Paired Afferent Stimulation in Children: Mechanisms of Developmental Plasticity

by

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Abstract

Plasticity may be enhanced in the developing brain, but mechanisms are poorly understood. Transcranial magnetic stimulation (TMS) offers increasingly sophisticated means of assessing neurophysiology and mechanisms of neuroplasticity \textit{in vivo} in adults. Paired associative stimulation (PAS) is an advanced modality that pairs sensory electrical peripheral nerve stimulation with TMS over the contralateral motor strip (M1). PAS induces rapidly evolving, long lasting, reversible and topographically specific increases in M1 excitability in adults consistent with NMDAR-dependent long-term potentiation (LTP). PAS has not been studied in the more plastic brains of children. Our aim was to determine the developmental profile of PAS neurophysiology in school-aged children, evaluating the putative correlation of PAS effect with age, and possible endogenous systems that may be modulators of PAS (i.e. Shot-Interval Intracortical Inhibition) and dictating plasticity. Twenty-eight children aged 6-18 years underwent PAS evaluations. Eighteen had significant PAS responses that were reproducible on repeat testing. Addition of inhibitory paired-pulse TMS appeared to block the PAS effect. The PAS effect did not correlate with age. PAS is safe and tolerable with effects comparable to adults. PAS may carry clinical and research utility in perinatal stroke and other pediatric brain injury populations.
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<td>ACH</td>
<td>Alberta Children’s Hospital</td>
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<tr>
<td>AMT</td>
<td>Active Motor Threshold</td>
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<tr>
<td>AMPAR</td>
<td>α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor</td>
</tr>
<tr>
<td>APB</td>
<td>Abductor Pollicis Brevis</td>
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<tr>
<td>APPIS</td>
<td>Arterial presumed perinatal ischemic stroke</td>
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<tr>
<td>APSP</td>
<td>Alberta Perinatal Stroke Program</td>
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<tr>
<td>BDNF</td>
<td>Brain Derived Neurotrophic Factor</td>
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<tr>
<td>BOLD</td>
<td>Blood Oxygen Level Dependent</td>
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<tr>
<td>CIMT</td>
<td>Constraint Induced Movement Therapy</td>
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<tr>
<td>CMCT</td>
<td>Central Motor Conduction Time</td>
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<tr>
<td>CNS</td>
<td>Central Nervous System</td>
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<tr>
<td>CPSP</td>
<td>Calgary Pediatric Stroke Program</td>
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<tr>
<td>CS</td>
<td>Conditioning Stimulus</td>
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<tr>
<td>CST</td>
<td>Corticospinal tract</td>
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<tr>
<td>CSVT</td>
<td>Cerebral Sinovenous Thrombosis</td>
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<tr>
<td>DTI</td>
<td>Diffusion Tensor Imaging</td>
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<tr>
<td>DWI</td>
<td>Diffusion weighted imaging</td>
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<tr>
<td>EMG</td>
<td>Electromyogram</td>
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<tr>
<td>EPSP</td>
<td>Excitatory Post-synaptic Potential</td>
</tr>
<tr>
<td>fMRI</td>
<td>Functional Magnetic Resonance Imaging</td>
</tr>
<tr>
<td>GABA</td>
<td>Gamma-Aminobutyric acid</td>
</tr>
<tr>
<td>HCP</td>
<td>Hemiparetic Cerebral palsy</td>
</tr>
<tr>
<td>IPSP</td>
<td>Inhibitory Post-synaptic Potential</td>
</tr>
<tr>
<td>LP</td>
<td>Lateral Posterior Nucleus</td>
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<tr>
<td>LTD</td>
<td>Long-Term Depression</td>
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<tr>
<td>LTP</td>
<td>Long-Term Potentiation</td>
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<tr>
<td>M1</td>
<td>Primary Motor Cortex</td>
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<tr>
<td>MCA</td>
<td>Middle cerebral artery</td>
</tr>
<tr>
<td>MEP</td>
<td>Motor Evoked Potential</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
</tr>
<tr>
<td>MVC</td>
<td>Maximal Voluntary Contraction</td>
</tr>
<tr>
<td>NAIS</td>
<td>Neonatal arterial ischemic stroke</td>
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<td>NIBS</td>
<td>Non-invasive Brain Stimulation</td>
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<tr>
<td>NMDAR</td>
<td>N-methyl-D-Aspartate Receptor</td>
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<tr>
<td>PAS</td>
<td>Paired Associative Stimulation</td>
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<tr>
<td>PEHI</td>
<td>Pediatric Edinburgh Handedness Inventory</td>
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<tr>
<td>PLIC</td>
<td>Posterior limb of the internal capsule</td>
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<tr>
<td>PPIS</td>
<td>Presumed perinatal ischemic stroke</td>
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<tr>
<td>PVI</td>
<td>Periventricular venous infarction</td>
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<tr>
<td>RMT</td>
<td>Rest Motor Threshold</td>
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<tr>
<td>rPAS</td>
<td>Rapid rate Paired Associative Stimulation</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>rTMS</td>
<td>Repetitive transcranial magnetic stimulation</td>
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<tr>
<td>S1</td>
<td>Primary Sensory Strip</td>
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<tr>
<td>S2</td>
<td>Secondary Sensory Strip</td>
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<tr>
<td>SEP</td>
<td>Sensory Evoked Potential</td>
</tr>
<tr>
<td>SICI</td>
<td>Short-Interval Intracortical Inhibition</td>
</tr>
<tr>
<td>SM1</td>
<td>Primary sensory motor strip</td>
</tr>
<tr>
<td>SRC</td>
<td>Stimulus Response Curve</td>
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<tr>
<td>ST</td>
<td>Sensory Threshold</td>
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<tr>
<td>STDP</td>
<td>Spike Timing Dependent Plasticity</td>
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<tr>
<td>TBS</td>
<td>Theta Burst Stimulation</td>
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<tr>
<td>tDCS</td>
<td>Transcranial Direct Current Stimulation</td>
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<tr>
<td>TMS</td>
<td>Transcranial magnetic stimulation</td>
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<tr>
<td>TS</td>
<td>Test Stimulus</td>
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<tr>
<td>VPM</td>
<td>Ventral Postereomedial Nuclei</td>
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<tr>
<td>VPN</td>
<td>Ventral Posterolateral Nuclei</td>
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CHAPTER 1 – BACKGROUND

Non-invasive brain stimulation technologies can both study and potentially treat neurological disorders. Transcranial magnetic stimulation (TMS) offers increasingly sophisticated means of assessing neurophysiology and mechanisms of neuroplasticity in vivo in adult human subjects. Paired associative stimulation (PAS) is an advanced TMS method that pairs sensory electrical stimulation of a peripheral nerve with TMS over the contralateral motor strip\(^1\). Paired Afferent Stimulation induces rapidly evolving, long lasting, reversible and topographically specific increases in motor cortex excitability in adults consistent with NMDA-receptor-dependent long-term potentiation\(^1\)\(^-\)\(^3\). PAS has not been studied in the more plastic brains of children. Our aim is to define the developmental profile of PAS neurophysiology in children and potential mechanisms dictating the fate of plasticity.

1. Developmental Plasticity

A fundamental first step in understanding the developmental profile of a child’s brain is to appreciate how it copes with external stimuli. The term neuroplasticity refers to the brain’s intrinsic ability to adapt and change in response to environmental, structural and functional experiences\(^4\). More specifically, plastic change occurs throughout normal development, including throughout the course of motor learning, as well as in response to injury or disease\(^4\). Resulting neural tissue alterations, oftentimes, outlive the initial stimulation (i.e. change) period. Previous research has shown that brain plasticity includes various mechanisms of neuronal reorganization leading to recruitment of convergent or non-convergent pathways, fortification of existing synaptic connections, as well as an increase in synaptogenesis\(^4\),\(^5\). Plasticity is well studied in the primary motor cortex (M1) because such changes occur rapidly and are measurable\(^6\).

Transcranial magnetic stimulation (TMS) is an ideal modality for further investigation of neuroplasticity in humans including long-term potentiation (LTP)-like mechanisms that may
potentially carry therapeutic utility in a myriad of neurological disorders. Clinically, the study of neuroplasticity may provide insight into the pathogenesis and potential treatment of many disorders including perinatal stroke, adolescent major depression, pediatric headache, Tourette’s syndrome, epilepsy, traumatic brain injury, attention deficit hyperactivity disorder (ADHD), as well as pre-surgical management and planning\(^{7-14}\). Research employing the use of TMS has been extensive in many neurological disorders to unveil potential mechanisms of disease progression, pathogenesis and treatment effects to combat disease. Adult studies have been extensive over the past three decades; however, work has been minimal in children with neurological disease. Our study marks the beginning of developmental plasticity work in children and potential application to neurological disorders. Common consensus among researchers and clinicians is that younger brains are more plastic. Understanding how malleable the pediatric brain is will help researchers and clinicians understand how a young brain will adapt to external factors leading to future therapy and treatment planning.

1.1 Sensorimotor Neurophysiology

Mapping within the cortex unveils fundamental information regarding connectivity within the brain to dictate simple peripheral functions. Generally, the primary motor cortex is responsible for basic motor functions such as picking up a ball, extending your right leg, closing one’s eye lid, etc. However, in order for this to happen, a motor signal from the primary motor cortex (i.e. the surface of the brain) must travel down a highway of neurons, namely the corticospinal tract (CST). The CST is comprised of upper and lower motor neurons. Upper motor neurons, otherwise known as Betz cells (pyramidal cells forming the cortex), reside in layer V of the
cortex, and carry motor information down the CST where they synapse with lower motor neurons in the ventral horn of the spinal cord. Synaptic transmission at the level of the spinal cord occurs through glutamate release. Lower motor neurons transmit a neuromuscular impulse towards an effector muscle, where peripheral motor neurons control synchronized movements. Coordinated function involves many different regions in the brain. One in particular is directly posterior to the M1 – the primary sensory cortex (S1). This region of the brain helps one sense the object they are touching. Furthermore, self-motivated movements not requiring the use of an object require this region of the brain for proprioception, perception of simple movements as well as sensation. These actions cause activation of this region of the brain. Figure 1 is a 3D reconstruction of my brain during a simple right-handed finger tap task illustrating left M1 as well as the left S1 activation during this task.

Figure 1. M1 S1 Cortices. During a simple right finger tap task in a functional magnetic resonance image sequence, the left M1 and S1 cortices respectively show high activation. The
nature of the task by act of moving the thumb and first finger towards one another and act of touching causes both cortices to light up.

Recent research using quadripulse TMS (i.e. pairing of four magnetic stimulations) attempted to elucidate the putative influences between S1 and M1. Under the facilitatory paradigm, stimulations were delivered over the M1 resulting in potentiated sensory evoked potentials (SEPs) evoked from the S1. These effects were long lasting, but did tend to fade over long periods of time. Hence, these data seem to suggest that the M1 may have bidirectional control of sensory cortical function through corticocortical connections.

Neuroimaging research employing the use of functional magnetic resonance imaging (fMRI) sought to determine whether motor performance had activity level increase in the M1 or in other areas surrounding the central sulcus and post central gyrus (i.e. S1). Changes in cerebral blood flow are detected and correlated with task-directed movements in order to correlate increases in activation in anatomy. Blood oxygen level dependent (BOLD) contrast is the main outcome of an fMRI scan and an increase in this signal is a measure of increased blood flow, which is correlated with an increase in recruitment of a given area of the brain during a given task. Self-motivated finger movements during a given fMRI scan indicated significantly higher mean intensities in the primary sensory-motor (SM1) regions contralateral to the hand performing the movement together rather than the M1 or S1 individually. These findings seem to suggest direct involvement of central sulcus areas (i.e. S1-M1 integration) in motor control.
Thalamocortical fibers are thought to connect the S1 and M1, or at least help both corticies, communicate\textsuperscript{18,19}. In order for sensory stimuli to reach the S1, peripheral sensory neurons have their cell bodies within dorsal root ganglia in the spinal cord, which receive neuronal impulses from various areas in the body. This is known as the dorsal white column-medial lemniscus system, which is responsible for fine touch, vibration, as well as disseminating proprioception. Signals from neurons within the dorsal column neurons synapse with axons in the thalamus. Thalamocortical fibers lie near the post central gyrus and central sulcus. In the S1 areas, thalamocortical projecting fibers from the ventral postereolateral (VPN) and postereomedial nuclei (VPM) as well as the lateral posterior nucleus (LP) project to the S1 and S2 areas\textsuperscript{20}. These fibers terminate immediately lateral to the post central gyrus\textsuperscript{20}. The projections from the VPM are responsible for touch\textsuperscript{20}. It has been postulated that there are also a series of non-specific projections from the thalamus that help the S1 and M1 communicate with one another to dictate motor function\textsuperscript{18-20}. This is therefore thought to create the thalamocortical sensorimotor circuit\textsuperscript{18}. There are also a number of small white matter fibers help with the S1 and M1 association.

A case report of a 63 year-old man with a metastatic brain tumor utilized diffusion tensor imaging (DTI) and fMRI to delineate the connections between S1 and M1 during awake surgery\textsuperscript{21}. In addition to electrical stimulation, which only shows which cortex is responsible for a given function, DTI and fMRI supported the notion that there are tracts running between the M1 and S1 during hand clenching\textsuperscript{21}. Total resection of the tumour resulted in mild hemiparesis (right), which recovered with time; however, during left hand activities, his right hand would
occasionally lapse in function causing him to drop certain items, during concentration\textsuperscript{21}. During fMRI of a right-handed task, the area of cortex that had increased BOLD signal was posterior to that pre-operation\textsuperscript{21}. On anatomical images, this shows a shift of function from the motor to the sensory cortex to compensate (i.e. connected) \textsuperscript{21}. Hence, activation of both afferent and efferent parallel fibers connecting the aforementioned primary cortices could potentially play a significant role in dictating plastic change within the pediatric brain\textsuperscript{2,17}. However, interpretation of this case report must be taken with caution since perilesional areas often change with installation of lost functions following resection. This may not indicate original connectivity pre-surgery. Nevertheless, a S1-M1 association in this scenario exists.

Contralateral (crossed) CST projections are the basic foundation for normal motor brain development; on the other hand, ipsilateral (uncrossed) CST projections also provide critical insight on motor development, predominantly following perinatal or early brain injury \textsuperscript{22}. Crossing of the CST occur at the cervico-medullary junction. Serial single-pulse TMS studies over M1 in new born children to the first two years of life have shown that with time, a balance incurs between contralateral and ipsilateral projections within early development\textsuperscript{23}. Furthermore, latency data also suggests that ipsilateral pathways have faster conductances than contralateral pathways before six months of age\textsuperscript{23}. This phenomenon tends to persist in proximal rather than distal muscles in majority of healthy children before ten years of age\textsuperscript{24}.
1.2 Transcranial Magnetic Stimulation

Transcranial magnetic stimulation (TMS) is a non-invasive brain stimulation technique, first described in 1985 \(^{25}\), allowing clinicians to measure and map brain function, analyze integrity of certain pathways, excitability of certain regions in the brain, and elucidate how certain cortical regions communicate with one another. The emergence of TMS radically changed how clinicians and scientists investigated neurophysiology of the M1, since it was previously confined to invasive studies prior to its discovery. Applications of TMS to the more plastic brains of children have been simple and few; less than 4% of published TMS studies since 2008 include children.

1.2.1 Cortical Effects

Magnetic energy discharged through a coil can take many different shapes. Coils can be circular, figure-of-eight shaped, or a double-cone-coil, which consists of two coils (Figure 2)\(^{26}\).
Figure 2. TMS Coils. (A) Flat iron coil is designed to use in a pediatric population. (B) Circular coil is designed to stimulate a larger area of cortex. The stimulus spread is much larger compared to other coils. (C) Double cone coil is designed to stimulate a focused area of cortex at greater depths. This design is ideal for stimulating the lower extremity. However, the basic biophysical principle causing activation of a select pool of neurons in a specific orientation is consistent. In a given piece of cortex, many neurons coexist in a number of different orientations, of which, cannot be recruited by magnetic energy. The fundamental unit within a muscle is coined a motor unit. It is comprised of a motor neuron encapsulated by skeletal muscle fibers, which are innervated by that axon. Coordinated contracted is accomplished through motor unit synchrony within a given muscle group; a motor pool accomplished synergistic movements. The central nervous system acts to recruit motor units based on increasing size and load. Increased voluntary contraction, or load placed on a given muscle group, will cause greater motor units to become recruited by the central nervous system; this is termed spatial recruitment. An alternative method the CNS employs to activate motor units is through rate coding. This refers to the frequency of neuronal firing, thus, as the
intensity of stimulus increases, the frequency of action potentials increases causing increased recruitment of motor units. This is paralleled to increased TMS stimulus intensity; higher TMS stimuli will lead to increased motor unit recruitment causing increased EMG responses in peripheral muscles. Short-latency responses of motor unites are typically recorded via EMG. Previous research suggests that increasing the strength of TMS increases the response probability of a tonically firing motor unit at the same time recruited new units, which discharged phasically during peak responses.

