The ipsilateral silent period as a measure of transcallosal inhibition: an investigation of individual and methodological factors influencing interhemispheric inhibition between motor cortices

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Contribution to the literature

This thesis presents the research of Travis Davidson in collaboration with his thesis supervisor Dr. François Tremblay. The sum of this work resulted in the following contributions to the literature.

Manuscripts to be submitted and included in this thesis:

Davidson T, Tremblay F: **Neurophysiological changes revealed in young athletes after a concussion in sport**. To be submitted to PloS ONE

Davidson T, Gerguis M, Tremblay F: **Relationship between transcranial magnetic stimulation intensity and ipsilateral inhibition induced in small hand muscles.** To be submitted to Brain Stimulation

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Abstract

The corpus callosum provides a physical and functional connection between the two hemispheres of the brain allowing interactions between homologous cognitive, sensory and motor areas. In humans, the integrity of transcallosal connections between motor cortices can be evaluated quickly and non-invasively using transcranial magnetic stimulation (TMS) via the ipsilateral silent period (iSP). While the technique has been known for 20 years, many issues remain unsolved regarding which methods are best to elicit the iSP as an index of transcallosal inhibition. In addition, there is still limited information regarding the influence of individualized factors such as age on iSP measurements. This thesis investigates how common physiological and methodological factors influence the iSP in order to establish this method as a reliable index to assess the integrity of the transcallosal pathway linking primary motor cortices.

In the first series of experiments, we used a previously described TMS protocol to elicit iSPs [1] to investigate changes in motor transcallosal inhibition in relation to individual factors linked to age, hand preference and history of concussions. A second series of methodological experiments examined the effects of stimulation intensity on the iSP and to determine its inter-session reliability.

Our first series of experiments provided evidence that advancing age and history of concussions in young athletes were each independently associated with alterations in transcallosal inhibition. This was evidenced by changes in the duration of transcallosal inhibition (DTI) and in the latency of transcallosal inhibition (LTI) derived from iSP measurements. These experiments also revealed that the degree of hand preference in young adults was reflected in measures of transcallosal inhibition, so that mixed-handed individuals (i.e., ambidextrous) exhibited evidence of more efficient transcallosal transmission than either strong right or left handed individuals.

The second series of experiments focusing on methodological aspects showed that the iSP duration (though not its onset) was influenced by stimulation intensity, increasing linearly with intensity up to 140% of the resting motor threshold (RMT). Our analysis further revealed that the probability of eliciting detectable iSP also increased with increasing intensity up to 130% RMT before reaching a plateau. A stimulation intensity of 130% of RMT appears to be optimal to elicit iSPs in healthy participants. In a subsequent study, we showed that iSP elicited at this stimulation intensity (i.e., 130% RMT) had good inter-session reliability. In light of these investigations, we recommend for future studies that, in addition to contraction of the homologous muscles of the opposite hand as proposed by Giovannelli et al 2009, that an intensity of 130% RMT should be used to elicit the iSP when assessing transcallosal inhibition between motor cortices.

List of Abbreviations

CC – corpus callosum

CST – corticospinal tract

DTI – duration of transcallosal inhibition

EMG – electromyogram

IHI – interhemispheric inhibition

iSP – ipsilateral silent period

LIHI – long-latency interhemispheric inhibition

LTI – latency of transcallosal inhibition

M1 – primary motor cortex

MEP – motor evoked potential

MT – motor threshold

mTBI – mild traumatic brain injury

RMT – resting motor threshold

SIHI – short-latency interhemispheric inhibition

TCT – transcallosal conduction time

TMS – transcranial magnetic stimulation

Table of contents

Chapter I – Introduction and literature review

General introduction

Motor control is a field of neuroscience concerned with the nature of movement and how it is generated. Multiple brain regions and neuronal pathways are involved in the generation of movement. The primary motor cortex (M1) is traditionally known as a key region for the planning and execution of voluntary movements [1]. The somatotopic arrangement of the M1 allows for selective control of proximal and distal muscles engaged in limb movements. [2, 3]. Research with transcranial magnetic stimulation (TMS) has shown that cortical interneurons involved in regulating excitability of descending motor projections are also involved in regulating excitability of inter-area and interhemispheric connections via the corpus callosum (CC) [4].

The CC has a critical role in ensuring effective transfer of information between the two hemispheres. The integrity of this transcallosal pathway can be quickly and non-invasively evaluated with TMS via the ipsilateral silent period (iSP) [5]. However, there is still a dearth of literature regarding the possible physiological and methodological factors that can influence the iSP.

The following section is a brief review of the current literature with a primary focus on interhemispheric communication of motor commands generated by the M1. I will begin with a short description of the major descending motor pathways and of the transcallosal pathway which is the main interhemispheric pathway. Then, I will examine the role of the different factors that can affect the transcallosal pathway. Finally, I will briefly review the principles of TMS applications including how this technique can be used to evaluate the integrity of the transcallosal pathway under different conditions.

1.1 The primary motor cortex and its descending projections

The human M1, which is located in the precentral gyrus, is the area associated with the generation of motor commands allowing postural adjustments and limb movements. In the 1950s, Wilder Penfield was one of the first to establish that the M1 had a somatotopic map of the different areas of the body, which is often referred to as the Penfield's motor homunculus [6]. From these early observations, it was clear that the precentral gyrus in humans had privileged access to the spinal cord through descending projections reaching the ventral horn cells. In fact, several pathways are now known to contribute to the transmission of central commands leading to activation of spinal motoneurons [7]. The three major descending pathways are the ventromedial brainstem pathway, the dorsolateral brainstem pathway and the corticospinal (corticobulbar) pathways. Among these, the corticospinal pathway is considered to be the most functionally important in primates for its critical role in controlling distal limb muscles. The corticospinal tract originates from several cortical areas, including the M1, the dorsal and ventral premotor cortices, supplemental motor area and cingulate cortex [8]. As these projections descend, about 85% of the corticospinal fibres decussate at the level of the lower medulla (i.e., pyramidal decussation) and project down the contralateral dorsolateral funiculus to form the lateral corticospinal tract (CST); thus explaining why the left hemisphere controls limb movements in the right side of the body and vice-versa for the left side of the body. The remaining 15% of the fibres do not cross and remain ipsilateral and form the ventral CST [9], which is more involved in the control of proximal and axial musculature. In humans, most corticospinal axons originating from M1 establish strong and secure connections with spinal motoneurons in the ventral horn innervating limb muscles [10]. Corticomotoneuronal projections refer to the CST fibres that make direct, monosynaptic excitatory connections with motoneurons in the ventral horn with its strongest projections innervating distal limb muscles such as the intrinsic hand muscles [11-13].

1.2 Overview of callosal pathways and functions

The two cerebral hemispheres connect through five major brain commissures the CC, the anterior commissure, the tectal commissure, the posterior commissure, and the hippocampal commissure [14]. The CC, the largest of the commissures, mediates the connections between the majority of cortical areas between the right and left hemispheres [15]. The CC contains a wide band of nerve fibers of more than 200 million axons that link both homotopic areas (i.e., areas in corresponding locations in the both hemispheres) and heterotopic areas (areas in different locations) [16].

Although no clear anatomical landmarks or boundaries can be identified, the CC can be divided into many sub-regions organized topographically depending on their functionality and morphology, with each section believed to have different roles on cognitive processes [17]. From anterior to posterior, these areas include the genu, rostral body, anterior midbody, posterior midbody, isthmus and splenium [18]. The genu at the anterior portion of the CC has the highest density of thin myelinated axons which interconnect the prefrontal cortices and sensory areas. The thick myelinated axons are found in the midbody region whose projections interconnect cortical areas in the parietal and temporal lobes. The thin area between the midbody and splenium is the isthmus, a region which is crucial in interconnecting primary sensory and motor areas. Fiber density increases once again in the splenium region, which interconnects visual areas of the occipital lobes [15, 19, 20]. The size of myelinated fibers affects callosal transmission, regions with thick myelinated fibers (e.g., midbody) exhibiting faster transfer time than regions containing thin myelinated fibers (e.g., genu) [21]. In sum, the CC is the main commissural pathway linking the two hemispheres and is crucial for the exchange of information between homologous cortical areas participating in cognitive, perceptual and motor abilities [22, 23].

In the motor domain, the posterior portion of the CC has been linked with functions related to bimanual coordination [24] and with the control of motor overflow associated with unilateral actions. The transcallosal projections have been found to aid in the execution of bilateral movements in three possible ways. Firstly, the CC could be involved in the transfer of motor commands through a feed-forward mechanism from one hemisphere to the other through either excitatory or inhibitory projections to allow smooth bimanual actions [25]. Secondly, the CC could be involved in relaying corollary discharge signals between homologous cortical areas, so that each hemisphere is kept informed of what the other is doing in terms of processing and output [26]. Finally, the CC could be critical in ensuring proper integration and unification of sensory signals converging on each hemisphere during bimanual actions. An imaging study found a bilateral force task led to greater levels of activation in frontal and parietal brain regions, most likely arising from interhemispheric inhibition as well as from the greater need for motor coordination and visual processing [27]. Therefore, lesions or impairments in callosal functions could impair bimanual coordination, leading to poor coordination patterns. The latter could take the form of antagonistic activity in the unused hand during a unimanual task. However, intermanual conflict has been observed mainly in young age after lesion of the CC and is normally short-lasting [28].

Actual mirror movements remain a rare occurrence; nevertheless, involuntary electromyographic (EMG) activity accompanying the production of unilateral voluntary movement can be seen in healthy individuals; a phenomenon known as “motor overflow”. Motor overflow has been linked to callosal functions, yet it remains uncertain if the unwanted EMG activity is due to facilitatory transcallosal influences from the active hemisphere to the resting hemisphere [29] or ipsilateral facilitation originating from the active hemisphere [30]. In this

respect, it is worth noting that the transcallosal pathway can exert both inhibitory and facilitatory influences, although mutual inhibition seems the predominant mode of interactions between motor cortices [31-33].

