than learning of a de novo task, as typically referenced with skill acquisition.

Given that motor adaptation involves relearning an already known movement pattern in response to novel environmental demands, the process strongly reflects the re-learning process of those post stroke early within any therapeutic intervention. This feature has made adaptation of key interest within motor learning research, particularly locomotor learning, despite its role as a short-term learning process. Locomotor adaptation affords the ability to learn and unlearn a given locomotor pattern rapidly depending on the environment, allowing for flexibility and efficiency [14]. The capacity of the nervous system to adapt to and store a new locomotor pattern that approximates an already stored walking pattern may provide insight into the ability of the damaged nervous system to regain a more normal walking pattern [23,26,27].

Current Evidence

Few studies have examined the motor learning capability of individuals post stroke [28–33] with most evidence confined to the upper extremity [28–30,34–38]. Based largely on research from the upper extremity [28–30,35], and few studies of the lower extremity [31–33], it is believed that those with brain damage as a result of stroke retain the ability to utilize trial and error practice to learn a novel motor task. Winstein et al. 1999 demonstrated that those with chronic stroke retain the ability to utilize augmented feedback in a manner similar to neurologically intact controls to learn a novel upper extremity task, however demonstrate greater errors and increased variability in their movements compared to

controls [29]. Notably, the researchers had subjects utilize their uninvolved upper extremity limiting the implications of these findings to post stroke rehabilitation in which the involved extremity is the main target of the therapeutic intervention. Platz and colleagues [30] also demonstrated that those post-stroke retain the ability to learn both simple and complex upper extremity motor tasks compared to neurologically intact controls. Subjects post-stroke however, demonstrated increased variability in their movements, increased errors and required increased time for performance during skill acquisition of a more complex maze coordination task and peg board task [30]. These studies highlight the capability of those post-stroke to perform and retain a novel motor task with use of the upper extremity. They also, however, call attention to an increase in variability and error during motor performance, in comparison to neurologically intact controls. It is plausible that this increased error and slowed performance may be increasingly detrimental in more complex functional tasks, such as locomotion, which may require increased practice to achieve learning and retention of the motor skill.

In comparison to the upper extremity, there is limited research exploring the acquisition and retention of lower extremity functional motor skills, despite an important link between post stroke ambulation and physical inactivity, community participation, and additional morbidity[8]. To date, literature to support lower-extremity motor-learning is limited and has been largely constrained to neurologically intact subjects [24,39,40]. However, recent evidence utilizing various locomotor adaptation paradigms indicates that those with chronic stroke and resultant hemiparesis retain the ability to learn a novel locomotor pattern [26,31–33]. Savin et al. (2013) required subjects with hemiparesis as well

as neurologically intact controls to overcome a novel swing phase resistance during treadmill walking[32]. They found that both neurologically intact and chronic stroke subjects were able to adapt both temporal and spatial parameters of gait. Those with chronic stroke however, differed in the rate of adaptation, requiring increased repetition during the late, slow phase of adaptation compared to controls. Tyrell et al. (2014) also found that those with chronic stroke retain the ability to acquire a novel locomotor pattern, however they require more days of practice to acquire the pattern compared to neurologically intact individuals[33]. Currently, the study by Tyrell and colleagues (2014) is the only study to demonstrate that learning, in addition to acquisition, of a novel locomotor pattern in slowed in those post-stroke[33]. The above cited studies indicate the ability of those post-stroke to utilize trial and error practice for skill learning, however indicate a difference in the rate of this learning. Given that the control of motor learning is suggested to occur in multiple brain areas that may be affected by stroke, it is plausible that rate delays may be a result of deficits in acquisition and retention of a motor skill within particular damaged cortical areas.

Given the evidence above it is likely that those with chronic stroke may retain the ability to utilize trial and error practice to learn a novel motor skill, however may require additional practice or different practice parameters in order to optimize skill learning. In the animal model the amount of practice needed to directly influence task dependent neuroplastic changes and demonstrate significant improvements in stepping quality is greater than 1,000 steps per session[41]. Corroborating this effect, Moore and colleagues (2010) previously demonstrated a dose- response relationship between the amount of

stepping practice and improved community ambulation in those post stroke[42]. Despite the apparent dose-response relationship, patients often receive a limited amount of locomotor practice within a physical therapy session limiting the potential for learning related neuroplastic changes[42]. This inconsistency highlights a crucial role for empirical evaluation of motor learning strategies which exploit neuroplasticity in the brain and provide efficient and effective rehabilitation strategies for those post-stroke.

Practice Paradigms

Constant vs. Variable Practice:

A fundamental principle of motor leaning is that performance improvement is dependent on the amount of practice (Schmidt R, 2011). The type of practice performed, however, may impact the acquisition as well as the retention of the skill. Motor learning studies of neurologically intact subjects demonstrate variable practice paradigms to improve motor learning relative to constant practice paradigms [43–45]. Within various upper extremity tasks in neurologically intact individuals, it has been demonstrated that task variability (variable practice) during initial performance results in improved retention while repetition of a task or blocked practice of several tasks (constant practice) during initial performance results in enhanced performance of the skill during the trial, however limits retention [43–45] and transfer of the skill to other tasks [43]. Retention and generalization of motor skills are two benefits of variable practice that have led this paradigm to be promoted for use in neurorehabilitation.

Variable practice is thought to exert its beneficial effects through a mechanism of contextual interference (CI) which refers to interference induced by the trial-to–trial variability of the practice schedule such that random or variable practice induces high interference while blocked or constant practice induces low interference [43]. It has been suggested that high interference, induced through variable practice, requires the learner to engage in increased cognitive processing during the task leading to a stronger motor memory representation allowing for increased retention [15]. Theoretical explanations for the contextual interference effect have been proposed with overlapping interpretations. The elaborative-distinctiveness hypothesis suggests variable practice provides an opportunity for comparison of tasks during the inter-trial interval which allows the learner to encode critical task relevant information resulting in a stronger memory representation. Constant or blocked practice, according to the elaborative-distinctiveness hypothesis, limits opportunities for inter-task comparison resulting in a less robust memory representation [46]. A forgetting-reconstruction hypothesis has also been put forth suggesting repetitive practice of the same task, as in constant practice, results in reliance on a previously constructed motor plan available in working memory requiring less cognitive processing. Variable practice of tasks however results in "forgetting" of the previous motor plan and reconstruction of the plan at the next presentation. Continued reconstruction of task parameters and movement strategies requires increased cognitive effort resulting in a stronger memory representation [47]. Despite the nuances of each hypothesis, both the elaborative distinctiveness and forgetting reconstruction hypothesis seem to identify variable practice to require increased cognitive effort that results in

slowed skill acquisition and poorer performance at immediate testing compared to constant practice, however, allows improved learning assessed at a later time point [15]. Lin et al. recently demonstrated the contextual interference effect in a group of healthy young subjects utilizing TMS to selectively interfere with encoding processes during variable and constant practice conditions. Single TMS pulses were applied over the primary motor cortex within the inter-trial interval. TMS disruption to M1 during the inter-trial interval diminished performance and learning in those who participated in variable practice implicating encoding within M1 during variable but not constant practice as a critical mechanism for the learning enhancements of variable practice [45].

Despite significant interest in practice structure within motor learning rehabilitation research and clinical practice, few studies have examined variable practice to enhance motor learning after neurological insult [28]. Furthermore, to our knowledge, a role for variable practice in complex motor learning tasks such as locomotion has yet to be addressed. It is plausible that the benefits of variable practice may not generalize to complex tasks such as locomotion (Wulf & Shea, 2002). Given that those with chronic stroke demonstrate increased errors and require increased practice [30,33,39] disruption of steady state practice during variable practice paradigms may limit the ability to acquire a novel locomotor pattern.

Location of cortical damage post-stroke may also play a determining role in the beneficial effects of variable practice. Schweighofer and colleagues (2011) demonstrated that the potential to exploit the benefits of variable practice in those post stroke was dependent upon the integrity of subjects visuospatial working memory[48]. Young

neurologically intact subjects as well as subjects at least 3 months post stroke were asked to learn 3 specific grip force patterns in either a variable or blocked schedule[48]. Visuospatial working memory was assessed prior to training with the figural memory subtest of the Wechsler memory test. Performance and learning was assessed immediately after and 24 hours post training. Corroborating previous results, neurologically intact subjects and those post stroke participating in variable practice demonstrated improved retention relative to those in the blocked condition. However, when subdividing subjects post stroke by visuospatial working memory, the benefits of variable practice were negated. Subjects post stroke who demonstrated impaired visuospatial working memory had improved retention in the blocked condition compared to those without working memory deficits. Consistent with previous contextual interference hypotheses, the authors suggest that those with decreased visuospatial working memory participating in constant practice "forget" the previous motor plan and must reconstruct the plan at each subsequent presentation [48]. "Forgetting" leads to enhanced cognitive processing which is thought to be a key mechanism allowing variable practice to exert its beneficial effects on retention[15]. Regardless of the mechanism involved, the study highlights the impact of stroke on neural function that may directly influence the role of various practice paradigms in motor skill acquisition and learning.

In addition to the variations attributable to the location and extent of cortical deficits, variable and constant practice have been found to rely on different neural substrates during skill acquisition and consolidation [15,45,49]. Kantak and colleagues (2010) utilized repetitive TMS (rTMS) to disturb M1 or the dorsolateral prefrontal cortex

during the consolidation phase immediately following variable or constant practice of an arm movement in healthy subjects [50]. Assessment of the effect of rTMS on motor memory consolidation and learning for constant and variable practice was assessed 24 hour later. Perturbation to M1 significantly attenuated learning for those in the constant practice group while rTMS applied to the dorsolateral pre-frontal cortex during consolidation limited the beneficial effects of the variable practice condition on learning [50]. TMS delivered to the contralateral M1 within the inter-trial interval, the interval of time following feedback just prior to performance of the next task, reduced learning of a rapid elbow flexion and extension task in healthy subjects receiving random practice. TMS to M1 had no effect on learning in those participating in constant practice [45]. The distinct neural substrates required for encoding and consolidation of a motor skill in variable and constant task practice may be differentially impacted as a result of stroke. Thus, the current proposal seeks to assess the role of variable and constant practice in locomotor adaptation in those post-stroke. Examination of these practice parameters may provide an enhanced understanding of the ability of those post-stroke to utilize trial and error practice to facilitate motor skill acquisition and learning as well as provide insight into optimal practice conditions for the promotion of walking recovery post stroke.

The Split Belt Treadmill:

As referenced above, the split-belt treadmill provides an excellent paradigm for exploring mechanisms of locomotor learning, particularly adaptation. The treadmill has two independent belts, one under each leg, so that subjects can walk with belts moving at the same speed, “tied”, or with the belts moving at different speeds “split”. By splitting

the treadmill belt speeds in a 2:1 ratio, the paradigm forces both neurologically intact and subjects post-stroke to alter their interlimb coordination while walking[40]. Initially, both spatial and temporal characteristics of step symmetry are altered, however over a period of ten to fifteen minutes this asymmetry will be reduced with the use of trial and error practice[40].This use of trial and error practice, or adaptation, to a perturbing environment provides important insight into the ability of the post-stroke CNS to temporarily store and recall a motor memory. When returning to a tied belt condition following 10-15 minutes of split belt walking both neurologically intact and subjects post stroke demonstrate after-effects indicating that the nervous system learned and stored a new locomotor pattern[40,51]. In addition, when gait asymmetry in those post stroke is initially exacerbated, after-effects produced can result in improved step length symmetry relative to baseline (tied-belt walking)[31]. This exaggeration of baseline asymmetry cues the nervous system to make a feedforward motor plan that re-establishes symmetry. This is an important concept for those with chronic stroke whose nervous system may no longer perceive gait deviations as errors, and require an exaggeration of this error in order to make a correction.

Thus, the split-belt treadmill paradigm allows exploration of various aspects of motor learning including adaptation and retention of a novel locomotor pattern, but also provides exploration of the capacity of the nervous system for error recognition and correction. Recent evidence suggests exaggeration of post stroke gait asymmetry using the split belt treadmill can lead to after effects resulting in a more symmetric pattern of walking on the treadmill as well as over ground[31]. With repeated exposure to split-belt

treadmill walking subjects post stroke demonstrate longer-term improvements in step length symmetry [52]. Consequently, the split belt treadmill can be utilized to facilitate improvements in asymmetric gait post stroke[52], or can be utilized as a specific probe of motor learning[53]. In the current proposal the split belt treadmill will be used as a probe of motor learning only, without reference to use as a therapeutic tool for intervention. The current proposal aims to use the split belt treadmill to assess characteristics of motor learning including variable or constant practice that may enhance or hinder motor learning in the chronic stroke population. Information regarding the advantages or disadvantages of the use of such practice may provide insight into optimal rehabilitations for enhancing locomotor learning post-stroke.

Mechanisms of Learning Dependent Cortical Plasticity: ***Brain Plasticity in Learning and Rehabilitation Post-Stroke, Brain Derived Neurotrophic Factor, and Genetic influences of BDNF on Motor Learning***

Brain Plasticity

In Learning:

The mechanisms which enable plasticity in the brain post-stroke are similar to those which occur in the healthy brain during learning [10,54]. As such, the neural constructs which enable motor skill learning, and the behavioral parameters which optimize such learning are of great interest to researchers in the field of rehabilitation [54]. Research on the neurobiology of learning and memory suggests that, for each new learning event, there is a required change in the nervous system to supports the learning (Hebb, 1949)

(Kandel ER, 2001). Animal models have corroborated this link between motor learning and the corresponding CNS changes, demonstrating alterations in motor map representations [2,13] as well as changes in gene expression, dendritic growth, synaptogenesis and increased neural excitability as a result of motor skill acquisition [3,55–57].

Motor learning paradigms involving skilled reaching in both the primate [13] and rodent model [3] have demonstrated the requirement of active learning, rather than repetitive movement, in order to facilitate plastic changes within the motor cortex with motor skill acquisition. In particular, monkeys trained to retrieve food pellets from small wells requiring increased manual dexterity, resulted in reorganization of movement representations of the motor cortex [13]. These changes were not present when the size of the well was increased, decreasing the skill demand required, demonstrating that simple repetition of a well learned task is not sufficient to induce plastic changes in the brain [13]. Similarly, Xu et al (2009) had rodents either perform a skilled seed reaching task or perform unskilled motor activity over the course of 16 days of training. Results revealed that animals participating in skilled reaching demonstrated an increase in dendritic spine formation within 1 hour post training on the first day of training. The number of dendritic spines formed were directly correlated with the number of successful reaches in the skilled reaching group. Over the course of training, synaptic reorganization was evidenced with an elimination of dendritic spines to control levels by day 16 of training. This selective pruning was learning specific in that pre-skill training dendritic spines were reduced at a greater percentage than new synaptic connections formed with training

[3]. The ability of motor skill learning to inhibit or facilitate neural connections provides a glimpse of the mechanisms regulating adaptive plasticity at the level of the individual synapse.

Recent advances in imaging technology has allowed similar, yet less invasive, exploration of cortical reorganization and functional modulation as a result of motor skill acquisition and learning in the human. Skill acquisition has been associated with changes in activation patterns in the motor cortex via fMRI as well as alterations in movement representation as revealed via transcranial magnetic stimulation. Long term practice of particular sensorimotor skills have been found to produce functional reorganization with increased cortical representation in the digits of the dominant hand of a skilled musician [4] or badminton player [6] relative to the non-skilled hand. On a much shorter time-scale, Perez et al. (2004), demonstrated enhanced corticospinal excitability in human participants trained to make skilled ankle movements relative to those trained to make generalized unskilled movements[7].

In Rehabilitation:

Alterations in motor map activation have been strongly correlated with the magnitude of functional recovery post stroke[58]. Motor skill acquisition as a result of rehabilitative training has been evidenced to induce alterations in motor maps and increase corticomotor excitability of the injured hemisphere as well as promote functional recovery in humans post stroke[5,59–61]. Functional MRI (fMRI) studies have demonstrated correlations between alterations in activation patterns of the ipsilesional sensorimotor cortex with improvements in motor function following rehabilitative training[59,60]. Leipert

and colleagues (2000) utilized transcranial magnetic stimulation (TMS) to map cortical motor output of the hand of both the injured and uninjured hemispheres in 13 subjects greater than 6 months post-stroke before and after rehabilitation training involving constraint induced movement therapy (CIMT). Following rehabilitative training of the impaired upper extremity, subjects post-stroke demonstrated an increased area of hand representation on the injured hemisphere corresponding with increased function of the impaired upper extremity. Follow-up examination revealed nearly identical cortical sizes for the hand representation at 6 months post training, with increased motor performance maintained[5]. More recent results corroborate the findings by Liepert and colleagues (2000) demonstrating a link between cortical reorganization assessed via TMS and functional improvements post stroke (Sawaki, et al., 2008). Similar morphologic and physiologic changes have been corroborated in the ischemic animal model. Adult primates with brain microlesions within the hand region of the motor map underwent CIMT, with restraint of the uninvolved upper extremity, or no intervention[2]. Primates receiving behavioral training demonstrated a 10% increase in the total hand area adjacent to original lesion compared to those without skill training who retained only 80% of the undamaged area of the hand representation[2]. These results, once again, corroborate the necessity of motor learning for facilitation of cortical reorganization.

As evidenced above, the underlying basis of cortical plasticity in learning has been extensively studied. Animal models have provided insight into the cellular and molecular events essential to motor skill learning, including structural and functional changes at the individual synapse and across the neuronal network. Until recently

however human studies have been limited in the ability to provide insight into the role of learning in neural plasticity beyond the systems level. Within the current proposal we seek to identify the role of brain derived neurotrophic factor (BDNF) as a key modulator of cortical plasticity within human motor learning utilizing non-invasive methods in neurologically intact and subject's chronic post-stroke.

Brain Derived Neurotrophic Factor

One particular molecular substrate implicated in modulation of nervous system plasticity is brain derived neurotrophic factor (BDNF). As one of four proteins within the neurotrophin family, it is well known for its role in survival, differentiation and maintenance of function of neurons[62–65]. Unlike other members of the neutrophin family however, BDNF has been found to be released in an activity dependent manner in response to neuronal activity making it a prime target for exploration of experience dependent neural plasticity[62,63,65].

Brain derived neurotrophic factor is broadly expressed in the adult brain, with intracellular BDNF found predominantly in glutamatergic neurons[62]. The role of BDNF in neuronal plasticity is dependent upon stimulation and release of the mature (mBDNF) versus the premature form (proBDNF). Brain derived neurotrophic factor is initially translated as a precursor protein (proBNDF) and subsequently proteolyticaly cleaved to generate a mature protein (mBNDF)[62,63]. Both the mature and pro forms of the protein are released in a constitutive as well as activity dependent manner. Activity dependent release of pro-BDNF however, is thought to be converted to mature BDNF

extracellularly, during high frequency stimulation as seen with long term potentiation[66]. Following activity dependent or constitutive release pro-BDNF binds to the p75 receptor with high affinity to elicit long term depression and apoptosis. Conversely binding of mBDNF to the tyrosine receptor kinase B (TRK-B) receptor facilitates cell survival, dendritic and axonal branching and synaptic efficacy at glutamatergic synapses through solicitation of various signaling cascades involved in synaptic plasticity and long term potentiation (LTP)[63,65]. The present study will focus on the activity dependent effects of the mature form of BDNF, found to be crucial for neural plasticity and learning.

The mature form of BDNF has become a major target of investigation in learning related neural plasticity secondary to its role in mediation of induction and maintenance of LTP [66], the most studied form of synaptic plasticity in learning and memory[67]. Increases in intracellular calcium induced through neuronal activity at glutamatergic synapses has been found to increase BNDF mRNA as well as stimulate release of the mBDNF protein[68]. Enhanced BDNF gene expression through the Ca2+- CaMKII pathway is a requisite for long term potentiation[66]. In vitro and in vivo work has demonstrated the functional and structural neuronal network changes corresponding to early and late long term potentiation occur through several BDNF mediated intracellular signaling cascades including mitogen activated protein kinase/extracellular signal-regulated protein kinase (MAPK/ERK), phosphatidylinositol 3-kinase (PI3k) and phospholipase C(PLCy)[63,65]. Through binding of these various pathways, BDNF has been shown to enhance the excitability of the post-synaptic cell and potential for synaptic transmission through increased calcium and neurotransmitter release, as well as promote

morphological changes including dendrite and axon branching for structural plasticity[62–65]. An in-depth discussion of the effects of BNDF signaling through the various pathways is beyond the scope of this introduction however, as a brief overview a graphical depiction ***(Figure 1.1)*** is provided to summarize the current known effects of these pathways on functional and structural synaptic plasticity.

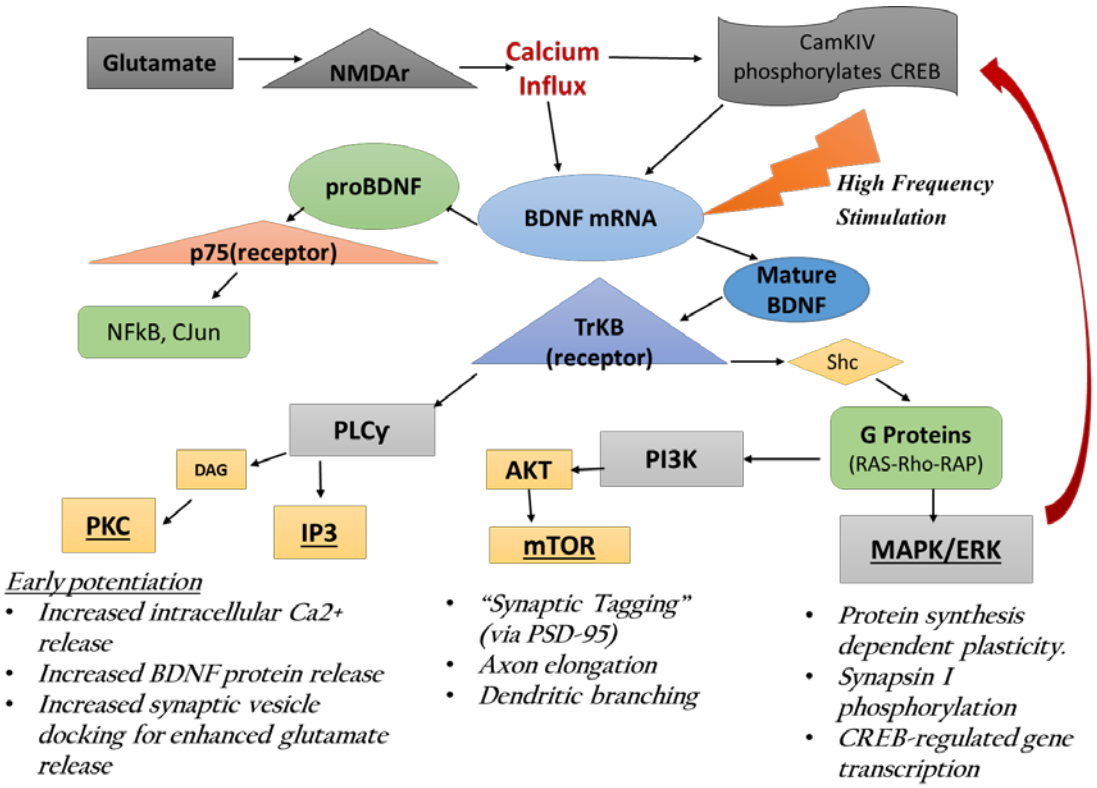

Figure 1.1 Mature BNDF-TrkB signaling activates the mitogen-activated protein kinase/extracellular signal-regulated protein kinase (MAPK/ERK), the phospholipase Cγ (PLCγ), and the phosphatidylinositol 3-kinase (PI3K) pathways to regulate multiple molecular events related to synaptic plasticity (Numakawa et al., 2010; Yoshii & Constantine-Paton, 2010).