Basic motor neuron classification is imperative to appreciate central and peripheral nervous system communication. TMS triggers efferent signals to travel from the CNS to peripheral muscle targets via intricate motor neuron systems. Somatic motor neurons originate in the CNS, and sprout projections to skeletal muscles. Axons embed in muscles of locomotion. Subdivisions of somatic motor neurons include alpha, beta and gamma neurons, which are subspecialties of within a motor unit. Alpha motor neurons diffusely exist throughout muscle fiber are their cell fibers exist in the ventral horn of the spinal cord. Beta motor neurons innervate muscle fibers of the muscle spindle whereas gamma motor neurons are located within the muscle spindle itself. Hence, an efferent stimuli from the CNS, possibly elicited by TMS, would target alpha motor neurons first, then beta and lastly, gamma in an outside in fashion to illicit a muscle response. Alpha motor neuron activation regulates muscle tone whereas gamma motor neuron activation regulates muscle stretch. Muscle stretch causes secondary activation of sensory neurons to detect degree of stretch resulting in a signal being sent back to the CNS to thereby resist further muscle stretch.
Magnetic energy radiating from a TMS coil acts to preferentially recruit pyramidal neurons. The principles behind this will be explained in further detail shortly. Placing a high energy wire on the scalp and subsequently passing a rapidly changing current through it results in the formation of a magnetic field, which penetrates the scalp, skull, dura, arachnoid, and pia to eventually reach the cortex causing recruitment of a select area of neuronal mater. Moreover, recruitment of a specific neuronal pool (topographical cortical area) is the result of eddy currents, which causes membrane depolarization either producing an excitatory post-synaptic potential (EPSP) and/or an inhibitory post-synaptic potential (IPSP).

TMS non-invasively causes neuronal depolarization preserving this natural physiological response. Specifically, many researchers have hypothesized that the neuron undergoes an influx of positive sodium ions resulting in nerve depolarization; this may be due to summation of EPSP’s as well as other facilitatory mechanisms. The corollary is also true for inhibition, with an influx of negative chloride ions due to a summation of IPSP’s as well as other inhibitory mechanisms. Ultimately, the neuron will fail to distinguish whether a rise in membrane potential results from a rapidly changing magnetic field or an imposed electric field intracellularly. Albeit changes being described referring to cellular levels at an individual neuron, TMS has the ability to cause these exact changes to a large population of neurons resulting in a substantial excitatory or an inhibitory response in an effector muscle, thus achieving a net effect. Moreover, these net effects can be measured by electromyography (EMG).
The skull boundary ensures the normal component (perpendicular to the skull) of current is essentially zero, thus the magnetic field within the cortex is considerably smaller that the imposed magnetic field from TMS. The magnetic field within the cortex rises instantaneously to cause neuronal depolarization, and then falls resulting in a reversal of its direction ensuring the normal component of the current is zero\textsuperscript{28}. Magnetic stimulation between the coil and the scalp creates a parallel circuit resulting in induced current flowing within the horizontal (i.e. pyramidal) fibers of the cortex. In our pediatric population, a 70mm figure-of-eight coil penetrates to a depth of 3 to 4cm to stimulate the cortex\textsuperscript{29}. A small figure-of-eight coil is typically used to stimulate the motor cortex, specifically the hand knob (Figure 3).

Figure 3. Hand Knob. The hand knob is medial from the vertex and is a large area of cortex within the M1. It becomes an ideal area of interest for TMS to study corticomotor neurophysiology and changes in plasticity.
Focal stimulation from this coil is ideal for mapping studies. Smaller coils typically have tightly wound wire packed into smaller wings, which are parallel to the scalp. This design allows for focal stimulation at a depth ideal for stimulating the hand knob, which is superior on the cortex. However, to provide contrast, in order to recruit lower extremity muscles, coil design changes. The coil typically has a slight angulated design with wire wrapped around larger wings providing strong magnetic pulses penetrating deeply into the cortex. Regardless of coil type or orientation, pyramidal neurons within layers III and V remain preferentially activated.

The fundamentals behind TMS stem from Faraday’s Law of Magnetic Induction. In TMS, Faraday’s Law of Induction describes how a current carrying wire is capable of creating a focused magnetic field. Subsequently, this magnetic field is able to penetrate the scalp and skull to stimulate a very focused area of the cortex leading to excitation and recruitment of cortical matter\textsuperscript{30}. By manipulating the magnetic field vector radiating from the coil, focal depolarization of human cortical matter achieves non-invasive, \textit{in vivo} electrical stimulation of motor systems\textsuperscript{22, 31-33}.

1.2.2 Single Pulse TMS

Single-pulse TMS recordings of any effector muscle via EMG record motor evoked potentials (MEP) bilaterally, thereby quantifying corticomotor effects. Measures such as difference in peak-to-peak MEP amplitudes and stimulus-response curves (see methods) elicited by single-pulse TMS can evaluate central motor neurophysiology\textsuperscript{34-36}. Increased temporal corticomotor excitatory spread is likely as a result of TMS compared to compound muscle action potentials.
Typically, CMAPs are obtained with suprathreshold peripheral nerve stimulation resulting in responses larger peak-to-peak amplitude, shorter duration and less polyphasic responses compared to the motor evoked potential (MEP) elicited by TMS\textsuperscript{27}. Consistency in morphology tends to decrease with MEP recordings elicited via TMS compared to CMAP due to the amount of temporal dispersion and variability in central nervous system cortical excitability\textsuperscript{27}.

Increasingly sophisticated TMS methods allow exploration of human physiology and direct correlation to animal models. An MEP created in peripheral muscles via TMS over the contralateral M1 is due to the activation of pyramidal fibers within the precentral gyrus. The first volley of an MEP is called the D-wave\textsuperscript{37,38}; this depicts direct activation of axonal pyramidal fibers or the proximal internodes of subcortical white matter. Low intensity TMS activates D-waves and upon summation, or with increasing intensity, subsequent recruitment of I-waves ensues (Figure 4)\textsuperscript{37,38}.
**Figure 4.** MEP Volleys. Recruitment of D-waves and I-waves occur with increasing TMS intensity in a top down fashion from the cortex down the corticospinal tract until recruitment of lower motor neurons within the spinal cord occur to cause contraction of a specific hand muscle. (A) Over time, I-waves are recruited with increasing TMS intensity. Reprinted with permission from Groppa et al\(^{27}\) (B) D-waves are the first volley in an MEP and are followed by an I-wave.

Three I-waves with differing latencies form – I1, I2, and I3\(^{37,38}\). Among these I-waves, the threshold needed for activation increases from I1 to I3\(^{37,38}\). The activation of these waves to elicit an MEP can be thought of as rungs on a ladder. The D-wave is the top rung and the I-waves from one to three are the lower rungs respectively, which represent the path down the CST originating from the M1. This area of the brain is the motor control center, a gateway of information to and from the brain to the entire body.

Over the two past decade, TMS studies have defined corticomotor projections and pathways from birth through the early years of development\(^{23,39}\). In children, MEP from M1 stimulation are present bilaterally at birth become evident and increase over the first 3 years of life;
however, to elicit these responses, TMS stimulator output must be maximal (in children under 6 years of age)\textsuperscript{23}. Previous research has effectively shown that motor thresholds in children under the age of 10 tend to be higher than adults, but reach adult levels during adolescence\textsuperscript{35}. Central motor conduction time (CMCT) is a measure that is used to help delineate possible reasons as to why TMS evoked MEPs are increasingly difficult to elicit in much younger children. Muscle contraction during TMS, otherwise known as active motor threshold (AMT), allows MEPs to be evoked at lower stimulator outputs. At a molecular level, a percentage of maximal voluntary contraction (MVC) primes neuronal membranes by slightly depolarizing it to reach a threshold, thus less stimulator output is required to create an all-or-none response to evoke an MEP. Hence, active CMCT is evident in much younger children (3-5 years of age), where as rest CMCT does not approach adult values until adolescence. It has been postulated that the discrepancy in latency signifies trans-synaptic activation of select corticomotor neuron pools via interneurons and fast pyramidal tract neurons during a percentage of MVC\textsuperscript{40, 41}. A sophisticated study compared muscle action potentials evoked at rest and during contraction using a round coil. Results indicate that latencies in pre-school children are much higher than the average adult and decrease with age as children approach adolescence\textsuperscript{42}. The general consensus is that the gradual decrease in CMCT with age is thought to be correlated with increased CNS myelination, leading to maturation of M1 neuronal and synaptic connections through pruning. This results in more effective and efficient recruitment of these respective pathways\textsuperscript{42}. A decrease in CMCT with age may correlate with significant PAS response in children. We intend to study the effects of PAS across a broad age range to determine PAS effect in children.
TMS is safe and well tolerated in children\textsuperscript{34,43-45}. Seizures associated with TMS have not been described in children including administration over seizure-affected hemispheres\textsuperscript{46-48}. Not only can non-invasive brain stimulation technologies measure and evaluate neurophysiology and neuroplasticity, previous research has suggested that it can potentially treat neurological disorders such as stroke. Application is safe in adult stroke\textsuperscript{49-57} and our neurophysiology studies and clinical trials in children with stroke further support safety and feasibility in this population\textsuperscript{58,59}. Despite such favourable characteristics and potential as a research tool, the application of TMS to understand developmental plasticity has been limited.

Single-pulse TMS methods assess basic neurophysiology such as cortical excitability and pathway integrity. Modifications of TMS methods can expand and elaborate this potential to interrogate plasticity mechanisms in human subjects.

### 1.3 Paired Associative Stimulation

Paired Associative Stimulation (PAS) is an advanced TMS method that pairs sensory electrical stimulation of a peripheral nerve with TMS over the contralateral motor strip\textsuperscript{1-3,60}. When the dual peripheral and central stimulation paradigm is practiced according to spike timing dependent plasticity (STDP) measures (short bursts of high frequency depolarization)\textsuperscript{2,61}, PAS results in rapidly evolving, long lasting, reversible and topographically specific increases in M1 excitability\textsuperscript{1-3,60}. At a specific inter-stimulus interval (ISI) between afferent and efferent stimulation, PAS has the ability to both facilitate and inhibit cortical changes via long-term potentiation (LTP) and long-term depression (LTD)\textsuperscript{62,63}, respectively. Previous research in adults
demonstrates that an ISI of 25ms (Figure 5) produces an enhancement of cortical activity and an ISI of 10ms produces an attenuation of cortical activity\textsuperscript{60,64}. Repeated stimulation enhances MEP amplitudes, likely via an NMDAR-mediated LTP mechanism\textsuperscript{1-3, 60, 65-67}.

The NMDAR is a ligand-gated ion channel that responds to the excitatory neurotransmitter glutamate. Cortical neurons, in this case in the motor cortex, that release glutamate and bind to the NMDAR are not sufficient to open the ion channel. An alkali-earth metal, namely Mg\textsuperscript{2+}, occupies the pore, and blocks flux of ions due to its large hydration shell. When glutamate binds, there is a conformational change of the NMDAR, but it is not sufficient to open the pore to release Mg\textsuperscript{2+}. The NMDAR is voltage sensitive; therefore, it needs a backpropagating depolarization, supplied by an activated AMPAR, to dilate the pore such that Mg\textsuperscript{2+} can pass through allowing Na\textsuperscript{+} and Ca\textsuperscript{2+} to flow freely through the NMDAR. The main current generated by the NMDAR is due to Na\textsuperscript{+}; however, small fraction is due to Ca\textsuperscript{2+}, which is more than enough to trigger plasticity events.
Figure 5. PAS<sub>25</sub>. Pairing of sensory peripheral nerve stimulation with TMS over the contralateral M1 is used to stimulate a motor response in the right APB muscle (Purple corticospinal tract). The afferent sensory stimulus at the median nerve precedes the efferent motor stimulus evoked by TMS at an interstimulus interval (ISI) of 25ms leading to LTP (Red corticospinal Tract). Crossing of the corticospinal tract occurs at the cerviomedullary junction.

Recently, elegant TMS research demonstrated that an ISI of 21.5ms rather than 25ms is more effective at eliciting an LTP response<sup>68</sup>. On a molecular basis, orthodromic sensory stimulation followed by a shorter interval for TMS leads to recruitment of early I-waves compared to the typical and well-established 25ms LTP paradigm, which recruits late I-waves. Recent research has shown that recruitment of early I-waves is better at substantiating facilitatory learning.
within the M1. Comparisons between PAS25 and PAS21.5 showed facilitation of MEPs; however, when cathodal (inhibitory) Transcranial Direct Current Stimulation (tDCS) over the cerebellum was delivered post-PAS intervention, LTP was abolished only in those subjects who received PAS25, whereas the effects remained in the PAS21.5 group. These data support the notion that indeed, different I-waves are recruited to show the same effect; however the timing of this recruitment will determine the longevity of LTP. Adult testing under the LTP paradigm showed facilitation lasting a minimum of 30-60 minutes in both healthy adults and those affected by stroke. Table 1 illustrates pivotal work that has been done to date.

**Preliminary Data**

We performed the above protocol on 6 healthy young adults. Preliminary data from our group on healthy adults depicts a robust post-PAS cortical change within the left M1. Specifically, our data shows a significant post-PAS effect size between baseline and post-effect MEP measurements. Data from 6 young adults ages 20-35 confirms feasibility and indicates reproducibility of robust PAS-induced changes in cortical excitability. Post-PAS mean MEP amplitudes increased up to 43% above baseline (p=0.054). Subsequent ancillary trials have also been conducted in order to ensure our PAS protocol produces optimal and consistent data. Preliminary data, combined with our unique experience in pediatric non-invasive brain stimulation, ideally positions us to explore the developmental profile of PAS plasticity.
### Table 1. Summary of PAS Clinical Trials in Healthy and Stroke-Affected Adults

<table>
<thead>
<tr>
<th>Study</th>
<th>Age</th>
<th>n</th>
<th>Muscle/Nerve</th>
<th>PAS</th>
<th>Testing</th>
<th>Outcome</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stefan, K. 2000.</td>
<td>19-37</td>
<td>22</td>
<td>APB/Median</td>
<td>90 pairs, 300%, 0.05Hz, ISI: 10, 25, 35, 100, 525, 5000ms</td>
<td>Baseline, 30min post, 60min post, 24h post</td>
<td>MEP, silent period, ISI, muscle</td>
<td>ISI 25 facilitation, APB ideal muscle</td>
</tr>
<tr>
<td>Stefan, K. 2002.</td>
<td>22-42</td>
<td>14</td>
<td>APB/Median</td>
<td>90 pairs, 0.05Hz, ISI: 10, 25ms</td>
<td>Baseline, post-measure</td>
<td>MEP, SI</td>
<td>NMDA involved in plastic change (DM block)</td>
</tr>
<tr>
<td>Stefan, K. 2004.</td>
<td>19-33</td>
<td>27</td>
<td>APB/Median</td>
<td>132 pairs, 0.2Hz, ISI: 10, 25ms</td>
<td>Base (2), Test 1, 2</td>
<td>MEP, SP, attention</td>
<td>Increase MEP amplitude with attention</td>
</tr>
<tr>
<td>Stefan, K. 2006.</td>
<td>19-34</td>
<td>63</td>
<td>APB/Median</td>
<td>90 pairs, 300%, 0.1Hz, ISI: 10, 25ms</td>
<td>Baseline, Cross-over test</td>
<td>MEP, motor performance</td>
<td>Increase MEP amplitude post-training</td>
</tr>
<tr>
<td>Quatarone, A. 2006.</td>
<td>26-38</td>
<td>10</td>
<td>APB/Median</td>
<td>600 pairs, 200%, 5Hz, ISI: 10, 25ms</td>
<td>Baseline, 30min post, 60min post</td>
<td>MEP, SICI, ICF, SAI, LAI</td>
<td>rPAS produces long lasting facilitation</td>
</tr>
<tr>
<td>Kujirai, K. 2006.</td>
<td>21-48</td>
<td>47</td>
<td>FDI/Median</td>
<td>50 pairs, 0.1Hz, ISI: 10, 25ms</td>
<td>Baseline, post – rest and active</td>
<td>PAS Type, MEP, REC, SICI</td>
<td>AP PAS method produces specific STDP effects</td>
</tr>
<tr>
<td>Rosenkranz, K. 2007.</td>
<td>26-38</td>
<td>6</td>
<td>APB/Median</td>
<td>200 pairs, 300%, 0.25Hz, ISI: 3, 10, 25ms</td>
<td>Baseline, test with motor practice</td>
<td>MEP, IO curve, SICI</td>
<td>Motor practice improved task performance, SICI reduced and IO steepened</td>
</tr>
<tr>
<td>Fathi, D. 2010.</td>
<td>21-79</td>
<td>48</td>
<td>APB/Median</td>
<td>240 pairs, 0.5Hz, ISI: 25ms</td>
<td>Baseline, post-measure</td>
<td>MEP, age</td>
<td>Increased MEP post-PAS among younger subjects</td>
</tr>
<tr>
<td>Rajji, T.K. 2011.</td>
<td>&gt;18</td>
<td>24</td>
<td>APB/Median</td>
<td>180 pairs, 300%, 0.1Hz, ISI: 10, 25ms</td>
<td>Baseline, Test 1-5, Motor Task</td>
<td>MEP, time, motor task</td>
<td>PAS improved motor memory and performance (maintained)</td>
</tr>
<tr>
<td>Shin, H.W. 2011.</td>
<td>23-28</td>
<td>9</td>
<td>APB/Median</td>
<td>225 pairs, 300%, 0.25Hz, ISI: 10, 25ms</td>
<td>Baseline, post-measure</td>
<td>MEP, REC, LICI, SICI, ICF, SI</td>
<td>SI 1mV ideal test</td>
</tr>
</tbody>
</table>
Subsequent cortical changes induced by PAS share numerous physiological properties common to LTP. Specifically, in humans, antidromic sensory impulses from the median nerve take approximately 20ms to reach the primary somatosensory cortex (S1)\textsuperscript{2,4}.