1.3 Transcranial magnetic stimulation

In 1985, Barker et al. [34] developed a method called transcranial magnetic stimulation (TMS) to magnetically stimulate the human cerebrum in a non-invasive and practically painless manner. This technique creates a magnetic field that can penetrate the cranium virtually unimpeded. The changing magnetic fields in the cortex engender the creation of an eddy current that can depolarize cerebral neurons thereby generating a neural response that can be either excitatory or inhibitory [4]. The introduction of this technique significantly advanced the neurophysiologic investigation of the cerebral cortex, especially of the motor cortex, which had been practically confined to animal studies [9].

TMS primarily stimulates corticospinal neurons transynaptically via interneurons prompting the creation of indirect waves (I-waves) which occur approximately 1.5 ms sequentially following a direct wave (D-wave) elicited by anodal transcranial electrical stimulation [35-37]. The 1.5 ms latency difference between the D-wave and the I-wave is consistent with a monosynaptic activation of layer V neurons originating from presynaptic axons. These presynaptic axons are most likely the superficial cortical axons of pyramidal neurons from layer II or III, which are believed to be the most excitable to transcranial stimulation. These neurons then have a monosynaptic excitatory connection with the large pyramidal tract neurons in layer V [38]. Thus, the I-wave might originate from monosynaptic activation of the layer V neurons by direct excitation of axons of layer 2 or 3 neurons [39].

When TMS was first developed it could only be applied in single monophasic pulses with a circular coil [34]. Modern stimulators either produce monophasic or biphasic waveforms. Biphasic waveforms are more powerful than monophasic ones when the stimulation energy is normalized to the square root of the maximal energy stored [40]. Although the circular coil is very useful as a general purpose coil, its site of stimulation is not very well defined. For this reason, traditional circular coils have generally been replaced with the figure-of-eight coil (also termed butterfly or double coil). This shape allows a focused stimulation of superficial cortical regions by inducing a current under the junction region of the eight that is roughly twice as large as that under the two wings [41]. Finally, the new stimulators allow for repetitive stimulation which has led to the development of different protocols; the most popular being paired pulse TMS and theta burst stimulation. All these protocols involve trains of repetitive stimuli to elicit excitatory or inhibitory responses in the stimulated region. There is a multitude of applications of these techniques, however the following will focus on the use of single pulse TMS to obtain reliable neurophysiological measures.

1.3.1 TMS measures of excitability for intrahemispheric processes

When applied as a single pulse over the M1, TMS can be used to assess the excitability of excitatory and inhibitory circuits within a given motor representation reflecting intra-hemispheric processes. In fact, several measures can be derived and the following sections will briefly explain their basis and characteristics.

Motor evoked potential (MEP). The MEP amplitude (peak-to-peak) is the most common measure of corticospinal excitability [42]. The amplitude of MEPs is an important index because it provides a direct measure of the intrinsic and extrinsic excitability of cortical and spinal

motoneurons [43]. The MEP latency, which corresponds to the time interval between the delivery of the TMS pulse and the onset of the MEP is affected notably by conduction velocities in the fast descending corticospinal fibers and by temporal dispersion of descending volleys [44]. It thus provides another index of the integrity and efficiency of the corticospinal projections.

The motor threshold (MT). The motor threshold (MT) is a common measure of cortical excitability and refers to the notion of minimal intensity to excite neural elements reliably. It can be measured using various methods but the most common is to determine the minimum intensity capable of eliciting MEPs of at least 50μV in about 50% of a series of consecutive stimulations [45]. The MT can be measured either at rest (i.e., resting motor threshold, RMT) or with minimal tonic contraction (i.e. active motor threshold, AMT). The RMT is thought to reflect the local density of a central core of excitatory interneurons and corticospinal neurons [46]. While the AMT conveys approximately the same physiologic information, it possesses more variability than RMT partially because of the difficulty in maintaining a constant level of contraction

Recruitment curve (RC). Another measure of corticospinal excitability is the RC (also known as input-output or stimulus-response curve), which is obtained by measuring MEPs at increasing stimulation intensity [47]. The RC reflects the progressive recruitment of less excitable neuronal elements adjacent to the hot spot, which is the optimal stimulation area to elicit MEPs [35, 48]. The slope of the RC provides an index of the strength of corticospinal projections [49].

Contralateral silent period (cSP). When a TMS pulse is delivered during a tonic contraction of the corresponding muscle, the MEP will be facilitated followed by a period of silence where EMG activity is absent or greatly reduced, called the contralateral silent period (cSP). The first initial part of the inhibitory period (~50 ms) is thought to be mediated primarily

by spinal mechanisms whereas the later longer part (50-200 ms) is mediated by cortical mechanisms [50-53]. The period of inhibition in the EMG activity is thought to be mediated primarily by $GABA_B$ receptors at the cortical level [54]. The cSP is similar to the MEP as in they both increase rapidly in response to increasing strength of stimulation until they eventually plateau. Further research quantifying this relationship found that they both fit into a Boltzmann function demonstrating a sigmoidal relationship with stimulation intensity [55, 56]. Although the RC curves of resting MEP and cSP are correlated to one another [57], they differ since they reflect different neurophysiologic mechanisms; specifically the MEP is affected by both changes of membrane and trans-synaptical excitability while the CSP reflects $GABA_B$-mediated inhibitory processes [58].

1.3.2 TMS measures of interhemispheric inhibition

While most TMS studies focus on measures of corticomotor excitability derived from one hemisphere, TMS can also be used to assess interactions between motor cortices through transcallosal connections. One approach involves the use of two coils, one on each hemisphere, in paired stimulation, while the other approach involves applying single pulse stimulation to the hemisphere ipsilateral to the contracting muscles.

1.3.2.1 Paired pulse interhemispheric inhibition (IHI)

The paired pulse interhemispheric inhibition (IHI) technique is a TMS -derived measure of transcallosal inhibition obtained using double coils stimulation paradigms (one on each hemisphere). The IHI is obtained by conditioning a test pulse with a second pulse at interstimulus interval between 6 and 50 ms to test hemispheric interactions (predominantly inhibition) [59, 60]. Similar to increased duration of iSP with stimulation intensity, the amount of IHI increases with increased conditioning intensity [61]. IHI can be further divided into short latency (SIHI) and long-

latency (LIHI) depending on the interstimulus interval used for conditioning (SIHI: 8-10 ms; LIHI: 40-50 ms). These two measures are believed to have different physiological origins and characteristics [62-64].

1.3.2.2 iSP

The iSP is characterized by a short attenuation or interruption of ongoing EMG activity in the muscle ipsilateral to the stimulated hemisphere [65]. It was first described by Wasserman et al. 1992 when high intensity single pulse stimulations (100% of stimulator output) were applied with different levels of activation in the target muscles. Although an iSP could be elicited in every muscle tested, differences did exist between muscles. The iSP was most consistently obtained in distal hand muscles (abductor policis brevis) while proximal muscles (biceps and deltoids) were often accompanied by ipsilateral MEPs [65]. It is important to note that iSP and ipsilateral MEPs are independent processes since the ipsilateral MEPs are conducted by ipsilateral oligosynaptic pathways instead of transcallosal pathways like the iSP [66, 67]. The iSP is of cortical origin as demonstrated by the lack of decrement in H-reflex amplitude, specifically the iSP is thought to reflect hemispheric interactions through transcallosal inhibition mediated by the posterior half of the CC since it is absent or delayed with patients with callosal agenesis or lesions [5, 65, 67]. More recent research has confirmed these findings in subjects who have suffered from callosal infarction. Specifically, Li, Lai and Chen (2013) found that lesions to the posterior half of the CC were associated with reduced transcallosal inhibition measured with iSP and IHI [68]. The ipsilateral inhibition likely a direct effect of excitatory transcallosal fibres from the stimulated hemisphere crossing to the contralateral side activating inhibitory interneurons for a net inhibitory action [37, 59, 69, 70]. The iSP can be divided into multiple parameters of transcallosal inhibition. The time period between the onset and offset of the iSP is known as the duration of transcallosal

inhibition (DTI) and is thought the reflect the amount of transcallosal inhibition [59]. The latency of transcallosal inhibition, reflecting the speed of signal transmission, is the time interval between the TMS stimulus and the iSP onset. Transcallosal conduction time (TCT) calculated as the difference between contralateral MEP latency and LTI is utilized to eliminate the peripheral element present in the LTI in order to better estimate the efficiency of signal transmission through the CC [71]. These measures of transcallosal inhibition are an effective means for the neurophysiological assessment of the functional the status of neural fibres crossing the middle and posterior body of the CC [46, 72].

The background level of electromyographic (EMG) activity is believed to influence the amount of inhibition in an iSP [59]; hence, the iSP is often elicited with a near maximal engagement of the target ipsilateral muscle to obtain optimal iSPs [65]. Additionally, the voluntary activation of the contralateral M1 associated with either real or imagined contraction of the contralateral target muscles significantly enhanced the iSP elicited in the homologous muscles of the opposite hand [73]. Engaging the contralateral M1 during ipsilateral stimulation of the opposite MI is a critical factor to study interhemispheric inhibition through the iSP. Furthermore, a consistent muscle activation of the ipsilateral and contralateral target muscles is essential to obtain a reliable iSP.

1.3.2.3 Differences between paired pulse IHI and iSP

The IHI and iSP are two distinct TMS-derived measures of interhemispheric inhibition mediated by the CC [68]. The nature of these measures is inherently different since the iSP is characterized by an interruption of volitional activity while the IHI reflects the inhibition of synchronized activation of the corticospinal system [59].