The molecular attributes of mBDNF have been directly linked to behavioral outcomes in the animal model. Disruption of BDNF synthesis or BDNF-TrkB receptor binding through pharmacologic intervention impairs enhancement of neural plasticity, motor skill acquisition and cognitive learning in the animal model [69–74]. Ying et al. (2008) evidenced the role of exercise to enhance BDNF levels as well as the role of BDNF to modulate functional recovery post spinal cord injury[72]. Animals that were provided with voluntary running wheels for 14 days had significantly higher levels of BDNF mRNA as well as significant improvements in symmetrical stepping following hemisection. Animals receiving a BDNF receptor inhibitor however, showed significant impairments in recovery post-hemisection as well as blunted levels of BDNF and its downstream molecular mediators of plasticity. Specifically pertinent to post-stroke rehabilitation, Ploughman (2009) provided evidence for the role of BDNF in motor-skill relearning in rats with a middle cerebral artery occlusion induced stroke[69]. Animals were given either an antisense BDNF oligonucleotide to block production of BDNF mRNA, or a saline vehicle and then provided with a graduated rehabilitation program consisting of forelimb skilled reaching and exercise. The rehabilitation significantly improved forelimb reaching in animals who received the saline vehicle. The antisense BDNF oligonucleotide, however, significantly negated BDNF mRNA as well as negated any beneficial effects of rehabilitation. Corroborating these results, Schäbitz and colleagues (2007) demonstrated the ability of BDNF to improve long term functional sensorimotor outcomes in male rats with photothrombotic stroke in the R parietal cortex. Rats were assigned to either ischemia + vehicle, sham + vehicle, ischemia+BDNF or sham +

BDNF. Following induction of stroke, animals were treated with either intravenous saline vehicle or BDNF for 5 days post ischemia. Four hours after the treatment, dividing cells were labled for immunohistochemical analysis. All animals underwent sensorimotor tests including the rotorod, adhesive tape removal and balance beam test pre-ischemic induction and at 2,3,4, 5 and 6 weeks following ischemia. Neurologic function at all time points was scored based on motor, sensory and reflex tests. Results demonstrated significantly increased neurogenesis in the dentate gyrus and increased migration of progenitor cells to the striatum in the ischemic hemisphere of BDNF treated rats. In addition, BDNF-treated ischemic animals had improved motor performance on the balance beam task, improved sensorimotor function as measured by the adhesive tape removal task and improved locomotor function as assessed via the Rotorod test compared with the ischemia+vehicle group.

Taken together these results indicate that BDNF is a requisite for induction of neural plasticity with motor learning and may mediate functional recovery following neurologic insult in the animal model. To date, equivalent data in humans demonstrating BDNF's direct involvement in motor learning and functional recovery post-stroke is non-existent. However, genetic abnormalities in the BDNF gene have been linked to altered cortical structure[75,76] and function[77–80] as well as learning[79,81,82] in the human population.

The Genetic Influences of BDNF on Motor Learning

In roughly thirty percent of humans [83] a single nucleotide polymorphism (SNP) exists on the BDNF gene resulting in a substitution of methionine (Met) for valine (Val) at

position 66 on the amino acid chain (Val66Met)[77]. The Val66Met SNP has been linked to decreased secretion of the activity dependent mature form of the BDNF protein within the CNS[77,84]. In humans the SNP is associated with altered brain structure and function[78] with decreased prefrontal and hippocampal volumes[76]. In addition, presence of the polymorphism has been associated with altered cortical activation and short term plasticity[79,80,85] as well as altered skill acquisition and learning [79,82,85–87].

Joundi et al. examined visuomotor adaptation in a group of healthy participants with and without the Val66Met polymorphism[82]. Participants with the polymorphism demonstrated a decreased rate of adaptation to a 60 degree visuomotor deviation during skill acquisition as well as during 24 hour retention test. Those with the polymorphism did not demonstrate a difference in mean error, measured as the absolute angular error between the initial outward movement of the cursor and the target angle, nor did they demonstrate a difference in de-adaption versus participants without the polymorphism. However, when the angular deviation of the cursor was increased to an 80 degree deviation, those with the polymorphism demonstrated a significant increase in errors compared to the Val66Val participants[82]. McHughen and colleagues also assessed the effect of the Val66Met polymorphism on short term motor learning and retention with a driving based motor learning task[85]. Participants utilized a steering wheel to guide a vehicle on a computer screen through a curving track. The subjects were instructed to keep the vehicle centered over a black line in the center of the track. Maintaining the vehicle on the black lined required turning the steering wheel prior to the visual car moving along the path trajectory. Deviation from the black line was recorded throughout

15 repetitions of skill acquisition and at a retention test four days later. Similar to performance in the previously cited visuomotor adaptation task[82], subjects with the polymorphism demonstrated greater error, measured through deviation from the path trajectory, during short term learning as well as decreased retention of the driving task, with increased errors during retention testing, compared to those without the polymorphism[85]. The above studies seem to indicate that those with the polymorphism appear to have a decreased rate of skill acquisition and decreased retention relative to those without the polymorphism, however those with polymorphism retain the ability to adapt to and learn novel visuomotor pertubation[82] and driving based motor learning task[85].

The blunted skill acquisition and retention demonstrated above, however has not been consistently demonstrated in other similar motor learning paradigms[81,87,88]. These contrasting results, however, may be due in part due to the differing level of complexity of the tasks studied[88] as well as attenuation of genotype effects in older adults[81]. Previous evidence suggests learning, rather than simple repetition, is required to elicit synaptic plasticity and cortical reorganization [13,55,56]. Given BDNF is a primary mediator of learning related neural plasticity [63,66] it is plausible that BDNF mediated neural plasticity is utilized during learning of a novel learning task rather than simple repetition [56,88]. Of the current paradigms tested, learning of a more complex, real-world, motor task rather than simple motor performance, has only been assessed in one protocol[85]. The population sampled within the above studies may also limit the detection of the effects of the BDNF polymorphism on motor skill learning, with difficulty in providing a novel and

challenging motor task to healthy subjects with various levels of previous motor skill experience. The behavioral effects of reduced mBDNF secretion may be elucidated more fully in the compromised central nervous system where neural plasticity is a requisite for functional recovery. As mentioned above, pharmacologic blocks of BDNF synthesis and TrkB receptor binding, have led to significant limitations in motor skill relearning in the ischemic animal model[69,89]

Currently, there is no literature to support or refute the direct role of BDNF is motor function recovery in the human post stroke. A few studies have provided correlative evidence for an influential role for the BDNF Val66Met polymorphism in functional recovery post stroke[90,91]. Siironen and colleagues (2007) found those with the polymorphism to have an increased incidence of poor recovery following subarachnoid hemorrhage, as measured by the Glascow Outcome Scale (GOS), relative to those without the polymorphism[90]. Neither of the aforementioned studies utilized an outcome measure that differentiated between cognitive and motor recovery. Only one study has currently been published in the chronic stroke population[92]. This study found an association between the BDNF polymorphism and deficits in visual memory in those with subarachnoid hemorrhage, but not cerebral infarct. The study did not assess a role for the polymorphism in post stroke motor function.

It is currently unknown whether the Val66Met interferes with motor learning in those with chronic stroke. The current proposal aims to address this gap in empirical knowledge by examining the impact of the Val66Met polymorphism in learning of a novel locomotor task in subjects with chronic stroke (> 6 months post stroke). The

knowledge gained will directly impact the implementation of motor rehabilitation in the chronic stroke population.

BDNF, Exercise and Post-Stroke Recovery: *Exercise as a "Primer" and A Role for BDNF*

Exercise as a "Primer"

Animal experiments suggest the role of exercise as a "homeostatic" mechanism that provides a fertile environment to support the formation of functionally appropriate synaptic connections during learning [3,55,56,93]. By enhancing molecular mediators such as BDNF, exercise may strengthen synaptic transmission, thus "priming" the nervous system for encoding of pertinent information [74,93,94]. Animal models have corroborated the molecular influences of exercise with enhanced cognitive and motor performance and learning and have indicated BDNF as a key mediator of these enhancements[56,72–74,95]. Various animal studies have indicated a role for exercise in enhancement of cognitive function mainly through assessment of hippocampal regulated spatial learning[73,74]. Intlekofer and colleagues (2013) demonstrated that voluntary wheel running prior to an object location memory task was sufficient to promote retention and spatial memory while sedentary animals demonstrated no retention of the task [74]. Enhancement of spatial learning was directly related to elevation of hippocampal BDNF mRNA demonstrating a clear role for exercise mediated BDNF increases in facilitation of learning[74].

A role for exercise has also been demonstrated in motor learning in animal models, particularly during recovery of motor skill functions following various injuries to the central nervous system[69,72,94,95]. Ying et al. (2008) demonstrated the role of exercise in induction of synaptic mediators including BDNF for functional recovery following a spinal cord injury in rats. Animals were given a C4 level hemisection followed by either a TrkB inhibitor blocking binding of the BDNF-TrkB complex or saline vehicle 7 days post injury. Animals were further divided into an exercise and control group, with exercising animals exposed to a voluntary running wheel for 14 subsequent days. Results demonstrated exercise to significantly elevate protein levels of CREB, synapsin I and BDNF to uninjured control levels. Sedentary animals and exercising animals receiving the BDNF-TrkB binding inhibitor demonstrated higher levels of asymmetry during treadmill locomotion as well as significantly blunted levels of proteins related to synaptic function relative to exercising animals receiving a saline vehicle. The results demonstrate a causal link between BDNF regulation and exercise in determining functional recovery in spinal cord injury. A contributory link has also been demonstrated in the ischemic stroke model[69,71]. Ploughman and colleagues (2007) have demonstrated the ability of voluntary wheel running, to upregulate BDNF protein levels in the hippocampus and cortex following focally induced ischemia in rats [94]. Blockade of BDNF mRNA, via an antisense BDNF oligonucleotide, has also been demonstrated to negate the ability of exercise and rehabilitation to upregulate BDNF gene expression as well as limit the recovery of skilled reaching with rehabilitation[69]. Although the exercise mediated increases in levels of BDNF and other synaptic mediators were not directly assessed in

relation to functional recovery as eloquently evaluated in spinal cord injury[72], together the above studies maintain a role for exercise in moderation of synaptic mediators and recovery of function post-stroke.

Direct causal evidence demonstrated in the animal literature has not been demonstrated in humans, however converging evidence has linked exercise with enhanced brain function in both neurologically intact humans as well as those post stroke[93,96–99]. Aerobic exercise has been found to increase cognitive function in healthy individuals as well as those post stroke [98–102]. A meta-analysis of 18 studies examining the role of exercise training interventions in healthy older adults found that participation in aerobic activity elicited the greatest improvements in the executive control domain of cognitive function with the majority of studies involving moderate intensity exercise of ~70% heart rate max[101]. Similar results have been noted for those with chronic stroke [98,100]. Kluding et al. (2010) demonstrated that a combination of aerobic and resistance training 2-3 times per week for 12 weeks improved executive function and memory in individuals with chronic stroke[98]. This study however was limited by small sample size and lack of a control group.

Evidence citing the effects of aerobic exercise on *motor* learning in healthy subjects, as well as those post stroke, is sparse in comparison to studies of cognitive performance and learning. Currently, only two studies have provided evidence to support the role of aerobic exercise in enhancement of motor learning in young healthy adults[103] and chronic stroke survivors[102]. Roig and colleagues (2012) demonstrated that cycling above anaerobic threshold for 3- 3 minute intervals, prior to or following practice of a

motor task, was enough to enhance retention of the motor skill at 1 and 7 days post practice in healthy young adults[103]. Currently, Quaney and Colleagues (2009), is the only group to examine the influence of aerobic exercise on post stroke motor learning[102]. Within this study chronic stroke survivors participated in either an 8-week aerobic cycling program in which subjects were asked to exercise for 45 minutes, 3 sessions per week, at an intensity of 70% of their heart rate max or an 8 week stretching program. Subjects in the aerobic exercise group demonstrated improved processing speed on a serial reaction time task, improved predictive force accuracy during a gripping tasks as well as improved ambulation and sit to stand transfer speed relative to those within the stretching group.

A Role for BDNF?

It is clear that BDNF plays a mediating role in the molecular control of neuroplasticity and learning in the animal model and alterations in secretion, as with the BDNF polymorphism, alter learning and plasticity in humans. Given this evidence it is plausible that increases in BDNF levels with exercise may mediate the beneficial effects of exercise on learning. Aerobic and anaerobic exercise has been noted to increase systemic BDNF in humans[99,104–106]. The relative increase in BDNF is dependent upon the type and intensity of exercise as well as the process of analysis, time of day, and inter-subject variability including medication usage and menstrual cycle[99,104,105,107,108]. Following aerobic exercise systemic BDNF levels have been noted to remain elevated for 10 to 60 minutes[104]. Increases in circulating BDNF assessed in the peripheral blood is

considered to reflect CNS levels as the protein undergoes bidirectional transport across the blood brain barrier[109] and as such return of peripheral BDNF levels to baseline levels following exercise is theorized to reflect uptake of BDNF into the CNS[104].

Few studies have concurrently assessed the relationship between exercise induced changes in BDNF and learning. Of these studies the majority have assessed cognitive function in healthy humans[99,106]. Specifically, evidence provided by Winter and colleagues (2007) suggest a relationship between exercise intensity, BDNF levels and cognitive learning[99]. Short intervals of anaerobic exercise, 3 repetitions of 3 minutes each, were able to elicit increases in BDNF levels to a greater degree than moderate intensity longer duration activity. These increases in circulating BDNF were correlated with enhanced short-term retention of a novel vocabulary.

It is plausible that BDNF may also mediate a relationship between exercise and *motor* learning, as indicated in the animal model, although this relationship has not been examined in neurologically intact humans nor those post stroke. Through an increase in BDNF production, it is postulated that exercise may promote molecular processes involved in neural plasticity and cortical reorganization thus "priming" the nervous system for learning. As such an optimal timing and intensity of exercise may be required to capitalize on this enhanced neuroplasticity and thus facilitate improvements in motor function[99,103]. A specific relationship between BDNF levels and motor learning has yet to be evaluated in neurologically intact subjects or those post-stroke, however limited evidence suggests intensity of training may facilitate alterations in neural excitability and

improved locomotor function in those post-stroke[61]. As such, exercise may provide a novel adjunct to meaningful task specific practice in post stroke rehabilitation through a BDNF mediated mechanism. The current proposal aims to assess the role of BDNF in mediating motor skill performance and retention of a novel locomotor task following high intensity exercise. Measurement of peripheral BDNF levels during high intensity exercise and throughout the initial task specific practice will allow exploration of the suggested mediating role of BDNF on motor learning. Knowledge of the regulation of BDNF in the periphery with motor learning and physical activity may provide insight into the optimal timing and intensity of exercise required for enhanced motor performance and/or learning. Exploration in neurologically intact adults will be the first step in identifying parameters of exercise that may enhance BDNF mediated plasticity for optimal motor rehabilitation post stroke.

Overarching Aim of the Project

The global aim of this proposal is to understand the key molecular and behavioral requisites of motor rehabilitation post-stroke. The split belt treadmill will be utilized to investigate practice constructs which may promote or limit locomotor learning in those post-stroke. Addition of high intensity exercise prior to a novel locomotor learning task in neurologically intact individuals will provide insight into the role of exercise in promotion of locomotor learning. Systematic assessment of BDNF regulation with locomotor learning, via genetic analysis and peripheral protein assessments, may provide a more mechanistic analysis of optimal learning paradigms for post-stroke rehabilitation.

The studies in this proposal are the first to directly examine the relationship between stroke, motor learning and BDNF.

1.2 Specific Aims and Hypotheses:

Stroke is the leading cause of long term disability in the United States[8]. The ability to recover motor skill function post-stroke relies largely upon the adaptive capacity of the brain following neurologic insult[9]. The mechanisms which enable neural plasticity post-stroke are similar to those which promote neural reorganization in the healthy brain during learning (Nudo, 2003). As such, parameters of neuro-rehabilitation which optimize motor learning and enhance neural plasticity are of great interest to clinicians and researchers in the field of stroke rehabilitation. Within the current proposal we seek to identify mechanisms impacting neural plasticity and motor learning and the specific rehabilitation parameters that may optimize these mechanisms for optimal locomotor learning post-stroke.

Aim 1. To determine the role of a single nucleotide polymorphism (SNP) in the BDNF gene in moderating motor learning post-stroke.

Induction of neural plasticity through motor learning has been demonstrated in the animal[3,13,56] and human[4,7]. Animal models have provided insight into the cellular and molecular events essential to motor skill learning and neural plasticity, including structural and functional changes at the individual synapse and across the neuronal network[2,3,13,55,56]. One particular molecular substrate implicated in modulation of nervous

system plasticity is brain derived neurotrophic factor (BDNF), known for its role in survival, differentiation and maintenance of neurons[62–65]. Thirty percent of humans [83] possess a single nucleotide polymorphism (SNP) on the BDNF gene (Val66Met)[77] that has been linked to decreased activity dependent release of the mature form of the BDNF protein in the animal model [77,84]. Presence of the polymorphism has been associated with altered cortical activation and short term plasticity[79,80,85] as well as altered skill acquisition and learning [79,82,85–87] in healthy humans. Despite an established relationship between neural plasticity and motor learning post stroke, the impact of the Val66Met polymorphism on motor learning post stroke has yet to be explored. The current aim will address this gap by examining the impact of the Val66Met polymorphism in learning of a novel locomotor task in subjects with chronic stroke (> 6 months post stroke).

H1.1. *Those with the BDNF polymorphism will have an altered rate of adaptation to the split-belt treadmill, compared to those without the polymorphism.*

H1.2. *Those with the BDNF polymorphism will demonstrate an altered magnitude of adaptation compared to those without the polymorphism.*

Aim 2. To determine if the rate and/or magnitude of motor learning in subjects with chronic stroke differs depending on practice paradigm (variable vs. constant).

Restoration of movement function post-stroke is thought to be a function of motor learning or relearning due to reorganization of neural connections within the brain (Nudo, 2003). The optimal characteristics of learning which promote functional recovery of walking are not well defined for the post-stroke population. Studies of neurologically

intact subjects indicate that variable practice conditions result in greater motor learning than blocked practice[43–45], however few studies have demonstrated these beneficial effects on learning after neurological insult [28]. Furthermore, to our knowledge, a role for variable practice in complex motor learning tasks such as locomotion has yet to be addressed. It is plausible that the benefits of variable practice observed in more simple, upper extremity tasks may not generalize to complex tasks such as locomotion (Wulf & Shea, 2002). The current aim will utilize the split belt treadmill to examine characteristics of task practice, specifically variable and constant practice, that may limit or promote learning of a novel locomotor pattern in the chronic stroke population.

H2.1. *On the initial day of practice, chronic stroke survivors who participate in variable speed ratios of split-belt walking will demonstrate a decreased magnitude of adaptation compared to those participating in a constant 2:1 speed ratio.*

H2.2. *On Day 2, following one day of practice, chronic stroke survivors who participate in variable speed ratios of split-belt walking on day 1 will demonstrate a faster rate of re-adaptation and an increased magnitude of retention of the split belt walking pattern compared to those who participate in a constant 2:1 speed ratio of split belt walking.*

Aim 3. To determine the influence of high intensity exercise prior to a motor learning task on circulating levels of peripheral BDNF and motor learning in neurologically intact subjects.

BDNF levels have been directly related to exercise enhanced motor performance in the neurologically injured animal model[69,72]; however literature concerning the role of BDNF in enhancement of motor learning in the human population is limited. Previous studies in healthy subjects have shown a relationship between intensity of an acute bout of exercise and increases in circulating BDNF[99,104]. Furthermore, the intensity of exercise has been shown to have a moderating influence on the relationship between peripheral BDNF levels and cognitive learning[99,106]. The current aim will examine the role of high intensity exercise on upregulation of peripheral BDNF levels as well as the role of high intensity exercise in mediation of motor skill performance and retention of a novel locomotor task in neurologically intact adults.

H3.1. *Subjects who participate in a single session of high intensity upper extremity cycling immediately prior to split-belt treadmill walking will demonstrate greater increases in peripheral BDNF levels, compared to subjects who participate in quiet rest prior to split-belt treadmill walking.*

H3.2. *On the initial day of practice, subjects participating in high intensity exercise prior to split-belt walking will demonstrate an increased rate and magnitude of adaptation to the split-belt treadmill, in comparison to those who do not participate in exercise immediately prior to split-belt treadmill walking. The increased rate and magnitude of adaptation in those participating in high intensity exercise will be greater for those without the Val66Met polymorphism.*

H3.3. *On Day 2, following one day of practice, those participating in high intensity exercise prior to split-belt walking will demonstrate an increased rate of*

re-adaptation and an increased magnitude of retention of the split belt walking pattern compared to those who do not participate in exercise immediately prior to split-belt treadmill walking. The increased rate of re-adaptation and magnitude of retention in subjects participating in high intensity exercise will be greater for those without the Val66Met polymorphism.

Chapter 2

THE PRESENCE OF A SINGLE NUCLEOTIDE POLYMORPHISM IN THE BDNF GENE AFFECTS THE RATE OF LOCOMOTOR ADAPTATION AFTER STROKE

In review at Experimental Brain Research

2.1 Abstract

Induction of neural plasticity through motor learning has been demonstrated in animals and humans. One particular molecular substrate implicated in modulation of nervous system plasticity is brain derived neurotrophic factor (BDNF). Thirty percent of humans possess a single nucleotide polymorphism on the BDNF gene (Val66Met), which has been linked to decreased activity dependent release of BDNF. Presence of the polymorphism has been associated with altered cortical activation, short term plasticity and altered skill acquisition, and learning in healthy humans. The impact of the Val66Met polymorphism on motor learning post-stroke has not been explored. The purpose of this study was to examine the impact of the Val66Met polymorphism in learning of a novel locomotor task in subjects with chronic stroke. It was hypothesized that subjects with the polymorphism would have an altered rate and magnitude of adaptation to a novel locomotor walking paradigm (the split-belt treadmill), compared to

those without the polymorphism. Twenty-seven individuals with chronic stroke participated in a single session of split-belt treadmill walking and tested for the polymorphism. Step length and limb phase were measured to assess adaptation of spatial and temporal parameters of walking. The rate of adaptation of step length asymmetry differed significantly between those with and without the polymorphism, while the amount of total adaptation did not. These results suggest that chronic stroke survivors, regardless of presence or absence of the polymorphism, are able to adapt their walking pattern over a period of trial and error practice, however the presence of the polymorphism influences the rate at which this is achieved.