Sensory neurons are unique in their function; their axons extend both centrally and peripherally via dorsal root ganglia with signal transduction beginning in a peripheral nerve fiber. Responses to temperature, cutaneous sensation, nocioception or mechanical deformation cause ion channels to open resulting in signal transduction. Central nervous system relay can then occur. In PAS, peripheral nerve stimulation relies on cutaneous sensation. There are several types of structural receptors within the skin, encapsulated and non-encapsulated receptors. Encapsulated receptors located within peripheral nerves are responsible for touch and vibration. Specifically, these are Meisner’s corpuscles, Pacinian corpuscles as well as Ruffini endings. The intensity of peripheral nerve stimulation is proportional to the frequency of action potential generation within the sensory neuron.

S1-M1 integration takes approximately 5ms; therefore, if TMS were applied 25ms after sensory stimulation, synchronous activation of M1 neurons would result from both the afferent and efferent inputs (i.e. facilitation)\textsuperscript{41,61,70,71}. Hence, similar to the STDP exemplar tested in animal models, “timing” is key within the PAS paradigm to elicit a desired response.

Understanding neuroplasticity mechanisms is critical to identifying new therapeutic targets. Recent evidence suggests the ability of PAS to modulate neural networks to enhance motor
function in healthy adults\textsuperscript{65}. Specifically, PAS has not only demonstrated enhanced motor skill learning with PAS25 paired with a motor skill task, but it has also been shown to enhance that learned skill over time, from hours to 1 week post-PAS treatment to improve motor outcome\textsuperscript{65}. The duration of effect outlasts the PAS intervention in a dose dependent fashion\textsuperscript{65}, confirming therapeutic potential in adults and suggesting potential utility in the more plastic brains of children.

### 1.4 Modulators of Plasticity

Plasticity is an intricate interplay of many determinants at the cellular and molecular level. Three main players that previous research has postulated to potentially dictate and modulate plasticity are the NMDA, AMPA and GABA receptors. Separately, as well as together, these three receptors have the ability to modulate plasticity in both animal models and potentially humans. In addition, genetic studies have unveiled many determinants of plasticity, most notably, brain-derived neurotrophic factor (BDNF), which may be a marker of an individual’s plastic potential. In the subsequent section, the role of each receptor will become clear and potential application to developmental neuroplasticity.

#### 1.4.1 - Long-Term Potentiation, the N-methyl-D-Aspartate Receptor and the AMPA Receptor

Long-term potentiation (LTP) is characterized as a lasting, albeit reversible, increase in synaptic strength. Synaptic plasticity, a determinant of LTP, is regulated in part by the \textit{N-methyl-D-Aspartate} receptor (NMDAR) at a molecular level. Specifically, its role as an ionotropic
glutamate receptor depends on two critical aspects: binding of glutamate, and cell membrane depolarization to alleviate magnesium (Mg$^{2+}$) block, and allow the flow of ions. The glutamate ligand is able to bind not only to the NMDAR, but also the AMPA receptor (AMPAR). Once glutamate binds to the AMPAR, positive ions, namely sodium (Na$^+$), are able to flow producing a current. The current generated by cation flow through the AMPAR sufficiently depolarizes the neuron enough such that the Mg$^{2+}$ block is diminished. Moreover, the influx of ions through the NMDAR, specifically calcium (Ca$^{2+}$), is the trigger for LTP. Defined, LTP is a long lasting enhancement in signal transduction between two neurons resulting from synchronous stimulation. This elucidates the necessity for both activity (glutamate release) and post-synaptic depolarization (Mg$^{2+}$ block relief) to initiate this form of plastic change. Hence, both NMDAR and AMPAR activation are needed in order for LTP to occur. Previous research has shown that increases in AMPAR content at synapses correlates with LTP activity; on the other hand, decreases in AMPAR correlate with long-term depression (LTD).

Despite the intracellular rise in Ca$^{2+}$ concentration triggering neurotransmitter release (i.e. glutamate) from the presynaptic terminal to their respective targets on the post-synaptic knobs, retrograde messengers are needed to facilitate antidromic signaling towards the dendritic spine (e.g. second messengers and enzymes). This form of synchronized signaling is termed spike timing dependent plasticity (STDP). The term “timing” is key to the outcome of STDP; poor timing risks dialyzing a key ion or second messenger out of the cell limiting facilitation of LTP. Under the STDP exemplar, if the short bursts of high frequency input stimulation occur before post-synaptic depolarization, then that particular input is
facilitated/stronger\(^5\). Elegant *in vivo* neurophysiological research in mice and rat models has demonstrated that the presynaptic trigger must come approximately 5-15ms before the post-synaptic back-propagating action potential at a high frequency using a current clamp paradigm\(^5\). The M1 to muscle pathway timing between the aforementioned animal model and the human system will not directly translate\(^2\).\(^4\). In order to achieve the same amount of facilitation between synapses in humans, “timing” will have to be adjusted. Paralleled M1 to muscle pathway in humans is governed by many physiological factors such as height, temperature, and myelination, all of which vary from animal models and will dictate timing differences within humans.

1.4.2 Neuromodulation and the GABA Receptor

Synaptic plasticity is a multifaceted process that is influenced by a multitude of different proteins and neurotransmitters. Neuromodulation describes complex physiological processes by which a neuron uses a collection of neurotransmitters to regulate a family of receptors. A key player in synaptic plasticity and regulation is the GABA neurotransmitter, which helps to maintain homeostatic neuronal function\(^72\). Sophisticated *in vitro* animal studies have shown that treatment of cortical slices with GABA antagonists preferentially modulates and blocks either GABA\(_A\) or GABA\(_B\) receptors depending on drug treatment resulting in limited spatial diffusion of GABA and duration of IPSPs \(^72\), \(^73\), \(^74\), \(^75\). Moreover, GABA is an inhibitory neurotransmitter, which acts at two main receptor sites, GABA\(_A\) and GABA\(_B\); both of which help to regulate negative ion concentration within the cell body of a neuron. GABA, derived from glutamate, binds to GABA\(_A\) resulting in an influx of chloride ions (Cl\(^-\)) down a concentration
gradient into the soma of the neuron\textsuperscript{76-79}. Negative membrane potential results in increased GABA\textsubscript{A} receptor expression resulting in hyperpolarization. Within the cortex, our primary output cells, namely pyramidal cells, release glutamate causing excitation. Without some form of regulation, or “gatekeeper”, uncontrolled excitation will lead to excitotoxicity of these pyramidal cells\textsuperscript{79}. Prevention relies upon GABA\textsubscript{ergic} interneurons, making connections upon and throughout the dendritic tree of pyramidal cells creating extremely close interconnections (2nm; synapse approximately 20nm). As a result, these GABA\textsubscript{ergic} interneurons are able to release timely amounts of GABA, which activate GABA\textsubscript{A} receptors to allow influx of Cl\textsuperscript{-} to maintain synaptic homeostasis and a optimal status for plastic events to take place\textsuperscript{76-79}.

As described, GABA activity suggests that it is only capable of modulating inhibition; however, activity dependent LTP also happens at GABA synapses. Hebbian plasticity applies to the development and formation of these neuronal circuits; neurons, including their receptors, that are wired together, will fire together. Increased synaptic efficiency will arise as a result of consistent presynaptic firing upon a postsynaptic counterpart; this is Hebbian plasticity. In contrast to the well-established inhibitory role of GABA in adults, neonatal hippocampal rodent studies have demonstrated excitatory neurotransmitter activity\textsuperscript{80-91}. Activation of GABA\textsubscript{A} receptors in immature neurons results in activation of Na\textsuperscript{+} channels causing positive ion influx\textsuperscript{72}. As a result, this activity is sensed by NMDA receptors leading to their subsequent activation via Mg\textsuperscript{2+} ion block alleviation\textsuperscript{81-92}. As shown \textit{in vitro}, GABA also has the ability to increase intracellular Ca\textsuperscript{2+} levels\textsuperscript{81-91,93}. Thus, this seems to suggest that through development, there is a timely and intricate shift from excitatory to inhibitory actions of GABA, possibly
supporting further the role of GABA as a homeostatic “gatekeeper” in regulating plasticity. GABA may prime plasticity mechanisms in the neonatal period, essentially kick-starting it through this intricate interplay between GABA, NMDA, and AMPA receptors, and after a certain point in time, switch it’s role to regulate the actions of the latter two receptors ensuring deleterious outcomes do not ensue\textsuperscript{72, 81-91, 94}. Further, previous research seems to suggest that around the neonatal period, AMPA receptors are still quite immature and their function is not yet fully established\textsuperscript{72, 81-91, 95}. It is important to note that this transition of GABA is only noticed in the “A” subset of the receptor, the inhibitory role of GABA\textsubscript{A} is established at birth\textsuperscript{72, 81-91, 96}. The role of GABA in plasticity very likely evolves during development and possibly plays a pivotal role in regulating plasticity.

TMS allows \textit{in vivo} investigation of cortical inhibition via GABA circuits within human M1. Paired-pulse TMS seems to reflect intracortical interneuron phenomena. Separating each pulse by a brief interval dictates which types of circuits, inhibitory or excitatory, are recruited. NIBS has allowed research to translate from animals to humans in a near seamless manner to further understand how plasticity works not only in adults, but throughout development. It has been postulated that substitution of single-pulse TMS with paired-pulse TMS under the SICI paradigm will preferentially modulate facilitatory mechanisms through persistent “leaky” GABA\textsubscript{A} release preventing LTP from occurring\textsuperscript{4, 97-101} (Figure 6). Repeated stimulation, according to adult evidence, attenuates LTP-like plastic events keeping MEP amplitudes at baseline measures; pediatric data is non-existent. Our data shows signs of inhibition. Inhibitory GABA\textsubscript{A} interneurons, in this case in the motor cortex, that release GABA, bind to ligand-gated chloride
channels on pyramidal neurons leading to opening. Influx of chloride ions brings membrane potential down closer to a more negative potential preventing depolarization (i.e. recruitment) of neurons to occur. These acts to regulate plastic events maintain a level of homeostasis.

Figure 6. PAS_{SICI}. Pairing of sensory peripheral nerve stimulation with paired-pulse TMS over the contralateral M1 is used to stimulate a motor response in the right APB muscle (Purple corticospinal tract). Subthreshold CS is followed by suprathreshold TS separated by an interstimulus interval of 2ms leading to recruitment of inhibitory interneurons. The afferent sensory stimulus at the median nerve precedes the efferent motor stimulus evoked by TMS at an interstimulus interval (ISI) of 25ms leading to LTP (Red corticospinal Tract). Crossing of the corticospinal tract occurs at the cerviomedullary junction.
Application of this paradigm to children will be a proof of principle that PAS is indeed possible. PAS has been extensive studied in adults. Moreover, it may delineate putative mechanisms behind disease progression or certain sequelae after a brain injury.

Paired-pulse paradigms elucidate corticocortical connections between different central systems within the brain and the intricate interplay between inhibitory and excitatory modulation that may dictate plasticity. Paired-pulse TMS delivers a conditioning stimulus (CS) followed by a test stimulus (TS); both stimuli are separated by an inter-stimulus interval (ISI) varying from 1 to 70ms. Intracortical inhibition paradigms explore regional inhibitory circuits within focal areas of motor cortex. For example, a subthreshold CS combined with a suprathreshold TS at an ISI of 2ms applied to M1 produces a consistent effect termed short-interval intracortical inhibition (SICI). Pediatric studies suggest SICI effects approach adult values by school-age. Possible mechanisms of action for SICI stem from adult neuropharmacology studies, These suggest SICI effects result from recruitment of inhibitory GABAergic interneurons. SICI is a well-established TMS paradigm in adults and children. Suprathreshold TS excites pyramidal neurons via excitatory interneurons, whereas the preceding subthreshold CS recruits inhibitory interneurons. Therefore, by combining these two stimuli, the TS elicits an MEP in order to assess inhibitory networks within that given area of cortex. A series of seminal experiments refined the ISI between the CS and TS in the SICI exemplar; a 2ms ISI produces the most significant inhibition. This is thought to be due to a release in GABA, which subsequently binds to GABA_A, upon arrival of a CS at 90% active motor threshold (AMT). SICI may modulate plasticity mechanisms and paradigms such as PAS have not been studied in children.
1.4.3 Genetics

Additional major determinants of endogenous human neuroplasticity are neuromodulators and genetics, for example, brain-derived neurotrophic factor (BDNF). Genetic, brain imaging and hematological studies have supported the notion that brain-derived neurotrophic factor (BDNF) is a fundamental factor in regulating synaptic plasticity. Specifically, two alleles (Val66Val), dictate a person’s plastic potential. A single base pair substitution in the pro-domain of the BDNF gene, leads to a Val66Met polymorphism causing significant decrease in an individual’s plastic potential, resulting in a decreased ability to adapt to change in environmental surroundings, synaptogenesis, synaptic fortification, and possible reorganization following injury.

Well-designed motor training research combined with single-pulse TMS to map M1 excitability pre- and post-training in patients with and without the BDNF polymorphism showed that subjects with the Met allele had decreases in M1 map area and lower mean MEP amplitudes compared to Val carriers. Although no differences in tapping, grip and pinch strength, or peg-board time were noticed between Val and Met carriers, those with the polymorphism had decreased responses to TMS stimuli possibly due to a reduction in BDNF expression. Animal studies have showed that transfection of the Val66Met polymorphism in mice reduces BDNF secretion in response to neuronal stimulation resulting in an inability of synapses to conduct LTP.107, 108.
Recent TMS evidence has shown that theta-burst stimulation (TBS), repetitive TMS (rTMS) and Paired associative stimulation (PAS) are all capable of producing lasting changes in cortical excitability in healthy patients supporting the notion of maintained plasticity\textsuperscript{109, 110}. Further, recent studies indicate that a single session of excitatory intermittent TBS (iTBS) in healthy volunteers with a BDNF polymorphism produces LTP-like effects\textsuperscript{109, 111}. Moreover, continuous TBS (cTBS) in this same cohort produces LTD effects, which mimic the same observations seen in healthy subjects without the polymorphism\textsuperscript{109, 111}. This suggests that the BDNF homeostatic effect on plasticity has the ability to be overridden and can be primed using specific non-invasive brain stimulation (NIBS) techniques. Even though previous studies suggest a genetic link to possible modulation of plastic-like events in the central nervous system (CNS), recent evidence suggests that BDNF polymorphism carriers may be capable of undergoing LTP\textsuperscript{109}. These data does not confirm that BDNF does not have a role in modulating plasticity, but it is enough evidence to cast reasonable doubt in the amount of emphasis placed on whether BDNF is a major player in motor cortex plasticity\textsuperscript{109, 112, 113, 3}. Overall, BDNF may modulate plasticity and provide insight upon the mechanism of plasticity in children\textsuperscript{114}.

\textbf{1.5 Perinatal Stroke}

Non-invasive brain stimulation carries therapeutic potential, producing lasting changes in brain activity. Such interventions are increasingly supported by evidence across a variety of adult neurological disorders, most notably stroke and depression. Again, applications in children have been few. However, previous work in children with stroke has demonstrated similar potential for TMS to both measure and modulate neuroplasticity\textsuperscript{58, 59}. Perinatal stroke is defined as a
venous or arterial occlusion leading to focal, unilateral brain injury usually presenting between 20 weeks gestation through to the 28th postnatal day\textsuperscript{115}. The relative risk for a term newborn is 1:2500, but current provincial data suggests the risk within Alberta may be as high as 1:1500\textsuperscript{115}. The three main ischemic perinatal stroke syndromes are neonatal arterial ischemic stroke (NAIS), arterial presumed perinatal ischemic stroke (APPIS) and periventricular infarction (PVI)\textsuperscript{116, 117}; an additional venous syndrome includes cerebral sinovenous thrombosis (CSVT) (Figure 7).

![Image](image.png)

**Figure 7.** Perinatal Stroke. NAIS (Diffusion and corresponding ADC map) and APPIS (FLAIR MR sequence) are arterial ischemic strokes acquired near term resulting in cortical and subcortical damage. PVI (T2 MR sequence) lesions are acquired \textit{in utero} prior to 34 weeks gestation damaging subcortical white matter in isolated areas. CSVT also results in motor dysfunction. In all cases, corticospinal tract damage occurs potentially compromising contralateral motor projections to the spinal cord and subsequent innervation to muscles, specifically in the upper extremity (i.e. HCP).
NAIS and APPIS are essentially the same condition, but differ in time of presentation. NAIS is diagnosed shortly after birth upon clinical presentation of a seizure prompting magnetic resonance imaging (MRI). Subsequent imaging, usually acute diffusion weighted imaging (DWI), will accurately determine the location of the arterial infarct. On the other hand, APPIS is diagnosed at least 4-6 months after birth; newborns present as normal and develop seizure or hemiparesis (weakness) later in life prompting further investigation (i.e. MRI) to confirm arterial infarct.