However, there is undoubtedly some overlap in the neuronal populations involved for each measure. For instance, mapping studies have found that both the iSP and IHI have similar optimal scalp locations which are the same as the optimal locations to obtain MEPs [59, 74]. Both measures are potentiated by an increasing stimulation intensity up to approximately 75% of stimulator output [75]. The iSP and IHI are also similarly increased during movement execution during ballistic compared to self-paced finger movements [76]. Additionally, Chen et al. 2003 found that iSP duration was strongly correlated with LIHI while it was not correlated with SIHI [63]. Overall, different neuronal populations or different sets of target neurons may be responsible for each of these measures of transcallosal inhibition; therefore, they should be considered complementary rather than equivalent means to evaluate motor callosal integrity.

1.4 iSP – reliability of measures and intensity of stimulation

Neurophysiological measures derived from TMS are known to be inherently variable owing to the multiple factors that can influence the outcomes at the methodological and individual level. Approximately 40–50% of this variability appears to originate from within-subjects variation [77]. While certain measures of corticospinal excitability and inhibition such as MEP latency and cSP possess excellent reliability, multiple trials are required to reliably estimate MEP amplitude and may still lack consistency between testing sessions [78-80]. An initial study conducted by De Gennaro et al. 2003 found that IHI had poor reproducibility between sessions, possibly due to the inherent variability of MEP amplitudes [81]. There is still very limited information with regard to reliability of transcallosal inhibition measures derived from either IHI or iSP measures. Establishing reliability is critical to assure that any changes observed between two separate groups

are genuine and are due to physiological changes rather than errors arising from methodological variability or experimental error [82].

One methodological factor well known to have a drastic effect on response output is the intensity of the stimulation. The duration of the iSP is influenced by the stimulation intensity, much like MEP and cSP [55, 56]. Both Meyer et al. 1995 and Chen et al 2003 found that higher absolute TMS stimulation intensity was associated with progressively longer iSP durations [63, 69]. To accommodate the high appearance threshold of the iSP [63, 65], Meyer et al. 1995 suggested using a higher absolute stimulation intensities of 80% of the maximum stimulator output to obtain iSP since they found that this stimulation intensity was able to consistently elicit an iSP in every healthy participant [69]. However, this intensity may be over-stimulating the patients causing extra discomfort and has not been shown to be reliable between sessions. In sum, there is still limited information with regard to the relationship between iSP and stimulation intensity, especially at lower near-threshold TMS intensities.

1.5 Impact of individuals factors influencing transcallosal function

TMS measures are also known to be influenced by physiological factors pertaining to the individual. In the next section, I will examine more specifically how factors, such as age, handedness and history of concussions can influence measures of transcallosal functions derived from TMS

1.5.1 Aging and transcallosal function

The size and width of the CC has been shown to vary between individuals depending on the developmental trajectory of that individual [83, 84]. Although the number of axons is fixed at birth, developmental morphological changes do occur owing to the myelination process which continues throughout puberty [85]. Maturation from childhood to adolescences is associated with increasing synaptic connectivity and a gradual increase in callosal myelination [86, 87]. Recent evidence has found that morphological growth of the CC peaked in the third decade of life [88]. However, diffusion tensor imaging studies found that the integrity of the transcallosal pathway peaked in the early 20's in the anterior regions and in the early 30's for posterior regions [89-91].

White matter integrity remains fairly stable until the 60's at which point it begins to decline [92]. Advancing age is associated with a reduction in global and regional white matter and gray matter across different cortical regions [93, 94], with frontal white matter being particularly affected compared to more posterior regions [95-97]. These anatomical changes in brain matter are coupled with degradation of multiple neuronal tracts; amongst them, the CC and the longitudinal tract that connects frontal regions to more posterior regions [98]. In general, the age effects on the neuronal tracts are more substantial on anterior than posterior, inferior than superior, and association than projection fiber tracts [97, 99]. However, the CST seems to be resistant to the effects of age [98].

Diffusion tensor imaging has revealed that aging is associated with decreased microstructural integrity in the transcallosal pathway as shown by lower fractional anisotropy and higher mean diffusivity in the CC in the older adults [18, 96]. Consistent with the anterior to posterior gradient of age-related neuronal deterioration, the genu (anterior region of the CC) experiences larger age-related changes compared to the posterior splenium [18, 96, 100]. In

addition, these changes in CC integrity were shown to be related to deficits in interhemispheric communication efficiency [101], which may explain the prolonged interhemispheric transmission times in older adults during sensorimotor tasks [102].

The development of interhemispheric inhibition has an inverted "U" characteristic with very young and older individuals presenting with shorter DTI and longer LTI if present at all [103, 104]. Older age is associated with a decrease in inhibitory influences as revealed by age-related decreases in GABA mediated inhibition involved in both the cSP and iSP [105, 106]. A study on age-related changes in iSP parameters found that these modifications were related to a decrease in transcallosal excitability, rather than a slowing in conduction velocity of transcallosal interneurons [106]. A study evaluating interhemispheric inhibition with LIHI found that transcallosal inhibition was influenced by age and the degree of ipsilateral M1 activation during the execution of a simple gripping task [107].

The age-related disruptions in the structural and functional integrity of the aging brain are marked by the progressive deterioration of cognitive functions [94, 108, 109] and motor function ranging from a simple motor tasks such as repetitive finger tapping [110], to more demanding timed tasks [111], steadiness tasks [112, 113] and dexterity tasks [114]. There is a dearth of evidence relating neurophysiological and behavioural measures despite both these measures being influenced by age.

Given the critical role of the CC with coordinating bimanual movements in young adults [115], it would be predicted that the age-related deficits in the CC could translate to impairments in bimanual control. In fact, research has demonstrated that these age-related alterations in callosal fiber integrity are correlated to bimanual coordination deficits associated with normal aging [116]. A recent study found that a larger amount of transcallosal inhibition, as reflected by larger and

longer iSPs, was related to poorer performance on a dominant hand force variability task in both age groups [108]. However, these observations are in contrast with findings from other studies on aging [117, 118] where higher levels of IHI were associated with better manual performance. Additionally, older adults did not show any deficits in bilateral transfer conditions during attention tasks suggesting that alterations in CC integrity may not always translate into behavioural deficits [102]. The conflicting results between studies could be explained by differences in the proportion of higher functioning individuals in the aged groups, since these seniors have a preserved capacity to modulate both intra- and interhemispheric inhibition networks [119].

In summary, advanced age is associated with a global reduction in white and gray matter as well as reduction in the integrity of multiple neural pathways including the CC [94]. Even though these neurophysiological changes seem to occur in parallel to age-related changes in cognitive and motor function [108], there is still a dearth of information concerning the functional consequences of age-related changes in CC integrity.

1.5.2 Hand preference and transcallosal function

The most apparent functional asymmetry in humans is the lateralized preferential use of one hand over another. In fact, this concept of handedness is clearly apparent with approximately 90% of individuals showing a rightward preference [120, 121]. While hand preference refers to the preferred hand to complete a given task, hand dominance refers to the hand that outperforms the other hand on a particular unimanual task [122].

Given the critical role of the M1 and its corticospinal projections in controlling intricate details of manual dexterity, it is expected that asymmetries in manual ability would be reflected at

the cortical level. Although structural imaging studies have produced rather inconsistent results with regard to asymmetries related to handedness, many studies have indicated a leftward asymmetry in the size of the M1 region in consistent right-handers [123-125]. The left M1 region also displays more profuse intrinsic connectivity in consistent right-handers, which may explain why hand preference is more lateralized in right handed individuals [126, 127]. Left-handers in contrast have been found to have less consistency with more heterogeneous cortical organisation for contralateral as well as ipsilateral activity according to structural and functional imaging studies [128-132].

There is anatomical evidence of asymmetries in interhemispheric connections between cortical areas [133]. For instance, studies have shown that transfer of visuomotor information from the right to the left hemisphere is faster in this direction than from the left to right hemisphere [134]. Evidence of asymmetries in relations to handedness has also been found in the transcallosal motor pathway [135]. At the macro-structural level, right-handed subjects were shown to have a larger overall area in CC coupled with enhanced fractional anisotropy and reduced mean diffusion at the microstructural level, when compared to left-handed subjects [136]. In contrast, recent research found that degree of handedness could be related to the speed and accuracy of interhemispheric interaction with less strongly lateralized individuals having larger CCs [137, 138]. Additionally, a recent study found that better performance in the dominant hand of right handers is related to greater callosal thickness. Bimanual performance had a weaker link with callosal thickness and non-dominant performance had no correlation with callosal thickness [139]. This provides further evidence of the importance of CC integrity for unilateral movements which is in agreement with neuroimaging studies showing that unilateral hand movements are accompanied by an active disengagement of the ipsilateral hemisphere [59, 140, 141]. Thus, the

CC seems a critical structure to examine to assess the neural correlates of handedness genotype and phenotype.

According to several reports, in both left and right handers, the dominant hand/hemisphere exhibits lower motor thresholds [142, 143], greater intra-cortical inhibition [144, 145] and greater mutual inhibitory influence than the non-dominant hand/ hemisphere [146], [147]. These findings, however, could not be confirmed in other similar reports [148-150]. One lingering confounding issue pertaining to the interpretation of laterality effects has been the problem of categorizing individuals into two simple categories, right and left, whereas people tend to show different degrees of laterality. Handedness, described only in terms of right and left dichotomy, does not seem to be associated with major asymmetries at the cortico-motor level. However, recent investigations examining the influence of the degree of handedness on motor cortical processes have shed a new light on the issue of laterality. For instance, a recent study by Bernard, Taylor and Seidler [151] found a direct relationship with the extent of laterality and interhemispheric transfer time which is a functional measure indicative of the integrity of the posterior CC [151, 152]. This laterality effect on CC integrity could partially explain why right-handers demonstrated asymmetries in intrahemispheric and interhemispheric inhibition on a bimanual task with asymmetrical force requirements which was not replicated by the left-handed participants who could have been less lateralized due to living in a world designed for right-handed individuals [153, 154]. Clearly, more studies are needed to determine the effect of preferential hand use on transcallosal inhibition.