2.2 Introduction

The mechanisms which enable plasticity in the brain post-stroke are similar to those which occur in the healthy brain during learning [10,54]. As such, the neural constructs which enable motor skill learning, and the behavioral parameters which optimize such learning are of great interest to researchers in the field of rehabilitation [54]. Research on the neurobiology of learning and memory suggests that, for each new learning event, there is a required change in the nervous system to support the learning (Hebb, 1949; Kandel ER, 2001). Animal models have corroborated this link between motor learning and the corresponding CNS changes, demonstrating alterations in motor map representations [2,13] as well as changes in gene expression, dendritic growth, synaptogenesis and increased neural excitability as a result of motor skill acquisition [3,55–57].

Recent advances in imaging technology have allowed similar, yet less invasive, exploration of cortical reorganization and functional modulation as a result of motor skill acquisition and learning in the human. Motor skill acquisition as a result of rehabilitative training has been evidenced to induce alterations in motor maps and increase corticomotor excitability of the injured hemisphere as well as promote functional recovery in humans post stroke [5,59–61]. In addition, functional MRI (fMRI) studies have demonstrated correlations between alterations in activation patterns of the ipsilesional sensorimotor cortex with improvements in motor function following rehabilitative training [59,60]. Although the underlying basis of cortical plasticity in learning has been extensively studied in the animal model, until recently, human studies have been limited in their ability to provide insight into the role of learning in neural plasticity beyond the systems level.

One particular molecular substrate implicated in modulation of nervous system plasticity is brain derived neurotrophic factor (BDNF). Unlike other members of the neutrophin family, BDNF has been found to be released in an activity dependent manner in response to neuronal activity making it a prime target for exploration of experience dependent neural plasticity [62,63,65]. The role of BDNF in neuronal plasticity is dependent upon stimulation and release of the mature form of BDNF (mBDNF). The mature form of BDNF has become a major target of investigation in learning-related neural plasticity secondary to its role in mediation of induction and maintenance of long term potientiation (LTP) [66]. Disruption of BDNF synthesis or BDNF-TrkB receptor binding impairs enhancement of neural plasticity, motor skill acquisition and cognitive learning in the

animal model [69,72–74]. Current evidence in the animal model indicates that BDNF is a requisite for induction of neural plasticity with motor learning [69,71,89]. Equivalent data in humans is currently non-existent, however, genetic abnormalities in the BDNF gene have been linked to altered neural plasticity [80,86] and learning [81,82] in the human population.

In roughly thirty percent of humans [83] a single nucleotide polymorphism (SNP) exists on the BDNF gene resulting in a substitution of methionine (Met) for valine (Val) at position 66 on the amino acid chain (Val66Met) [77]. The Val66Met SNP has been linked to decreased secretion of the activity dependent mature form of the BDNF protein within the CNS [77,84]. In humans, the SNP is associated with altered brain structure and function [78] and with decreased prefrontal and hippocampal volumes [76]. In addition, presence of the polymorphism has been associated with altered cortical activation and short term plasticity [79,80,85,86] and altered skill acquisition and learning [79,82,85,86]. In particular, neurologically intact individuals with the polymorphism appear to have a decreased rate of adaptation to a visuomotor perturbation and a decreased rate of readaptation 24 hours later compared to those without the polymorphism [82].

To date, studies examining the influence of the polymorphism on motor learning have been confined to neurologically intact individuals [81,82,85–88]. Of these studies, all have utilized upper extremity paradigms to examine motor learning, with limited application to complex, real world motor tasks [85]. It is not known whether the polymorphism may also influence learning of a more complex lower extremity task such as locomotion in non-neurologically intact participants, such as those with stroke.

The split-belt treadmill paradigm has previously been well-characterized as a tool to probe short–term locomotor learning in neurologically intact and individuals post-stroke [14,26,40,52]. The short-term learning process of locomotor adaptation involves re-learning an already well-known movement pattern, similar to the re-learning process of those post-stroke early within a therapeutic intervention. Given neural plasticity and motor learning are inherently linked to functional recovery post-stroke, the behavioral effects of altered mBNDF secretion, through presence of the SNP, may be more apparent in this population.

Therefore, in the current study we sought to examine the impact of the Val66Met polymorphism in learning of a novel locomotor task in subjects with chronic stroke (> 6 months post stroke) utilizing the split-belt treadmill paradigm. We hypothesized that subjects with the polymorphism would demonstrate a slowed rate of adaptation to the novel locomotor walking pattern, compared to those without the polymorphism. Additionally, we hypothesized that subjects with the polymorphism would demonstrate a reduced amount of total adaptation relative to those without the polymorphism as well as a limited ability to return to their individual baseline walking (a)symmetry.

2.3 Methods

Participants

Participants at least 6 months post-stroke were recruited from Delaware and surrounding states with the assistance of local physical therapists, physicians and advertising. All participants provided written informed consent, with the study protocol

approved by the University of Delaware Human Subjects Review Board. To be included, subjects must have sustained one single stroke at least 6 months prior to study participation, the ability to ambulate independently with or without bracing, and walk for at least 4 minutes at a self-selected speed without assistance from another person. In addition, to be included participants provided written informed consent to supply a saliva sample for genetic testing for the BDNF Val66Met polymorphism. Exclusion criteria included history of cerebellar stroke, presence of cerebellar signs (ataxic gait or decreased coordination during rapid alternating hand or foot movements), neurologic conditions other than stroke, sensorimotor neglect, intermittent claudication, inability to walk outside the home prior to the stroke, or orthopedic problems of the lower extremities or spine that limited walking. In addition, those with a coronary artery bypass graft or myocardial infarction within 3 months, or unexplained dizziness within 6 months of study participation were excluded.

Instrumentation and Procedures

All subjects participated in a single session of split-belt treadmill walking in which the belts were set to a 2:1 speed ratio. Prior to split-belt treadmill walking, subjects were asked to walk on the treadmill with the belts tied at a 1:1 ratio at their fastest speed possible for 1 minute, followed by a speed half of their fastest possible for 2 minutes in order to assess baseline step and limb phase asymmetry. To achieve the subject's fastest possible speed the treadmill was increased by 0.1 m/s until the subject reported inability to tolerate a further increase or the researcher felt the subject would be unsafe at a faster speed. All subjects participated in split-belt treadmill walking for 10 to 15 minutes,

consisting of walking at a constant 2:1 speed ratio. For each participant the split-belt configuration (which leg was placed on the fast vs. slow belt) was chosen to provide an exaggeration of baseline asymmetry. The fast belt speed was set to the subject's fastest walking speed achieved on the treadmill and the slow belt was set to half of this speed. Subjects ambulated with this speed ratio throughout the entire session.

All participants walked on a split-belt treadmill instrumented with two independent six degree of freedom force platforms (AMTI, Watertown, MA) from which ground reaction force data was continuously collected at 1000Hz. Kinematic data was continuously collected using an 8-camera Vicon Motion Capture System (Vicon MX, Los Angeles, CA) at 100Hz. Retro-reflective markers (14-mm diameter) secured to rigid plastic shells were placed on the pelvis, bilateral thighs and bilateral shanks. Single markers were placed on the most prominent superior portion of the bilateral iliac crests, greater trochanters, medial and lateral knee joint lines, medial and lateral malleoli, bilateral heels, and the first and fifth metatarsal heads. During walking all subjects were instructed to gently rest fingertips on the treadmill handrail, and were given verbal cues, as necessary, to avoid excessive use of the handrail while walking.

All subjects wore a safety harness around their chest for fall prevention; however the harness did not provide body weight support. Blood pressure, heart rate and rating of perceived exertion (RPE) [110] were monitored throughout the treadmill walking session and subjects were provided with optional standing or sitting rest breaks. During optional rest breaks, subjects were not permitted to dismount from the treadmill.

Genotyping

Each subject provided a 2 mL saliva sample in a DNA Self-Collection Kit (DNA Genotek, Kanata, Canada) containing a DNA stabilizing buffer. The samples were sent to DNA Genotek (GenoFIND Services, Salt Lake City, UT) for processing. Genotek created a set of primers to amplify the region surrounding the SNP (Val66Met: rs6265) of the BDNF gene and then examined the sample for the presence or absence of the Val66Met polymorphism. Extracted DNA results of genotyping were sent to the primary investigator with remaining saliva samples destroyed following analysis.

Data Analysis

All kinematic and kinetic data were exported from Vicon-Nexus software, and further processed using Visual 3D (C-Motion, Inc, Germantown) and Matlab (MathWorks, Natick, MA). Gait events of foot strike and lift off were determined for each limb individually using an automatic algorithm in Visual 3D. Foot strike was identified when the vertical ground reaction force exceeded 20 Newtons for at least 8 frames, and lift-off identified when the vertical ground reaction force dropped below 20 Newtons for at least 8 frames. All gait events were visually checked for accuracy.

Dependent variables

Spatial and temporal parameters of gait have been found to respond differently during split-belt walking [33,53,111]. Therefore, both spatial (step length) and temporal (limb phasing) variables were evaluated. Both variables were calculated for each leg

continuously throughout treadmill walking. The spatiotemporal measure of step length was calculated as the sagittal distance between the right and left heel markers at foot strike. Step length was labeled as Left or Right based on leading leg. Stride by stride symmetry data for step length was calculated as:

$$\frac{\text{(Step Length of Leg on Slow Belt-Symmetrical Step Length)}}{\text{Symmetrical Step Length}}$$

Where symmetrical step length = (paretic step length + non-paretic step length)/2 [33,112].

Based on the above calculations, a value of 0 would indicate that the subject has achieved perfect symmetry based on their individual stride length. A negative value denotes the leg on the slow belt has a decreased step length relative to perfect symmetry. This method is preferred over the calculation of a ratio (paretic/non-paretic) because it prevents extremely large values when the denominator of the ratio is small due to a "step to" gait pattern in which one leg does not pass the other leg [113].

The temporal measure of limb phasing was calculated as previously reported [33,112]. Briefly, a calculation of limb phase for each leg provides a measure of the difference in time between the contralateral limb's peak flexion and the ipsilateral limb's peak extension, normalized by the ipsilateral limb's stride duration. Stride-by-stride limb phase symmetry was calculated by dividing the limb phase value for the leg on the slow belt by the contralateral limb phase value.

Locomotor adaptation to the split-belt treadmill paradigm, through trial and error practice, has previously been well characterized [14,26,40]. By splitting the treadmill belts in a 2:1 ratio, the split-belt paradigm requires both neurologically intact, and subjects post-stroke to alter their coordination while walking [26,40]. Initially characteristics of gait symmetry, including limb phasing and step length, are altered, however over a period of ten to fifteen minutes this asymmetry is reduced through use of trial and error practice [26,40].

To evaluate differences in locomotor adaptation in those with (MET) and without (VAL) the Val66Met polymorphism we examined: the *Rate of Adaptation*, the *Magnitude of Total Adaptation*, and *Return to Baseline*. Calculations were performed for both step and limb phase symmetry.

Rate of Adaptation. For each variable the rate of adaptation was calculated by first removing baseline (a)symmetry from each raw symmetry value to provide a value that reflects the deviation from the individual's baseline (a)symmetry pattern [33,112,114]. A value of 0 reflects a pattern identical to baseline (a)symmetry. Subtraction of the baseline (a)symmetry pattern allows for comparison of data across subjects who may demonstrate different levels of baseline asymmetry. In order to account for individual differences in the initial asymmetry at the start of the split-belt paradigm (initial perturbation) individual stride data was normalized by initial perturbation [114]. Normalization was achieved by dividing each symmetry value by the initial perturbation value, where initial perturbation was defined as the average of the first 3 strides during adaptation [114]. This normalization allows individual subject data to be scaled to a proportion of the initial perturbation [114].

Individual stride data, was separated into “Early” and “Late” adaptation. A value of 30 strides was selected to represent *Early* adaptation for step length asymmetry. Previous literature indicates that adaptation to limb phase asymmetry occurs on a much shorter timescale than step length adaptation [33,111]. In order to accurately capture rapid adjustments in limb phase asymmetry we utilized the first 10 strides to assess *Early* adaptation for limb phase. *Late* adaptation for both step and limb phase asymmetry were represented by the last 100 strides of adaptation for each individual subject.

Group (presence vs. absence of Val66Met polymorphism) averages of stride by stride data for *Early* and *Late* adaptation were then compared through a linear regression.

Magnitude of Total Adaptation. To evaluate the total amount of adaptation for both step length and limb phase symmetry during split-belt treadmill walking, the magnitude of total adaptation was calculated as follows:

Magnitude of Total Adaptation = Mean of Initial 10 Strides – Mean of Last 10 Strides.

This calculation represents the difference between the (a)symmetry pattern utilized at the start of adaptation and the (a)symmetry pattern utilized at the end of adaptation. A larger positive number would indicate a larger amount of adaptation.

Return to Baseline. To assess whether subjects were able to fully adapt back to their baseline (a)symmetry, the amount of adaptation relative to their individual baseline was calculated as follows:

Return to Baseline = Mean of Last 10 strides – Mean of tied slow (baseline)

This calculation represents the difference between the (a)symmetry pattern achieved at the end of adaptation and the subject's baseline (a)symmetry pattern with the belts tied at a 1:1 speed ratio. A value of 0 would indicate the subject has completely adapted to the split-belt treadmill and has returned back to their baseline (a)symmetry pattern, despite the continued split-belts.

Statistical Analysis

All statistical analyses were completed with SPSS v22.

In order to test our hypothesis that subjects with the polymorphism (Met) would demonstrate a slowed ***Rate of Adaptation*** compared to those without the polymorphism (Val), a linear regression was performed for *Early* and *Late* adaptation separately. In the regression analyses, the n would then be the number of steps. To ensure that averaging across individuals for adaptation within a group was appropriate, each individual's stride data over time was examined for the nature of the relationship using a modified Box-Cox test for linearity (Draper, 1998). To ascertain the appropriate use of a linear model (Osborne, 2010) an *apriori* decision was made to utilize group data when greater than 70% of subjects within each group (Val vs. Met) met the criteria for a linear relationship. A linear relationship was defined as 95% confidence interval around the Box-Cox lambda containing one. Moderated regression was then used to test if the relationship between the change in asymmetry and stride number during both *Early* and *Late* adaptation differed

with presence or absence of the polymorphism. Normality of the data was assessed using Kolmogorov-Smirnov test.

To test our hypothesis that subjects with the polymorphism would demonstrate a reduced ***Magnitude of Total Adaptation*** *and* ***Return to Baseline***, group differences (presence vs. absence of Val66Met polymorphism) were assessed utilizing an ANCOVA, with the initial perturbation value as the covariate to adjust for individual differences in the initial perturbation [114].

2.4 Results

A total of twenty seven participants, 11 with the polymorphism (67.75+/- 9.5 yr) and 16 without the polymorphism (67.0+/- 6.7 yr), participated in this study. There were no significant differences in baseline demographics or clinical scores between subjects with and without the polymorphism, all $p > .05$, *see Table 2.1*. Subjects with and without the polymorphism also did not differ in amount of initial perturbation to the split belt treadmill for step length and limb phase asymmetry ($p=.312$ and $p=.187$ respectively).

Table 2.1 Subject Characteristics

	VAL66VAL ***(n=16)***	**VAL66Met** ***(n=11)***
Age (years) (mean±std)	65.10 ± 9.26	61.45 ± 6.19
Time since Stroke (months) (mean±std	43.8 ± 42.45	32.18 ± 21.9
Total Fugl Meyer (mean±std)	22.50 ± 4.62	23.30 ± 5.30
Fast speed on treadmill (m/s) (mean±std)	0.64 ± 0.24	0.78 ± 0.19
Baseline Step Length (a)symmetry (m) (mean±std)	0.14 ± 0.15	0.07 ± 0.08
Baseline Limb Phase (a)symmetry (m) (mean±std)	0.90 ± 0.24	0.90 ± 0.20

Rate of Adaptation

Figure 2.1 illustrates the pattern of changes in step length asymmetry with exposure to the split-belt treadmill in the group of participants with (MET) and without (VAL) the Val66Met polymorphism. When the treadmill belt speeds are set to a 2:1 speed ratio with the paretic leg walking on the slow belt and non-paretic leg walking on the fast belt, both groups of subjects (Val and Met) demonstrate an increase in step length asymmetry relative to their baseline. With a period of trial and error practice ("Adaptation"), both groups demonstrate the ability to reduce this asymmetry despite the belts still moving at a 2:1 speed ratio. The two groups, however, demonstrate two divergent patterns of adaptation. Those with the polymorphism (MET) demonstrate a slowed rate of initial adaptation relative to those without the polymorphism (VAL). In addition, those with the polymorphism (MET) continue to adapt their step length asymmetry throughout

adaptation, while those without the polymorphism (VAL) appear to plateau near their baseline symmetry.

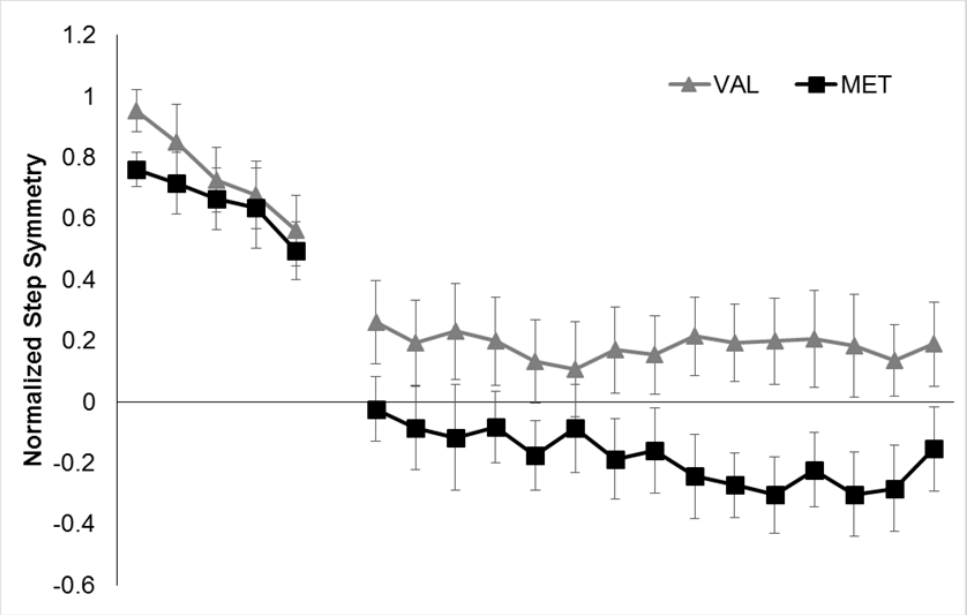

Figure. 2.1 Normalized step symmetry over the first 50 and last 150 strides of adaptation. Each data point represents the group average of 10 symmetry values for VAL (gray) and MET (black) groups. Error bars = standard error.

These qualitative results are supported by the quantitative data. The results of the linear regression show that those with the polymorphism (MET) demonstrate a slowed rate of step length adaptation relative to those without the polymorphism (VAL) within *Early* adaptation (Figure 2.2; p=0.000).

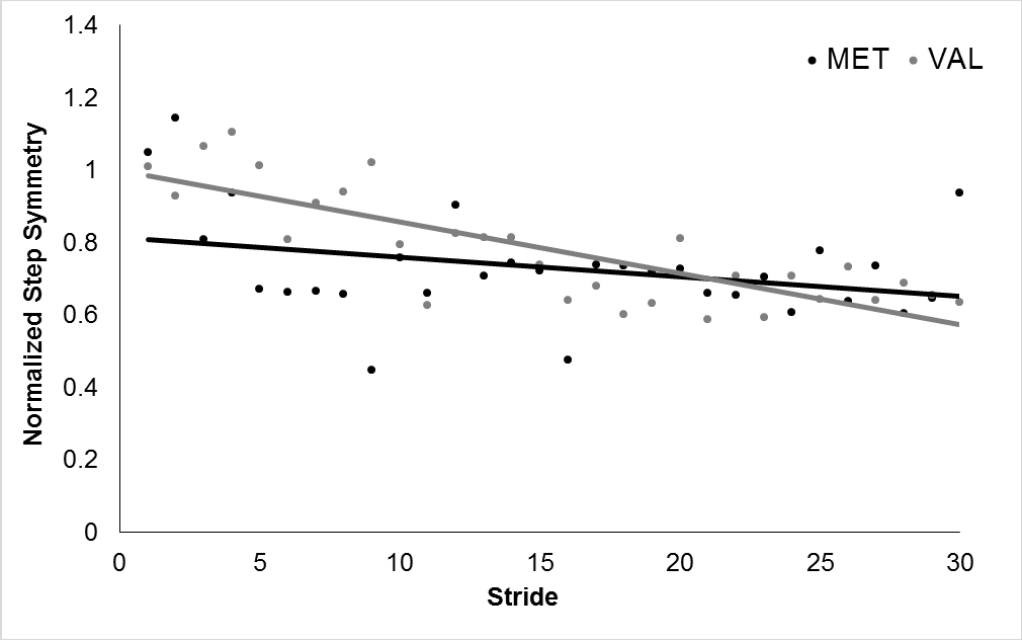

Figure 2.2 Early Adaptation. Group averaged stride by stride data for normalized step asymmetry over the first 30 strides of adaptation for VAL (gray) and MET (black) subjects. Each data point represents the average of the individual step length asymmetry value per group for each stride.

In the first block, Group (presence vs. absence of the polymorphism) and Stride (group step symmetry values for each of the first 30 strides) were able to predict change in asymmetry within *Early* adaptation (R^2=0.331; p= 0.000; Table 2.2). Addition of the interaction term (Group x Stride) significantly improved the model (ΔR^2=0.108; p=0.002; Table 2.2), indicating that the groups differed in how step length asymmetry was reduced over time. For *Late* adaptation, the first block of Group and Stride, predicted change in asymmetry (R^2 =0.711; p= 0.000; Table 2.3). Addition of the interaction term (Group x Stride) improved the model (ΔR^2 =.006; p=0.041; Table 2.3), indicating a small

difference in the relationship between stride and asymmetry for VAL and MET subjects. In contrast to rate of step length adaptation, there were no differences in the rate of limb phase adaptation for those with and without the polymorphism.

Table 2.2 Sequential linear regression model predicting change in asymmetry over the first 30 strides (*Early* adaptation) for those with (MET) and without (VAL) the polymorphism.