Pathophysiology of arterial ischemic strokes (AIS) in children is thought to be due mainly to thromboembolism within a cerebral artery leading to focal ischemia\textsuperscript{118}. A number of potential risk factors lead to focal thrombosis within cerebral arteries including vasculitis due to immunological or bacterial etiologies including varicella infection, hematological compromise due to a prothrombotic state, narrowing of blood vessels (stenosis), or a combination of the aforementioned factors\textsuperscript{116-120}.

PVIs are periventricular white matter medullary venous infarctions that are localized around the corticospinal tract\textsuperscript{121}. These sub-cortical lesions usually occur \textit{in utero} between 24-34 weeks gestation as a result of a germinal matrix hemorrhage. Neurodevelopmental deficits usually present in approximately 75% of perinatal stroke survivors\textsuperscript{122-130}. Severity of deficits depends on a variety of factors: duration of ischemia and timing of perfusion restoration, availability of collateral blood supply, volume and function of affected brain structures, maturational status of the brain, as well as concurrent disease processes and metabolic demands of ischemic brain
tissue\textsuperscript{118}. As a result of the ischemia, hypoxia ensues. Depletion of ATP results in a shift from oxidative metabolism to glycolysis. This causes glucose to metabolize to lactate leading to acidosis, thus making the ischemic injury worse.

One of the major neurological sequelae children, who have had a stroke in the perinatal period, is hemiplegic cerebral palsy (HCP)\textsuperscript{131-133}. Defined, HCP is often generalized as a permanent unilateral motor weakness characterized by a decrease in muscle tone. Motor deficits in stroke-induced HCP are prominent in 30-60\% of NAIS\textsuperscript{127,134,135} cases and in greater than 80\% of APPIS and PVI cases\textsuperscript{117,124,127,136}. Unfortunately, research in this research in this area has been highly limited leaving the mechanisms, treatment and prevention strategies largely unknown. Once safety and feasibility of PAS in healthy children has been established, further studies may be prompted in an injured brain. Such a focal injury early in life represents an ideal model of motor impairment and human developmental plasticity.

Many adult plasticity and stroke treatment paradigms have been modeled off of the synaptic competition model\textsuperscript{7}. Specifically, this model is based on targeting lower motor neuron pools in the spinal cord, which have been shown to ultimately dictate motor function\textsuperscript{7}. The competition aspect of this model is between contralateral and ipsilateral corticospinal tract projections, which act to establish functional synapses with these neuron pools\textsuperscript{7}. Through development, one is born with contralateral and ipsilateral innervations in both hands\textsuperscript{7}. Subsequent contralateral supremacy and ipsilateral pruning of these projections produces normal motor function\textsuperscript{7}. Perinatal injury likely disrupts this normal contralateral formation allowing ipsilateral
projections to persist and establish control of the ipsilesional periphery in severe HCP individuals. TMS over the non-lesioned hemisphere can help to identify these connections and cortical stimulation may modulate these corticomotor projections. PAS carries both diagnostic and treatment potential under the synaptic competition model exemplar. Single-pulse TMS can establish whether or not ipsilateral corticomotor projections are present. PAS paradigms can delineate if a diseased brain is capable of learning and memory. Adapted PAS models such as rapid rate-PAS (rPAS), which boosts the effects of repetitive-TMS (rTMS), potentially carry the therapeutic potential of non-invasive brain stimulation to further heights.

Recent research suggests that PAS under a paired-pulse prototype, such as SICI, has the ability to prevent LTP from occurring through the recruitment of GABA circuits. The relevance of this in healthy children is that it will help elucidate whether or not PAS25 functions correctly in healthy children, explain the mechanistic role of developmental plasticity, and it may have potential to describe why plasticity mechanisms are dampened in children with a brain injury such as perinatal stroke. Children with perinatal stroke often suffer HCP, but other comorbid conditions include developmental delays such as language difficulties. Epilepsy may also develop (~30% of children); furthermore, children who develop epilepsy are often prescribed medications that are GABA agonists in an attempt to slow seizure frequency and progression. PAS-SICI, which will be described in more detail in a subsequent section, may delineate inhibitory mechanisms present in healthy and diseased brains. If these inhibitory circuits are being recruited by seizure medications, PAS-SICI may show that plasticity mechanisms may be depressed due to increases in GABA. Thus, children who have had a stroke, develop epilepsy,
and are being prescribed these particular medications, their brains may not be at their prime
during motor therapy or other types or TMS treatments, subsequently discouraging boosts in
plasticity, which aid in treatment of motor deficits. Accordingly, PAS has potential as a
biomarker of plasticity to assess which stroke children have healthier plasticity mechanisms and
would perform better under motor therapy, other TMS treatment modalities (i.e. rTMS), and
new clinical trials.

1.6 Perinatal Brain Injury Leading to Subsequent Plastic Reorganization: An Integrated Model

Margaret Kennard coined the idea of plastic reorganization and coined it the Kennard
Principle\textsuperscript{138}, it states that children are better able to adapt to changes within the environment
as well as internal changes out of their control when compared to adults. Early experiments in
primates nicely demonstrated that those given cortical lesions at an early age show little
sensory and motor impairment than those who succumb the same injury later in life\textsuperscript{139-141}.
Focal perinatal brain injury such as stroke and traumatic brain injury maximize the potential to
fully understand the mechanisms behind age-dependent plasticity. As mentioned previously,
perinatal stroke encapsulates the Kennard principle in a human model. Common consensus
suggests that the brain has inherent mechanisms in place to repair, reorganize, and restore
function in a continuous fashion after an injury ensues. Elegant work in animals and humans
has led to the development of a plastic reorganization model from which novel therapeutic
interventions to combat sequelae, like HCP, can be developed. Simply put, the model consists
of the lesioned and an unlesioned M1 connected via a bridge representing inter- and intra-
hemispheric connections to modulate both motor cortices, as well as their projections to spinal
motor neuron pools leading to peripheral function of extremities. This integrated model has been used in numerous adult stroke studies and is an emerging area of interest in pediatric and perinatal stroke.

1.7 Optimal Opportunity in Developmental Neuroplasticity

TMS studies conducted both by our group as well as in animals\textsuperscript{142,143} have evidence suggesting that plastic motor organization continues well beyond youth. Emerging trials are even suggesting evidence of plasticity in the aging brain, albeit to a lesser degree\textsuperscript{144}. Before non-invasive brain stimulation can be applied to young infants, who seem to have the highest degree of plasticity, neurophysiology, efficacy and safety parameters must be determined in older children. Thus, school-age children (age 6-18) represent an ideal cohort to which brain stimulation and brain plasticity can be explored.

1.8 Aims and Hypotheses

Aim 1 – Determine PAS effects in children

\textit{Hypothesis:} PAS-induced plasticity effects can be demonstrated in most school-aged children.

Aim 2 – Determine the developmental profile of PAS in children.

\textit{Hypothesis:} PAS-induced plasticity effects are inversely correlated with age.

Aim 3 – Determine the effects of SICI on PAS effects in children.

\textit{Hypothesis:} SICI attenuates PAS effects through decreased corticomotor exciability.
CHAPTER 2 – MAPPING DEVELOPMENTAL NEUROPLASTICITY

**Title:** Understanding Developmental Motor Plasticity in Children using Paired Associative Stimulation

**Abbreviated Title:** Paired Associative Stimulation in Children

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2. Abstract

Brain plasticity may be greater in children, but mechanisms have yet to be determined. Paired associative stimulation (PAS) combines peripheral sensory stimulation with Transcranial Magnetic Stimulation (TMS) over the primary motor cortex (M1). PAS induces rapid, reversible, topographically specific increases in M1 excitability consistent with NMDAR-dependent long-term potentiation. PAS has not been studied in children. Healthy right-handed children (6-18yrs) were recruited. Median nerve stimulation was delivered 25ms prior to suprathreshold left M1 stimulation (0.2Hz, ~7minutes). Primary outcome was change in motor evoked potential (MEP) amplitude from baseline to five time-points post-PAS (0/15/30/45/75 minutes) expressed as area under the curve. Effects were categorized as definitive, possible, or no effect. Reproducibility over time was evaluated. Secondary outcomes included stimulus response curves (SRC), and safety and tolerability evaluations. Of 30 children (20 male, mean age 12yrs), 11 (53%) showed definitive PAS effects (7 possible, 10 no effect). Two children were excluded due to a high rest motor threshold. PAS effect could be seen across all time points among responders (p=0.004). SRC scores were significantly increased following PAS (n=9, p=0.022). No correlation between PAS effect and age was seen. Reproducibility of PAS effect among responders was high (p>0.05). Tolerability scores were favorable with no adverse events. PAS effects are robust and reproducible in children. PAS paradigms appear safe and tolerable in children and effects may be higher in children as compared to adults. PAS may provide insight on endogenous developmental plasticity and therapeutic targets in children with cerebral palsy and motor disorders.
2.1 Introduction

The potential for brain plasticity may be greater in children, but mechanisms are not understood. Non-invasive brain stimulation (NIBS) technologies can both study and potentially treat neurological disorders. Transcranial magnetic stimulation (TMS) has predominated to date with increasingly sophisticated means of assessing motor neurophysiology and mechanisms of neuroplasticity in adult human subjects. Despite established safety and tolerability, applications to understand plasticity neurophysiology in children have been simple and few\(^{145}\).

Paired associative stimulation (PAS) is an advanced TMS method that pairs electrical stimulation of a peripheral nerve with TMS over the contralateral primary motor strip (M1)\(^1\). PAS induces rapidly evolving, long lasting, reversible and topographically specific increases in corticomuscular excitability in adult human M1\(^1-3\). Adult and animal studies have demonstrated that PAS-induced plastic change appears to respect the principles of Hebbian plasticity and long-term potentiation (LTP)\(^{62,63}\). Such TMS methods can elucidate the putative mechanisms underlying neuroplasticity ranging from simple strategies such as blocking PAS with NMDA-receptor antagonists\(^1,146\) to using paired-pulse TMS paradigms to recruit inhibitory networks to modify PAS effects\(^{66,106,109}\). A recent study suggests PAS may decline with age in adults, but the developmental profile in children has never been studied\(^{66}\).

Understanding PAS effects in children may also have direct therapeutic implications. A recent study of healthy adults suggests administering a 45 minute PAS protocol may enhance motor learning with effects lasting at least a week\(^{66}\). If established, clinical applications of such non-
invasive enhancement of motor learning are readily apparent. Cerebral palsy is the leading cause of motor disability in children. Perinatal stroke accounts for most hemiparetic cerebral palsy (HCP) and, as a focal injury early in life, represents an ideal model of human developmental motor plasticity\textsuperscript{147}. Such models are now informing new clinical trials including non-invasive brain stimulation (\url{www.clinicaltrials.govNCT01189058})\textsuperscript{148}. PAS also has the potential to serve as an individualized biomarker of endogenous plasticity; a valuable, but currently unavailable tool to predict treatment responses and select patients within such trials.

In this open label, unblinded, prospective, interventional study, our aim was to assess the prevalence, characteristics, age effects and reproducibility of PAS in healthy school-aged children. We hypothesized that PAS-induced plasticity effects are common, reproducible, and inversely correlated with age.

**2.2 Methods**

*Study Population*

Subjects were recruited through the Calgary Pediatric Stroke Program (CPSP) at the Alberta Children’s Hospital (ACH), Calgary. Three streams of recruitment included: (1) Department of Pediatrics members (n=\textasciitilde210) were invited via email to participate with their healthy children via email, (2) Families already consented within the Alberta Perinatal Stroke Project (APSP) healthy controls program were contacted consistent with previously granted permissions, and (3) advertisements describing the study and providing contact information were posted throughout our institution to recruit from the general public.
Inclusion criteria for eligible healthy children included: (1) age 6-18 years; (2) right-handed by self/parent report; (3) developmental maturity consistent with protocol; (4) informed consent and, where applicable, child assent. Subjects were excluded for any of the following: (1) any neurological, developmental, or psychiatric condition; (2) any chronic medical conditions (defined as requiring ongoing care from a specialist or prescribed medications), (3) regular, previous or anticipated use of any neuroactive medications; (4) any TMS contraindications according to accepted guidelines and pediatric considerations including any implanted metal or electronic devices.

Methods were approved by the Conjoint Health Research Ethics Board at the University of Calgary.

*Single-Pulse TMS – Motor Threshold*

Subjects were seated in a comfortable chair with a soft pillow to rest their hands upon in order to ensure the muscle of interest was relaxed. Single pulse TMS was applied over the left M1 to stimulate the resting abductor pollicis brevis (APB) muscle using a flat figure-of-eight-shaped, 70mm magnetic coil (BiStim2 Magstim Co, UK). Single-pulse TMS was performed by first placing the coil against the child’s scalp tangentially in the region of the hand motor cortex to create a 45° angle with respect to the midline at the vertex. Stimulations were then applied at low levels (starting at 30% stimulator output) and increased slowly (increments of 5%) to facilitate comfortable acclimatization. This was continued until consistent APB MEPs were elicited on the
screen and visual inspection of the subject’s hand. Grid mapping in small increments (~0.5-1 cm) was then performed to determine the optimal coil location for APB stimulation (most consistent and maximal MEP amplitude). This “hotspot” was marked directly on the scalp with a soft-tip pen for future reference.

At the same site, the rest motor threshold (RMT) was then determined according to published standards as the stimulator intensity required to elicit an MEP response of at least 50μV (peak-to-peak amplitude) within the relaxed APB muscle in at least five of 10 consecutive trials. This value was recorded as RMT. A second threshold was then determined that elicited higher peak-to-peak amplitudes of 1mV in the relaxed APB. This was termed 1mV MT and used as the individualized suprathreshold stimulus-intensity for subsequent measurements and PAS testing.

Additional measures included a stimulus response curve (SRC). SRC is a TMS protocol that randomly administers single-pulse stimulations at intensities from 100% RMT to 150% RMT in 10% increments. SRC is a potential additional measure of M1 excitability at one given time point and was evaluated at baseline and immediately after PAS as an additional measure of change. Our SRC protocol consisted of 60 stimulations (10 stimulations per 10% increment). Mean MEP amplitudes per each 10% increment were plotted on a threshold versus average MEP graph. A subject’s SRC score was pre- and post-PAS was calculated as an area under the
curve to show degree of change over stimulus intensity. The change in SRC score indicated the
degree to which cortical excitability had changed pre- and post-PAS.

*Electromyography (EMG) Recordings*

EMG activity was recorded from the right APB muscle, at rest, using two Ag/AgCl surface
electrodes (Covidien Kendall Foam Electrodes – Conductive Adhesive Hydrogel) placed at the
belly of the muscle and at the meta-carpal phalangeal joint, respectively. This target muscle
(and subsequent methods below) were based on, and are consistent with, the most accepted
methods of published PAS adult studies\(^1\)-\(^3\), \(^60\), \(^65\)-\(^67\). One PAS study interrogated the extensor
carpi radialis, flexor carpi radialis, as well as the APB. The APB muscle yielded the most reliable
and robust PAS effects post – intervention using an ISI of 25ms with respect to the other two
muscles. This high-powered, pivotal study guided future adult studies as well as ours regarding
the APB as the muscle of choice.

Motor evoked potentials (MEP) obtained from this muscle were amplified by a factor of 1000,
band pass filtered between 20-2000 Hz, and recorded using a CED 1401 signal analog/digital
converter (Cambridge Electronic Design, Cambridge, UK). EMG data were visualized using Signal
6.0 where MEP from the right hand were amplified and viewed over a 300ms window. TMS
given over the M1 hotspot elicits a peripheral muscle response in the APB resulting in an MEP
being seen within a 50-100ms time window. EMG data was recorded and stored for future
analysis.
Individual MEP recordings were visualized for quality and artifacts. Tracings with evidence of pre-stimulation muscle activity (i.e. muscle activation at time of stimulation) were excluded. A standardized script within Signal was then applied to automatically determine the primary measure of peak-to-peak amplitude of all elicited MEP. Stimulations with no deflection from baseline were recorded as zero and included in the group analysis. For each epoch of M1 TMS (e.g. 40 trials for baseline, 10 trials per SRC interval), all valid MEP amplitudes were averaged to generate the primary variable of interest.

**Peripheral Nerve Stimulation – Sensory Threshold**

Soft, replaceable surface electrodes (CareFusion Disposable Electrodes) were placed on clean, dry, palpable landmarks indicating the location of the right median nerve (lateral to palmaris longus tendon and medial to flexor carpi radialis tendon). The median nerve was chosen for peripheral nerve stimulation since it directly innervates the APB muscle and most previous adult literature used median nerve stimulation\(^1\)-\(^3,\) \(^60,\) \(^64-67,\) \(^149\). Manual peripheral nerve stimulations (Digitimer Constant Current Stimulator Model DS7A) were administered at subthreshold intensities until the subject reported to the investigator when he/she first sensed the stimulation for the first time. Stimulation intensity (mA) was recorded and then multiplied by a factor of three. Consistent with established PAS protocols demonstrating efficacy, safety and favourable tolerability, this 300% sensory threshold was used as the sensory stimulus\(^2,\) \(^60,\) \(^65,\) \(^67,\) \(^149\).
**Intervention – PAS**

The primary intervention was PAS, which combined right median nerve stimulation with TMS over the left M1 (Figure 8).