1.5.3 Concussion and transcallosal function

Traumatic brain injuries (TBI) have been in the spotlight because of their high incidence in military personnel and contact sports including recent high profile cases such as Sidney Crosby (hockey), Klay Thompson (basketball) and Tim Tebow (football). TBI is a very common neurological disorder with an estimate that between 1.6 and 3.8 million North American athletes experience some form of TBI in the context of a sport-related injuries each year [155]. In Canada, mild TBI (mTBI) rates have been estimated at ~ 653/100,000 based on a diagnosis made by primary care providers or secondary review by an expert [156]. This is believed to still be an underestimation of the actual incidence since it does not include individuals who do not seek hospital-based care [157]. The majority of sports-related head injuries (70-90%) are classified as mTBI, these brain injuries are more commonly known as "concussions" [157].

A concussion (i.e., mTBI) has been traditionally defined as a trauma to the head resulting in post-traumatic amnesia (PTA) of less than 24 hours, Glasgow Coma Scale of 13–15, and no evidence of intracranial abnormality on CT [158]. The most recent consensus statement on concussion following the 4th International Conference on concussion in Sport held in Zurich (Zurich concussion consensus) defined concussion as "a complex pathophysiological process affecting the brain, induced by biomechanical forces" [159]. Concussion is caused by rotational or angular forces being transmitted to the brain either by a direct impact to the head, face, neck or by an impact elsewhere on the body where an impulsive force is transmitted to the head [159-161]

Concussion may result in neuropathological changes, but the acute clinical symptoms largely reflect a functional disturbance rather than a structural injury since no no abnormalities are seen in standard structural neuroimaging [159]. Concussions are associated with various rapid

onset short-lived neurological dysfunctions that often resolve spontaneously resulting from cerebral metabolic dysfunction and axonal shearing [159, 162, 163]. Although loss of consciousness and post-traumatic amnesia may result from a concussion, it is not a prerequisite for the concussion diagnosis [164, 165]. Other common signs and symptoms include behavioural changes (e.g. irritability), cognitive impairments (e.g. slowed reaction time or memory impairments), sleep disturbances (e.g. insomnia), somatic symptoms (e.g. headache or nausea), cognitive symptoms (e.g. feeling foggy) and emotional symptoms (e.g. mood swings) [159].

In some cases, signs and symptoms may evolve over a number of minutes or hours and symptoms may be prolonged leading to post-concussion syndrome (PCS). PCS refers to a set of non-specific symptoms that are reported following a concussion or mTBI and that persist beyond the expected recovery period typically 7-10 days [159, 166]. Despite the Zurich Consensus stating that post-concussion symptoms lasting more than 10 days should be considered persistent [159], there is a considerable lack of consensus regarding the definition of PCS among physicians [167]. The incidence of PCS is therefore difficult to establish with inconsistent diagnostic criteria with reports showing as few as 10% of patients symptomatic seven days after sport-related concussion [168] to as many as 43% still with symptoms three months after being hospitalized for mTBI [169].

Although macrostructural damage to the CC is not associated with concussions, microstructural integrity of the CC can be impaired with brain injury [170]. A recent meta-analysis of diffusion tensor imaging studies found that mTBI is associated with a decrease in the integrity of the CC [171]. Subregional analysis found a posterior to anterior gradient of injury with the splenium being the most affected followed by the midbody and the genu being relatively spared [171]. These posterior areas are more susceptible to torque injury since the posterior body and the splenium are fixated to the overlying dura [172].

TMS studies in concussed patients have found elevated motor thresholds, longer MEP latencies, decreased MEP amplitude and greater asymmetry between the hemispheres for these measures in the acute stage of a TBI [173, 174]. Such abnormalities tend to normalize within two weeks after the injury [173, 174]. Long term effects of concussions include an abnormal prolongation of the cSP related to the number of concussions and severity of the symptoms [175-178]. In related studies, cholinergic function in the motor cortex, assessed by short-latency afferent inhibition (SAI), was found to be significantly affected in subjects who had sustained severe brain injuries and who continued to experience memory impairments [179], but not in asymptomatic formerly concussed athletes [178]. Transcallosal inhibition, evaluated by the iSP, was found to be significantly reduced in a subset of individuals who had recovered from mild to severe brain injuries [180], but this has yet to be evaluated in asymptomatic formerly concussed individuals.

Conclusion

In summary, the CC is the main interhemispheric pathway relaying information between hemispheres with the posterior third region (splenium) being responsible for transferring motor and sensory information. The CC has important implications for bimanual coordination with hand preference influencing both the macrostructural and microstructural integrity of the transcallosal pathway. Additionally, both normal aging and mild traumatic brain injury are associated with physiological and anatomical alterations in callosal functions. TMS is a useful non-invasive tool to quickly evaluate transcallosal inhibition, notably through the iSP. Therefore, the iSP can be used to investigate changes in motor callosal functions with respect to individual factors related to hand preference, aging or history of concussion, for which there is still limited information in the current literature.

Objectives of the present work

The overall objective of the present thesis was to investigate factors influencing hemispheric interactions between motor cortices using the iSP as an index of transcallosal inhibition. Our first series of experiments examined the influence of specific individual factors with regard to the respective effects of age, hand preference and a history of concussion on transcallosal inhibition. It was hypothesized that each of these factors, namely advancing age, strong preference for one hand and antecedents of concussion would be associated with alterations in motor transcallosal inhibition, as reflected the iSP. Our second set of experiments focused on methodological aspects inherent to the iSP as a test to assess transcallosal inhibition between motor cortices. In these experiments, we specifically addressed the role of stimulation intensity in influencing ipsilateral inhibition elicited in small hand muscles and the reliability of iSP measures when assessed between sessions and between raters. From these last series of experiments, we can formulate recommendations as to the optimal stimulation parameters to obtain reliable measures of iSP in small hand muscles from TMS.

Chapter II – Research Papers

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Age and hemispheric differences in transcallosal inhibition between motor cortices: an ispsilateral silent period study

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Abstract

Background: In this study, we investigated age and hemispheric differences in transcallosal inhibition (TCI) in the context of active contraction using the ipsilateral silent period (iSP). We also examined whether age-related changes in TCI would be related to corresponding changes in manual performance with age. Participants consisted of right-handed individuals from two age groups (young adults, n=13; seniors, n=17). The iSP was measured for each hemisphere using suprathreshold TMS pulses delivered over the primary motor cortex ipsilateral to the maximally contracting hand while the homologue muscles of the opposite hand were lightly contracting (~15% of the maximum). Manual performance was assessed bilaterally for both grip strength and fine dexterity.

Results: Our results yielded two main findings. First, TCI measures derived from iSP were strongly influenced by age, whereas differences between hemispheres were only minor. Second, correlation analyses revealed that age-related variations in TCI measures were related to changes in manual performance, so that left-to-right TCI correlated with right hand performance and vice-versa for the opposite hand/hemisphere.

Conclusion: Overall, these results concur with other recent reports indicating that mutual inhibition between motor cortices tends to decline with age. In this respect, our observations are in line with the notion that the balance of normally predominantly inhibitory interactions between motor cortices is shifted toward excitatory processes with age.

Background

Coordination of movements relies on both the activation of each independent hemisphere and the communication between hemispheres which is mediated via the corpus callosum [1]. This transcallosal pathway is essential for the interhemispheric transfer of perceptual, sensory and motor information underlying complex and integrated behaviors [2]. Although transcallosal connections can be facilitatory, mutual inhibition appears to be the primary mode of action between the two primary motor cortices [3]. This mutual inhibition has been shown to be finely modulated depending on task demands, unilateral actions leading to increased inhibitory drive from the active hemisphere, whereas bilateral actions lead to more balanced inhibition between hemispheres allowing for coordinated actions of the two extremities [4,5]. In the primary motor cortex (MI), interhemispheric inhibition can be assessed non-invasively with transcranial magnetic stimulation (TMS) using either paired-pulse paradigms or via the ipsilateral silent period (iSP) [6]. With paired-pulse paradigms, the target hemisphere is first conditioned by applying suprathreshold TMS on the opposite hemisphere at short (8–12 ms) or long (e.g., 40 ms) inter-stimulus intervals leading to two corresponding periods of inhibition of test motor evoked potentials (MEPs), i.e. short-latency (SIHI) and long-latency (LIHI) interhemispheric inhibition. The iSP assesses transcallosal inhibition by applying focal TMS to the motor cortex ispsilateral to the test hand while the target muscle is activated voluntarily, leading to a brief interruption of the ongoing muscle activity [7]. This interruption of voluntary muscle activity is thought to reflect transcallosal inhibition (TCI) mediated by the MI

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opposite to the one being active for maintaining the contraction [7]. As stressed by Chen et al. [8], although SIHI/LIHI and iSP both reflect interactions between motor cortices, they should be considered complementary rather that equivalent measures of TCI since their effects appear to be mediated by different neuronal populations.