Model #	Predictors	Model *p*	ΔR^2	ΔR^2 *p*
1	Group Stride	.000	.331	.000
2	Group Stride Group x Stride	.000	.108	.002

Table 2.3 Sequential linear regression model predicting change in asymmetry over the last 100 strides (*Late* adaptation) for those with (MET) and without (VAL) the polymorphism.

Model #	Predictors	Model *p*	ΔR^2	ΔR^2 *p*
1	Group Stride	.000	.711	.000
2	Group Stride Group x Stride	.000	.006	.041

Given the significant group differences in the rate of *Early* step length adaptation, it was important to examine how the groups may differ in the pattern of this adaptation. To assess differences in the pattern of *Early* step length adaptation between those with and without the polymorphism, stride by stride symmetry data were group averaged as the first, second, and third 10 symmetry values within the first 30 strides (Figure 2.3). Each group of ten symmetry values within the first 30 strides were then compared using a repeated measures ANOVA for each group individually. Those without the polymorphism (VAL) demonstrated a significant decrease in step length asymmetry within the first 30 strides, while those with the polymorphism (MET) did not (Figure 2.3; $p = 0.015$ and $p = 0.522$, respectively).

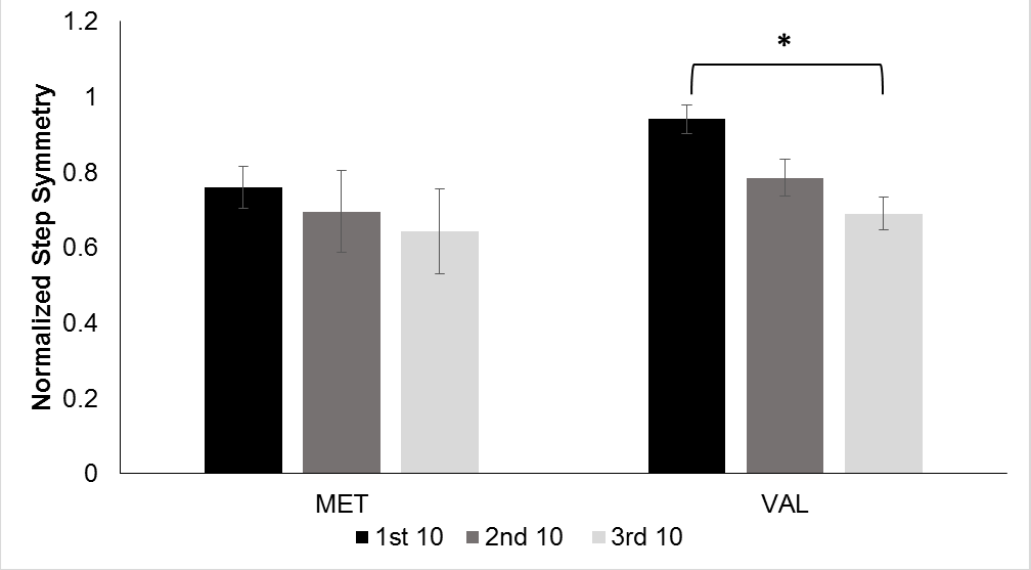

Figure 2.3 Early Adaptation. Group step symmetry data for the first, second and third ten step symmetry averages over the first 30 strides. *p=0.015

Magnitude of Adaptation

Despite a slowed rate of step-length adaptation, those with the polymorphism did not demonstrate a reduced ***Magnitude* of *Total Adaptation*** relative to those without the polymorphism. The groups did not differ significantly in the total amount of adaptation for step length or limb phase (Table 2.4). In addition, subjects with the polymorphism were able to achieve a magnitude of step and limb phase (a)symmetry relative to baseline (***Return to Baseline****)* that did not differ significantly from that achieved by those without the polymorphism (Table 2.4).

Table 2.4 Non significant step and limb phase symmetry variables. Average and standard deviation for *Total Adaptation* (average of first 10 symmetry values - last 10 symmetry values) and *Return to Baseline* (average of last 10 symmetry values - baseline symmetry values).

Step Symmetry (m)		
	Total Adaptation	Return to Baseline
VAL	0.19 ± 0.11	0.02 ± 0.10
MET	0.15 ± 0.08	-0.02 ± 0.08
Limb Phase Symmetry		
	Total Adaptation	Return to Baseline
VAL	0.27 ± 0.20	0.20 ± 0.25
MET	0.31 ± 0.24	0.15 ± 0.13

2.5 Discussion

The results of this study demonstrate that chronic stroke survivors, regardless of presence or absence of the BDNF Val66Met polymorphism, are able to adapt their walking pattern over a period of trial and error practice, however the presence of the polymorphism influences the rate at which this is achieved. Specifically, our results suggest that the process of modifying a spatial parameter of gait to a novel locomotor task is slowed in those with the polymorphism. The current study provides a crucial first step in identifying mechanisms of neural plasticity and motor learning that may influence the response to rehabilitation interventions and identifies a potential biomarker for individualization of rehabilitation post-stroke.

Within the current study chronic stroke survivors with the BDNF Val66Met polymorphism demonstrated a slowed rate of step length adaptation to a novel locomotor task, however were able to achieve a similar amount of total adaptation relative to those without the polymorphism. Similar findings have been reported within a visuomotor adaptation task in neurologically intact individuals [82]. Specifically, Joundi et al. found that neurologically intact participants with the polymorphism demonstrated a decreased rate of adaptation to a 60 degree visuomotor deviation during skill acquisition as well as during a 24 hour retention test. The subjects, however, did not differ significantly in mean error at the end of adaptation, indicating subjects with and without the polymorphism had similar levels of total adaptation [82]. Together these findings indicate that subjects with and without stroke with the BDNF Val66Met polymorphism are able overcome slowed adaptation with repetition. This is in line with previous evidence

showing that intense training on a marble navigation task can overcome deficits in motor map plasticity in those with the polymorphism [115].

How are subjects with the polymorphism able to achieve similar amounts of total step length adaptation despite differing rates of step length adaptation? As shown in Figure 1, subjects with the polymorphism show a subtle continued reduction in step length asymmetry over the last 100 strides, while subjects without the polymorphism show no change. While the results of the regression confirm these differences (Table 2.3), the change in R^2 is very small and would generally not be considered meaningful. Nevertheless, it appears that these small changes in late adaptation in those with the polymorphism allowed them to achieve similar amounts of total step length adaptation as those without the polymorphism. The contrasting behavioral patterns during both *Early* and *Late* adaptation, may be due in part to a deficit in error processing in those with the Val66Met polymorphism [79].When performing a stimulus-response flanker task, neurologically intact subjects with the polymorphism demonstrated a decreased neural response to error and thereby a lessened behavioral response [79]. It is plausible that decreased error recognition in those with the polymorphism limited the drive to detect and reduce the "error" signal induced through the exaggeration of step length asymmetry in the current study. This lack of drive may have been demonstrated behaviorally through a reduced rate of adaptation. If present, this reduced error recognition could also limit the ability to plateau at one's previous baseline. Although not significant in the current study, qualitatively, subjects with the polymorphism appear to continue past their baseline asymmetry (Figure 2.1). This is an important concept for those with chronic

stroke whose nervous system may no longer perceive gait deviations as errors, and require an exaggeration of this error in order to make a correction. As such, the polymorphism may present an additional obstacle for motor learning in those post-stroke.

The current results demonstrate deficits in the rate of adaptation to spatial (step length) but not temporal (limb phase) parameters of gait, in those with the polymorphism. This discrepancy between the spatial and temporal variables is not entirely surprising. Differences in adaptation rates of temporal versus spatial characteristics of gait have been previously demonstrated in neurologically intact subjects [111] and in those with stroke (Tyrell et al, 2014). Temporal characteristics of gait appear to be much more resistant to manipulations of practice structure[111] as well as developmental stage [114]. In addition, a previous study of subjects with chronic stroke adapting to the split-belt treadmill showed a slowed rate of adaptation compared to neurologically intact subjects for step length, but not for limb phase (Tyrell et al, 2014). These differences in temporal versus spatial gait characteristics have been postulated to be due to differing sites of neural control [24,53] with temporal characteristics thought to be under greater subcortical control. As such, temporal characteristics may be more resistant to manipulation with the split-belt paradigm, regardless of presence or absence of the polymorphism.

Within the current paradigm chronic stroke subjects demonstrated a slowed rate of step length adaptation with continued use of trial and error practice throughout treadmill walking. It is currently unknown if providing additional practice would result in a plateau in adaptation in the subjects with the polymorphism. In a recent study of longer-term learning of the split-belt walking pattern, it was shown that although those

with chronic stroke took an additional day of practice to reach a stable plateau in learning, compared to neurologically intact controls, they were able to learn the pattern with this additional practice [33]. It may be that chronic stroke survivors with the BDNF Val66Met polymorphism require even more practice, or different practice parameters, to achieve longer term learning of a novel walking pattern. Longer-term studies in the post-stroke population are needed to better understand the impact of the BDNF polymorphism on post-stroke motor learning.

2.6 Conclusions

The goal of this study was to examine the role of a single nucleotide polymorphism (SNP) in the BDNF gene in moderating motor learning post-stroke. To our knowledge this is first study to address the role of BDNF in motor learning post-stroke. The results suggest that chronic stroke survivors, regardless of presence or absence of the polymorphism, are able to adapt their walking pattern over a period of trial and error practice. The process of locomotor adaptation, however, is slowed in those with the Val66Met polymorphism. These results have important implications for motor learning and rehabilitation post-stroke because they identify a population that may benefit from increased practice or differing practice parameters to facilitate optimal motor learning. In addition, the current results identify a potential biomarker that may be utilized to further individualize treatment approaches within rehabilitation post-stroke.

Chapter 3

VARIABLE AND CONSTANT PRACTICE IN LOCOMOTOR LEARNING AFTER STROKE

3.1 Abstract

Although significant effort is concentrated toward gait retraining during stroke rehabilitation [116], thirty-three percent of community-dwelling individuals post-stroke continue to demonstrate gait asymmetries following participation in conventional rehabilitation [117]. The optimal characteristics of learning which promote functional recovery of walking have yet to be defined for the post-stroke population. Studies of neurologically intact subjects indicate that variable practice conditions result in greater motor learning than blocked practice [43–45], however few studies have demonstrated these beneficial effects on learning after neurological insult [28]. To our knowledge, a role for variable practice in complex motor learning tasks such as locomotion has yet to be addressed. The purpose of this study was to examine characteristics of task practice, specifically variable and constant practice, that may limit or promote learning of a novel locomotor pattern in those with chronic stroke. We hypothesized that chronic stroke survivors who participated in variable practice would demonstrate a decreased adaptation to a novel locomotor walking paradigm (the split-belt treadmill) on an initial day of

practice compared to chronic stroke survivors participating in constant practice. However, with a subsequent day of testing, subjects participating in variable practice would demonstrate a faster rate of re-adaptation and increased retention of the novel locomotor pattern compared to subjects participating in constant practice. Thirty-two chronic stroke survivors participated in two 15 minute sessions of split-belt treadmill walking. Step length and limb phase were measured to assess adaptation and retention of spatial and temporal parameters of walking. Subjects participating in variable practice demonstrated similar adaptation to those participating in constant practice. The magnitude of retention of the split-belt walking pattern also did not differ between groups. The results suggest that variable practice does not limit the ability of chronic stroke survivors to adapt to a novel locomotor pattern. However, in contrast to studies in neurologically intact subjects, variable practice does not appear to impart any additional benefit to learning of the novel walking pattern in chronic stroke survivors.

3.2 Introduction

Few studies have examined the motor learning capability of individuals post stroke [28–33] with most evidence confined to the upper extremity [28–30,34–38]. In comparison to the upper extremity, there is limited research exploring the acquisition and retention of learning of lower extremity functional motor skills. This is particularly surprising given that the ability to regain ambulatory function post-stroke is a common goal of stroke survivors (Bohannon RW, 1998) and rehabilitation professionals [116].

Recent evidence utilizing various locomotor adaptation paradigms indicates that those with chronic stroke and resultant hemiparesis retain the ability to adapt their walking to accommodate a novel locomotor pattern [26,31–33]. Savin et al. (2013) required subjects with hemiparesis as well as neurologically intact controls to overcome a novel swing phase resistance during treadmill walking[32]. They found that both neurologically intact and chronic stroke subjects were able to adapt temporal and spatial parameters of gait. Those with chronic stroke however, differed in the rate of adaptation, requiring increased repetition during the late, slow phase of adaptation compared to controls. Tyrell et al. (2014) also found that those with chronic stroke retain the ability to acquire a novel locomotor pattern when walking on a split-belt treadmill with the belts moving at two different speeds, however they adapted their walking pattern more slowly compared to neurologically intact individuals[33]. In addition, stroke survivors also required more days of practice to acquire the novel locomotor pattern in comparison to neurologically intact controls. Currently, the study by Tyrell and colleagues (2014) is the only study to demonstrate that learning of a novel locomotor pattern in slowed in those post-stroke[33].

Given this limited evidence, it is likely that those with chronic stroke may retain the ability to utilize trial and error practice to learn a novel motor skill, however may require additional practice or different practice parameters in order to optimize learning. Empirical evaluation of the parameters of motor learning which enhance locomotor learning and provide efficient and effective rehabilitation strategies for those post-stroke is currently lacking.

Motor learning studies of neurologically intact subjects demonstrate that variable practice paradigms improve motor learning relative to constant practice paradigms [43–45]. Within various upper extremity tasks in neurologically intact individuals, it has been demonstrated that task variability (variable practice) during initial performance results in improved retention while repetition of a task or blocked practice of several tasks (constant practice) during initial performance results in enhanced performance of the skill during the trial, however limits retention [43–45]. Retention and generalization of motor skills are two benefits of variable practice that have led this paradigm to be promoted for use in neurorehabilitation.

Despite significant interest in practice structure within motor learning rehabilitation, few studies have examined variable practice to enhance motor learning after neurological insult [28,48]. Furthermore, to our knowledge, a role for variable practice in complex motor learning tasks such as locomotion has yet to be addressed. Given that those with chronic stroke demonstrate increased errors and require increased practice [30,33,39] disruption of steady state practice, which has been shown to be important for retention (Huang et al, 2011), during variable practice paradigms may limit the ability to acquire a novel locomotor pattern.

Therefore, the purpose of the current study was to examine the impact of task characteristics, specifically variable and constant practice, on locomotor learning in subjects with chronic stroke (>6 months post stroke). We hypothesized that chronic stroke survivors who participated in variable speed ratios of split-belt walking would demonstrate a decreased magnitude of adaptation on the initial day of practice compared

to subjects participating in a constant 2:1 speed ratio. However, with a subsequent day of testing, subjects participating in variable practice would demonstrate a faster rate of re-adaptation and increased retention of the novel locomotor pattern compared to subjects participating in constant practice.

3.3 Methods

Participants

Participants at least 6 months post-stroke were recruited from Delaware and surrounding states with the assistance of local physical therapists, physicians and advertising. All participants provided written informed consent, with the study protocol approved by the University of Delaware Human Subjects Review Board. To be included, subjects must have sustained one single stroke at least 6 months prior to study participation, demonstrated the ability to ambulate independently with or without bracing, and walk for at least 4 minutes at a self-selected speed without assistance from another person. Exclusion criteria included history of cerebellar stroke, presence of cerebellar signs (ataxic gait or decreased coordination during rapid alternating hand or foot movements), neurologic conditions other than stroke, sensorimotor neglect, intermittent claudication, inability to walk outside the home prior to the stroke, or orthopedic problems of the lower extremities or spine that limited walking. In addition, those with a coronary artery bypass graft or myocardial infarction within 3 months, or unexplained dizziness within 6 months of study participation were excluded.

Instrumentation and Procedures

All subjects participated in two consecutive days of split-belt walking. All subjects walked on a split-belt treadmill instrumented with two independent six degree of freedom force platforms (*Bertec* Co., Columbus OH, USA) from which ground reaction force data was continuously collected at 1000Hz. Kinematic data was continuously collected using an 8-camera Vicon Motion Capture System (Vicon MX, Los Angeles, CA) at 100Hz. Retro-reflective markers (14-mm diameter) secured to rigid plastic shells were placed on the pelvis, bilateral thighs and bilateral shanks. Single markers were placed on the most prominent superior portion of the bilateral iliac crests, greater trochanters, medial and lateral knee joint lines, medial and lateral malleoli, bilateral heels, and the first and fifth metatarsal heads. During walking all subjects were instructed to gently rest fingertips on the treadmill handrail, and were given verbal cues, as necessary, to avoid excessive use of the handrail while walking.

All subjects wore a safety harness around their chest for fall prevention; however the harness did not provide body weight support. Blood pressure, heart rate and rating of perceived exertion (RPE) [110] were monitored throughout the treadmill walking sessions and subjects were provided with optional standing or sitting rest breaks. During optional rest breaks, subjects were not permitted to dismount from the treadmill.

Prior to split-belt treadmill walking on Day 1, subjects were asked to walk on the treadmill with the belts tied at a 1:1 ratio at their fastest speed possible for 1 minute, followed by a speed half of their fastest possible speed for 2 minutes in order to assess baseline step and limb phase asymmetry. To determine the subject's fastest possible

speed, the treadmill speed was increased by 0.1 m/s until the subject reported inability to tolerate a further increase in speed or the researcher felt the subject would be unsafe at a faster speed. Following tied belt walking, subjects then participated in split-belt treadmill walking for 15 minutes. For each participant the split-belt configuration was set with the paretic leg placed on the slow belt. On the initial day of split belt walking, half of the participants were assigned to CONSTANT practice, while half were assigned to VARIABLE practice.

Subjects in the CONSTANT condition were required to walk on the split-belt treadmill at a constant 2:1 speed ratio (Figure 3.1). The fast belt speed was set to the subject's fastest walking speed achieved during baseline testing as described above. The slow belt was set to half of the fast belt speed. This 2:1 speed ratio was maintained throughout the entire session on Day1.

Subjects in the VARIABLE condition were required to walk on the split-belt treadmill at three different speed ratios; 2:1, 1.5:1 and 2.5:1 (Figure 3.1). The 2:1 speed ratio was determined as described above for the CONSTANT group and is identified as the *base speed ratio*. The 2.5:1 speed ratio was calculated based on 80% of the fastest speed collected for the base speed ratio. The 1.5:1 ratio was calculated based on 90% of the fastest speed collected for the base speed ratio. VARIABLE condition ratios were calculated as a percentage of the fastest speed possible to allow greater variations in speed ratios while avoiding the possibility of the participant experiencing a speed faster than they were capable of safely performing. The speed ratios were changed within the VARIABLE condition every 2.5 minutes so that the subject experienced each of the

ratios twice (Figure 3.1). The subjects were not informed of the change in ratio and the treadmill continued to operate as the change in ratio occurred. All subjects started and concluded the 15 minutes of treadmill walking at their base speed ratio (2:1).

On the second day, both groups (CONSTANT and VARIABLE) participated in 15 minutes of split-belt treadmill walking at their individual base speed ratio (2:1) only. Subjects did not participate in tied belt walking on the second day or after split-belt walking on the first day (Figure 3.1).

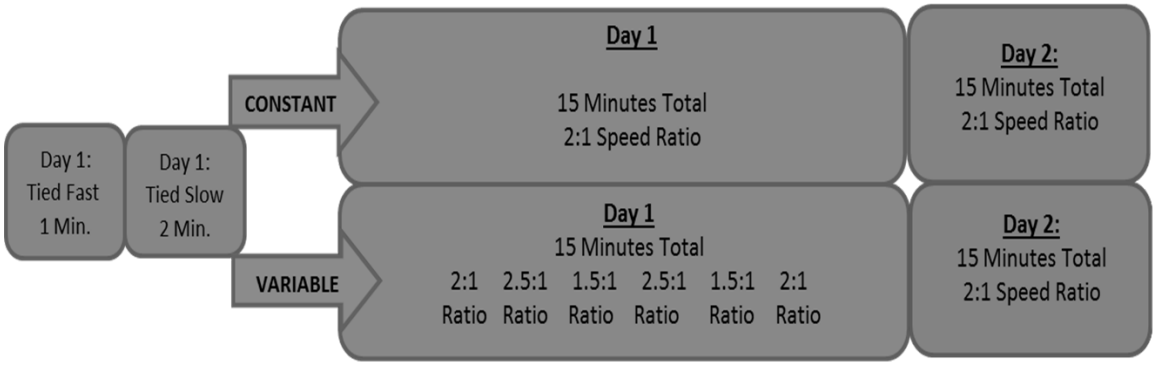

Figure 3.1 Experimental Protocol

Data Analysis

All kinematic and kinetic data were exported from Vicon-Nexus software, and further processed using Visual 3D (C-Motion, Inc, Germantown) and Matlab (MathWorks, Natick, MA). Gait events of foot strike and lift off were determined for each limb individually using an automatic algorithm in Visual 3D. Foot strike was

identified when the vertical ground reaction force exceeded 20 Newtons for at least 8 frames, and lift-off identified when the vertical ground reaction force dropped below 20 Newtons for at least 8 frames. All gait events were visually checked for accuracy.

Dependent variables

Spatial and temporal parameters of gait have been found to respond differently during split-belt walking [33,53,111]. Therefore, both spatial (step length) and temporal (limb phasing) variables were evaluated within the current study. Both variables were calculated for each leg continuously throughout treadmill walking. The spatiotemporal measure of step length was calculated as the sagittal distance between the right and left heel markers at foot strike. Step length was labeled as Left or Right based on leading leg. Stride by stride symmetry data for step length was calculated as:

$$\frac{\text{(Step Length of Leg on Slow Belt-Symmetrical Step Length)}}{\text{Symmetrical Step Length}}$$

Where symmetrical step length = (paretic step length + non-paretic step length)/2 [33,112]

Based on the above calculations, a value of 0 would indicate that the subject has achieved perfect symmetry based on their individual stride length. A negative value denotes the leg on the slow belt has a decreased step length relative to perfect symmetry. This method is preferred over the calculation of a ratio (paretic/non-paretic) because it prevents extremely large values when the denominator of the ratio is small due to a "step to" gait pattern in which one leg does not pass the other leg [113]. The temporal measure of

limb phasing was calculated as previously reported [33,112]. Briefly, a calculation of limb phase for each leg provides a measure of the difference in time between the contralateral limb's peak flexion and the ipsilateral limb's peak extension, normalized by the ipsilateral limb's stride duration. Stride-by-stride limb phase symmetry was calculated by dividing the limb phase value for the leg on the slow belt by the contralateral limb phase value.

To evaluate differences in locomotor adaptation between the different practice conditions, we examined the *Magnitude of Total Adaptation*, and *Return to Baseline*. To assess learning differences across days between the practice conditions we evaluated the *Magnitude of Retention* and the *Rate of Re-Adaptation* on Day 2. Calculations were performed for both step and limb phase symmetry.

Magnitude of Total Adaptation. To evaluate the total amount of adaptation for both step length and limb phase (a)symmetry during split-belt treadmill walking, the magnitude of total adaptation was calculated as follows:

Magnitude of Total Adaptation = Mean of Initial 10 Strides – Mean of Last 10 Strides.