![Image](image-url)

**Figure 8.** Schematic illustrating the setup of peripheral nerve stimulation of the median nerve along with EMG recordings from the right APB muscle.

Rationale for this study comes from evidence of PAS in healthy and stroke-affected adults suggesting equivalency and possible superiority of this approach over similar paradigms (see table 1 above). Consistent with previous PAS studies in adults, subjects underwent 90 pairs of sensory peripheral median nerve stimulation with TMS at $S_{1mV}$ over the contralateral motor strip at 0.2 Hz at an ISI of 25ms to potentially induce cortical facilitation\(^1\text{-}^3, 60, 64\text{-}^67, 149\). Numerous adult studies have shown that sensory stimulation 25ms subsequent to efferent TMS is within an optimal window to promote a substantial increase in motor plasticity\(^1\text{-}^3, 60\). Pediatric SSEP data suggests an optimal sensory stimulus of approximately 23ms; however recent adult data
suggests robust PAS effects can be elicited a wider sensory stimulus window. Typically, orthodromic sensory stimulation takes approximately 20ms to reach the S1 region of the cortex and additional 5ms for M1 integration\textsuperscript{2, 4, 17}. Height is a minor determinant in human SSEP, particularly in creating variance within PAS plasticity effects. Thus, an ISI of 25ms was considered applicable and most comparable for the evaluation of PAS in our pediatric population.

During the PAS pairing of approximately 7 minutes, subjects were instructed to fixate and pay attention to the hand receiving the stimulation (right). Previous adult PAS studies have shown that effects are maximal with subject attention to the stimulated hand as compared to attending to the contralateral hand or no focused attention\textsuperscript{3}. To ensure attention, subjects were prompted to continue looking and thinking about their right hand by the investigator every 20-30 seconds throughout the entire intervention. Additional distractions, including television, were turned off during the 7 minutes to ensure complete attention was maintained and to maximize PAS potential.

MEPs elicited by 0.2Hz single-pulse TMS were analyzed pre- and post-PAS in order to determine possible effects of PAS on motor cortex excitability. Subsequent post-PAS measurements occurred immediately after PAS; 40 single-pulse TMS measurements at 15/30/45/75 minutes after PAS (Figure 9).
Figure 9. PAS Protocol. Healthy children are recruited and their baseline PAS effects are determined. Each visit consists of pre and post-PAS measurements using 40 single-pulse TMS. PAS consists of 90 pairs of sensory stimulation at 300% sensory threshold and TMS at 0.2Hz with ISI of 25ms.

Previous adult PAS studies suggest significant inter-individual variability in PAS responsiveness\textsuperscript{150, 151}. Despite this, the same studies have not explicitly defined objective criteria for defining “significant” PAS effects within individuals. Therefore, we developed a novel approach whereby the mean MEP amplitude at each time interval was compared to baseline for potential significant increase (Paired t-test). As the hypothesis was unidirectional and predefined, no correction for multiple comparisons was performed. Subjects exhibiting significant increases in post-PAS MEP at ≥2 time points were classified as definite responders (see example in Figure 10, results below). Those exhibiting significant increase at only one time point were classified as possible responders. Those without any statistically significant increases were deemed non-responders. This last category could include subjects with post-PAS curves with many or even all points of greater numerical value than baseline. Therefore, our more strict criteria likely produced a \textit{minimum} estimate of PAS effects, but the increased objective validity of this scoring system was deemed important.
**Handedness**

Healthy children and their parents recruited for phase 1 of the trial reported right-handedness. Previous studies suggest that handedness has a high degree of variability between reporting and empiric evidence\textsuperscript{152}. Robust adult evidence suggests that a simple, ten-task activity session, namely the Edinburgh Handedness Inventory, can accurately identify hand dominance in young to geriatric adults. An adapted handedness inventory employing child friendly tasks was developed with a team of highly trained occupational therapists to effectively determine handedness in a pediatric population. Tasks included asking which hand a child would throw a ball, brush their teeth, build a block tower, hold scissors and cut paper, and touch their nose with. Older children were able to understand each activity with less explanation; however, younger children required more explanation. All children returning for PAS testing in phase 2 underwent handedness measures (n=18). Scores greater than 50 are generally considered indicative of right-handedness in adults\textsuperscript{152}.

**Reproducibility**

Previous adult studies have limited documentation of the consistency or reproducibility of PAS effects over time within subjects. Subjects who show significant PAS effects during phase 1 of the study were invited 2-4 weeks later to participate in phase 2 of our interventional, open-label investigation. Phase 2 consisted of two groups. Participants invited back were randomized 1:1 to either Group A or Group B to investigate reproducibility of PAS effects or endogenous
mechanisms dictating plasticity. Group A underwent a re-test of PAS to determine if PAS effects were reproducible. Group B participants were recruited for an additional study seeking to elucidate endogenous mechanisms within the brain dictating plasticity in children. Reproducibility testing followed the same PAS protocol outlined above.

TMS is safe, tolerable and an overall enjoyable experience among children\textsuperscript{145}. PAS has not been systematically evaluated in children, safety and tolerability measures were also obtained. An adapted safety and tolerability measure\textsuperscript{153} was employed to identify and post-intervention side effects such as headache, parasthesia, pre-syncpe, nausea, and muscle pain. Children were asked to rate the severity of any side effects mild, moderate or severe. Subjects also ranked their PAS experience against 7 other common childhood experience to quantify relative tolerability including watching television, going to a birthday party, getting a shot at the doctor, etc. Safety and tolerability measures were obtained from all participants in phase 1 and all returning participants in phase 2 (n=45).

\textit{Data Collection and Management}

All subjects were assigned a unique study number subsequently used for all data collection and storage. A master code with patient names and study numbers existed in a single, separate, secure location with access only by the primary investigators. Essential demographic information was recorded on standardized data capture forms. Neurophysiology data was collected through the ACH Pediatric TMS Laboratory and securely stored\textsuperscript{58}. 
Statistical Analysis

Descriptive analysis examined the three groups of definite responders, possible responders and non-responders. Mean RMT, 1mV test threshold, sensory threshold and 300% sensory test threshold were analyzed across the three PAS responder groups. Error was reported as standard error of the mean (SEM).

MEP values within subjects were converted to a ratio \( \frac{(Post - PAS \text{ Mean MEP})}{(Baseline \text{ Mean MEP})} \) to correct for inter-individual variance in gross MEP amplitude measurements (mV). To test the primary hypothesis that PAS effects can be demonstrated in school-aged children, we compared standardized baseline MEP values to post-PAS scores across PAS responders as well as between responders and non-responders. Post-hoc testing included t-test comparisons at individual time points and was considered only if the preceding ANOVA analysis violated sphericity.

In order to test for effect of PAS across all time points, we did a repeated-measures analysis of variance (ANOVA) with time as the within-patient factor and intervention (PAS effect) as the between patient factor. Individual PAS data was plotted as a curve with respect to time and converted to a PAS score by calculating the area under the curve. This single variable was designed to capture the net effect of PAS, incorporating both the degree and time of MEP enhancement. A Kaplan-Meir curve was created to descriptively show how many PAS responders there were with respect to the entire healthy control cohort. Each step in the curve
represented how many children had their first response at that given time point for TMS measurement post-PAS.

Paired t-test was used to assess differences across pre-and post stimulus response curves following PAS intervention. Area under the curve was used to quantify effect of SRC. The relationship between age and PAS effect was examined and elucidated using bivariate spearman’s rank correlation coefficient.

Analyses were completed using SPSS 19.0.

Data from preliminary trials in young adults conducted at the ACH and variables seen in adult PAS literature were used to estimate sample size. For the primary aim hypothesizing that PAS will occur in children, we defined a clinically significant effect size of 33% increase in MEP amplitude (1.675±0.242). With paired t-test analysis, significance level alpha was set at 0.05, and power at 90%, estimated a sample size of 22 subjects would be required. Anticipating that 1 in 3 subjects may not manifest a PAS effect, we aimed to recruit 33 subjects. TMS tolerability was a possible issue, thus we anticipated a 20% drop out rate prior to beginning the study.

2.3 Results

Population

Thirty-one families were contacted for potential recruitment. Thirty families agreed to participate (96%). Thirty children were recruited and initiated the study; two children were
subsequently excluded due to high RMTs that precluded measurement of the required 1mV threshold. The final population for this study was 28 children.

Population demographics are summarized in Table 2 and 3. Mean age of participants was 12.1±3.9 years (range 6-18 years) and 67% were male (n=20). Of the 18 participants invited to participate for the phase 2 reproducibility arm, all self reported being right-handed. Of the 9 children who were randomized into group A of phase 2, Pediatric Handedness Assessments had shown 7/9 children were right handed with a mean assessment score of 82.9±15.0 (range 50-90). The remaining two children yielded scores indicating potential ambidexterity; mean scores were 35±5.0 (range 30-40).
**Table 2. PAS Participant Demographics**

<table>
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<th>PAS Number</th>
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*RMT – rest motor threshold, AMT – active motor threshold; participants highlighted in red were excluded due to high RMT*
Table 3. Phase 2 Return Participant Demographics and Neurophysiology

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<th>AMT (%)</th>
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<th>Sensory Threshold (mA)</th>
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*RMT – rest motor threshold, AMT – active motor threshold*
Figure 10. PAS Recruitment. 30 children aged 6-18 were recruited prospectively into the Alberta Perinatal Stroke Project Healthy Control Cohort to participate in our PAS study. Of those 30 children, 2 were excluded due to high rest motor thresholds. Of the remaining 28 children, 18 had significant PAS effects (possible and definite PAS responders) and the remaining 10 and negligible effects. In phase 2, 9 children were randomized to test for PAS reproducibility and remaining 9 children were randomized to a PAS mechanism study test arm.

Of 30 children, 11 (36%) showed definitive PAS effect, 7 possible, 10 were non-responders and 2 were excluded due to high rest motor thresholds.

Excitability: Effects of PAS

A case depicting a typical PAS effect and corresponding SRC curve is shown in Figure 11a and 11b. A steady increase in corticomotor excitability was seen immediately following PAS. Following this, at the 15 minute time interval, a sharp increase in excitability was noted, and sustained over time indicating a boost in corticomotor excitability has occurred and was
maintained. Steady increases in excitability were seen at each stimulus step in the SRC curve between pre- and post-PAS supporting an increase in cortical excitability due to PAS.
Figure 11. PAS Case. A) Case depicting a typical PAS response. Post-PAS intervention, a slow increase in excitability is typically noticed. A subsequent spike at the 15 minute interval ensues and persists up to the 75 minute interval. B) This is corroborated by the SRC curves; the post-SRC curve is significantly higher at all stimulus intervals post-PAS.
Comparisons were made with respect to the non-responder group rather than baseline ratios. Non-responder groups had PAS effects greater than zero (p=0.0001) indicating a genuine PAS effect. One-way t-test analysis confirmed non-responder PAS effect was significantly different from zero. Comparison between non-responder, probable and definite responder group show an increased PAS effect trend, respectively, towards the definite responder group (Figure 12). The non-responder group differed significantly from the probable group (p=0.037) as well as the definite group (p=0.0001). Furthermore, the probable group differed significantly from the definite group (p=0.015).

![PAS Effect vs. Responder Groups](image)

**Figure 12.** Comparison of PAS scores between dichotomized PAS responder groups showed an increased trend of effect towards those participants with multiple within subject MEP differences with respect to baseline. PAS effect in the definite sub-group was sustained over time.

Comparison of PAS scores pre and post-PAS intervention accounts for effect across time. Repeated measures ANOVA analysis was conducted including probable and definite PAS
responders in order to delineate any significant differences between MEP amplitude and time post-PAS intervention. A general trend towards increased excitability followed by a plateau effect was seen cumulatively among all PAS responders (Figure 13); Mauchley’s Test of Sphericity: p=0.002, F(3.27,52.38)=2.94, p=0.037.

![PAS Phase 1 Average MEP vs. Time Trend](image)

*Figure 13.* PAS Effect over Time. Of 28 children, 18 showed significant PAS effects, rmANOVA analyses showed average MEP amplitude, represented as a ratio, differed significantly across all time points cumulatively across all responders (p=0.037).

**Excitability: Motor and Sensory Thresholds**

Rest and 1mV motor thresholds from the left M1 were obtained from 28 participants. Mean RMT was 58.9±14.5% of stimulator output (range 32-92%), and mean 1mV motor threshold was 66±12.9% of stimulator output (range 37-89%). 1mV thresholds from non-responders (68.9±14.3%), probable (64.7±13.0%), to definite (63.9±12.2%) participants, trended towards increased excitability (p=0.109). Although not reaching significance, we thought this was an important result to note (Figure 14a. and 14b.). No significant differences in rest motor
threshold or 1mV test threshold were noticed in those children who returned for PAS reproducibility studies.
Figure 14. PAS Neurophysiology. (A) No significant trends were noted between non-responders and PAS responders in RMT. 1mV test threshold category showed a potential trend where definite PAS responders had lower thresholds compared to non-responders. (B) No significant differences were noted between non-responders and PAS responders in sensory threshold and 300% sensory threshold categories.
Baseline and test sensory thresholds from the right median nerve were obtained from all 30 participants. Mean baseline sensory threshold was 2.3±2.1mA stimulator output (range 1.4-4.0mA) and mean 300% test sensory threshold was 6.9±6.3mA stimulator output (range 4.2-12mA). Select participants had their test sensory thresholds adjusted below 300% values for comfort and above 300% values if no peripheral nerve stimulation MEP was detected. No definite trends between PAS effect groups were noted.

*Excitability: Stimulus Response Curve*

Stimulus response curve (SRC) is based on a percentage of RMT. Of 28 participants, we were able to obtain pre and post-PAS SRC measurements on 9 children. Not all 9 children were PAS responders according to our predefined criteria outlined in section 2 of our methods. Seven of nine children were PAS responders. The two remaining children were non-responders according to our pre-defined criteria. Participant 3004 had a corresponding decrease in post-SRC score indicative of a decrease in cortical excitability; however, participant 3027 had a consistently high post-SRC score. Table 3 shows pre- and post-SRC scores of our 9 participants with SRC measurements.
Table 4. Summary of Pre- and Post-SRC Scores of Participants Randomized to Group A of Phase 2

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*SRC – stimulus response curve

The remaining 21 children had high RMTs, which did not permit completion of the full dose escalation to 150% RMT. Cumulative pre-SRC and post-SRC curves are shown in Figure 15. Paired t-test confirmed a significant increase in pre-PAS SRC score (167.4±27.8) compared to post-PAS SRC score (204.0±27.9) cumulatively across the five stimulus points (p=0.022).
Figure 15. SRC. Of 28 children, SRC was collected in 9 children. Pre and Post-SRC curves significantly differed post-PAS intervention. Area under the curve analysis yielded a significant increase cumulatively in corticomotor excitability post intervention across all stimulus intensities after PAS (p=0.022).

A Kaplan-meir curve was created to show the relationship between first PAS effect and timing post-intervention (Figure 16). Most children either responded immediately after PAS or at the 15 minute time interval; fewer children responded at subsequent time points.
Figure 16. PAS Responder Demographics. Of the 28 children tested, 18 responded to PAS. Kaplan Meier analysis depicts time of first response post intervention to PAS. Majority of children responded either immediately post-PAS or at the 15 minute time interval.

PAS Reproducibility

Of 28 children studied in phase 1, 18 showed PAS effects post intervention. Nine children were subsequently randomized to the test, re-test arm of the phase 2 portion to investigate reproducibility of PAS.

Using the same selection criteria to determine a PAS responder, 7 of 9 children satisfied criteria on repeat testing. In the remaining two children, increases above baseline were seen and PAS increased, but the strict criteria were not met. Comparison of PAS scores between the 9 children randomized to the re-test arm between phase 1 and phase 2 showed no significant differences in PAS effect (Figure 17a. and 17b.). Therefore, reproducibility was supported.
Comparison of PAS effect between the non-responder group and the probable and definite groups of children combined yielded significant differences (p=0.0001). When comparisons between these groups were limited to solely definite responders, significance was maintained (p=0.0001).

Comparison of PAS effect between non-responder group and the probable and definite group of children randomized to the reproducibility arm of phase 2, yielded significant differences (p=0.001). Including probable and definite responders in this analysis, this broadens the SEM. When comparison between these groups were limited to exclusively definite responders, significance was maintained (p=0.0001) (Figure 17a., 17b., and 17c.). It is important to note that the definite responders for re-testing do not make up the entire subchort of the test retest arm. The difference in reproducibility representation in Figure 17a., 17b., and 17c. represent subcohort sample size differences based on PAS responder selection criteria for analysis.
Figure 17. PAS Effect and Reproducibility. A) Comparison of PAS scores across 18 children with respect to non-responders showed a significant increase in corticomotor excitability. Comparison of the nine children from phase 1, who were randomized for re-testing in phase 2, showed no significant increase in excitability compared the responders from phase 1, thus PAS can be deemed reproducible. B) Comparison of PAS scores across definite responders with respect to non-responders also showed a significant increase in corticomotor excitability. C) Comparison of PAS scores across those participants randomized from the PAS responder group from phase 1 to the reproducibility arm of phase 2. Comparison between those two groups using paired t-test yield non-significant differences in PAS scores suggesting reproducibility in PAS methods.