While TMS investigations have highlighted the critical role of interhemispheric inhibition in the acquisition and transfer of motor skills [9], there is still limited information as to how this process changes as people advance in age. Indirect evidence of changes in interhemispheric inhibition with age comes from observations of difficulties experienced by older adults in bimanual tasks along with the presence of motor overflow during performance of unimanual actions [reviewed in 10]. This is paralleled by reports from functional neuroimaging studies showing that task-related activation patterns in sensorimotor areas are typically less lateralized and more widespread in older adults than in young adults [11]. Such changes in brain activation patterns are consistent with reports of structural alterations in the corpus callosum with age affecting its integrity in terms of the quantity and quality of white matter [12,13]. As suggested by Seidler and colleagues [10], such observations point to a shift in the balance of mutual inhibition between motor cortices with age towards excitatory processes. In agreement with this view, Talelli et al. [14] reported that the degree of LIHI from the left MI to the right MI during right hand grip was progressively reduced with advancing age, there was even a switch from inhibition to facilitation in the very old participant. Interestingly, the age-related reduction was not observed for SIHI, a differential effect the authors ascribed to putative physiological differences between SIHI and LIHI. In line with this, a recent investigation by Fling and Seidler [15] using the iSP as an index of transcallosally-mediated inhibition associated with voluntary contraction, also failed to detect age differences although young participants tended to show longer iSP durations than old participants. Thus, although observations from behavioral and neuroimaging studies seem compatible with an age-related shift in the balance of mutual inhibition between motor cortices, recent findings from TMS studies remains controversial in this regard.

Another related controversial topic with regard to hemispheric interactions pertains to laterality issues associated with manual asymmetries. Given anatomical and physiological evidence pointing to a leftward asymmetry in the organization of the hand motor representation in strong right-handers [16], one would expect that the balance of inhibition between motor cortices would favour the dominant (left) over the non dominant (right) hemisphere in most individuals. Surprisingly, very few TMS studies have examined this issue specifically. In line with the existence of a leftward asymmetry, early investigations by Netz et al. [17] showed that levels of SIHI from the dominant hemisphere was greater than that elicited from the non-dominant hemisphere in right-handers. More recent investigations, however, showed that such asymmetries in interhemispheric inhibition observed in the resting state are not necessarily present in the active state [18]; highlighting the importance of examining interhemispheric interactions in the context of voluntary contraction.

In the present study, we investigated mutual inhibition between motor cortices using the iSP as an index of transcallosally mediated inhibition in healthy young and senior adults. We first asked whether differences existed between the dominant and non dominant MI in strong right-handed participants and whether these differences were affected by age. Then, we asked whether age-related variations in the strength of TCI from one hemisphere would be related to changes in performance of the contralateral hand with age.

Results

Manual performance and corticomotor excitability

Right-left differences in manual performance and in basic measures of corticomotor excitability are described in Table 1 for the two groups. As expected, both young and senior participants exhibited significantly better performance in terms of dexterity and grip strength with their right dominant hand (Young, $t_{12}>3.75$, $p<0.003$; Senior, $t_{16}>3.52$, $p<0.01$) when compared to the left hand. It is also apparent in Table 1 that young participants clearly outperformed their senior counterparts. In terms of corticomotor excitability, both age groups showed a tendency for higher resting motor threshold (rMT) in the left hemisphere (i.e., right hand in Table 1) than in right hemisphere; a difference that reached significance only in the senior group ($t_{15}=3.01$, $p=0.008$). Apart from this difference, the other paired comparisons revealed no significant right-left differences in either MEP characteristics (amplitude and latency) or in contralateral silent period (cSP) duration in both groups. Finally, as for manual performance, age differences were also clearly evident in corticomotor excitability, all measures pointing to a decrease (e.g., elevated rMTs) in the senior group. These age differences in excitability were accounted for when examining age-related variations in ispsilateral inhibition, as described in the next section.

Age and hemispheric differences in ispsilateral inhibition

Variations in measures of ispsilateral inhibition with respect to age and hemisphere are illustrated in Figure 1. Note that the final analysis of iSP data excluded two senior participants. The first case had incomplete data due

Table 1 Characteristics of the participants with respect to demographics, hand function and basic measures of corticomotor excitability (All values represent Mean ± SD)

	Young (n=13)	Senior (n=17)
Demographics		
Age (years)	22.4 ± 3.0	73.0 ±7.6
Gender (n)	9 M, 4 F	6 M, 11 F
Edinburgh Handedness score (/20)	15.7 ± 3.4	17.7 ± 2.6
Hand Function		
Dexterity	RH: 55.5 ± 4.9**	RH: 88.5 ± 33.6**
(GPT in s)	LH: 67.5 ± 6.9	LH: 100.3 ± 38.9
Pinch	RH: 10.1 ± 2.6**	RH: 7.2 ± 1.6
Strength (kg)	LH: 9.1 ± 2.4	LH: 6.7 ± 1.7
Corticomotor excitability [a]		
rMT	RH: 61.0 ± 11.3	RH:70.1 ± 11.5**
(% output)	LH: 57.6 ± 8.3	LH: 65.9 ± 13.0
MEP amplitude (mV)	RH: 5.6 ± 1.8	RH: 3.6 ± 1.2
	LH: 5.5 ± 1.8	LH: 3.5 ± 1.5
MEP Latency (ms)	RH: 20.2 ± 1.8	RH: 21.8 ± 2.1
	LH: 20.1 ± 1.5	LH: 21.7 ± 2.3
cSP duration (ms)	RH: 141.5 ± 34.8	RH: 115.9 ± 24.2
	LH: 148.9 ± 37.9	LH: 115.3 ± 26.0

[a]All measures were derived from the hand contralateral to the hemisphere stimulated during the cSP/iSP assessment procedure. TMS data from one senior was incomplete (n=16).
Abbreviations: *GPT* Grooved Pegboard Test, *RH* Right Hand, *LH* Left Hand, MEP, *rMT* resting Motor Threshold, Motor Evoked Potential, *cSP* contralateral Silent Period.
**Significant right-left differences at p<0.01 in paired t-test comparisons.

to poor tolerance for TMS at high intensity. The second case was an outlier, as detected with the Grubb's test (Z=3.67, p<0.01), with very long iSPs. Two main observations can be made from inspection of Figure 1. First, only relatively small differences were observed between hemispheres in the two age groups. This is evident in Figure 1A showing examples of iSP recordings obtained from each hemisphere in a typical senior and young participants. Second, age had a major impact on TCI with seniors showing delayed onset latency of transcallosal inhibition (LTI), decreased iSP area and prolonged transcallosal conduction time (TCT). The impact of age can be easily appreciated by looking at the mean variations in LTI, iSP area and TCT measured in each age group, as illustrated in Figure 1 (B, C and D). The ANOVA confirmed that "hand/hemisphere" had little influence on iSP measures, with only a marginal trend noted for iSP area ($F_{1,26}$=3.8, p=0.06) owing to the interhemispheric difference observed in the young group (Figure 1C). The large influence of "Age Group" on TCI measures ($F_{1,26}$>20, p<0.001) was also confirmed, this factor alone accounting for >40% of the total variance observed in LTI, iSP area and TCT measures. For all TCI measures, no interactions were found between "hand/hemisphere" and "Age Group" ($F_{1,26}$<1, p>0.49).

The large "Age" effect found in the primary analysis prompted a secondary analysis to better delineate the impact of this factor and also to address possible influences arising from age differences in corticomotor excitability. For this secondary analysis, all right and left TMS measures obtained in each participant were averaged to get single mean values. Then, chronological age, rMT, MEP amplitude, MEP latency and cSP duration were entered as co-variates into univariate analyses of co-variance (ANCOVA) to examine their respective impact on each measure of TCI (i.e., LTI, iSP area and TCT). This series of ANCOVAs confirmed the significant impact of chronological age ($F_{1,22}$>4.5, p<0.05), this factor alone accounting for respectively 17%, 24% and 23% of the variance in LTI, iSP area and TCT measures. Besides a significant effect of MEP latency on LTI measures ($F_{1,22}$=11.1, p=0.002), which was expected given that LTI depends to a large extent on MEP latency, no other co-variates had a significant effect on measures of TCI ($F_{1,22}$<3.6, p>0.07).

Relationships between transcallosal inhibition and manual performance

The results of the correlation analysis examining the relationships between measures of interhemispheric inhibition and age-related variations in unimanual performance in the right and left hands are described in Table 2. As evident in the Table 2, the associations were generally stronger for the dexterity than for the grip strength test and this for both hands. The nature of these relationships can be further appreciated in Figure 2. It can be seen, for instance, that age-related variations in LTI derived from each hemisphere were strongly related to dexterity of the contralateral hand; delayed onset being associated with slower performance (Figure 2A). Likewise, age-related variations in iSP area explained a significant proportion of the variance observed in contralateral grip strength, especially for the left hand with right to left TCI (Figure 2B).