This calculation represents the difference between the (a)symmetry pattern utilized at the start of adaptation and the (a)symmetry pattern utilized at the end of adaptation. A larger positive number would indicate a larger amount of adaptation.

Return to Baseline. To assess whether subjects were able to fully adapt back to their baseline (a)symmetry, the amount of adaptation relative to their individual baseline was calculated as follows:

Return to Baseline = Mean of Last 10 strides – Mean of tied slow (baseline)

This calculation represents the difference between the (a)symmetry pattern achieved at the end of adaptation and the subject's baseline (a)symmetry pattern with the belts tied at a 1:1 speed ratio. A value of 0 would indicate the subject has completely adapted to the split-belt treadmill and has returned back to their baseline (a)symmetry pattern, despite the continued split-belts.

Magnitude of Retention. If subjects have learned something about how to walk on the split-belt treadmill on Day 1, with re-exposure to the split-belt paradigm, on Day 2, subjects should have less step length or limb phase asymmetry [33,53]. To assess this reduction in "error" from Day 1 to Day 2 the magnitude of retention was calculated as follows:

Magnitude of Retention = Mean of first 10 strides Day 1 – Mean of first 10 strides Day 2

This calculation represents the difference between the initial adaptation on Day 1 relative to the initial adaptation on Day 2. A positive number would indicate that the subject was less perturbed by the split-belt treadmill on Day2 in comparison to Day1 and therefore has learned something about the split-belt treadmill paradigm.

Rate of Re-Adaptation. For each variable the rate of adaptation on Day1 and rate of re-adaptation on Day 2 was calculated by first removing baseline (a)symmetry from each raw symmetry value to provide a value that reflects the deviation from the individual's baseline (a)symmetry pattern [33,112,114]. A value of 0 reflects a symmetry pattern identical to baseline (a)symmetry. Subtraction of the baseline (a)symmetry pattern allows for comparison of data across subjects who may demonstrate different levels of baseline asymmetry. A value of 30 strides was selected to represent *Early* adaptation and *Early* re-adaptation to step length asymmetry. Previous literature indicates that adaptation to limb phase asymmetry occurs on a much shorter timescale than step length adaptation [33,53,112]. In order to accurately capture rapid adjustments in limb phase asymmetry we utilized the first 10 strides to assess *Early* adaptation and Early re-adaptation for limb phase. Group (CONSTANT vs. VARIABLE) averages of stride by stride data for *Early* adaptation on Day 1 and *Early* re-adaptation on Day 2 were compared through linear regression for each group separately.

Statistical Analysis

Normality of the data distributions were assessed with the Kolmogorov-Smirnov test for normality. All statistical analyses were completed with SPSS v22.

To test our hypothesis that subjects who participate in variable speed ratios of walking would demonstrate a reduced ***Magnitude of Total Adaptation*** and ***Return to Baseline*** on Day1, group differences (VARIABLE vs. CONSTANT) were assessed. Differences in limb phase for each dependent value were found to be non-normally distributed for the dependent measures. As such group differences in limb phase were

assessed utilizing the Kruskal-Wallis analysis of ranks for each dependent variable. Differences in step length (a)symmetry for each dependent measure were assessed utilizing independent samples t-tests.

In order to account for any differences in practice amount on Day 1 between groups (VARIABLE vs. CONSTANT), the total number of strides during split-belt walking was assessed (Table 3.1). If the groups (VARIABLE vs. CONSTANT) demonstrate significant differences in the total amount of steps taken, an ANCOVA will be utilized to assess ***Return to Baseline*** and ***Magnitude of Total Adaptation*** with total steps added as a covariate.

To test our hypothesis that participation in variable practice on Day 1 would result in an increased ***Magnitude of Retention*** on Day 2, the initial adaptation (mean of first 10 strides) on Day 1 was compared to the initial adaptation on Day 2. A repeated measures analysis of variance (ANOVA) was utilized to compare the mean differences within each group (VARIABLE vs. CONSTANT). Differences in the mean reduction in asymmetry between groups from Day 1 to Day 2 were assessed through analysis of the interaction effect within the repeated measures ANOVA. Analyses were performed for both step length and limb phase (a)symmetry.

In order to test our hypothesis that participation in variable practice on Day 1 would allow a faster ***Rate of Re-Adaptation*** on Day 2, a linear regression was performed for *Early* adaptation on Day 1 and *Early* re-adaptation on Day 2. Each individual's stride data for *Early* adaptation and *Early* re-adaptation was examined through use of the

modified Box-Cox test in SPSS (Draper, 1998) to ascertain the appropriate use of a linear regression (Osborne, 2010). An *apriori* decision was made to utilize group data within the linear regression when greater than 70% of subjects within each group (VARIABLE and CONSTANT) fell within the 95% confidence interval for utilization of a linear fit. The linear regression was then utilized to assess the relationship between the change in *Early* asymmetry from Day 1 to Day 2 for those in the CONSTANT and VARIABLE practice paradigms.

A power analysis for sample size estimation was performed using G-Power [118], utilizing the effect size required to detect a meaningful difference in the ***Magnitude of Retention*** from Day1 to Day2 from previous literature[33]. With a power of .80 and alpha level of p=.05 a total sample size of 24 (N= 12 per group) would be required to detect a difference in the magnitude of retention between groups.

3.4 Results

A total of thirty two subjects participated in the study with sixteen participants in both the VARIABLE (58.72 +/- 11.28 yr) and CONSTANT (62.28+/- 9.7 yr) groups. Table 3.1 contains participant demographics and baseline clinical scores. Groups did not differ significantly on baseline demographics or clinical scores (all measures $p<.05$).

Table 3.1 Subject Characteristics

	CONSTANT ***(n=16)***	**VARIABLE** ***(n=16)***
Age (years) (Std. Dev.)	62.28 (± 9.7)	58.72 (± 11.28)
Time since Stroke (months) (Std. Dev.)	46.37 (± 42.09)	34.06 (± 29.01)
Total Fugl Meyer (Std. Dev.)	21.12(± 5.30)	19.86 (± 5.15)
Fast speed on treadmill (m/s) (Std.Dev.)	0.7 (± 0.25)	0.81 (± 0.28)
Total strides day 1 (Std. Dev.)	555.43 (± 108.20)	587.50 (± 133.44)

Adaptation

Figure 3.2 illustrates the pattern of changes in step length asymmetry with exposure to the split-belt treadmill for subjects participating in VARIABLE and CONSTANT practice. At "baseline", with both treadmill belts set to the same speed, subjects in the CONSTANT and VARIABLE practice groups demonstrate an asymmetric walking pattern relative to perfect symmetry (perfect symmetry= 0). When the treadmill belt speeds are set to a 2:1 speed ratio with the paretic leg walking on the slow belt and non-paretic leg walking on the fast belt, both groups demonstrate an increase in step length asymmetry relative to their baseline. The subject participating in the constant practice structure shows a pattern of adaptation similar to what has been previously reported [51]. With a period of trial and error practice ("Adaptation"), subjects in the CONSTANT

group demonstrate the ability to reduce step length asymmetry despite the belts still moving at a 2:1 speed ratio. Subjects participating in VARIABLE practice also utilize trial and error practice to reduce step length asymmetry over the course of 15 minutes on the first day. However, the VARIABLE subject (Figure 3.2) shows additional exaggerations of step length asymmetry each time the speed ratio is changed. Despite these repeated exaggerations, the subject in the VARIABLE group appears to have adapted back to their baseline walking pattern by the end of practice on Day 1.

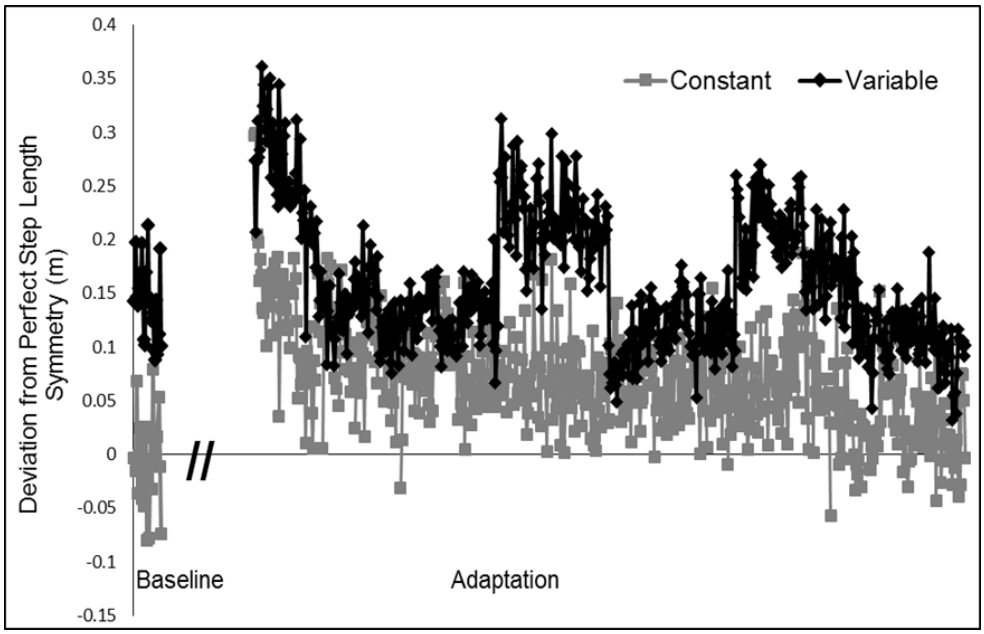

Figure 3.2. Adaptation to Step Length Asymmetry for individual subjects participating in Constant and Variable practice. Individual stride by stride data for step asymmetry during tied belt walking at a 1:1 speed ratio (Baseline) and during split belt walking on Day 1 (Adaptation) for an individual CONSTANT subject (gray) and individual VARIABLE subject (black). The start of split-belt walking on Day 1 is depicted by double hash marks along the horizontal axis. A value of 0 represents perfect symmetry.

This qualitative pattern is confirmed through analysis of the ***Magnitude of Total Adaptation*** and ***Return to Baseline*** for the group data. The ***Magnitude of Total Adaptation*** on Day 1 does not differ significantly depending upon the practice structure (CONSTANT and VARIABLE) for step length (Table 3.2; $p = 0.883$) or limb phase (Table 3.2; $p = 0.491$) (a)symmetry. Similarly, there is no difference between groups in the asymmetry at the end of adaptation relative to baseline (***Return to Baseline***) for step length or limb phase (a)symmetry Table 3.2; $p = 0.718$ and Table 3.2; $p = 0.196$ respectively).

Table 3.2 Non significant step and limb phase symmetry variables. Average and standard deviation for *Total Adaptation* (average of first 10 symmetry values - last 10 symmetry values) and *Return to Baseline* (average of last 10 symmetry values - baseline symmetry values).

Step Symmetry (m)		
	Total Adaptation	Return to Baseline
Constant	0.17 ± 0.07	0.02 ± 0.10
Variable	0.18 ± 0.10	0.00 ± 0.12
Limb Phase Symmetry		
	Total Adaptation	Return to Baseline
Constant	0.27 ± 0.18	0.22 ± 0.23
Variable	0.28 ± 0.26	0.33 ± 0.28

Learning

On a second day of practice, those in both the CONSTANT and VARIABLE practice conditions participated in split belt walking at a 2:1 speed ratio. If participants learned something about how to walk on the split-belt treadmill, one would expect subjects to demonstrate a faster rate of re-adaptation and/or decreased magnitude of initial asymmetry upon re-exposure to the split-belt paradigm [33,53]. Figure 3.3A and Figure 3.3B demonstrate this result for individual subjects in the CONSTANT (A) and VARIABLE (B) groups.

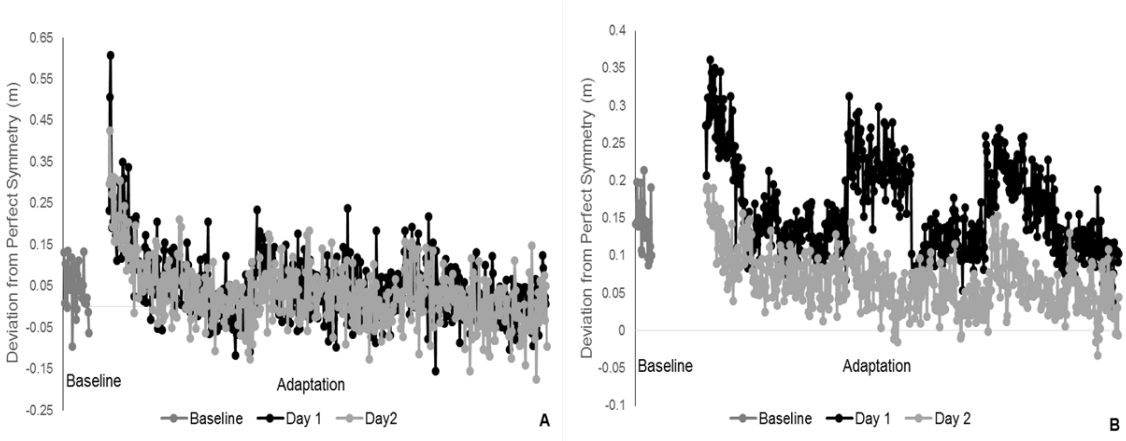

Figure 3.3 Adaptation to Step Length Asymmetry for individual subjects participating in Constant **(A)** and Variable **(B)** practice paradigms on both days. Individual stride by stride data for step asymmetry during tied belt walking at a 1:1 speed ratio (Baseline) and during split belt walking on Day 1 and Day2 (Adaptation). A value of 0 represents perfect symmetry.

The group data supports these individual results. Subjects in both the CONSTANT and VARIABLE practice groups demonstrate a reduction in the initial step length and limb phase asymmetry upon re-exposure to the split-belt treadmill on Day 2 (*p*=.000 for both). The magnitude of this reduction in asymmetry from Day 1 to Day 2, defined as the ***Magnitude of Retention***, did not differ between groups for step and limb phase (a)symmetry (Figure 3.4A; p=0.117, η^2_p=0.08and Figure 3.4B; p= 0.435, η^2_p=0.021 respectively).

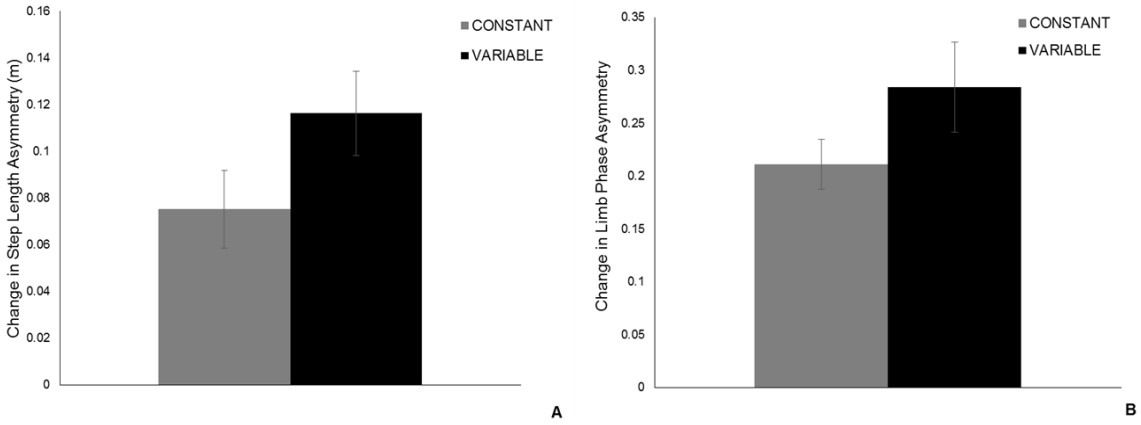

Figure 3.4 Magnitude of Retention for step length **(A)** and limb phase **(B)**. Error bars = standard error.

The results of the linear regression assessing rate of step length adaptation and re-adaptation between Day 1 and Day 2 showed similar results for subject's participating in CONSTANT and VARIABLE practice conditions. For the CONSTANT practice group,

in the first model, Day (Day1 vs. Day2) and Stride (group step (a)symmetry values for each of the first 30 strides) predicted the change in asymmetry across strides (R^2=0.900; p= 0.000; Table 3.3). Addition of the interaction term (Day x Stride) did not significantly improve the model (R^2=0.001; p= 0.544; Table 3.3).

Table 3.3. Sequential linear regression model predicting change in step length (a)symmetry over the first 30 strides for *Early* adaptation on Day 1 and *Early* re-adaptation on Day 2 for subjects participating in CONSTANT and VARIABLE practice.

Rate of *Early* Adaptation: CONSTANT practice				
Model #	**Predictors**	**Model *p***	**ΔR^2**	**ΔR^2 *p***
1	Day Stride	.000	.900	.000
2	Day Stride Day x Stride	.000	.001	.544
Rate of *Early* Adaptation: VARIABLE practice				
Model #	**Predictors**	**Model *p***	**ΔR^2**	**ΔR^2 *p***
1	Day Stride	.000	.947	.000
2	Day Stride Day x Stride	.000	.007	.005

For the VARIABLE practice group, in the first model Day and Stride predicted the change in asymmetry across strides (R^2=0.947; p= 0.000; Table 3.3). Addition of the

interaction term (Day x Stride) significantly improved the model (ΔR^2=0.007; p=0.005; Table 3.3), but this addition explained very little additional variance.

The results of the linear regression assessing rate of limb phase adaptation and re-adaptation between Day 1 and Day 2 also show similar results for subject's participating in CONSTANT and VARIABLE practice conditions. For the CONSTANT practice group, in the first model, Day (Day1 vs. Day 2) and Stride (group limb phase (a)symmetry values for each of the first 30 strides) predicted the change in asymmetry across strides (R^2=0.796; p= 0.000; Table 3.4). Addition of the interaction term (Day x Stride) significantly improved the model (ΔR^2=0.054; p=0.029; Table 3.4), but this addition, again, explained very little additional variance. For the VARIABLE practice condition, in the first model, Day and Stride predicted the change in asymmetry across strides (R^2=0.874; p= 0.000; Table 3.4). Addition of the interaction term (Day x Stride) did not significantly improve the model (R^2=0.009; p= 0.292; Table 3.4).

Table 3.4. Sequential linear regression model predicting change in limb phase (a)symmetry over the first 30 strides for *Early* adaptation on Day 1 and *Early* re-adaptation on Day 2 for subjects participating in CONSTANT and VARIABLE practice.

Rate of *Early* Adaptation: CONSTANT Practice				
Model #	**Predictors**	**Model *p***	**ΔR^2**	**ΔR^2 *p***
1	Day Stride	.000	.796	.000
2	Day Stride Day x Stride	.000	.054	.029
Rate of *Early* Adaptation: VARIABLE Practice				
Model #	**Predictors**	**Model *p***	**ΔR^2**	**ΔR^2 *p***
1	Day Stride	.000	.874	.000
2	Day Stride Day x Stride	.000	.009	.292

3.5 Discussion

The results of this study demonstrate that chronic stroke survivors are able to utilize variable practice to adapt and learn a novel locomotor pattern and that variable practice does not reduce the amount of adaptation during practice. Variable practice, however, does not appear to confer additional benefit to the retention of a novel locomotor pattern in subjects post-stroke. The current study is the first to assess the effects of practice characteristics on learning of a complex lower-extremity task in subjects post-stroke.

The current results demonstrate that variable practice of a novel locomotor pattern does not impede locomotor adaptation within the session. Chronic stroke survivors who participated in variable practice of the novel locomotor pattern demonstrate a similar amount of total adaptation and were able to return to similar levels of walking (a)symmetry over 15 minutes of practice, in comparison to subjects participating in constant practice. The current finding is particularly interesting given subjects in the variable practice group were required to repeatedly adjust their walking pattern to additional exaggerations of asymmetry throughout the 15 minutes of walking. This finding contrasts with previous studies in neurologically intact adults that suggest that variable practice impairs within session performance [43–45].

Unlike previous findings in neurologically intact adults, variable practice does not appear to confer an advantage for retention of what was learned with the current novel walking task. Subjects participating in both variable and constant practice demonstrate a reduced initial perturbation on Day 2 indicating they learned and retained something about walking on the split-belt treadmill. While the variable practice group does appear to have a larger magnitude of retention for both step length and limb phase (Fig. 4) this difference was not significant and the effect size was small (0.08 and 0.021 respectively). Similarly, rate of adaptation on Day 1 and Day 2 was not different in either the constant or variable practice group. That is, despite a significant increase in the variability explained with the addition of the interaction term in the regression for the variable practice group for step length and for the constant practice group for limb phase, the changes in the variability explained were extremely small and likely not meaningful.

To our knowledge, only three studies have assessed the role of variable versus constant practice in those with chronic stroke [28,48,119]. Of these studies, Hanlon (1996) demonstrated improved retention of a 5 step movement sequence within the hemiparetic upper extremity following random practice versus constant practice. Cauraugh & Kim (2003), demonstrated similar improvements in a functional test of manual dexterity, force modulation and force production with an upper extremity reaching task regardless of practice paradigm [119]. Lastly, Schweighofer and colleagues (2011) demonstrated that variable practice conferred improved retention over constant practice in those post-stroke, however this effect was modulated by the integrity of the subject's visuospatial working memory. All of the aforementioned studies have utilized upper extremity tasks, with a noticeable conflict of results. In comparison to previous results in chronic stroke survivors, the current study is the only study to assess variable and constant practice effects when learning a complex lower extremity task.

It has been suggested that the benefits of variable practice in simple tasks may not generalize to complex task (Wulf & Shea, 2002). It is possible that the current results confirm this suggestion. It is, however, also possible that the current paradigm tested did not provide an adequate stimulus to elicit the benefits of variable practice. Variable practice is thought to exert its beneficial effects through a mechanism of contextual interference (CI) with trial-to–trial variability during variable practice inducing high interference [43]. With repetition of each speed ratio provided for 2.5 minutes (Figure 3.1), it is possible that the variability of the task was not great enough to induce high contextual interference, limiting the benefits of variable practice. It is also possible that

specific deficits to motor and cognitive processes post-stroke may limit the benefits of variable practice in motor skill acquisition and learning. Previous evidence in neurologically intact individuals indicates that separate neural substrates may be engaged during acquisition and consolidation of a motor learning task depending on practice structure [45,49]. Lin et al. demonstrated that TMS disruption to M1 during the inter-trial interval diminished performance and learning during variable, but not constant practice, implicating encoding within M1 as crucial for learning enhancements of variable practice [45]. Likewise, cortical damage as a result of cerebral infarct may differentially impact motor learning. Schweighofer and colleagues (2011) demonstrated that the potential to exploit the benefits of variable practice in those post-stroke was dependent upon the integrity of subjects visuospatial working memory [48]. In that study, both neurologically intact subjects and those post stroke demonstrated improved retention of an upper extremity task with variable compared to blocked practice. However, when those with stroke were subdivided into groups based on visuospatial working memory, the benefits of variable practice were negated in those with visuospatial deficits [48].