Excitability and Age

Bivariate Spearman’s correlation analyses between PAS score and age did not yield any significant results (Figure 18). Even though no general trend towards PAS effect and age was noted, these results indicate PAS still occurs in most school-aged children.
Figure 18. PAS Effect and Age. No correlation was found between PAS effect and age suggesting that PAS occurs in most school-aged children.

No significant correlation between handedness and age was noted in our healthy child cohort, although a large proportion of our return participants showed right hand dominance at an early age (Figure 19).
Figure 19. Handedness and Age. Although bivariate correlation was not significant between handedness and age, right handed preference was noted at an earlier age in our cohort.

Safety and Tolerability

All PAS procedures, both phase 1 and phase 2, were well tolerated with no serious adverse events reported. One child reported severe neck pain upon initial introduction of TMS and during the entire PAS session; however, neck pain seemed to dissipate during breaks. Three children had reported symptoms of headache, all of which were mild to moderate in severity. None needed prophylactic treatment. Three children also had reported discomfort resulting from peripheral nerve stimulation upon immediate exposure; however this dissipated after a few seconds. Two children reported mild discomfort during the start of the session; this dissipated shortly after and became an overall enjoyable experience. Lastly, two children felt
symptoms of nausea and presyncope, which could be attributed to not having a meal prior to their PAS session and also by starting at their hand constantly for 7 minutes during PAS. Tolerability scores were very favorable among the entire cohort and were comparable to watching television (Figure 20).

![Figure 20. PAS Tolerability. PAS ranked close in popularity to watching television. Overall, PAS was very well tolerated. Most children, if not all, were very willing and eager to return for a second session and to future TMS studies.](image)

2.4 Discussion

We sought to assess and identify the developmental profile of PAS in children and whether or not this effect is reproducible. Overall, increases in corticomotor excitability induced by PAS do occur in children and rates are comparable, if not higher, than adult populations. We demonstrate the feasibility of measuring neurophysiological systems and neuroplasticity in children using PAS. While no definitive correlation was seen between PAS effect and age, our results suggest that PAS plasticity mechanisms are already present in most school-aged
children. This prospective, open label, unblinded, well-powered interventional study is the first dedicated pediatric PAS study and will lay the foundation for future studies to map out developmental plasticity in children with potential hopes of potential therapeutic applications.

We provide new data supporting the ability of a simple non-invasive brain stimulation protocol to generate and measure plastic change in real time in the developing brains of school-aged children. The ability to map developmental plasticity in real time within a relatively short interval has not been previously demonstrated in a pediatric population. Not only have we shown that a boost in plasticity can be induced immediately after PAS in the pediatric brain, but it can be sustained over a substantial period of time. Determining feasible paradigms to measure and map developmental plasticity in children is both scientifically and clinically relevant. Our results provide an original assessment of potential mechanisms to explain differences in plasticity in younger brains with potential therapeutic relevance to children with motor disabilities.

One rationale for studying PAS in children was the possibility of gaining new insight to differences in plasticity mechanisms of the young brain. The perception that younger brains are more plastic is commonly considered, but a statement that should be used with caution\textsuperscript{154}. In the only adult study examining the relationship between PAS and age, an inverse association was suggested\textsuperscript{66}. Our hypothesis that PAS effects would be inversely correlated with age was not supported by our results. One possible explanation is extrapolation of protocol ISI measures from adult studies. Specifically, an adult ISI may not account for pediatric height and
myelination, which may dictate corticomotor excitability. The combination of our large sample size and quantified PAS score that allowed a direct correlation analysis between age and PAS effects suggests a strong relationship does not exist, at least between the ages of 6 and 18 years. However, the rate of PAS responsiveness does appear to be higher than in adults. Though difficult to compare directly due to inexact responder criteria in adult studies (see below), our observed rates of 64% appear higher than many adult studies where rates averaged more like 30-40%\textsuperscript{150}. At a minimum, the consistency of PAS effects across our sample suggests the cortical mechanisms underlying PAS are likely established by school-age. From here, additional brain stimulation paradigms may be developed in order to further understand plasticity properties in the brains of young children.

Our pediatric PAS results also carry potential clinical translation with therapeutic potential in children with motor disorders. Recent studies have shown that combining PAS with upper extremity motor tasks can enhance motor memory and learning in healthy adults and those who have suffered a stroke\textsuperscript{65}. Perinatal stroke is the leading cause of hemiparetic cerebral palsy and affects >10000 Canadian children\textsuperscript{155}. Such motor disability lasts for decades and current treatment options to enhance function are limited. However, new models combining animal and human evidence have identified central therapeutic targets focused on M1\textsuperscript{7}. These are already being translated into novel clinical trials using brain stimulation\textsuperscript{7}, but PAS may also represent an even simpler way to directly target M1 plasticity and potential enhance motor learning. PAS measures could also represent a biomarker of endogenous plasticity, a valuable
tool in patient selection and prediction of response within such clinical trials that does not currently exist.

Another advance in our methods was the use of the SRC as a potential measure of plastic change. This TMS measure is well established as a marker of motor cortex excitability,\textsuperscript{148, 156} but has not been employed in previous adult PAS studies. Even though we were only able to collect SRC data on 9 children (due to high rest motor thresholds in some children), multiple subjects demonstrated significantly increased post-SRC curve slopes even though their inter-individual mean MEPs did not differ significantly in at least one time point post-PAS from baseline measures to be considered a possible or definite responder according to our predefined criteria. Participant 3027 (see table 3 in section 3 of results above) had a consistently high level of cortical excitability as noted by post-SRC score possibly indicative of a slightly elevated level of excitability not noted through single-pulse TMS. Interestingly, this may suggest that SRC is a more sensitive measure of changes in corticomotor excitability following PAS intervention. Larger studies would be required to evaluate this, but our results suggest SRC should be considered as an outcome in PAS experiments.

We documented changes in motor thresholds between our non-responders and PAS responders; notably definite responders had lower 1mV test thresholds when compared to non-responders. Albeit not significant, this trend seems to suggest cortical recruitment in responders is not only easier, but at a mechanistic level, NMDA Mg\textsuperscript{2+} ion blockade inhibition is much easier to alleviate potentially allowing LTP facilitation to occur.
Previous adult PAS studies have been the absence of objective criteria and methods to define “significant” PAS effects and differentiate responders from non-responders. We considered this a substantial gap in the literature that was necessary to address in order to better characterize PAS in children. We therefore developed simple, novel, statistics-driven PAS responder criteria to define definitive, probable and non-responders. That our non-responder group still demonstrated elevations in their post-PAS MEP curves suggest we in fact under-estimated PAS effects across the population. We consider this an advantage as it provides assurance that our responders are demonstrating the most robust PAS effects without contamination by chance results.

Our study provides the first safety and tolerability data for PAS use in children. This can be an issue since a comparator is lacking in the literature; however, a systematic review of current safety guidelines would provide more insight on pediatric PAS, consistent with adult PAS. This foundational study now opens the door for researchers and clinicians to utilize PAS as a tool in children for measuring plasticity in real time and potentially for neurorehabilitation.

Limitations with previous adult studies for quantification of PAS effect were noticed. Looking for a net effect across multiple time points has used various methods such as repeated measures ANOVA. While valuable, this does not create a single quantified measure that simultaneously considers both the extent and duration of effect (in this case, MEP amplitude enhancement). Instead, our area under the curve method (PAS score) appeared to achieve
these characteristics. We suggest that these two simple modifications in methodology to better define and quantify PAS effects should be considered in future studies.

Additional possible limitations include a modest sample size for the phase 2 reproducibility studies although our power calculations, previous adult PAS studies\textsuperscript{1-3, 60, 65-67} and positive results support the validity of our results. The same arguments support the validity of the SICI results in the other randomized subset of PAS children.

Consistent with most pediatric TMS studies\textsuperscript{145}, an additional challenge in younger children is the age-dependent nature of rest motor thresholds in the primary motor cortex. In younger children, higher rest motor thresholds limit the amount of data which can be collected during a given session, particularly if suprathreshold stimulations are needed as in the case of our PAS protocol (e.g. 1mV thresholds, stimulus response curves to 150\% RMT). In the youngest children, very high RMT even terminated the entire session in several cases. Options to address these challenges are limited but might include higher stimulator outputs, employing slight voluntary contraction of the target muscle to use lower (active) motor thresholds, or testing new PAS paradigms that use threshold or even subthreshold stimulation levels.

We limited enrolment to right-handed children to be consistent with most adult TMS neurophysiology studies including PAS experiments\textsuperscript{1-3, 60, 65-67}. Each hemisphere is functionally specialized to conduct certain functions. Paul Broca, a French neurosurgeon, first localized speech and language production to a particular area in the left hemisphere\textsuperscript{157-159}. Handedness,
a broad term used loosely in clinical practice, is mainly used to define the hand used for writing. Broca later discovered that handedness was localized to the contralateral hemisphere. This seemed quite simple for right-handed humans since this is where language was nearly always localized in the left hemisphere. However, elegant research later showed that many left-handed humans localize language differently, some with dominance to the right hemisphere. Modern neuroimaging including functional MRI has better clarified what is likely a continuum of lateralization for many brain functions including both language and motor skills. TMS and other studies have demonstrated that M1 neurophysiology is distinctly different between the “dominant” and contralateral sides. For these many reasons, our study was specifically limited to right-handed children to control for potential variability in neurophysiology. Adapted Edinburgh Handedness Assessments showed two children with potential ambidexterity. These children were young, 6 and 7 years of age, indicating they may be in the process of developing handedness, unlike older children. No significant correlation between handedness and age was noted in our healthy child cohort, although a large proportion of our return participants show right handedness at an early age.

In summary, we show that PAS occurs in most school-aged children. Effects are robust and reproducible. PAS methods are well tolerated and safe in children. Use of statistical criteria to define PAS responsiveness, quantified PAS scores, and the use of stimulus response curves may be advantageous in future PAS studies. PAS has future potential to better understand normal developmental motor plasticity and modulate it for therapeutic benefit in children with motor disability.
CHAPTER 3 – SHORT INTERVAL INTRACORTICAL INHIBITION ATTENUATES PLASTICITY

Title: Short-Interval Intracortical Inhibition Blocks Paired Afferent Stimulation Induced Plasticity in Children

Abbreviated Title: Paired Pulse PAS Blocks Increases in Excitability

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3. Abstract

Plasticity may be enhanced in the developing brain but mechanisms are poorly understood and difficult to study in humans. Paired Afferent Stimulation (PAS) combines peripheral nerve stimulation with transcranial magnetic stimulation (TMS), producing rapid, sustained changes in primary motor cortex (M1) excitability. PAS effects were recently demonstrated to be robust and reproducible in school-aged children. Paired-pulse TMS can induce short interval intracortical inhibition (SICI), likely through GABAergic interneurons. We hypothesized that SICI would attenuate PAS plasticity effects in children. Healthy right-handed children (6-18yrs) were recruited and administered a standard PAS protocol (median nerve stimulation at 300% sensory threshold, interstimulus interval 25ms, and M1 suprathreshold (1mV) single pulse TMS applied in combination for 90 stimulations over 7 minutes). Primary outcome was change in right abductor pollicus brevis motor evoked potential (MEP) amplitude from baseline to five time-points post-PAS (0/15/30/45/75 minutes) expressed as area under the curve. A subset of PAS responders received a second PAS protocol with the addition of a SICI conditioning stimulus during PAS. Change in PAS effect before and after SICI was compared (paired t-test). Of 28 children (20 male, mean age 12yrs), 18 showed definitive PAS, 9 of which were randomized to PAS-SICI. These 9 showed a robust original PAS effect (mean PAS score 153.9±53.2) that was significantly reduced on the PAS-SICI protocol (mean PAS score 80.1±15.9, p=0.003). Post PAS-SICI scores were lower than baseline (mean PAS score 80.1±15.9, p=0.035). Tolerability scores were favorable with no adverse events and were comparable across PAS interventions. SICI attenuates PAS-induced increases in corticomotor excitability in children. PAS-SICI paradigms appear safe and tolerable. PAS and its modulation by other TMS methods may provide insight
on endogenous developmental plasticity mechanisms and therapeutic targets in children with cerebral palsy and other motor disorders.

3.1 Introduction

Plasticity may be more robust in the developing brain, but human mechanisms are poorly understood and difficult to study in vivo. Non-invasive brain stimulation (NIBS) technologies can both study and potentially treat neurological disorders. Transcranial magnetic stimulation (TMS) has predominated to date with increasingly sophisticated means of assessing motor neurophysiology and mechanisms of neuroplasticity in adults. Despite established safety and tolerability, applications to understand the putative mechanisms underlying plasticity in children have been limited.

Paired associative stimulation (PAS) is a TMS paradigm that couples peripheral nerve stimulation with TMS over the contralateral primary motor cortex (M1). PAS induces rapidly evolving, long lasting, reversible and topographically specific increases in corticomuscular excitability in adult human M1. Adult and animal studies have suggested that PAS-induced plasticity respects the principles of Hebbian plasticity and long-term potentiation (LTP). Such TMS methods can elucidate putative mechanisms underlying neuroplasticity including strategies such as blocking PAS with NMDA-receptor antagonists. A recent study suggests PAS may decline with age in adults. We recently demonstrated that PAS effects are robust and reproducible in school-aged children.
Additional TMS methods may facilitate such plasticity studies. Paired-pulse TMS paradigms can evaluate regional, and functional cortical circuitry including inhibitory as well as excitatory modulators of plasticity\textsuperscript{162}. Short-interval intracortical inhibition (SICI) is a well-established TMS paradigm that pairs a subthreshold conditioning (CS) and suprathreshold test stimuli (TS) at short interstimulus intervals (e.g. 2ms)\textsuperscript{162, 163}. Neuropharmacological studies in adults suggest SICI effects are mediated by GABA\textsubscript{ergic} interneurons\textsuperscript{146}. SICI effects are well established to occur in both healthy\textsuperscript{35} and brain-injured\textsuperscript{59} school-aged children. Such paired-pulse TMS paradigms may recruit inhibitory networks to modify PAS effects\textsuperscript{66, 106, 109}. One adult study adding SICI to a PAS protocol demonstrated an attenuation of PAS effects\textsuperscript{106}. The effects of SICI on PAS plasticity in children are unknown.

Understanding PAS and its modulation is clinically relevant, particularly to disorders of motor function. A recent study of healthy adults suggests a 45 minute PAS protocol may enhance motor learning with effects lasting at least a week\textsuperscript{66}. Cerebral palsy is the leading cause of motor disability in children. Perinatal stroke accounts for most hemiparetic cerebral palsy and is an ideal model of developmental plasticity where brain stimulation trials are now underway\textsuperscript{147}. Many perinatal stroke children have symptomatic epilepsy treated with medications that often include GABA agonists, underscoring the need to understand plasticity mechanisms and how they are modulated by neurotransmitter systems.

In this open label, unblinded, prospective, interventional study, we hypothesized that SICI would attenuate PAS-induced plasticity in children.
3.2 Methods

Study Population

Subjects were recruited through the Calgary Pediatric Stroke Program (CPSP) at the Alberta Children’s Hospital (ACH), Calgary. Three streams of recruitment included: (1) Department of Pediatrics members (n=210) were invited via email to participate with their healthy children via email, (2) Families already consented within the Alberta Perinatal Stroke Project (APSP) healthy controls program were contacted consistent with previously granted permissions, and (3) advertisements describing the study and providing contact information were posted throughout our institution to recruit from the general public.

Inclusion criteria for eligible healthy children included: (1) age 6-18 years; (2) right-handed by self/parent report; (3) developmental maturity consistent with protocol; (4) informed consent and, where applicable, child assent. Subjects were excluded for any of the following: (1) any neurological, developmental, or psychiatric condition; (2) any chronic medical conditions (defined as requiring ongoing care from a specialist or prescribed medications), (3) regular, previous or anticipated use of any neuroactive medications; (4) any TMS contraindications according to accepted guidelines and pediatric considerations\textsuperscript{145} including any implanted metal or electronic devices.

The study is designed around two phases: In Phase 1, healthy children are recruited and their baseline PAS effects are determined. In Phase 2, children return weeks later and are
randomized to either a re-test procedure or a PAS-SICI procedure to prevent LTP from occurring. The re-test consists of the same PAS protocol. PAS responders were invited to participate in phase 2, 18 children were eligible and contacted to return.

All methods were approved by the Conjoint Health Research Ethics Board at the University of Calgary.

PAS methods were adapted as previously described (Damji et al., unpublished, see chapter 2).

Single-Pulse TMS – Motor Threshold
Subjects were seated in a comfortable chair with a soft pillow to rest their hands upon in order to ensure the muscle of interest was relaxed. Single-pulse TMS was applied over the left M1 to stimulate the resting abductor pollicis brevis (APB) muscle until the optimal coil location for APB stimulation was located (most consistent and maximal motor evoked potential amplitude). This “hotspot” was marked directly on the scalp with a soft-tip pen for future reference.