Discussion

In the present study, we investigated age and hemispheric differences in mutual inhibition between motor cortices during voluntary activation using the iSP to probe TCI. Two main findings emerge from our observations. First, TCI indices derived from iSP characteristics (i.e., LTI, iSP area and TCT) were strongly influenced by age differences, whereas differences between hemispheres were only marginal. Second, correlation analyses revealed significant

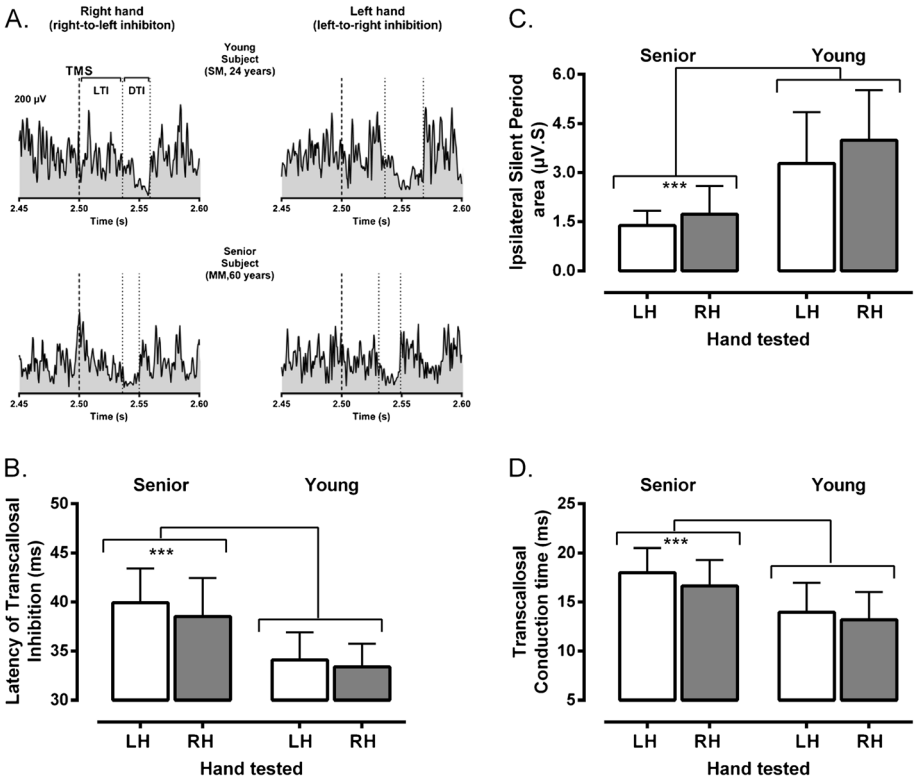

Figure 1 Examples of ispsilateral inhibition and mean group differences. A. Examples of rectified and averaged EMG traces (n=5 trials) depicting ipsilateral silent periods elicited in response to transcranial magnetic stimulation (TMS) in two participants, young and senior. As illustrated, several measures of transcallosal inhibition could be derived from iSP recordings. The latency of transcallosal inhibition (LTI) was computed as the time interval between the cortical stimulus (thick dotted line) and the iSP onset (first thin dotted line), which was determined as the first sustained decline in EMG activity when compared to mean pre-stimulus level (horizontal dotted line). The duration of transcallosal inhibition (DTI) was estimated as the time interval between the iSP onset and offset (second thin dotted line), which as determined as the point where the EMG activity returned to pre-stimulus level. Finally, the depth of ispsilateral inhibition was estimated by calculating the iSP area, as depicted by the blank area delimited by the two vertical dotted lines (DTI) and below the horizontal line in the recordings. Note that timing measurements are given only for illustrative purposes, as the real estimates were derived from a trial-by-trial analysis. **B**. **C** and **D**. Mean variations (± 1 SD) in measures of ispsilateral inhibition (**B**, LTI: Latency of transcallosal inhibition; **C**, iSP area: ispsilateral silent period area, **D**, TCT: transcallosal conduction time) derived from each hand/ hemisphere for the two groups of participants. Note again the major differences between age groups as indicated by the asterisks (p<0.001).

relationships between indices of TCI derived from each hemisphere and performance of the contralateral hand in dexterity and grip strength tests, so that left-to-right TCI correlated with right hand performance and vice-versa for right-to-left TCI and left hand performance. In the following discussion, we address the significance of these findings for the study of aging and its impact on motor systems.

Age and hemispheric differences in TCI

Contrary to evidence suggesting an asymmetry in the balance of mutual inhibition in favor of the dominant MI in right-handed individuals [17,19,20], only minor differences in TCI were found between the two hemispheres in our two groups of participants. In this respect, our results appear consistent with those of De Gennaro et al. [3], who found no difference in interhemispheric inhibition between the two hemispheres in resting state using bifocal TMS in young adults. Our observations for the senior group are also in line with those of Lewis & Perrault [18] who also failed to detect differences in paired-pulse SIHI between the dominant and non dominant hemisphere in their group of healthy senior adults, who served as controls for stroke patients. Interestingly, the absence of hemispheric asymmetry reported in Lewis & Perrault's study was evident only when the target hand (contralateral to the test hemisphere) was active, irrespective of whether the ipsilateral hand was active or not. In the present study, the fact that iSP was tested during concurrent contraction of both hands might have been critical in attenuating possible asymmetries in the level of mutual inhibition in relation to manual dominance. It remains that the issue of hemispheric asymmetries in relation to manual dominance remains a controversial topic in the TMS literature

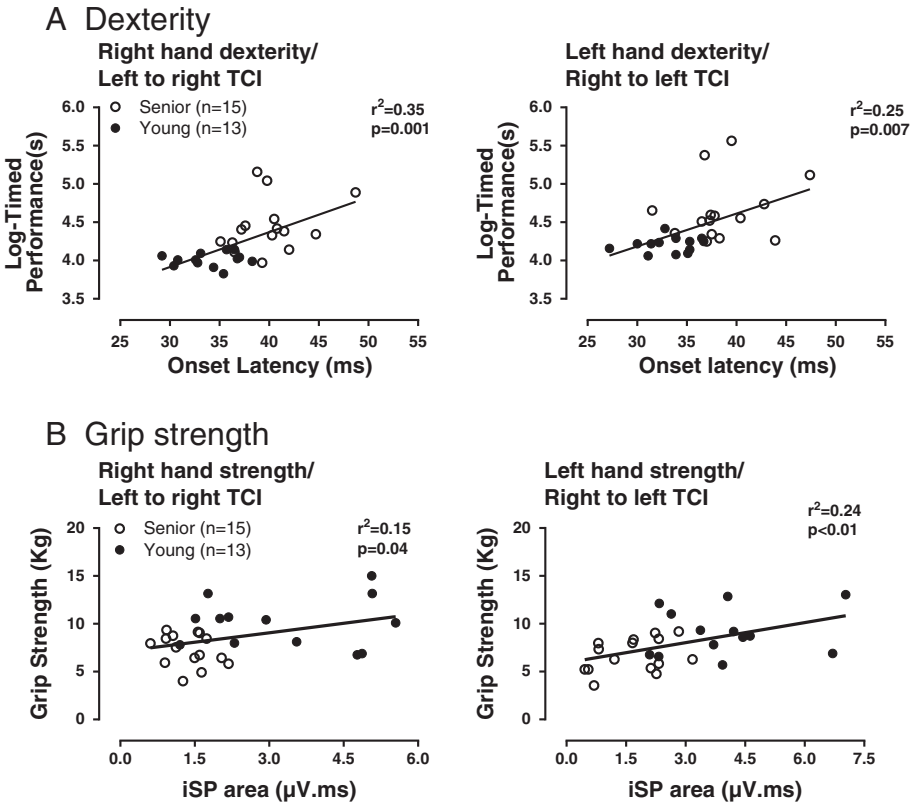

Figure 2 Illustrations of relationships found between measures of transcallosal inhibition (TCI) derived from each hemisphere and age-related variations in unimanual performance in dexterity (A) and strength (B) tests. In both **A** and **B**, the relationships are given with respect to the direction of TCI induced from one hemisphere to the other and performance of the contralateral hand controlled by the stimulated hemisphere; so that left to right TCI is correlated with performance of the right hand. **A**. Relationships between onset latency of TCI and contralateral dexterity, as measured with the Grooved Pegboard Test after log-transformation of timed performance. **B**. Relationships between the ipsilateral silent period area (iSP) and contralateral grip strength, as measured with a pinch dynamometer. Note that correlational analysis omitted two subjects in the senior group, one with incomplete data and one detected as an outlier.

Table 2 Associations between measures of transcallosal inhibition and age-related variations in unimanual performance of the right hand and left hand

Direction of motor transcallosal inhibition[a]	Manual performance	
	Dexterity (GPT)	Grip strength (Pinch dynamometer)
Left to right	Right Hand	
LTI	r= 0.59***	r= −0.37
TCT	r= 0.50**	r= −0.46*
iSP area	r= −0.46*	r= 0.38*
Right to left	Left Hand	
LTI	r= 0.50**	r= −0.33
TCT	r= 0.42*	r= −0.44*
iSP area	r= −0.57**	r= 0.49**

[a] The direction of transcallosal inhibition corresponds to the ispsilateral inhibition induced from the stimulated hemisphere towards the opposite hemisphere during voluntary contractions.
Abbreviations: *GPT* Grooved Pegboard Test, *LTI* Latency of Transcallosal Inhibition, *iSP* ispsilateral Silent Period area, *TCT* Transcallosal Conduction Time.
Significant correlations are marked with asterisks: *p<0.05, **p<0.01, ***p<0.001.

[e.g., see 21] and more research is needed with larger groups of right- and left-handers to address the question.

Contrasting with the relatively minor difference found between hemispheres, a major difference was found between age groups, senior participants showing delayed LTI, prolonged TCT and reduced iSP area when compared to young participants. Given that our group of seniors exhibited signs of decreased corticomotor excitability, it is possible that the observed age difference in TCI might have reflected impaired motor activation or peripheral nerve dysfunction. This possibility seems unlikely, however, for several reasons. First, with regard to motor activation, the work of Giovannelli et al. [22] has clearly demonstrated that TMS-induced ispsilateral inhibition is little affected by the intensity of contraction in the test hand, the most important factor being rather the presence of light activity in the opposite hand. During testing, all our participants, and especially seniors, were encouraged to produce their maximum effort in the ispsilateral hand, while lightly contracting the other hand. Thus, by basing our assessment on Giovannelli's method, we made sure that conditions would be optimal to elicit ipsilateral inhibition in each age group, in spite of individual variations in muscle activation. Second, as demonstrated by our secondary analysis (ANCOVAs), age-related variations in various indices of corticomotor excitability had little influence on measures of ispsilateral inhibition. In fact, the only significant interaction found was between LTI and contralateral MEP latency, which was expected. However, the computation of TCT, which largely removes the influence of MEP latency, showed that age differences were still highly significant. To summarize, while our seniors exhibited typical signs of corticomotor aging [e.g., 23], these changes could hardly account for the large age effect observed on iSP measures; pointing to central alterations in TCI as the primary cause.