3.6 Conclusions

Within rehabilitation, variable practice is often utilized to promote motor relearning and functional improvements in individuals post stroke. The use of this practice type is largely based on evidence from motor learning studies in neurologically intact individuals. The results of the present study indicate however, that variable practice

confers little benefit over constant practice in learning a novel locomotor task after stroke. Specific neurologic deficits, as a result of stroke may directly impact the role of various practice paradigms in motor skill acquisition and learning. Further studies are needed to differentiate characteristics of practice and the practice parameters that may enhance or hinder motor learning post-stroke.

Chapter 4

THE INFLUENCE OF HIGH INTENSITY EXERCISE AND THE VAL66MET POLYMORPHISM ON CIRCULATING BDNF AND MOTOR LEARNING.

4.1 Abstract

BDNF has been directly related to exercise-enhanced motor performance in the neurologically injured animal model; however literature concerning the role of BDNF in the enhancement of motor learning in the human population is limited. Previous studies in healthy subjects have shown a relationship between intensity of an acute bout of exercise and increases in peripheral BDNF. Furthermore, the intensity of exercise has been shown to have a moderating influence on the relationship between peripheral BDNF levels and cognitive learning. The current study sought to examine the role of high intensity exercise on upregulation of peripheral BDNF levels as well as the role of high intensity exercise in mediation of motor skill performance and retention of a novel locomotor task in neurologically intact adults. In addition, we explored the impact of a single nucleotide polymorphism in the BDNF gene (Val66Met) in moderating the relationship between exercise and motor learning. We hypothesized that participation in high intensity exercise prior to practicing a novel walking task (split-belt treadmill walking) would elicit increases in peripheral BDNF as well as promote an increased rate

and magnitude of within session learning and retention on a second day of exposure to the walking task. Within session learning and retention would be moderated by presence or absence of the Val66Met polymorphism. Fifty four neurologically intact participants participated in two sessions of split-belt treadmill walking. Step length and limb phase were measured to assess learning of spatial and temporal parameters of walking. Serum BDNF was collected prior to and immediately following high intensity exercise or 5 minutes of quiet rest on Day 1. The results demonstrated that high intensity exercise does not provide an additional benefit to learning of a novel locomotor pattern in neurologically intact adults, despite increases in circulating BDNF. In addition presence of a single nucleotide polymorphism on the BDNF gene did not moderate the magnitude of serum BDNF increases with high intensity exercise, nor did it moderate the interaction of high intensity exercise and motor learning.

4.2 Introduction

Animal experiments suggest the role of exercise as a "homeostatic" mechanism, providing a fertile environment to support the formation of functionally appropriate synaptic connections during learning [3,55,56,93]. Through upregulation of molecular mediators of neural plasticity, exercise may strengthen synaptic transmission, thus "priming" the nervous system for encoding of pertinent information [74,93,94]. Animal models have corroborated the molecular influences of exercise with enhanced cognitive and motor performance and learning and have indicated brain derived neurotrophic factor (BDNF) as a key mediator of these enhancements[56,72–74,95].

Brain derived neurotrophic factor has been identified as a requisite for induction of neural plasticity with motor learning and has been evidenced to mediate functional recovery following neurologic insult in the animal model[69,71,89]. The mature form of BDNF has become a major target of investigation in learning related neural plasticity secondary to its role in mediation of induction and maintenance of long term potientiation (LTP) [66]. Current evidence in the animal model demonstrates a role for exercise mediated BDNF increases in facilitation of spatial learning[74] and recovery of motor skill function[69,72,94,95]. Blockade of BDNF mRNA, via an antisense BDNF oligonucleotide, has been demonstrated to negate the ability of exercise and rehabilitation to upregulate BDNF gene expression as well as limit recovery of skilled reaching with rehabilitation in the ischemic animal[69].

Direct causal evidence of BDNF's moderating role in the relationship between exercise and learning, demonstrated in the animal literature, has not been demonstrated in humans. However, converging evidence has linked exercise with improved cognitive function in healthy individuals as well as those post stroke [98–102]. Evidence citing the effects of aerobic exercise on *motor* learning, however, is sparse in comparison to studies of cognitive performance and learning[103]. Although theorized to moderate the influence of exercise on learning in humans, few studies have concurrently assessed the relationship between exercise induced changes in BDNF and learning. Of these studies all have assessed cognitive function in healthy humans [99,106] with conflicting results. It is plausible that BDNF may mediate a relationship between exercise and *motor* learning, as indicated in the animal model, although this relationship has not been examined.

Aerobic and anaerobic exercise has been noted to increase systemic BDNF in humans[99,104–106]. However, thirty percent of humans [83] possess a single nucleotide polymorphism (SNP) on the BDNF gene (Val66Met) [77]. This polymorphism has been linked to decreased activity dependent release [77,84] of BDNF within the animal model. In healthy humans, presence of the polymorphism has been associated with altered cortical activation and short term plasticity[79,80,85] as well as altered skill acquisition and learning (Beste et al., 2010; Joundi et al., 2012; Kleim et al., 2006; McHughen et al., 2010). It is currently unknown whether presence of the Val66Met polymorphism would attenuate release of BDNF in response to exercise in humans [77,84], and if this attenuation would impact learning.

The split-belt treadmill paradigm has previously been well-characterized as a tool to probe short–term locomotor learning in neurologically intact and individuals post-stroke [14,26,40,52]. Splitting the treadmill belts in a 2: 1 or 3:1 ratio elicits an asymmetry in subject's locomotor pattern and requires subjects to utilize trial and error practice to return to their baseline walking pattern. The rate and magnitude of reduction of this asymmetry within- session, as well as across sessions has previously been utilized to explore differences in short term locomotor learning in in various populations ([14,27,33

Therefore, in the current study we utilized the split-belt treadmill paradigm to examine the role of BDNF in mediating within session learning and retention of a novel locomotor task following high intensity exercise. We hypothesized that participation in a single session of high intensity upper extremity cycling would elicit increases in peripheral BDNF levels relative to quiet rest. In addition, we hypothesized that high

intensity exercise prior to a novel walking task (split-belt treadmill walking) would enhance the rate and magnitude of within-session learning as well as retention on a second day of exposure to split-belt walking. We postulated the benefits of high intensity exercise on motor learning would be greater for subjects without the Val66Met polymorphism.

4.3 Methods

Participants

Neurologically intact subjects between the ages of 21 and 35 were recruited as a sample of convenience for participation. All subjects provided written informed consent, with the study protocol approved by the University of Delaware Human Subjects Review Board. To be included, subjects must have demonstrated the ability to walk without assistance and without assistive devices, the ability to understand spoken instruction and communicate with investigators, a resting heart rate between 40-100 beats per minute and a resting blood pressure between 90/60 to 170/90. In addition, to be included participants provided written informed consent to supply a saliva sample for genetic testing for the BDNF Val66Met polymorphism. Exclusion criteria included any neurologic condition, intermittent claudication, total joint replacement and orthopedic problems in the lower limbs or spine that limited walking.

Instrumentation and Procedures

Subjects were randomly assigned to an Exercise + Learning or Learning condition. Subjects assigned to the Exercise + Learning condition participated in a short bout of high intensity exercise on an upper body ergometer (UBE) (SCIFIT Systems, Inc., Tulsa, OK) prior to split-belt walking on Day 1. The high intensity exercise consisted of pedaling for 1 minute with high resistance immediately followed by 1 minute with resistance decreased by half, at speeds sufficient to achieve 80% of their maximum heart rate. Subjects were provided a timed 1 minute rest break, and then repeated the upper-body cycling protocol. Subjects in the Learning group were asked to quietly rest for 5 minutes prior to treadmill walking to account for time differences between groups (*See* Figure 4.1). Determination of resistance and revolutions per minute (RPM) for those participating in the Exercise + Learning condition was established at a separate session, on a separate day, prior to experimental testing. Determination was obtained by having the subject pedal while the researcher increased the resistance on the UBE by 0.5 levels every 5 seconds until 80 percent of the individual's heart rate max was achieved. The highest resistance that the subject could maintain, while maintaining 80 percent of their maximum heart rate for 1 minute of cycling, was utilized as the resistance during Day 1 of testing.

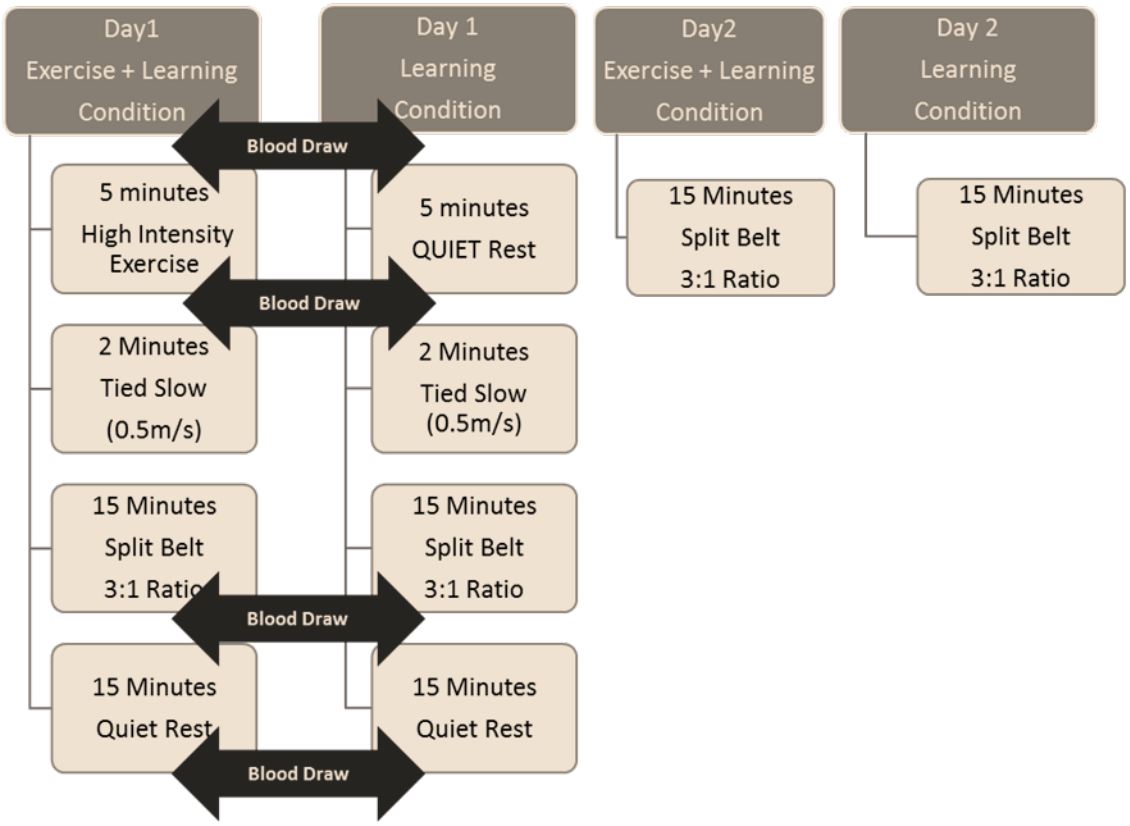

Figure 4.1 Experimental Protocol.

All subjects participated in two sessions of split-belt treadmill walking on two consecutive days. Prior to split-belt treadmill walking on Day 1 subjects were asked to walk on the treadmill with the belts tied at a 1:1 ratio at 0.5 m/s for 2 minutes in order to assess baseline step and limb phase asymmetry. All subjects then participated in split-belt treadmill walking for 15 minutes, consisting of walking at a constant 3:1 speed ratio of 1.5:0.5 m/s. Subjects ambulated with this speed ratio throughout the entire session (Figure 4.1). Subjects returned for a second day of split-belt walking at the same 3:1 ratio for 15 minutes. Subjects did not participate in acute exercise or treadmill walking with

the belts “tied” at the end of the session on Day 1 or prior to the split-belt walking session on Day 2.

All participants walked on a split-belt treadmill instrumented with two independent six degree of freedom force platforms (Bertec, Columbus, OH) from which ground reaction force data was continuously collected at 1000Hz. Kinematic data was continuously collected using an 8-camera Vicon Motion Capture System (Vicon MX, Los Angeles, CA) at 100Hz. Retro-reflective markers (14-mm diameter) secured to rigid plastic shells were placed on the pelvis, bilateral thighs and bilateral shanks. Single markers were placed on the most prominent superior portion of the bilateral iliac crests, greater trochanters, medial and lateral knee joint lines, medial and lateral malleoli, bilateral heels, and the first and fifth metatarsal heads. During walking all subjects were instructed to gently rest fingertips on the treadmill handrail, and were given verbal cues, as necessary, to avoid excessive use of the handrail while walking.

All subjects wore a safety harness around their chest for fall prevention; however the harness did not provide body weight support. Blood pressure, heart rate and rating of perceived exertion (RPE)[110] were monitored throughout the treadmill walking sessions and subjects were provided with optional standing or sitting rest breaks. During optional rest breaks, subjects were not permitted to dismount from the treadmill.

Serum BDNF collection

On Day 1, all subjects were asked to provide four blood samples to obtain levels of serum BDNF and lactate at specific time points throughout the session (Figure 4.1). To obtain blood samples, a venous catheter (IV) was inserted in the subjects arm prior to any activity by a registered nurse experienced in IV placement. Immediately before the intense exercise for subjects in the Exercise+Learning group or before 5 minutes of quiet sitting for subjects in the Learning group, a 7mL blood sample was collected to determine baseline levels of the above defined variables. A second and third 7mL sample was obtained immediately prior to and immediately following treadmill walking. A final 7 mL sample was obtained 15 minutes after the end of treadmill walking. Serum samples were allowed to clot for 30 minutes at room temperature and then centrifuged at 3,000 rpm for 15 minutes. Samples were then divided into several aliquots in microcentrifuge tubes designated for lactate and serum BDNF and stored at -80 C until assayed.

Serum samples were analyzed for levels of circulating BDNF utilizing commercially available Enzyme Linked Immunosorbent Assay (ELISA) kits. To detect BDNF levels in serum across time points a Human BDNF Quantikine ELISA kit was utilized (R&D Systems; Minneapolis, MN). The protocol put forth by R&D Systems was followed to determine serum BDNF protein concentrations.

To verify intensity of exercise, lactate levels were assessed utilizing a commercially available analysis kit (Lactate Colorimetric Assay Kit II, Bio Vision Incorporated, Milpitas, CA). Analysis was completed following the protocol established by the manufacturer (BioVision Incorporated). Optical density values derived from the

microplate reader for serum samples were subtracted from a 0 lactate sample control. The levels of lactate present in each sample were determined through the use of a standard curve generated with the assay by measuring the optical density of a series of known concentrations of lactate.

Genotyping

Each subject provided a 2 mL saliva sample in a DNA Self-Collection Kit (DNA Genotek, Kanata, Canada) containing a DNA stabilizing buffer. The samples were sent to DNA Genotek (GenoFIND Services, Salt Lake City, UT) for processing following study participation. Genotek created a set of primers to amplify the region surrounding the SNP (Val66Met: rs6265) of the BDNF gene and then examined the sample for the presence or absence of the Val66Met polymorphism. Extracted DNA results of genotyping were sent to the primary investigator with remaining saliva samples destroyed following analysis. Researchers were blinded to subject's genotype during study participation.

Data Analysis

All kinematic and kinetic data was exported from Vicon-Nexus software, and further processed using Visual 3D (C-Motion, Inc, Germantown) and Matlab (MathWorks, Natick, MA). Gait events of foot strike and lift off were determined for each limb individually using an automatic algorithm in Visual 3D. Foot strike was identified when the vertical ground reaction force exceeded 20 Newtons for at least 8 frames, and lift-off identified when the vertical ground reaction force dropped below 20 Newtons for at least 8 frames. All gait events were visually checked for accuracy.

Dependent variables

Spatial and temporal parameters of gait have been found to respond differently during split-belt walking [33,53,111]. Therefore, both spatial (step length) and temporal (limb phasing) variables were evaluated within the current study. Both variables were calculated for each leg continuously throughout treadmill walking. The spatiotemporal measure of step length was calculated as the sagittal distance between the right and left heel markers at foot strike. Step length was labeled as Left or Right based on leading leg. Stride by stride symmetry data for step length was calculated as:

$$\frac{\text{(Step Length of Leg on Slow Belt-Symmetrical Step Length)}}{\text{Symmetrical Step Length}}$$

Where symmetrical step length = (paretic step length + non-paretic step length)/2[33,112]. Based on the above calculations, a value of 0 would indicate that the subject has achieved perfect symmetry based on their individual stride length. A negative value denotes the leg on the slow belt has a decreased step length relative to perfect symmetry. This method is preferred over the calculation of a ratio (paretic/non-paretic) because it prevents extremely large values when the denominator of the ratio is small due to a "step to" gait pattern in which one leg does not pass the other leg [113].

The temporal measure of limb phasing was calculated as previously reported [33,112]. Briefly, a calculation of limb phase for each leg provides a measure of the difference in time between the contralateral limb's peak flexion and the ipsilateral limb's peak extension, normalized by the ipsilateral limb's stride duration. Stride-by-stride limb

phase symmetry was calculated by dividing the limb phase value for the leg on the slow belt by the contralateral limb phase value.

For both step length and limb phasing, each symmetry value was calculated to reflect deviation from an individual's baseline (a)symmetry pattern. This was performed by subtracting the average of the last 30 strides of the baseline condition from each raw symmetry value[33,112,114]. Subtraction of the baseline symmetry pattern from each raw symmetry value allows for comparison of data across subjects who may demonstrate different levels of baseline asymmetry. A value of 0 therefore reflects a pattern identical to baseline (a)symmetry. In order to account for individual differences in the initial asymmetry at the start of the split-belt paradigm individual stride data was normalized by initial perturbation[114]. Normalization was achieved by dividing each symmetry value by the initial perturbation value, where **initial perturbation** was defined as the average of the first 3 strides during adaptation[114]. This normalization allows individual subject data to be scaled to a proportion of the initial perturbation [114].

High Intensity Exercise and Serum BDNF

To evaluate the influence of high intensity exercise on peripheral BDNF and lactate the following variables were assessed:

***Magnitude Change in BDNF*.** To assess the influence of high intensity cycling on circulating levels of serum BDNF, the magnitude change in serum BDNF was calculated as follows:

Magnitude Change in BDNF = Serum BDNF post-exercise (or quiet rest) – baseline Serum BDNF

Magnitude Change in Lactate. To verify intensity of the cycling task, the change in concentration of lactate was assessed from pre to post high intensity cycling or quiet rest. The magnitude change in lactate was calculated as follows:

Magnitude Change in Lactate = Lactate post-exercise (or quiet rest) – baseline lactate concentration

Within-Session Learning

To evaluate differences in within session learning between subjects participating in high intensity exercise or quiet rest prior to split-belt walking and the potential interaction of exercise and the presence of the Val66Met polymorphism we examined: the *Magnitude of Total Adaptation* and the *Return to Baseline* as well as the *Percent Change of Early Asymmetry*.

Magnitude of Total Adaptation. To evaluate the total amount of adaptation for both step length and limb phase (a)symmetry during split-belt treadmill walking, the magnitude of total adaptation was calculated as follows:

Magnitude of Total Adaptation = Mean of initial 10 strides – Mean of Last 10 strides.

This calculation represents the difference between the (a)symmetry pattern utilized at the start of adaptation and the (a)symmetry pattern utilized at the end of adaptation. A larger positive number would indicate a larger amount of adaptation.

Return to Baseline. To assess whether subjects were able to fully adapt back to their baseline (a)symmetry, the amount of adaptation relative to their individual baseline was calculated as follows:

Return to Baseline = Mean of last 10 strides – Mean of tied slow (baseline)

This calculation represents the difference between the (a)symmetry pattern achieved at the end of adaptation and the subject's baseline (a)symmetry pattern with the belts tied at a 1:1 speed ratio. A value of 0 would indicate the subject has completely adapted to the split-belt treadmill and has returned back to their baseline (a)symmetry pattern, despite the continued split-belts.

NOTE: In order to test our hypothesis that subjects participating in exercise prior to split belt walking would demonstrate an increased rate of adaptation to the split belt paradigm compared to those participating in quiet rest, we proposed to utilize the methods as identified within Aim 1 and Aim2 and as outlined in the dissertation proposal document. However, in Aim 3, the criteria were not met to perform a linear regression on group data. In particular, each individual's stride data over Early adaptation was examined for linearity using a modified Box-Cox test (Draper, 1998). *Less than 70% of subjects within each group met the criteria for a linear relationship as determined by the*

modified Box-Cox test. In addition, the majority of individual subject data did not meet a specific curve fit. Therefore, a new variable, ***Percent Change of Early Asymmetry,*** *was defined to capture the rate of reduction in asymmetry within Early adaptation.*

Percent Change of Early Asymmetry. To assess the reduction of asymmetry within *Early* adaptation the percent change relative to the initial perturbation was calculated as the difference between the average of the first 3 strides and last three strides of Early adaptation divided by the average of the first 3 strides of Early adaptation. A value of 30 strides was selected to represent *Early* adaptation for step length asymmetry. Previous literature indicates that adaptation to limb phase asymmetry occurs on a much shorter timescale than step length adaptation[33,111]. In order to accurately capture rapid adjustments in limb phase asymmetry we utilized the first 10 strides to assess *Early* adaptation for limb phase.

Retention

The first step to examine retention of learning is to examine retention within groups. To do this we compared the outcomes of interest on Day 1 and Day 2 within groups. These outcomes include ***Percent Change of Early Asymmetry*** and the ***Magnitude of Early Asymmetry***, defined as the average of the first 10 strides on each day.

To test our specific hypotheses about group differences, we compared mean differences in outcomes of interest across groups as defined below.

Magnitude of retention. If subjects have learned something about how to walk on the split-belt treadmill on Day 1, with re-exposure to the split-belt paradigm, on Day 2, subjects should have less step length or limb phase asymmetry. To assess this reduction in "error" from Day 1 to Day 2, the magnitude of retention was calculated as follows:

Magnitude of Retention = Magnitude of Early Asymmetry Day1 – Magnitude of Early Asymmetry Day2

This calculation represents the difference between the initial adaptation on Day 1 relative to the initial adaptation on Day 2. A positive number would indicate that the subject was less perturbed by the split-belt treadmill on Day2 in comparison to Day1 and therefore has learned something about the split-belt treadmill paradigm.

Magnitude of Percent Change. If subjects have learned something about how to walk on the split belt treadmill from Day 1, with re-exposure to the split-belt paradigm, on Day 2, subjects should demonstrate a more rapid adjustment of their initial asymmetry relative to Day 1. For each variable, the ***Percent change of Early asymmetry*** on Day 2 was calculated as described above. The Magnitude of Percent Change was calculated as follows:

Magnitude of Percent Change= Percent change of Early asymmetry Day1- Percent change of Early asymmetry Day 2

Statistical Analysis

Normality of the data distributions were assessed with the Kolmogorov-Smirnov test for normality. All statistical analyses were completed with SPSS v22.