At the same site, the rest motor threshold (RMT) was then determined according to published standards as the stimulator intensity required to elicit an motor evoked potential (MEP) response of at least 50μV (peak-to-peak amplitude) within the relaxed APB muscle in at least five of 10 consecutive trials. This value was recorded as RMT. A second threshold was determined that elicited larger peak-to-peak amplitudes of 1mV in the relaxed APB. This was
termed 1mV MT and used as the individualized suprathreshold stimulus-intensity for subsequent measurements and PAS testing.

Additional measures included a stimulus response curve (SRC). SRC is a TMS protocol that randomly administers single-pulse stimulations at intensities from 100% RMT to 150% RMT in 10% increments. Our SRC protocol consisted of 60 stimulations (10 stimulations per 10% increment). Mean MEP amplitudes per each 10% increment were plotted on a threshold versus average MEP graph. A subject’s SRC score was pre- and post-PAS was calculated as an area under the curve to show degree of change over stimulus intensity. The change in SRC score indicated the degree to which cortical excitability had changed pre- and post-PAS.

*Electromyography (EMG) Recordings*

EMG muscle activity was recorded from the right APB muscle, at rest, using duel Ag/AgCl surface electrodes (Covidien Kendall Foam Electrodes – Conductive Adhesive Hydrogel) placed at the belly of the muscle and insertion point, respectively. Upon TMS stimulation, MEP were recorded from the APB and amplified by a factor of 1000, band pass filtered between 20-2000 Hz, and recorded using a CED 1401 signal analog/digital convertor (Cambridge Electronic Design, Cambridge, UK). MEP data was analyzed peak-to-peak pre and post-intervention to determine effect.
Peripheral Nerve Stimulation – Sensory Threshold

Surface electrodes CareFusion Disposable Electrodes) were placed on clean, dry, palpable landmarks indicating the location of the median nerve. Manual median nerve stimulations were delivered at subthreshold intensities until the subject reported cutaneous sensation to the investigator for the first time. Stimulation intensity (mA) was recorded and multiplied by a factor of three. As per established PAS protocols demonstrating efficacy, safety and favorable tolerability, this 300% sensory threshold was used as the sensory stimulus$^{2,60,65,67,149}$.

Intervention – PAS-SICI

The primary intervention for phase 2 of our open-label, interventional study remains similar to the protocol outlined in chapter two; however, the TMS portion of the intervention was adjusted to a paired-pulse paradigm in order to study endogenous pathways within the pediatric brain. Rationale included adult PAS-SICI methods in healthy adults suggesting equivalency of this approach in children in its application$^{106}$. SICI has been applied to children on many accounts and results have been reproducible.

During PAS, pairing of SICI and peripheral median nerve stimulation remained separated by an ISI of 25ms in accordance to pediatric SSEP data. Rather than single-pulse TMS paired with peripheral nerve stimulation, SICI was substituted in order to attenuate increases in cortical excitability. Paired-pulse SICI consists of a CS at 90% AMT followed by a TS at 120% RMT. Pulses are separated by an ISI of 2ms; the optimum time window to recruit inhibitory circuits within
the brain. Previous adult TMS studies have shown 2ms is the ideal ISI to recruit such circuits although paired pulse methods have a range of ISIs from 1-70ms\(^{102}\). Further pre measures were required including AMT. AMT is defined as the stimulator intensity required to produce MEP \(\geq 200\mu V\) in 5/10 trials during 20% voluntary muscle contraction of the APB (Figure 21). Maximum voluntary contraction (MVC) was determined using a GwINSTEK GDS-1022 oscilloscope and subsequent 20% MVC was used to determine AMT.

![Phase 2](image)

**Figure 21.** PAS-SICI. PAS Responders from Phase 1 are invited to participate in Phase 2 of the study. Children are randomized to receive PAS-SICI. PAS-SICI consists of 90 pairs of sensory stimulation at 300% sensory threshold and paired-pulse TMS consisting of a subthreshold conditioning stimulus at 90% AMT followed by a suprathreshold conditioning stimulus at 120% RMT separated by a 2ms ISI at 0.2Hz. The peripheral nerve stimulation and TMS are separated by an ISI of 25ms.

Previous research, similar to PAS, does not explicitly state how to classify a given participant as a responder to paired-pulse interventions. Despite this, the same studies have not explicitly defined objective criteria for defining “significant” paired-pulse PAS effects within individuals. Therefore, we developed a novel approach whereby the mean MEP amplitude at each time interval was compared to baseline for potential effects (Paired t-test). As the hypothesis was unidirectional and predefined, no correction for multiple comparisons was performed. Subjects
exhibiting non-significant increases in post-PAS\textsubscript{SICI} MEP at ≥2 time points were classified as definite responders (see example in Figure 22, results below). Those exhibiting non-significant increase at only one time point were classified as possible responders. Those without any statistically significant increases were deemed non-responders. This last category could include subjects with post-PAS curves with many or even all points of greater numerical value than baseline reaching statistical significance. Therefore, our more strict criteria likely produced a \textit{minimum} estimate of PAS\textsubscript{SICI} effects, but the increased objective validity of this scoring system was deemed important.

PAS responders from phase 1 of this interventional trial were randomized 1:1 for test re-test reproducibility (n=9) as well as PAS-SICI (n=9). Safety and tolerability as well as handedness measures were conducted post-intervention. Neurophysiology measures, keeping with phase 1, were collected at five time points (0/15/30/45/75 minutes) post PAS-SICI.

\textit{Data Collection and Management}

All subjects had a unique study number assigned during phase 1 of the study, which carried over during phase 2. A master code with patient names and study numbers existed in a single, separate, secure location with access only by the primary investigators. Essential demographic information was recorded on standardized data capture forms. Neurophysiology data was collected through the ACH Pediatric TMS Laboratory and securely stored\textsuperscript{58}. 
**Statistical Analysis**

Descriptive analysis studied three groups – definite responders, possible responders, and non-responders. Mean age, and pediatric Edinburgh handedness inventory scores were analyzed amongst 9 participants in the PAS-SICI arm of phase 2. Error was reported as standard error of the mean (SEM).

Comparisons were made with respect to the non-responder group rather than baseline values. Raw MEP values within subjects were converted to ratios to limit high variance (refer to formula in chapter 2 methods); therefore, baseline values for across each subject remained the same and lacked any variance. Thus, comparing probable and definite responder PAS affect groups against non-responder groups ensured variance was taken into consideration.

To test the primary hypothesis that SICI blocks PAS-induced plasticity, paired t-tests were used to assess differences across pre- and post PAS-SICI scores as well as differences between PAS response and PAS-SICI responses. Area under the curve was used to quantify the effect of PAS-SICI.

Analyses were completed using SPSS 19.0.

**Handedness**

Healthy children and their parents recruited for phase 1 of the trial reported right-handedness. Pediatric Edinburgh Handedness Inventory (PEHI) outlined in chapter 2 was used to assess
handedness in all children returning for PAS testing for phase 2 (n=18). Scores greater than 50 are generally considered indicative of right-handedness in adults\textsuperscript{152}.

\textit{PAS-SICI}

Previous adult studies have extensively studied inhibitory and excitatory circuits within the brain, but the role to which they modulate plasticity is still a young field. An elegant study analyzed the effects of intracortical inhibition in tandem with peripheral nerve stimulation with the hope of achieving attenuation of induced plasticity\textsuperscript{106}. In adults, prolonged attenuation of plasticity via postulated GABA\textsubscript{ergic} mechanisms were achieved through PAS-SICI\textsuperscript{106}. In our cohort, subjects who showed significant PAS effects during phase 1 of the study were invited 2-4 weeks later to participate in phase 2 of our interventional, open-label investigation. Phase 2 consisted of two groups. Participants invited back were randomized 1:1 to either Group A or Group B to investigate reproducibility of PAS effects or endogenous mechanisms possibly dictating plasticity. Group A underwent a re-test of PAS to determine if PAS effects were reproducible. Group B participants were recruited for an additional study, which sought to elucidate endogenous mechanisms within the brain dictating plasticity in children. PAS-SICI testing was similar to phase 1 with the exception of paired pulse TMS (i.e. SICI) being substituted for single pulse TMS in our PAS paradigm to achieve attenuation of cortical excitation.
3.3 Results

Population

Thirty-one families were contacted for potential recruitment. Thirty families agreed to participate (96%). Thirty children were recruited and initiated the study; two children were subsequently excluded due to high rest motor thresholds that precluded measurement of the required 1mV threshold. The final population for this study was 28 children.

Table 5. PAS-SICI Participant Demographics and Neurophysiology

<table>
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<th>PAS Number</th>
<th>Age</th>
<th>Randomization (1=re-test, 2=SICI)</th>
<th>Adapted Pediatric Edinburgh Handedness Inventory (PEHI)</th>
<th>RMT (%)</th>
<th>AMT (%)</th>
<th>1mV Threshold (%)</th>
<th>Sensory Threshold (mA)</th>
<th>300% Sensory Threshold (mA)</th>
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*RMT – rest motor threshold, AMT – active motor threshold

Of 18 children showing positive PAS effects in phase 1 of the study, 9 were randomized to the PAS-SICI arm of phase 2. Mean age of these subjects was 12.9±3.9 years (range 6-18 years) and 68% were male. Pediatric Handedness Assessments had shown 8/9 children were right handed with mean a mean assessment score of 77.5±19.1 (range 50-100). The remaining child yielded a score indicating potential ambidexterity; individual score was 30.
Excitability: Effects of PAS

A representative case depicting typical PAS-SICI effects and corresponding SRC curves are shown in Figure 22a and 22b. Participant 3009 had a PAS score of 232.82. This score represents effect size with respect to baseline measure pre-PAS as well as increases in cortical excitability over time. Post-randomization to PAS-SICI, participant 3009 had a significant decline in their PAS score to 84.19. Pre- and post-SRC scores from phase 1 were 68.61 and 88.75, respectively. When compared to phase 2, scores showed stagnated excitability – 143.78 and 143.44, respectively. In the original PAS experiment, a marked and sustained increase in MEP amplitude was observed at each time point (22a). As noted in the SRC curves, pre- and post-SRC curves overlap one another indicating no changes in corticomotor excitability. With addition of SICI to the same protocol, no change is seen with post MEP values remaining stable across all time points. No point was significantly different from baseline (i.e. would be a “non-responder by our PAS definitions”).
Figure 22. PAS-SICI Case. A) Case depicting a typical PAS-SICI response. Post-PAS intervention, negligible increases or decreases in excitability are typically noticed immediately post-intervention and over time. B) This is corroborated by the SRC curves; the post-SRC curve is overlies the pre-SRC curve indicative of brain activity remaining at baseline.
The addition of SICI appeared to attenuate previously observed PAS effects. Based on our predefined “responder” criteria, 8 of 9 children who were PAS responders in phase 1 failed to qualify in phase 2 with the only difference being the addition of SICI. The one subject who still met criteria also showed a decrease in his PAS score (see below). Moreover, non-responder groups had PAS effects greater than zero (p=0.0001) indicating that the PAS effect is authentic and unlikely underestimating the effect size of PAS. One-way t-test analysis confirms non-responder PAS effect is significantly difference from zero. Comparison of PAS-SICI effect between non-responder group and the probable and definite group of children combined yielded significant decrease in excitability (p=0.035). When comparison between these groups is limited to solely definite responders, significance is maintained (p=0.044). Comparison of PAS effect between non-responder group and the probable and definite group of children randomized to the reproducibility arm of phase 2, yielded significant difference (p=0.0001). When comparison between these groups is limited to exclusively definite responders, significance is maintained (p=0.0001). In all cases, decreases in corticomotor excitability were noted between comparator groups. These results are not only novel in the sense they are pediatric, but also different from what has been reported in adult literature\(^{106}\) (Figure 23-26).
Figure 23. PAS Effect and Endogenous Mechanism. Comparison of PAS scores across 18 children with respect to non-responders showed a significant increase in corticomotor excitability. Comparison of the nine children from phase 1, who were randomized for PAS-SICI in phase 2, showed a significant decrease in excitability compared to the responders from phase 1, thus PAS-SICI can attenuate LTP-like mechanisms in the pediatric brain via an endogenous mechanism.
Figure 24. PAS Effect and Endogenous Mechanism in Definite Responders. Comparison of PAS scores across definite responders with respect to non-responders also showed a significant increase in corticomotor excitability. Those definite responders from phase 1 were randomized 1:1 to the PAS-SICI intervention in phase 2 of the study. Not all definite responders made up the PAS-SICI intervention cohort, hence the population represents a smaller sample size. Comparison of PAS scores of definite responders within the PAS-SICI arm with respect to their response in phase 1 showed a significant decrease in corticomotor excitability, thus PAS-SICI can attenuate LTP-like mechanisms in the pediatric brain via an endogenous mechanism.
Figure 25. PAS Effect of PAS-SICI Responders from Phase 1 versus their PAS-SICI Intervention in Phase 2. Comparison of PAS scores across those participants randomized from the PAS responder group from phase 1 to the PAS-SICI arm of phase 2. Comparison between those two groups using paired t-test yield a significant decrease in corticomotor excitability between those participants randomized from phase 1.
Figure 26. PAS Effect, Reproducibility and Endogenous Mechanism. Comparison of PAS scores across 18 children with respect to non-responders showed a significant increase in
corticomotor excitability. Comparison of the nine children from phase 1 randomized to the reproducibility arm of Phase 2 showed no significant increase or decrease in corticomotor excitability indicative of PAS reproducibility between PAS sessions. Comparison of the nine children from Phase 2 randomized to the PAS-SICI arm of phase 2 showed a significant decrease in corticomotor excitability indicative of attenuation of LTP-like plasticity within the pediatric brain possibly though GABA like mechanisms. B) Comparison of PAS scores across definite responders relative to non-responders also supports the same trends noted in figure 23a. The nine definite participants in this comparison were randomized 1:1 to both the reproducibility and PAS-SICI arms of phase 2.

Excitability: Motor and Sensory Thresholds

Rest, active, and 1mV motor thresholds from the left M1 were obtained in the 9 participants. Mean RMT was 50.7±10.3% of stimulator output (range 37-65%), mean active motor threshold (AMT) was 42.1±8.3% of stimulator output (range 32-54%), and mean 1mV motor threshold was 63.9±11.1% of stimulator output (range 45-80%). Comparison of RMT values from phase 1 and 2 for those participants randomized to PAS-SICI showed low variability. Mean RMT from phase 1 was 58.9±14.5% of stimulator output (range 43-72%). Comparison of 1mV motor threshold values from phase 1 and 2 for those participants randomized to PAS-SICI showed low variability as well. Mean 1mV from phase 1 was 66±12.9% of stimulator output (range 49-87%).

Baseline and test sensory thresholds from the right median nerve were obtained from 9 participants. Mean baseline sensory threshold was 2.0±0.5mA stimulator output (range 1.4-2.9mA) and mean 300% test sensory threshold was 6.1±1.5mA stimulator output (range 4.8-8.7mA). Select participants had their test sensory thresholds adjusted below 300% values for comfort and above 300% values if sensory MEPs were absent. No definite trends between PAS effect groups were noted.
Safety and Tolerability

All PAS procedures, both phase 1 and phase 2, were well tolerated with no serious adverse events reported. Specifically in phase 2, one child reported mild neck pain upon initial introduction of TMS; however, this improved as the session progressed. Three children had reported symptoms of headache, all of which were mild to moderate in severity. None needed prophylactic treatment. Three children also had reported discomfort resulting from peripheral nerve stimulation upon immediate exposure; however this dissipated after a few seconds. Two children reported mild discomfort during the start of the session; this dissipated shortly after and became an overall enjoyable experience. Lastly, two children felt symptoms of nausea and presyncope, which could be attributed to not having a meal prior to their PAS session and also by starting at their hand constantly for 7 minutes during PAS. Tolerability scores were very favorable among the entire cohort, across both phases, and were comparable to watching television (Figure 24).
Overall, PAS was very well tolerated across both phases. Most children, if not all, were very willing and eager for their return and for future TMS studies.

3.4 Discussion

We provide new evidence that introduction of SICI using paired-pulse TMS antagonizes PAS effects in school-aged children. Our results indirectly support previous evidence that activation of cortical inhibitory circuits can modulate the LTP-like effects of NMDA via possible recruitment of GABAergic interneurons. Our results seem to correlate well with a previous study conducted in adults\(^{106}\), however we have found novel results in children showing decreases in cortical in excitability when SICI is paired with peripheral nerve stimulation at a particular inter stimulus interval. This prospective, open-label, interventional study is the first dedicated pediatric short-interval intracortical inhibition (SICI) study in children seeking to elucidate the suppositious mechanisms underlying plasticity in children. Subsequent foundational research
will arise with potential hopes of developing therapeutic applications in children with brain injury.

Our primary objective was to assess the effects of a paired-pulse paradigm previously studied in adults called SICI on developmental motor plasticity in children. Overall, adult studies have shown that paired-pulse TMS when paired with peripheral nerve stimulation cause the brain to remain at a steady state and attenuate increases in cortical excitability\textsuperscript{1-3, 60, 65-67, 149}; however, such studies have yet to be done in the more plastic brains of children. Data from our study supports that PAS paired-pulse methods have the ability to decrease excitability in children and potentially block the PAS effect via potential recruitment of inhibitory circuits that are GABA\textsubscript{ergic} to mediate neuroplasticity\textsuperscript{106}.