In fact, our observations appear to be congruent with the proposal of Seidler's group [10] that there is a shift with age in the balance of mutual inhibition between motor cortices towards excitatory processes. However, as noted earlier, these investigators [15] found only a trend when comparing the strength of ispsilateral inhibition in young and old adults. Their observation that young adults tended to show deeper levels of ipsilateral inhibition than older adults seems consistent with the age difference reported here. In more direct line with our results, Boudrias et al. [24] observed a strong age effect when comparing measures of interhemispheric inhibition derived from bi-focal TMS in young and older adults. In their study, a clear distinction was seen between young and older adults in that most seniors showed evidence of transcallosal facilitation rather than the typical inhibition. In the same vein, McGregor et al. [25] found that measures of ispsilateral inhibition were significantly reduced in older subjects when compared to young. Interestingly, the largest reductions were seen in the group of sedentary seniors, where iSP durations were on average 50% shorter than in young adults; a range comparable to the averaged reduction in iSP area reported here (57%). In physically active seniors, the reduced ipsilateral inhibition was less pronounced (~25%), which led McGregor et al. to conclude that engaging in regular physical activity could help to maintain levels of interhemispheric inhibition. Although we did not specifically control for activity level in our study, it is worth noting that the outlier senior who showed exceptionally long iSP duration (see Results) was also highly active as judged by self-report. It would be interesting for future studies to investigate how interactions between advancing age and levels of physical activity influence hemispheric interactions, but the small sample size used in the present study precludes any conclusion in this regard. Nevertheless, both the present findings and the results of recent TMS studies concur with the notion that transcallosally mediated inhibition becomes less efficient with age in line with reports describing structural alterations in the integrity of callosal fibres between motor cortices with advancing age [15,24,26].

Regarding the physiological mechanisms underlying the observed changes in iSP measures with age, the reduced iSP area points to a decrease in the excitability of ispsilateral transcallosal inhibitory neurons. Such a decrease would be consistent with reports of age-related reductions in the excitability of local inhibitory circuits mediating short-interval intra-cortical inhibition (SICI) [27] and short-latency afferent inhibition [28] reported in the motor cortex of seniors. As shown by Avenzino et al. [29], local interneurons involved in transcallosally mediated inhibition share common properties with those controlling excitability of pyramidal tract neurons; and thus, any alterations in intra-cortical excitability with age could also affect inhibitory connections between motor cortices. In parallel, the delayed LTI and prolonged TCT found in seniors are consistent with reports of structural alterations in callosal connectivity between motor cortices with age, as stated earlier. In fact, a growing body of evidence [12,30] is now emerging linking preserved task-related functional connectivity between hemispheres in seniors with integrity of transcallosal connections. Indeed, as suggested by our correlational analysis, integrity of transcallosal connections seems to be important in allowing older adults to maintain certain levels of performance, as discussed below.

Relationship between ipsilateral inhibition and manual performance

The result of our correlation analysis revealed significant relationships between our different measures of TCI in

each hemisphere and performance of the contralateral hand, so that left to right TCI (left hemisphere stimulated) correlated with right hand performance and vice versa for the left hand. Interestingly, these associations were particularly strong for the dexterity task, which is consistent with the purported role of TCI in preventing motor overflow when task demands require fine unilateral control of one hand [31]. While correlations with grip strength were not as strong, good quality relationships were still found, for example, between iSP area and left hand; suggesting a role of TCI in unimanual force production. In line with this, Fling and Seidler [4] recently reported an inverse relationship between measures of ispsilateral inhibition and the ability of young individuals to suppress motor activity in the resting hand (i.e., motor overflow) during a unimanual force production task. Although we did not monitor motor activity in the resting hand during our tests of manual performance, our observations on the association between measures of TCI and manual performance are consistent with those of Fling and Seidler with regard to the role of TCI in allowing fine independent control of unimanual performance. In a related study from the same group of investigators [13], the association between functional motor activation and performance of a precision task with the dominant hand was examined in young and old adults. Much like in the present study, the authors found that increased ispsilateral motor recruitment (and presumably less efficient TCI) was associated with poorer task performance. The fact that this association was found only for the older group and not in younger subjects does not invalidate the comparison with the present findings since both their results and ours converge to show that proper levels of TCI is an important factor in leading to fine motor performance in the context of precision tasks, especially as people advance in age. In fact, there is ample evidence from TMS studies that levels of intra- and interhemispheric inhibition are critical for the performance of fine motor tasks [14,32-34]. With regard to aging specifically, the observation that our group of seniors exhibited various levels of impaired ispsilateral inhibition and that these impairments were in part reflected in their dexterity performance would be consistent with other studies in which deterioration in motor performance and in interlimb coordination with age was associated with a decreased ability to modulate inhibition at the central level [14,34-36].

Conclusion

The present study examined age and hemispheric differences in mutual inhibition between motor cortices using the iSP as a marker of transcallosally mediated inhibition. Consistent with previous studies, we report a major difference with regard to age, whereas differences between hemispheres were only marginal. In addition, we show that measures of TCI derived from each hemisphere correlated well with age-related variations in manual performance of the contralateral hand. Overall, these results appear congruent with the hypothesis proposed by Seidler and colleagues [10] suggesting a shift with age in the overall balance of normally predominantly inhibitory interhemispheric interactions toward excitatory processes. Possible limitations of the present study include the small number of participants in each age group and the fact that older participants were considered as active seniors, which may not be representative of the population of seniors in general.

Methods

The study procedures were approved by the Research Ethics Board at the Bruyère Research Institute, Ottawa, Ontario, Canada. Written informed consent was obtained prior to participation from all participants in accordance with the *Declaration of Helsinki*. All assessments were performed in a controlled laboratory environment. Each participant received a small honorarium for his or her participation.

Participants

Two groups of participants, young and senior were recruited for this study. The young group (n=13) was recruited from the student population at University of Ottawa, whereas the senior group (n=17) was recruited from the community in the Ottawa-Gatineau area. All participants were right-handed, as determined by the Edinburg Handedness Inventory [37]. Prior to the experimental session, all participants completed a medical questionnaire to determine their general health status and to ensure that there were no contra-indications to TMS or antecedents of conditions likely to affect their performance in the tests. In addition, sensory function of the hand was assessed using a Rydel-Seiffer tuning fork to rule out the presence of undiagnosed neuropathies. All participants exhibited vibration thresholds in line with their norms for their age range [38]. The demographic characteristics of the participants are listed in Table 1.

Manual performance: Grooved Pegboard Test and pinch strength

For manual performance, participants were comfortably seated in front of a table. All tests were applied bilaterally and administered by the same experimenter (TD). The order of testing with each hand was determined randomly before the testing session. Manual dexterity was assessed with the grooved pegboard test (GPT, Lafayette Instr, IN 47903), which consists of inserting pegs

into keyholes in a specific order as fast as possible. After instructions and practice trials, the GPT was administered once to each hand and performance was recorded as the time in seconds to complete the task. The second test consisted of grip strength assessment. For this test, a small pinch gauge (PG-60, B & L Engineering, Santa Ana, CA 92705) was used to assess the thumb-index finger pinch strength. This test also provided an index of the muscle activity elicited by the first dorsal interosseous muscles (FDI) during maximal voluntary contraction. Participants were presented with the gauge and were asked to press as hard as they can for the duration of a tone, which lasted 3 s. Three trials were performed for each hand with a 60 s rest between trials. The average provided a measure of pinch strength and of maximal activation in the FDI. To avoid any interference associated with fatigue, the strength test was always performed at the end of the testing session, after neurophysiological testing was completed.

Electromyographic recordings

Electromyographic (EMG) activity was recorded using 10-mm auto-adhesive surfaces electrodes (Ag/AgCl, Kendall Medi-Trace™ 130) placed over the FDI muscles of each hand using a tendon-belly montage. EMG signals were amplified and filtered with a time constant of 10 ms and a low-pass filter of 1 kHz (AB-621G Bioelectric amplifier, Nihon-Kohden Corp., CA 92610). Signals were digitized at rate of 2 kHz (BNC-2090, National Instrument Corp.) and further relayed to a laboratory computer running custom software to control acquisition.

Transcranial magnetic stimulation and resting motor threshold (rMT)

Magnetic stimulation was delivered via a Rapid^2 stimulator (Magstim Co. Dyfed, UK) connected to a figure-eight coil (90 mm outer loop diameter). All testing was performed with the participants comfortably seated in a recording chair. Participants were fitted with a Waveguard TMS compatible cap (ANT North America Inc, WI 53719) to allow for consistent coil placement. A U-shaped neck cushion was also used to restrain head movements and prevent neck fatigue. TMS testing sessions began by first determining the "hotspot" for the FDI and then by determining the rMT for the stimulated hemisphere. This procedure was performed sequentially on each hemisphere with the order of testing between the two alternating between participants. To determine the optimal site to evoke MEP's in the contralateral target muscle (FDI), the approximate location of the hand motor area on the stimulated hemisphere was explored in 1-cm steps until reliable MEP's could be evoked. This site was then marked with a red dot to ensure consistent coil positioning. After localization of this stimulation "hotspot", the rMT was determined using the procedure described by Mills and Nithi [39]. Starting from suprathreshold intensity, the stimulation intensity was gradually decreased in 1% steps until no MEP's could be evoked in 10/10 consecutive trials (lower threshold). Then, the intensity was increased in 1% increments to find the minimal intensity that would produce reliable peak-to-peak amplitude MEPs of at least 50 μV for 10/10 consecutive trials (upper threshold). The rMT was defined for each participant as the median intensity between the upper and lower threshold values. EMG was continuously monitored on an oscilloscope, at high gain (0.1 mV/div), to ensure the absence of any muscle activity during the procedure. As mentioned above, the same procedures were repeated for the opposite hemisphere.