High Intensity Exercise and Serum BDNF

To test our hypothesis that participation in a single session of high intensity cycling would elicit increases in peripheral BDNF levels relative to quiet rest, group differences (Exercise + Learning vs. Learning) for the ***Magnitude Change in BDNF*** were evaluated. To evaluate the impact of the Val66Met polymorphism on changes in serum BDNF following exercise, group differences (presence (Met) vs. absence (Val) of the polymorphism) for the ***Magnitude Change in BDNF*** were evaluated within the Exercise + Learning group. To verify the intensity of the cycling task, group differences (Exercise + Learning vs. Learning) for the ***Magnitude Change in Lactate*** were assessed. Differences in the magnitude change for both serum BDNF and lactate were found to be non-normally distributed for the dependent measures, therefore group differences were assessed utilizing the Kruskal-Wallis analysis of ranks.

Within-session learning

We hypothesized that subjects participating in high intensity exercise prior to split-belt walking would demonstrate an increased ***Magnitude of Total Adaptation*** and ***Return to Baseline***, in comparison to subjects who did not participate in exercise immediately prior to split belt walking and that this effect would be greater in those without the Val66Met polymorphism. To test this hypothesis a two-way analysis of variance (ANOVA) was utilized to compare the mean differences between groups

(Exercise + Learning vs. Learning and presence (Met) vs. absence (Val) of Val66Met polymorphism) for the ***Magnitude of Total Adaptation*** and ***Return to Baseline***. The interaction between the presence or absence of the polymorphism and the effects of high intensity exercise were assessed through analysis of the interaction effect within the two-way ANOVA. Analyses were performed for both step length and limb phase symmetry. ***Return to Baseline*** for Limb Phase was found to be non-normally distributed therefore the main effects of exercise condition and presence vs absence of the polymorphism were tested with the Mann-Whitney U independent samples test. The interaction effect was examined through the Kruskal-Wallis analysis of ranks.

We hypothesized that subjects participating in high intensity exercise prior to split-belt walking would demonstrate a faster adjustment of their initial asymmetry relative to subjects who did not participate in exercise immediately prior to split-belt treadmill walking and that this effect would be greater for those without the Val66Met polymorphism. To test this hypothesis the ***Percent Change of Early Asymmetry*** was assessed utilizing a two-way ANOVA for both step length and limb phase (a)symmetry. Main effects of the two-way ANOVA were examined for mean differences between groups (Exercise + Learning vs. Learning and presence (Met) vs. absence (Val) of Val66Met polymorphism). The interaction between polymorphism (Val vs. Met) and exercise condition polymorphism was examined through interaction effect within the two-way ANOVA.

Retention

We hypothesized that following one day of practice, subjects in the Exercise + Learning condition would demonstrate an increased magnitude of retention of the split belt walking pattern compared to subjects in the Learning condition and that this effect would be greater for those without the Val66Met polymorphism. Both step length and limb phase (a)symmetry values were found to be non-normally distributed, therefore nonparametric tests were utilized to examine the above hypothesis. To first assess retention from Day 1 to Day 2 within groups, the ***Magnitude of Early Asymmetry*** on Day 1 was compared to the ***Magnitude of Early Asymmetry*** on Day 2 utilizing the Wilcoxin-Signed Ranks Assessment test. To assess the differences across groups (Exercise+Learning vs Learning and presence vs absence of the polymorphism) in the ***Magnitude of Retention***, the Mann-Whitney U test was used. The interaction effect between the presence or absence of the polymorphism and exercise condition was assessed with the Kruskal-Wallis test. Analyses were performed for both step and limb phase (a)symmetry.

We hypothesized that following one day of practice, subjects in the Exercise + Learning condition would demonstrate a more rapid adjustment of their (a)symmetry relative to Day 1 and that this effect would be greater for those without the Val66Met polymorphism. To first assess retention from Day 1 to Day 2 within groups, the ***Percent Change of Early Asymmetry*** on Day 1 was compared to the ***Percent Change of Early Asymmetry*** on Day 2 for both step length and limb phase. Step length (a)symmetry was found to be non-normally distributed, therefore the percent change of early asymmetry from Day 1 to Day 2 was assessed utilizing the Wilcoxin-Signed Ranks Assessment. To

assess differences in the ***Magnitude of Percent Change*** between groups for step length the Mann-Whitney U was utilized. The interaction effect between polymorphism and exercise condition was assessed with the Kruskal-Wallis test. Limb phase (a)symmetry was normally distributed, therefore, a repeated measures analysis of variance (ANOVA) was utilized to compare differences in the ***Percent Change of Early Asymmetry*** from Day 1 to Day 2 within groups (Exercise +Learning vs. Learning; Val vs. Met). Differences in the ***Magnitude of Percent Change*** between groups were assessed with a univariate ANOVA.

4.4 Results

A total of fifty-four subjects participated in the study with twenty-seven participants each in both the Exercise + Learning (24.51 +/- 2.83yr) and Learning (23.88+/- 2.40yr) conditions. Within the Exercise + Learning condition, 16 subjects were identified to have the Val66Met polymorphism (Met), while 11 subjects did not have the polymorphism (Val). Within the Learning condition, 10 subjects were identified as Met, and 17 subjects were identified as Val.

High Intensity Exercise and Serum BDNF

Serum BDNF and lactate levels for subjects participating in high intensity upper extremity cycling (Exercise + Learning) versus quiet rest (Learning) prior to split belt walking on Day 1 are shown in Figure 4.2 A and B. Secondary to technical issues, lactate was assessed in a total of 27 subjects in the Exercise + Learning and 22 subjects in the

Learning group. Subjects in the Exercise + Learning group demonstrated a significant increase in peripheral serum BDNF levels (***Magnitude Change in BDNF)*** (p=0.000) as well as lactate (***Magnitude Change in Lactate***) (p=0.000) compared to those in the Learning group. Subjects with (MET) and without (VAL) the polymorphism participating in exercise prior to split-belt treadmill walking demonstrated similar increases in serum BDNF from pre to post exercise (Fig 4.2 C. p=.577).

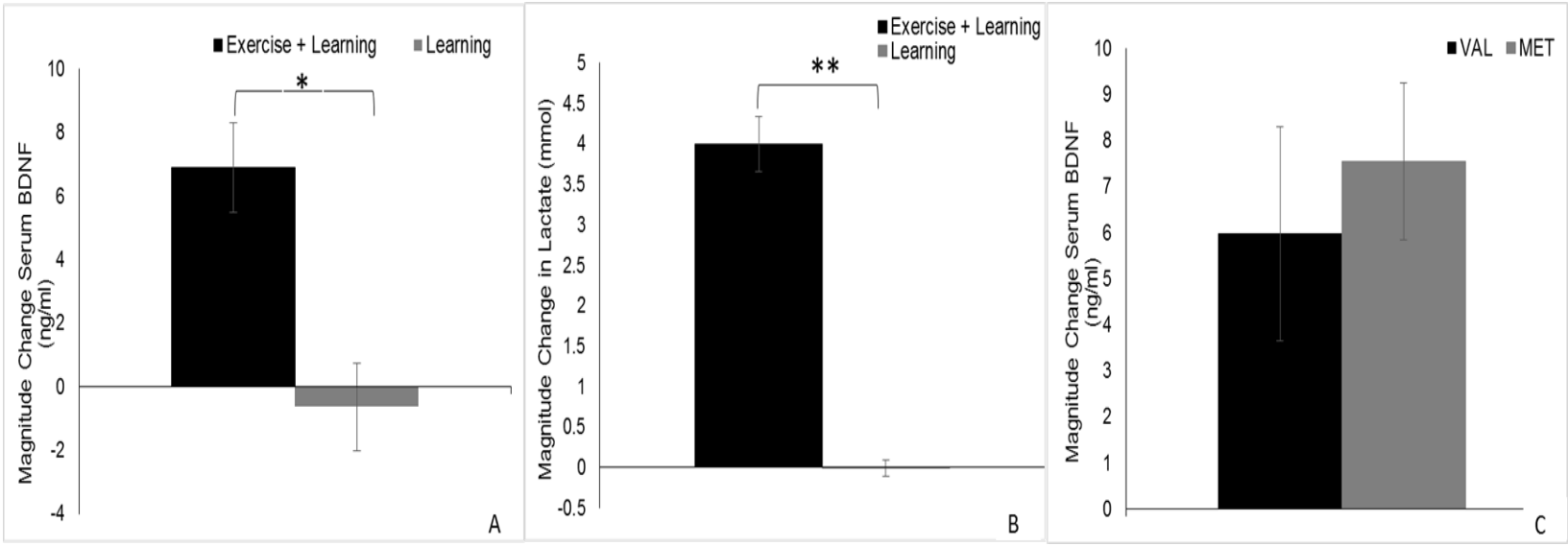

Figure 4.2 Magnitude change in peripheral serum BDNF (A) and Lactate (B) following high intensity upper extremity cycling (black) versus quiet sitting (gray). Data represents group averages. The magnitude change in peripheral serum BDNF following high intensity upper extremity cycling for those with (MET)(Gray) and without (VAL)(Black) the Val66Met BDNF polymorphism (C). Error bars = standard error. *p=. 000; **p=.000

Within session learning

Figure 4.3 illustrates the pattern of changes in step length asymmetry with exposure to the split-belt treadmill for subjects in the Exercise + Learning and Learning groups. At "baseline", with both treadmill belts set to the same speed, subjects in both groups demonstrate a walking pattern near perfect symmetry (perfect symmetry= 0). With initial exposure to the split belt paradigm, with belts set to a 3:1 speed ratio, participants demonstrate an increased step length asymmetry. Utilizing trial and error practice, participants reduce this asymmetry to return to a walking pattern similar to baseline walking. This pattern of adaptation is similar for subjects participating in high intensity exercise prior to split belt walking (Exercise + Learning) and those participating in quiet rest prior to split belt walking (Learning) and is similar to previous studies utilizing the split-belt treadmill[40,53].

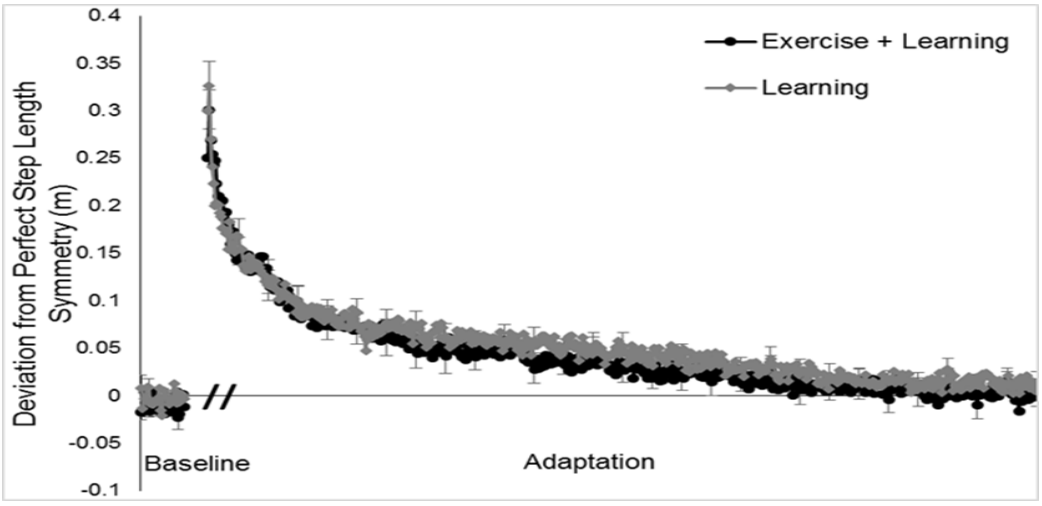

Figure 4.3 Adaptation to Step Length Asymmetry. Group averaged stride by stride data for step length (a)symmetry during tied belt walking at a 1:1 speed ratio (Baseline) and during split belt walking (Adaptation) for Exercise + Learning (Black) and Learning (Gray) conditions on Day 1. The start of split-belt walking on Day 1 is depicted by double hash marks along the horizontal axis. A value of "0" represents perfect symmetry. Error bars = standard error.

This qualitative pattern is confirmed through analysis of the ***Magnitude of Total Adaptation*** and ***Return to Baseline*** for the group data for both step length and limb phase (a)symmetry. The ***Magnitude of Total Adaptation*** on Day 1 does not differ significantly for those participating in high intensity exercise prior to split belt walking vs. quiet rest (Exercise + Learning vs. Learning) nor does it differ significantly for those with and without the polymorphism (Val vs. Met) for step length or limb phase (Table 4.1; all $p > 0.05$). The interaction between exercise condition and presence vs. absence of the polymorphism was also non-significant (Table 4.1; all $p>0.05$). Similarly, there is no difference between groups in the amount of (a)symmetry at the end of adaptation relative

to baseline (***Return to Baseline***) for step length or limb phase (a)symmetry (Table 4.1; all $p > 0.05$). The interaction between exercise condition and presence vs. absence of the polymorphism for ***Return to Baseline*** was also non-significant for both step length and limb phase (a)symmetry (Table 4.1; all $p>0.05$).

Percent Change of Early Asymmetry on Day 1 is not significantly different between subjects participating in high intensity exercise prior to split belt treadmill walking versus subjects participating in quiet rest (Exercise + Learning vs. Learning), nor does it differ significantly for those with and without the polymorphism (Val vs. Met) (Table 4.1; all $p > 0.05$). The interaction of exercise condition and presence vs. absence of the polymorphism for ***Percent Change of Early Asymmetry*** was also non-significant for step length and limb phase (a)symmetry (Table 4.1; all $p>0.05$).

Table 4.1 Non significant step and limb phase symmetry variables. Average and standard deviation for: *Magnitude of Total Adaptation* (average of first 10 symmetry values - last 10 symmetry values); *Return to Baseline* (average of last 10 symmetry values - baseline symmetry values); *Percent Change of Early Asymmetry* (average of initial 3 strides – last 3 strides of Early Adaptation/ initial 3 strides).

STEP SYMMETRY						
	All Subjects		VAL		MET	
	Exercise + Learning	Learning	Exercise + Learning	Learning	Exercise + Learning	Learning
Total Adaptation	0.93±.29	0.81±.22	1.01±.29	0.85±.24	0.87±.29	0.74±.19
Return to Baseline	-0.02±.21	-0.02±.20	-0.07±.23	-.04±.22	0.00±.20	0.00±.17
Percent Change of Early Asymmetry	49.29±26.2	53.07±24.4	39.60±36.9	54.25±27.9	55.95±12.9	51.06±18.3
LIMB PHASE SYMMETRY						
	All Subjects		VAL		MET	
	Exercise + Learning	Learning	Exercise + Learning	Learning	Exercise + Learning	Learning
Total Adaptation	0.71±.32	0.69±0.40	0.63±.44	0.74±.09	0.77±.19	0.62±.63
Return to Baseline	0.06±.30	0.10±.30	0.13±.40	0.09±0.23	0.02±.20	0.10±.41
Percent Change of Early Asymmetry	18.24±11.9	16.69±14.8	20.0±13.8	19.39±15.6	17.32±10.8	12.19±12.8

Retention

On a second day of practice, subjects in both exercise conditions (Exercise + Learning vs. Learning) participated in split belt walking at a 3:1 speed ratio. If participants learned something about how to walk on the split-belt treadmill, one would expect subjects to demonstrate a faster rate of re-adaptation and/or decreased magnitude of initial asymmetry upon re-exposure to the split-belt paradigm [33,53]. Subjects in both the Exercise + Learning and Learning groups, with and without the Val66Met polymorphism demonstrate a reduction in the initial step length and limb phase asymmetry upon re-exposure to the split-belt treadmill on Day 2 (all $p < .05$). The magnitude of this reduction

in asymmetry from Day 1 to Day 2, defined as the ***Magnitude of Retention***, did not differ between exercise conditions (Exercise + Learning) or polymorphism status (Val vs. Met) for step and limb phase (a)symmetry, (Fig 4.4A and 4.4B; $p > .05$ for all). The interaction of exercise condition and presence vs. absence of the polymorphism on magnitude of retention was also non-significant for step length ($p=0.475$) and limb phase (a)symmetry($p=0.190$).

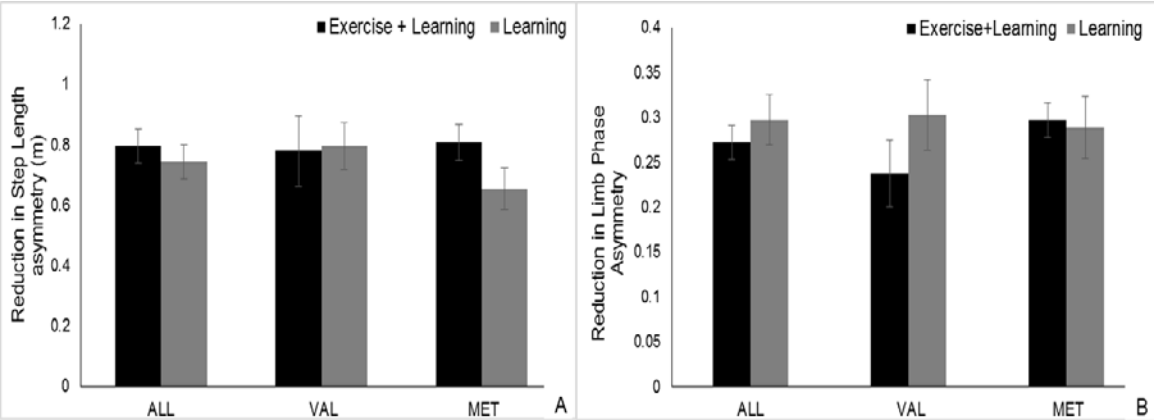

Figure 4.4 Magnitude of Retention for step length (A) and limb phase (B). Error bars = standard error.

In addition to the magnitude of retention, subjects in both the Exercise + Learning and Learning groups demonstrated significant differences in the ***Percent Change in Early Asymmetry*** from Day 1 to Day 2. With re-exposure to the split-belt paradigm subjects in both the Exercise +Learning and Learning groups, with and without the

polymorphism, demonstrate a faster reduction in their initial asymmetry relative to Day 1(all p<.05; Figures 4.5 & 4.6).

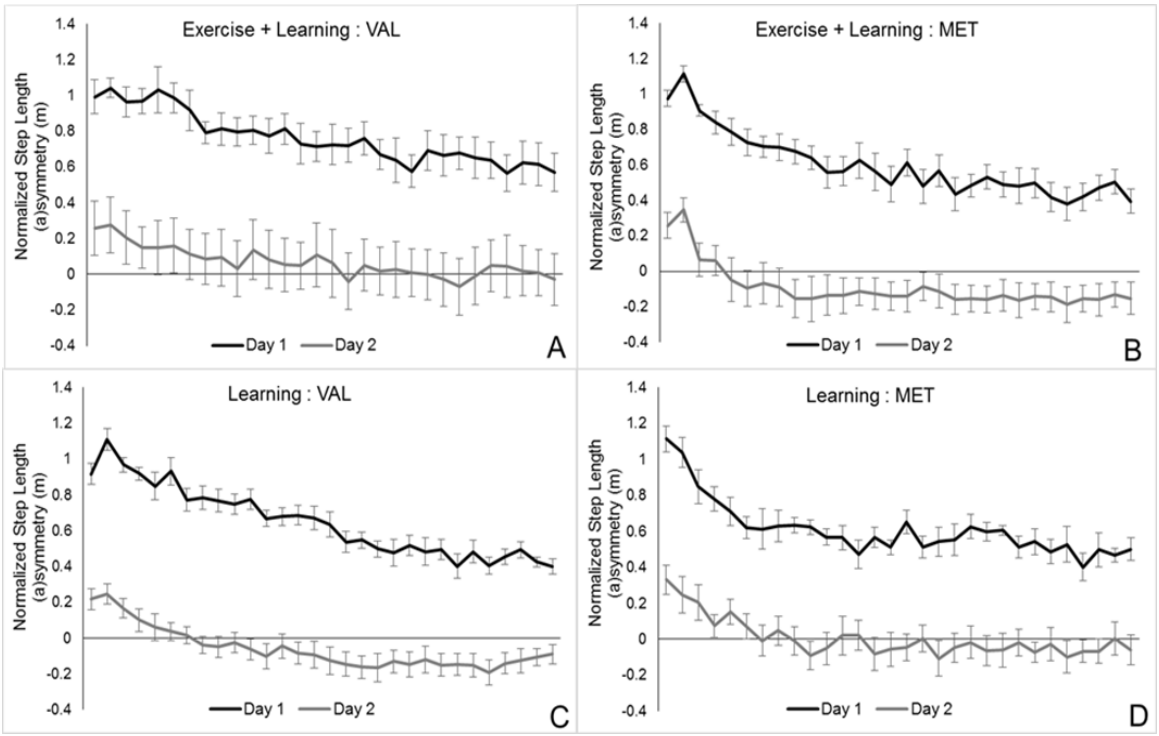

Figure 4.5 Group averaged stride by stride data for step length (a)symmetry within Early adaptation on Day 1 (Black) and Day 2 (Gray) for VAL (A) and MET (B) subjects participating in high intensity upper extremity exercise prior to split belt walking on Day 1 and for VAL (C) and MET(D) subjects participating in quiet rest prior to split belt walking on Day 1. Error bars= standard error.

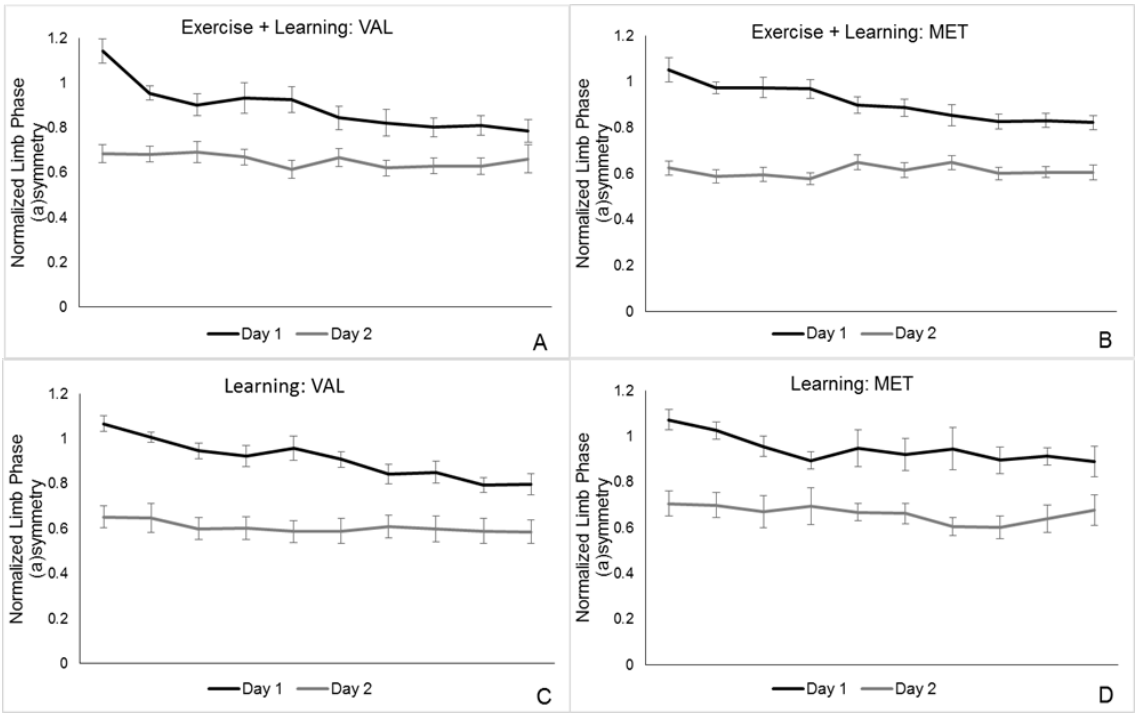

Figure 4.6 Group averaged stride by stride data for limb phase (a)symmetry within Early adaptation on Day 1 (Black) and Day 2 (Gray) for VAL (A) and MET (B) subjects participating in high intensity upper extremity exercise prior to split belt walking on Day 1 and for VAL (C) and MET(D) subjects participating in quiet rest prior to split belt walking on Day 1. Error bars= standard error.