In adults, application of PAS-SICI prevented increases cortical excitability; that is, MEP amplitudes remain unchanged post-intervention with respect to baseline measures. This effect was reversed when the test stimulus intensity was adjusted according to the conditioning stimulus. As such, the test stimulus was much higher that the 1mV threshold since thresholding was done in the presence of a conditioning stimulus, thus suprathreshold stimulation was much higher during this particular PAS-SICI paradigm. In this present study, we investigated the role of PAS-SICI under a 1mV test stimulus, and we found that PAS-SICI in children not only attenuates plasticity mechanisms, but also causes significant inhibition. To ensure PAS causes increases in plasticity and that PAS-SICI is indeed attenuating an LTP-like phenomenon, we have
robust data supporting that PAS causes significant increases in corticomotor plasticity and is reproducible in a second session.

Following PAS, MEP amplitudes significantly increased in our healthy control responder cohort following the first intervention. Children randomized to PAS-SICI, MEP amplitudes significantly decreased across the entire sub-cohort. Hence, applying a paired-pulse paradigm, namely SICI, inhibitory GABA interneurons may be recruited acting to attenuate cortical excitability within the left M1. However, another putative mechanism maybe a lack of magnesium block alleviation within NMDA due to PAS-SICI possibly resulting in decreased corticomotor excitability. Our findings demonstrate that NIBS techniques can modulate and identify neuroplastic mechanisms via nonpharmacological means in order to modulate cortical plasticity.

The potential uses and applications of PAS and PAS-SICI are significant in identifying abnormal plasticity in children with neurological disorders. A possible therapeutic application is to use PAS as a diagnostic measure to understand plasticity mechanisms in the developing brain. As a mentioned previously, paired-pulse TMS measures help us understand inhibitory and excitatory systems within the brain. SICI helps us to understand possible GABA mechanisms within the brain and how it might actually suppress plasticity.

To relate this back to perinatal stroke, another comorbid condition is epilepsy. Unfortunately, epilepsy, secondary to perinatal stroke, is a common condition. Epilepsy, in general, occurs in
5:1000 live births; however, the occurrence post stroke in neonates is significantly higher\textsuperscript{164}. Etiology is not definitive or descriptive, children present as symptomatic and idiopathic\textsuperscript{164}. Seizures often present during sleep and are debilitating, presenting as status (i.e. life threatening). In addition to abnormal diffusion weighted imaging, electroencephalogram (EEG) shows abnormal discharges that are continuous and bisynchronous (i.e. has two spikes and waves of differing frequencies) during non-REM sleep\textsuperscript{164}. This is termed continuous spike wave sleep (CSWS)\textsuperscript{164}. This may be a clinical sign of abnormal firing potentially causing abnormal connections within the brain forming. This is the basis behind epilepsy and CSWS, which may signify bad prognosis for the brain\textsuperscript{164}. Children, who have seizures as a result of their stroke, often get prescribed GABA agonists, which essentially pump excessive amounts of GABA into their brains to control seizures. Our PAS paradigm may elucidate the role of GABA, if this mechanism is dictating plasticity, in suppressing plasticity mechanisms and may show that certain medications may be doing the same due to GABA release and these children who have had a perinatal stroke may not be primed for other types of therapy. Ultimately, in children that have suffered a stroke during the perinatal period, we want to maximize their plastic potential for motor recovery. However, this may be hindered through administration of a GABA agent for their comorbid condition. Clinicians prescribing such agents may ameliorate this plastic potential and PAS-SICI may shed light upon this by paving a new path to prescribe children non-GABA seizure medications to treat their epilepsy whilst maintaining their plastic potential for optimal motor recovery during occupational therapy as well as other types of non-invasive brain stimulation treatments.
Our study provides further support of safety and tolerability of PAS use in children. This can be an issue since a comparator is lacking in the literature; however, a systematic review of current safety guidelines would provide more insight on pediatric PAS, consistent with adult PAS. This foundational study now opens the door for researchers and clinicians to utilize PAS as a tool in children for measuring plasticity in real time and potentially for neurorehabilitation.

Compared to adult studies, our sample size post-randomization is a limitation worth noting. Previous adult studies recruited 13 participants to note significant changes in cortical excitability post-PAS_{SICI} intervention. Even though we were able to note significant decreases in cortical excitability with 9 participants, this discrepancy in sample size is noteworthy. We suggest for future studies that more participants be recruited to further support the results of this primary pediatric study.

Additional limitations relate to whether our PAS-SICI paradigm was ideal to create attenuation of cortical excitability in the pediatric brain. SICI has been very well characterized in adults where the ISI has been titrated between a very short interval to determine the optimal time point to create ideal, momentary inhibition within the brain^{163}. However, studies are limited as to what the exact interval between peripheral nerve stimulation and SICI should be to create attenuation of PAS-induced plasticity. Further, ancillary studies are needed to determine TMS stimulus strength for optimal attenuation within this paradigm. For this study, our best practice guideline was extrapolated from one previous adult study with robust PAS-SICI data supporting
attenuation of cortical excitability. Further, this group has also played a pivotal role in characterizing the role of SICI in TMS literature.

Lastly, a major bias within this study is operator bias during PAS-SICI. During our protocol, the TMS operator views MEP responses per stimulation to ensure the hotspot is maintained; however this introduces immense bias since the operator can monitor the size of each MEP response to ensure the desired response is achieved. This introduces a chicken or the egg scenario. One side of this is the operator can blind themselves from the computer screen to ensure they are not monitoring responses for a desired effect. On the contrary, the hotspot may be lost in the process and the effect will not be achieved. Even though the hotspot is marked with a felt pen, there is a degree of variability around that spot since one does not mark the hotspot exactly with the felt pen each time from subject to subject. In our study, we did not blind during operation and made a conscious effort to not bias ourselves by monitoring responses for desired effect.

In summary, we demonstrate that a SICI paired-pulse paradigm can antagonize PAS effects in children. This suggests endogenous plasticity mechanisms might be both measured and modulated using non-invasive brain stimulation in school-aged children. Our results indirectly support an interaction between possible GABAergic inhibitory and NMDA, LTP-like excitatory systems in M1. The role of PAS-SICI in pediatric brain injury must also be explored and will open many doors for future clinical trials seeking to elucidate the role of PAS-SICI as a clinical
biomarker to guide clinical practice for neurorehabilitation as well as best practice guidelines in epilepsy.

CHAPTER 4 – DISCUSSION

In this study, we examined the developmental profile of PAS in 28 school-aged children. We successfully completed our primary aim by demonstrating that PAS is feasible in most school-
aged children. We also provide novel data to suggest that there are sub-groups of children that respond substantially better to PAS compared to others and have developed a unique criteria to classify participants into distinct groups to elucidate this distinction. Age does not seem to dictate PAS effect; therefore, PAS effect seems to be a broad phenomenon that can be exhibited in most school-aged children. We were also able to demonstrate PAS is a reproducible and robust effect as well as that paired-pulse PAS, namely PAS-SICI, is able to clarify potential endogenous neuroplastic mechanisms within the pediatric brain.

Results collected from this prospective, open label, unblinded, interventional study, correlate well with adult studies over the past two decades\textsuperscript{1-3, 60, 65-67, 149}. We were able to find significant PAS responses in most school-aged children as young as 6 years to eldest being 18 years of age. Specifically, effects were consistent and robust across all possible and definite PAS responders. Unlike previous adult studies\textsuperscript{68}, we developed a unique classification criterion to determine in which participants were selected as PAS responders for future studies and analyses. Further, PAS effect sizes in our pediatric population seemed to mimic adult responses, if not, larger and more consistent. Our unique criterion for determining PAS responders allowed us to select these participants and randomize them into two treatments arms in phase 2 of our study to determine reproducibility of PAS as well as an underlying mechanism for neuroplasticity. Arm 1 of phase 2 supported our theory that PAS is indeed a reproducible phenomenon. It remains unknown how PAS functions in an injured pediatric brain. More careful exploration of this is needed in the future to understand therapeutic and rehabilitation applications of PAS in early developmental plasticity.
Measurements obtained from group B of phase 2 elucidated a role of inhibitory GABA systems in mediating developmental neuroplasticity in children. Specifically, we were able to show not only an attenuation of corticomotor excitability, potentially preventing increases in excitability, and also significant depression post-intervention. Compared to a previous adult study\textsuperscript{106}, depression of corticomotor excitability in our pediatric cohort is a novel finding suggesting inhibitory systems, namely GABA, may be functioning at higher levels to modulate plasticity mechanisms compared to adults. Combining our new PAS data with the above evidence from a different population provides important insight into understanding the neuroplasticity within the brain and the fundamentals behind governing its development in the pediatric brain.

4.1 PAS Effect

We quantified PAS effect across 28 healthy participants and its effect over time in phase 1 of this interventional study. Adult literature strongly suggests PAS responses are highly variable and effect is seen in approximately one in every three cases\textsuperscript{150,151}. Taking this into account, we thought it appropriate to develop a novel criterion to determine which participant is a responder to PAS in order to tease out a true PAS effect in our pediatric cohort. Overall, 18 participants had significant PAS effects with respect to their baseline measures. Further, these differences were maintained across time in the entire sub group of responders indicating that increases in brain plasticity can be maintained in children. In contrast, an age association was not seen; however, PAS effect is seen across a broad range of ages supporting the notion that PAS can occur in school-aged children. Compared to adult studies\textsuperscript{1-3,60}, our pediatric study
measured more post-intervention neurophysiology as well as a longer time periods post-PAS while maintaining significance of effect. This provides insight into the possibility of the greater plastic potential of the pediatric brain and supports maintenance of this change. Our results provide in vivo evidence to suggest that LTP-like mechanisms exist within the pediatric brain and motor plasticity can be altered in real-time during a short intervention; these changes can also be maintained for at least 75 minutes. The ability to map developmental plasticity in real time within a relatively short interval has not been previously demonstrated in a pediatric population. Determining feasible paradigms to measure and map developmental plasticity in children is both scientifically and clinically relevant. We suggest that PAS may provide an accurate, clinically relevant plasticity parameter shedding light upon many neurological dilemmas. Plasticity has historically been a difficult variable to assess; however, our results point to a utility where it can be assessed with ease. The possibility also points to using PAS to assess plasticity and neurophysiological properties in an injured brain; future studies should be considered and inclusion of children who have suffered a perinatal stroke as well as a wide variety of brain injury syndromes.

4.2 PAS Reproducibility

We were able to delineate PAS reproducibility and understand PAS variability in a pediatric cohort by randomizing our PAS responders from phase 1 into a two-arm test design. Group A consisted of a test, re-test PAS protocol to elucidate reproducibility. Raw data within subjects were plotted as a curve relative to time and area under the curve was calculated to create a PAS score. Reproducibility scores from participants randomized to group A were compared to
their respective scores from phase 1 and insignificance supported strong reproducibility of PAS. Few adult studies have looked at reproducibility of effect, but the data suggests that PAS effects are robust. To address this issue, responders were randomized to test for reproducibility. Irrespective of time of day, and alertness, our results indicate that PAS response is replicable indicating that plasticity mechanisms are inherent, maintained over long periods of time and are not transient.

4.3 Developmental Plasticity Mechanism

A major aim of our study was to identify a putative mechanism dictating LTP-like plasticity in the pediatric brain. Most previous PAS studies in healthy adults as well as adults who have suffered a stroke, mechanisms have been linked to the NMDA receptor where M1 plasticity occurs rapidly. Specifically, previous research has attempted to clarify a potential NMDA mechanism of plasticity using pharmacology. Dextromethorphan, an active ingredient in many cough medicines worldwide, is known to be an NMDA-antagonist. An elegant PAS study nicely showed after administering a calculated dose of dextromethorphan to participants that PAS effects diminished. Moreover, dextromethorphan is speculated to act at the PCP site of the NMDA receptor rendering it inactive, disenabling influx of calcium ions leading to plastic-like events. A major limitation of employing neuropharmacology in children is dosing to create desired effect. Dosing of dextromethorphan leading to NMDA-antagonism, according to literature, is 1mg/kg. Unlike adults, who are able to consume pill medications, children, oftentimes, have difficulties. Alternatively, liquid medications can be offered; however, with this dosing, excessive amounts would have to be consumed and is not feasible. A second critical
limitation is some side effects of excessive consumption of dextromethorphan. Clinically, consumption of excessive dextromethorphan may cause hallucinations and psychotic outbreaks in children. An alternative method was chosen to elucidate a potential mechanism governing neuroplasticity in children.

Paired-pulse TMS has been extensively studied in adults, and recently, in children to clarify the integrity of inhibitory and excitatory systems within the brain\textsuperscript{99}. Recent adult evidence has shown that PAS combined with paired-pulse TMS has the ability to attenuate PAS effects non-invasively and nonpharmacologically in adults possibly providing insight on a potential endogenous mechanism of plasticity in adults\textsuperscript{106}.

Our study is the first to our knowledge that has looked to understand developmental plasticity in children and study a potential endogenous mechanism. The remaining 9 responders in phase 2 were randomized to a PAS-SICI paradigm. Our results differed from previously reported in adult studies; corticomotor excitability was not only attenuated, but significantly decreased compared to our non-responder group. Rat hippocampal data suggests that inhibitory interneurons are sequestered during subthreshold stimulation, resulting in the release of GABA. This neurotransmitter, derived from glutamate, diffuses to bind to GABA\textsubscript{A} receptors located on pyramidal neurons, which also have NMDA receptors, resulting in the influx of chloride ions bringing membrane potential to a negative value resulting in inactivity. In children, PAS-SICI may result in excessive chloride influx causing an overall net negative membrane potential causing a decrease in M1 excitability. Sensitivity in younger populations may be higher to such
NIBS, thus even though plasticity mechanisms are being attenuated, it can be said a different type of plasticity is at work. Homeostatic mechanisms regulating LTP-like plasticity – LTD-like plasticity. We suggest this PAS utility may have clinical significance; it may guide clinical practice in epilepsy, a common sequelae of perinatal stroke. Our PAS paradigm may elucidate the role of GABA in suppressing plasticity mechanisms. Overall, in children that have suffered a stroke perinatally, we ideally want to maximize their plasticity for motor recovery. Yet, this may be hindered through administration of a GABA agent for their comorbid condition. Clinicians prescribing such agents may block this plastic potential and PAS-SICI may shed light upon this and adjust clinical guidelines by paving a new path to prescribe children non-GABA seizure medications to treat their epilepsy whilst maintaining their plastic potential for optimal motor recovery during occupational therapy as well as other types of non-invasive brain stimulation treatments.

4.4 Safety and Tolerability

Overall, PAS was well tolerated across phase 1 and phase 2. Children ranked PAS as enjoyable as watching television. Common complaints were a stiff neck amongst a couple of children and one case of mild tingling; all symptoms resolved immediately after PAS. No other adverse reactions were noted. Many of the participants were keen on returning for future TMS studies when available within the Calgary pediatric Stroke Program (CPSP). Our data are a critical component, not only for PAS literature in pediatrics since it is the first of its kind, but on a broader scope, since it helps to build safety criteria within pediatric TMS.
4.5 Future Directions

Continuing research is providing foundational knowledge of developmental neuroscience, recovery of focal perinatal brain injury and insights on potential treatment and rehabilitation tools. Perinatal brain injury is a focused period of insult in development, but the period after is critical since the pediatric brain is incredibly malleable and responsive to a myriad of treatment options and therapy. The use of PAS in pediatrics has been naïve in the literature to date. Our group has extensively studied PAS in healthy children; however, the next step is to determine how an injured brain reacts to PAS. Comprehensive data from both adult and pediatric populations will lead to new therapeutic targets that will advance treatment opportunities for children with neurological deficits. Using PAS along with intensive motor therapy may lead to increased motor memory, and learning thus improving motor outcome in children with upper extremity deficits due to hemiparetic cerebral palsy as a result of perinatal stroke. Adult studies have shown that PAS along with specific upper extremity motor task can improve motor function up to one week post-intervention\textsuperscript{65}. Rapid rate-PAS (rPAS) has the potential to strengthen changes induced by other types of NIBS such as rTMS\textsuperscript{137}. In adult stroke, inhibitory rTMS over the non-lesioned hemisphere improved motor function in the chronic stage post-stroke\textsuperscript{56, 59, 148}. Potentially, applying rPAS after rTMS post motor therapy daily may fortify the effects of rTMS extending the effects of the treatment. Studies have yet to determine this in adults; however, if positive results are yielded, pediatric studies would be prompted shortly after. Therefore, the potential for PAS in pediatrics is numerous in potential and opportunity. The order to which one must tackle this research must be timely and orderly.
4.6 Conclusions

Our study of PAS as a tool to measure developmental motor plasticity in school-aged children supports the following conclusions: 1) PAS is safe and feasible in school-aged children. 2) Increases in plasticity induced by PAS occur in children; responder rates appear comparable to adult populations, if not higher. 3) There does not appear to be a correlation with age; however this may suggest PAS plasticity mechanisms are established by school-age. 4) PAS-SICI is an appropriate paradigm to evaluable inhibitory mechanisms within the brain and GABA may be responsible for mediating LTP-like developmental neuroplasticity. 5) Increases in plasticity induced by PAS are reproducible. 6) Further study of PAS effect, reproducibility, and mechanisms will further advance the study of developmental plasticity.

Reference List


Ref Type: Generic


Ref Type: Abstract