The ***Magnitude of Percent Change*** was not significantly different between subjects participating in high intensity exercise prior to split belt treadmill walking versus subjects participating in quiet rest (Exercise + Learning vs. Learning), nor did it differ significantly for those with and without the polymorphism (Val vs. Met) (all $p > 0.05$; Figure 4.7). The interaction of exercise condition and presence vs. absence of the polymorphism on rate of adaptation was also non-significant for step length (p=0.171) and limb phase (a)symmetry (p=0.164).

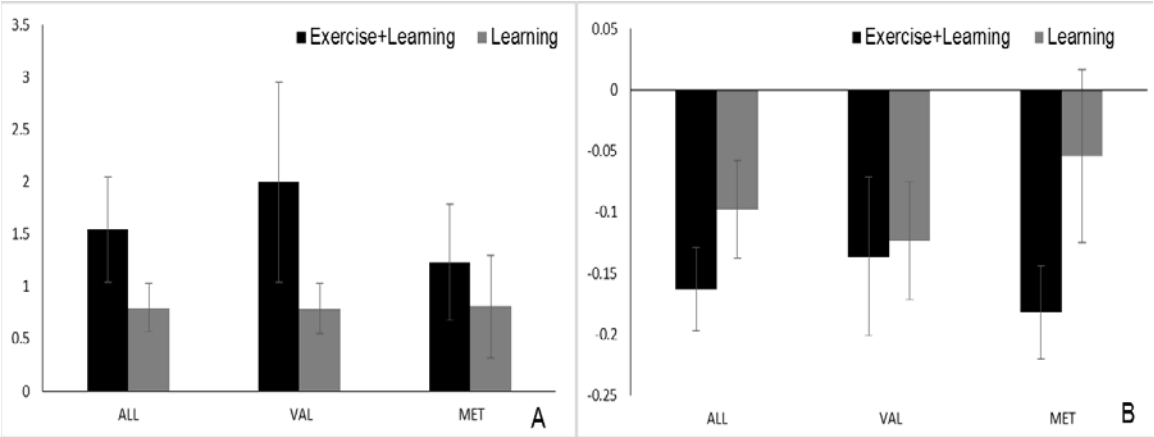

Figure 4.7 Magnitude of Percent Change for step length (A) and limb phase (B) for subjects participating in high intensity exercise (Black) or quiet rest (Gray) prior to split-belt walking on Day 1. Error bars= standard error.

4.5 Discussion

The current study is the first to assess the relationship between high intensity exercise, peripheral BDNF, the BDNF Val66Met polymorphsim and motor learning. There are several novel and important findings of this study that significantly advance our understanding of the role of exercise and BDNF in motor learning. First, the results of this study demonstrate that although high intensity exercise prior to a motor learning task resulted in increased peripheral serum BDNF, this exercise does not provide additional benefit to learning of a novel locomotor pattern in neurologically intact adults. The current results also demonstrate that presence of a single nucleotide polymorphism on the BDNF gene (Val66Met) does not influence the magnitude of upregulation of

peripheral serum BDNF with high intensity exercise, nor does it interfere with learning of a novel locomotor pattern.

Similar to previous evidence in humans [99,104–106], we found that a short bout of high intensity upper extremity cycling elicited a significant increase in serum BDNF relative to quiet rest. Surprisingly however, the magnitude of this increase did not differ between subjects with and without the Val66Met polymorphism. Previous evidence in the animal model indicates that presence of a gene mutation in the prodomain of BDNF results in decreased activity dependent secretion of the BDNF protein within neuronal cell populations [77,84]. This has led many to suggest that the presence of the Val66Met polymorphism results in decreased secretion of activity dependent BDNF in the human[120]. Our results showing that the increase in serum BDNF with exercise was similar in those with and without the polymorphism, does not directly refute this hypothesis. Rather, it may simply be that serum BDNF, which reflects peripheral, circulating BDNF, does not provide an adequate reflection of BDNF within the central nervous system. Indeed, in the animal model described above, while those animals with the gene mutation show decreased activity dependent secretion of the BDNF protein within neuronal cell populations, this was not observed in endothelial or smooth muscle cell populations [84]. This indicates that, in animals, the effects of the polymorphism may be isolated to the central nervous system. Our measures of serum BDNF suggest this may also be the case in humans.

Our results show an increase in serum BDNF following high intensity exercise, without a concomitant increase in locomotor learning. Previous evidence has indicated a

role for exercise in enhancement of brain function[93,96–99,101]. It has been postulated that these improvements, mainly cited in cognitive function, are related to increases in peripheral BDNF levels noted with aerobic and anaerobic exercise in humans[99,104–106]. Our results, along with the results by Ferris et al. (2007) conflict with this hypothesis[106]. A lack of relationship between serum BDNF levels and increases in locomotor learning, as well as the lack of moderation in serum BDNF by the Val66Met polymorphism noted in the current study indicates that systemic increases in BDNF do not necessarily reflect central neural processes, as often hypothesized(Knaepen et al., 2010; Winter et al., 2007). As such, the utilization of serum BDNF as supplementary marker of intervention efficacy on cognitive and motor function should be approached with caution.

Our results suggest that high intensity exercise did not provide an additional benefit to learning of a novel locomotor pattern, conflicting with the results of the one previous study of the effects of exercise on motor learning[103]. The current results also appear to conflict with evidence indicating a role for exercise in enhanced cognitive learning [93,96–99,101]. It possible however, that the current findings are a result of the population tested. Direct evidence supporting a role for exercise enhanced "brain health" comes from animal studies indicating an induction of growth factors and cellular cascades which enhance structural and functional neural plasticity[93]. These physiologic enhancements are believed to be represented behaviorally through enhanced cognitive and motor performance following exercise, however these effects have been most clearly demonstrated in the aging and neurologically impaired populations [93,98,100–102]. It is possible that although exercise elicits physiologic changes to allow for enhanced

learning, this “priming” effect is not captured behaviorally in a young, neurologically intact population.

This same argument could be made for the effects of BDNF on learning. Zhang et al. (2012) demonstrated that increased basal BDNF levels were associated with better cognitive function in patients with schizophrenia however this relationship was not evident for healthy controls [121]. In addition, Leckie et al. (2014) demonstrated that increasing age moderated the effect of increased BDNF levels on task-switching performance. In a study of 90 older adults, BDNF levels were found to mediate task switching performance for subjects over the age of 71 only[122]. Additionally, in a previous study by our group (Helm et al. *in review*) we demonstrate that the Val66Met polymorphism impacts the rate of locomotor adaptation in subjects with chronic stroke, while in the current study, a main effect of polymorphism was not found in neurologically intact individuals. It is plausible that increases in BDNF in healthy, neurologically intact individuals, do not confer additional benefit to learning, while in a population with a neurologic deficit, the influence of altered secretion of BDNF and/or the influence of the Val66Met polymorphism is more evident.

4.6 Conclusions

The findings of the current study indicate that exercise, and concurrent increases in serum BDNF, do not enhance learning of a novel locomotor pattern, at least in a young, neurologically intact population. Assessment of the effect of exercise on motor

learning in those with neurologic deficit is warranted, as the current findings may be the result of the population tested, limiting the detection of a "priming" effect on locomotor learning. Within the current study, the presence of the Val66Met polymorphism did not impact activity dependent release of BDNF within the periphery. In addition, increases in serum BDNF did not influence locomotor learning. Together these results suggest that assessment of peripheral BDNF in the serum may not provide an accurate reflection of the role of BDNF in central processes.

Chapter 5

CONCLUSIONS

The main goal of this dissertation has been to identify specific molecular and behavioral characteristics that may optimize motor learning and rehabilitation post-stroke. The split-belt treadmill was utilized to investigate practice constructs which may promote or limit locomotor learning post-stroke. In addition, the role of exercise in promotion of locomotor learning was assessed through addition of high intensity exercise prior to a split-belt treadmill walking in neurologically intact individuals. A role for BDNF in moderation of motor learning was examined through genetic analysis and peripheral protein assessments in both neurologically intact individuals and individuals post-stroke.

In the following sections, the specific aims and hypotheses will be reviewed and the respective findings will be summarized.

5.1 Aim 1 Findings

Thirty percent of humans possess a single nucleotide polymorphism on the BDNF gene (Val66Met) that, in the animal model[77,84], has been linked to decreased activity dependent release of BDNF. Presence of the polymorphism has been associated with

altered cortical activation and decreased short term plasticity [79,80,85,86], as well as altered skill acquisition and learning [79,82,85,86] relative to those without the polymorphism. The impact of the Val66Met polymorphism on motor learning post-stroke has not been explored and may provide a potential biomarker for further refinement and individualization of rehabilitation post-stroke. **Aim 1 of this dissertation was to examine the impact of the Val66Met polymorphism in learning of a novel locomotor task in subjects with chronic stroke (> 6 months post stroke).**

Hypothesis 1.1. *Those with the BDNF polymorphism will have an altered rate of adaptation to the split-belt treadmill, compared to those without the polymorphism.*

As predicted, subjects with the Val66Met polymorphism (MET) demonstrated a slowed rate of step length adaptation relative to those without the polymorphism (VAL) within early adaptation. In addition, subjects with the polymorphism continued to adapt their step length asymmetry throughout adaptation, while those without the polymorphism plateaued near their baseline (a)symmetry late in the adaptation period. In contrast to rate of step length adaptation, there were no differences in the rate of limb phase adaptation for those with and without the polymorphism.

Hypothesis 1.2. *Those with the BDNF polymorphism will demonstrate an altered magnitude of adaptation compared to those without the polymorphism.*

The magnitude of adaptation was determined by assessment of the total amount of adaptation during split belt walking (*Magnitude of Total Adaptation*), as well as the ability of subjects to fully adapt back to their baseline (a)symmetry pattern (*Return to Baseline*). Despite a slowed rate of step-length adaptation, subjects with the

polymorphism did not demonstrate a reduced *Magnitude of Total Adaptation* relative to those without the polymorphism. The groups did not differ significantly in the total amount of adaptation for step length or limb phase. In addition, subjects with the polymorphism were able to achieve a magnitude of step and limb phase (a)symmetry relative to baseline (*Return to Baseline)* that did not differ significantly from that achieved by those without the polymorphism. The current results conflict with Hypothesis 1.2.

5.2 Aim 2 Findings

The optimal characteristics of learning which promote functional recovery of walking are not well defined for the post-stroke population. Studies of neurologically intact subjects indicate that variable practice conditions result in greater motor learning than blocked practice[43–45], however few studies have demonstrated these beneficial effects on learning after neurological insult [28]. **Aim 2 of this dissertation was to examine characteristics of task practice, specifically variable and constant practice, that may limit or promote learning of a novel locomotor pattern in the chronic stroke population.**

Hypothesis 2.1. *On the initial day of practice, chronic stroke survivors who participate in variable speed ratios of split-belt walking will demonstrate a decreased magnitude of adaptation compared to those participating in a constant 2:1 speed ratio.*

The magnitude of adaptation was determined by assessment of the total amount of adaptation during split belt walking (*Total Adaptation*), as well as the ability of subjects

to fully adapt back to their baseline (a)symmetry pattern (*Return to Baseline*). Despite repeated exaggerations of gait asymmetry during the initial session of split-belt walking, subjects participating in variable practice did not demonstrate a reduced magnitude of *Total Adaptation* relative to subjects participating in constant practice for step length or limb phase (a)symmetry. In addition, subjects participating in Variable practice were able to achieve a magnitude of step and limb phase (a)symmetry relative to baseline (*Return to Baseline)* that did not differ significantly from that achieved by subjects participating in Constant practice. The current results conflict with Hypothesis 2.1.

Hypothesis 2.2. *On Day 2, following one day of practice, chronic stroke survivors who participate in variable speed ratios of split-belt walking on day 1 will demonstrate a faster rate of re-adaptation and an increased magnitude of retention of the split belt walking pattern compared to those who participate in a constant 2:1 speed ratio of split belt walking.*

On the second day of split-belt treadmill walking, those in both the Constant and Variable practice conditions demonstrated a faster rate of re-adaptation and a decreased magnitude of their initial asymmetry upon re-exposure to the split-belt paradigm, indicating both groups had learned something about how to walk on the split-belt treadmill. Surprisingly, the type of practice performed did not affect the rate of re-adaptation or magnitude of retention on Day 2. These results indicate that variable practice does not confer an advantage to learning of a novel locomotor pattern, conflicting with Hypothesis 2.2.

5.3 Aim 3 Findings

BDNF levels have been directly related to exercise enhanced motor performance in the neurologically injured animal model[69,72]; however literature concerning the role of BDNF in enhancement of motor learning in the human population is limited. Previous studies in healthy subjects have shown a relationship between intensity of an acute bout of exercise and increases in circulating BDNF[99,104]. Furthermore, the intensity of exercise has been shown to have a moderating influence on the relationship between peripheral BDNF levels and cognitive learning[99,106]. **Aim 3 of this dissertation was to determine the influence of high intensity exercise prior to a motor learning task on circulating levels of peripheral BDNF and motor learning in neurologically intact subjects.**

Hypothesis 3.1. *Subjects who participate in a single session of high intensity upper extremity cycling immediately prior to split-belt treadmill walking will demonstrate greater increases in peripheral BDNF levels, compared to subjects who participate in quiet rest prior to split-belt treadmill walking.*

As predicted, subjects who participated in a short bout of high intensity upper extremity cycling prior to split-belt walking demonstrated a significant increase in serum BDNF levels from pre to post- exercise, relative to subjects who sat quietly prior to split-belt walking. The magnitude change in serum BDNF levels for those participating in high intensity exercise did not differ between subjects with (MET) and without (VAL) the Val66Met polymorphism in the BDNF gene.

Hypothesis 3.2. *On the initial day of practice, subjects participating in high intensity exercise prior to split-belt walking will demonstrate an increased rate and magnitude of adaptation to the split-belt treadmill, in comparison to those who do not participate in exercise immediately prior to split-belt treadmill walking. The increased rate and magnitude of adaptation in those participating in high intensity exercise will be greater for those without the Val66Met polymorphism.*

The magnitude of adaptation was determined by assessment of the total amount of adaptation during split belt walking (*Total Adaptation*), as well as the ability of subjects to fully adapt back to their baseline (a)symmetry pattern (*Return to Baseline*). Despite participation in high intensity exercise prior to split-belt walking on Day 1, subjects in the Exercise+Learning group demonstrated a similar amount of *Total Adaptation* and achieved a similar magnitude of (a)symmetry relative to baseline (*Return to Baseline*) as compared to subjects in the Learning group. In addition, the *Percent Change of Early Asymmetry* on Day 1 was similar between the groups (Exercise + Learning vs. Learning). No significant differences were found across groups for the magnitude of adaptation and *Percent Change of Early Asymmetry* regardless of presence or absence of the Val66Met polymorphism. The current results conflict with Hypothesis 3.2.

Hypothesis 3.3. *On Day 2, following one day of practice, those participating in high intensity exercise prior to split-belt walking will demonstrate an increased rate of re-adaptation and an increased magnitude of retention of the split belt walking pattern compared to those who do not participate in exercise immediately prior to split-belt treadmill walking. The increased rate of re-adaptation and magnitude of retention in*

subjects participating in high intensity exercise will be greater for those without the Val66Met polymorphism.

Subjects in both the Exercise + Learning and Learning groups, with and without the Val66Met polymorphism demonstrate a reduction in the initial step length and limb phase asymmetry upon re-exposure to the split-belt treadmill on Day 2 indicating they have learned something about how to walk on the split belt treadmill. The magnitude of this reduction in asymmetry from Day 1 to Day 2 (*Magnitude of Retention)* did not differ between exercise conditions (Exercise + Learning). The *Magnitude of Percent Change* was also similar across exercise conditions (Exercise+Learning vs. Learning). In addition, the influence of high intensity exercise on the *Magnitude of Retention* and *Magnitude of Percent Change* was not differentially impacted by the presence or absence of the Val66Met polymorphism. The current results conflict with Hypothesis 3.3.

5.4 Concluding Comments and Future Directions

The overall goal of this dissertation was to identify key molecular and behavioral requisites of motor learning post-stroke. The current series of studies provides several novel and important findings.

Within rehabilitation, variable practice is often utilized to promote motor relearning and functional improvements in individuals post stroke. The use of this practice type is largely based on evidence from motor learning studies in neurologically intact individuals. The results of the study in Aim 2 indicate however, that variable practice confers little benefit over constant practice in learning a novel locomotor task after

stroke. It is possible that the current results reinforce the previous suggestion that variable practice benefits may be confined to simple tasks, rather than complex tasks[43], such as the one utilized in the current study. It is also possible, however, that specific neurologic deficits as a result of stroke may directly impact the role of various practice paradigms in motor skill acquisition and learning. Studies of neurologically intact individuals indicate that the neural substrates utilized during acquisition and consolidation of a learning task are dependent on the practice structure[45,49]. Furthermore, Schweighofer and colleagues (2011) demonstrated that the presence or absence of visuospatial memory deficits post stroke dictated the benefits of variable and constant practice[48]. Given this evidence it is plausible that the area and extent of cortical damage post-stroke may differentially impact motor learning. Further studies are needed to differentiate characteristics of practice and the practice parameters that may enhance or hinder motor learning post-stroke. In addition, stratification based on location of stroke, or presenting neurologic deficits, may allow for an enhanced examination of optimal rehabilitation paradigms for specific cohorts of patients.

Aim 3 of this dissertation provided the first study to assess the relationship between high intensity exercise, peripheral BDNF, and motor learning. In addition, the study provided an assessment of the moderating influence of the BDNF Val66Met polymorphism in regulation of serum BDNF levels and locomotor learning in neurogically intact controls. There were several novel and important findings of this study that significantly advance our understanding of the role of exercise and BDNF in motor learning. The results of this study demonstrate that presence of a single nucleotide

polymorphism on the BDNF gene (Val66Met) does not influence the magnitude of upregulation of serum BDNF with high intensity exercise, nor does it interfere with learning of a novel locomotor pattern. A lack of relationship between serum BDNF levels and increases in locomotor learning, as well as the lack of moderation in serum BDNF by the Val66Met polymorphism noted in the current study indicates that systemic increases in BDNF do not necessarily reflect central neural processes, as often hypothesized[99,104]. As such, the current results indicate that use of serum BDNF as a supplementary marker of intervention efficacy on cognitive and motor function should be approached with caution. In addition, the results of this study demonstrate that although high intensity exercise prior to a motor learning task resulted in increased peripheral BDNF this exercise does not provide additional benefit to learning of a novel locomotor pattern in neurologically intact adults. It is possible however that the influence of exercise and BDNF, although eliciting physiologic changes, may not be captured behaviorally in a young, neurologically intact population.

The above suggestion is made more salient secondary to the results presented in Aim 1 of the dissertation. Although the Val66Met polymorphism did not influence learning of a novel locomotor pattern in neurologically intact controls within the Aim 3 study, the current results within Aim 1indicate that the polymorphism does impact the rate of learning in chronic stroke survivors. Aim 1 of this dissertation is the first study to examine the role of a single nucleotide polymorphism (SNP) in the BDNF gene in moderating motor learning post-stroke. The results suggest that chronic stroke survivors, regardless of presence or absence of the polymorphism, are able to adapt their walking

pattern over a period of trial and error practice. The process of locomotor adaptation, however, is slowed in those with the Val66Met polymorphism. These results have important implications for motor learning and rehabilitation post-stroke because they identify a population that may benefit from increased practice or differing practice parameters to facilitate optimal motor learning. In addition, the current results identify a potential biomarker that may be utilized to further individualize treatment approaches within rehabilitation post-stroke.

The current series of studies identify several avenues of research necessary to the development of optimal neurorehabilitation treatments. In addition, this series of studies highlights the fact that mechanisms of motor learning and neural plasticity frequently exploited to benefit one population, may not necessarily benefit another. Stratification of chronic stroke subjects by neurologic deficit, and genotype, may offer additional insight into the capacity of motor learning and functional recovery in specific patient populations post-stroke.

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Appendix

INTERNAL REVIEW BOARD APPROVAL

RESEARCH OFFICE

210 Hullihen Hall
University of Delaware
Newark, Delaware 19716-1551
Ph: 302/831-2136
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DATE:	March 18, 2010
TO:	Darcy Reisman
FROM:	University of Delaware IRB
STUDY TITLE:	[160478-1] Locomotor Adaptation Following Stroke
IRB REFERENCE #:	
SUBMISSION TYPE:	Other
ACTION:	APPROVED
APPROVAL DATE:	March 17, 2010
EXPIRATION DATE:	April 15, 2011
REVIEW TYPE:	Full Committee Review

Thank you for your submission of Other materials for this research study. The University of Delaware IRB has APPROVED your submission. This approval is based on an appropriate risk/benefit ratio and a study design wherein the risks have been minimized. All research must be conducted in accordance with this approved submission.

This submission has received Full Committee Review based on the applicable federal regulation.

Please remember that informed consent is a process beginning with a description of the study and insurance of participant understanding followed by a signed consent form. Informed consent must continue throughout the study via a dialogue between the researcher and research participant. Federal regulations require each participant receive a copy of the signed consent document.

Please note that any revision to previously approved materials must be approved by this office prior to initiation. Please use the appropriate revision forms for this procedure.

All SERIOUS and UNEXPECTED adverse events must be reported to this office. Please use the appropriate adverse event forms for this procedure. All sponsor reporting requirements should also be followed.

Please report all NON-COMPLIANCE issues or COMPLAINTS regarding this study to this office.

Please note that all research records must be retained for a minimum of three years.

Based on the risks, this project requires Continuing Review by this office on an annual basis. Please use the appropriate renewal forms for this procedure.