Contralateral and ipsilateral silent period (cSP/ iSP)

The cSP and iSP were measured concurrently using the approach described by Giovannelli et al. [22], which involved maximal contraction of the hand ispsilateral to the stimulated hemisphere coupled with light contraction of the contralateral hand. As shown by these authors, voluntary activation of the contralateral hand, irrespective of the level of contraction significantly prolonges the iSP in the test hand when compared to rest and this, without affecting the level of background EMG in the ispsilateral hand. For cSP/iSP testing, participants were instructed to press as hard as they could on the pinch dynamometer using the thumb and index fingers with one hand, while lightly squeezing a soft exercise ball with their opposite hand. The latter contraction corresponded, on average, to ~15% of the maximal voluntary contraction in both age groups. Participants were trained to maintain the contractions for 3 s in sync with a tone (550 Hz) generated by the computer. The subjects were told to focus on the maximally contracting hand ipsilateral to the stimulation and were given verbal feedback to maintain contraction. In each trial, TMS was delivered on the hemisphere ipsilateral to the maximally contracting hand at 2 s in the course of the trial using an intensity equivalent to 120% of the rMT. The intertrial interval was 10–15 s. Short pauses were allowed to prevent fatigue. Five trials were performed on each hemisphere, the order of testing between hemispheres alternating between participants. A schematic representation of the testing paradigm for the cSP/iSP assessment is provided in Figure 3.

TMS data analysis

Data from the cSP/iSP trials were analyzed off-line from visual inspection of stored EMG traces using custom software. All visual analyses were performed by the same investigator (TD) using numerically coded files, which

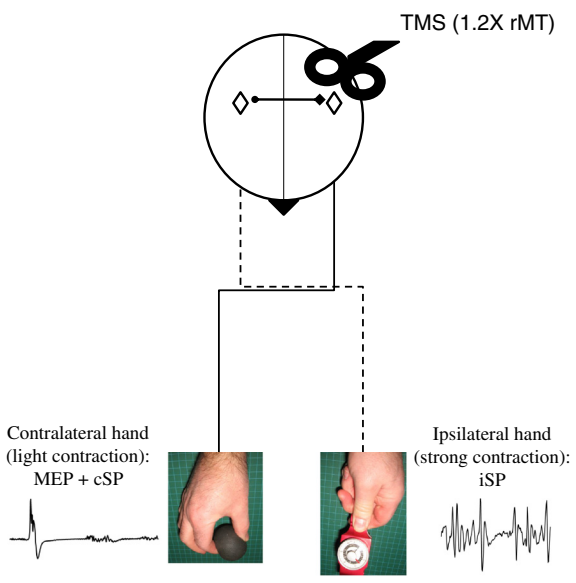

Figure 3 Schematic representation of the testing paradigm used to assess the contralateral and ispsilateral silent period (cSP/iSP). In the illustration, the iSP is elicited in the left hand during maximal effort in response to left transcranial magnetic stimulation (TMS), while the cSP with the associated motor evoked potential (MEP) is elicited in the right hand during light effort. (rMT: resting motor threshold).

were saved separately from the demographic data, to allow for blinding with respect to the age of the participant. Visual analysis of silent periods has been shown to have good inter and intra-rater reliability [40,41]. For cSP measures, EMG traces obtained from the contralateral hand were examined individually to determine the duration of the SP and measure the characteristics of the associated MEP in terms of amplitude (peak to peak) and latency. The cSP duration was determined as the time from the MEP onset till the first sign of EMG recovery. Mean values were computed by averaging individual trials. The same approach was used to analyze iSP trials using EMG traces obtained from the ispsilateral hand. For iSP measures, each individual file was analysed twice with at least one week interval between runs to ensure intra-rater reliability. In each file, two main indices of TCI were derived from the EMG trace of the ipsilateral hand. First, to get an index of the interhemispheric transfer time, the onset latency of transcallosally mediated inhibition (LTI) was measured. The LTI was defined as the time interval from the cortical stimulus until the 1st sign of sustained decline (>25% of mean EMG level for at least 5 ms) in the EMG activity level. Maximal and mean EMG levels were measured 100 ms prior to stimulation. The second index consisted of estimating the depth of ispsilaterally-induced inhibition by computing the iSP area. The latter was determined by first rectifying the EMG trace and then by computing the integral of the area delimited by the iSP onset and offset. As stated above, the iSP onset was determined as the first time point where the signal of the EMG activity clearly fell under the mean level observed before the cortical stimulus. The iSP offset was determined as the first time point after iSP onset at which the EMG level returned to the mean level. The reliability analysis showed a strong to very strong level of agreement, as reflected in intra-class correlation coefficients, between the two set of measurements for both the LTI (right hand, 0.93; left hand, 0.97) and iSP area measures (right hand, 0.82; left hand, 0.80). Individual examples of EMG traces analysed to derive iSP measurements are shown in Figure 1A for a participant in each age group. Finally, as a co-result of this analysis, we derived a third index of TCI by computing the transcallosal conduction time (TCT), i.e., the duration of the stimulus transfer to one hemisphere to the other, by subtracting the MEP latency obtained from the contralateral hand from the iSP onset latency (i.e., LTI, see Figure 3).

The statistical analysis was performed in three steps. First, right-left differences in manual performance and in basic measures of corticomotor excitability were examined in each age group with paired t-tests. For these paired comparisons, the significance level was set at $P<0.01$ to reduce the risk of type I error owing to multiple comparisons. The second step consisted of examining variations in each index of TCI (LTI, iSP area and TCT, respectively) using analyses of variance (ANOVA) for repeated measures with "Hand/Hemisphere" as the within-subject factor and "Age Group" as the between-subjects factors. For this analysis, the significance level was set at $P<0.05$ to detect main effects and interactions. The final step consisted of examining relationships between manual performance and measures of TCI using Pearson's correlation. For these analyses, timed performance for the dexterity test (i.e., GPT) was log transformed to normalize the distribution which was skewed owing to the presence of participants in the senior group (4/17) with very slow performance (>120 s). The significance level for the correlations was set at $P<0.05$. All statistical tests were performed using SPSS software version 17.0 for Windows® (Chicago, IL, USA). Figures were prepared using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego California USA, www.graphpad.com).

Abbreviations

ANOVA: Analyses of variance; DTI: Duration of transcallosal inhibition; EHI: Edinburgh inventory index; EMG: Electromyography; FDI: First dorsal interosseous; GPT: Grooved pegboard test; iSP: Ipsilateral silent period; iSP area: Area under the curve during the ipsilateral silent period; LH: Left hand; LIHI: Long-latency interhemispheric inhibition; LTI: Latency of transcallosal inhibition; MEP: Motor evoked potential; MI: Primary motor cortex; RH: Right Hand; rMT: Resting motor threshold; SIHI: Short-latency interhemispheric

inhibition; TCI: Transcallosal inhibition; TCT: Transcallosal conduction time; TMS: Transcranial magnetic stimulation.

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
TD assisted with the study design, carried out the data collection, analyzed all data, and drafted the manuscript. FT conceived the study, aided with data collection and in drafting and editing of the manuscript. All authors read and approved the final manuscript.

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Hemispheric Differences in Corticospinal Excitability and in Transcallosal Inhibition in Relation to Degree of Handedness

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Abstract

In this study, we examined hemispheric differences in corticospinal excitability and in transcallosal inhibition in a selected group of young adults (n = 34) grouped into three handedness categories (RH: strongly right-handed, n = 17; LH: strongly left-handed, n = 10; MH: mixed-handed, n = 7) based on laterality quotients (LQ) derived from the Edinburgh Handedness Inventory. Performance measures were also used to derive a laterality index reflecting right-left asymmetries in manual dexterity ($Dext_{li}$) and in finger tapping speed ($Speed_{li}$). Corticospinal excitability was assessed in each hemisphere by means of transcranial magnetic stimulation (TMS) using the first dorsal interosseus as the target muscle. TMS measures consisted of resting motor threshold (rMT), motor evoked potential (MEP) recruitment curve (RC) and the contralateral silent period (cSP) with the accompanying MEP facilitation. Hemispheric interactions were assessed by means of the ipsilateral silent period (iSP) to determine the onset latency and the duration of transcallosal inhibition (i.e., LTI and DTI). Analysis of hemispheric variations in measures of corticospinal excitability revealed no major asymmetries in relation to degrees of laterality or handedness, with the exception of a rightward increase in rMTs in the LH group. Similarly, no clear asymmetries were found when looking at hemispheric variations in measures of transcallosal inhibition. However, a large group effect was detected for LTI measures, which were found to be significantly shorter in the MH group than in either the LH or RH group. MH participants also tended to show longer DTI than the other participants. Further inspection of overall variations in LTI and DTI measures as a function of LQs revealed that both variables followed a non-linear relationship, which was best described by a 2nd order polynomial function. Overall, these findings provide converging evidence for a link between mixed-handedness and more efficient interhemispheric communication when compared to either right- or left-handedness.

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Introduction

The concept of handedness comes from the observation that most humans exhibit a preference for one hand over the other, with ~90% of the population showing a rightward preference [1]. The question thus arises as to whether this asymmetrical hand use is reflected at the level of sensori-motor organization and in terms of neural control. Given the critical role of the primary motor cortex (M1) and its corticospinal projections in controlling fine aspects of manual dexterity, one would assume that asymmetries would also be reflected at this level. Yet, such a simplistic assumption has proven difficult to establish. At the anatomical level, structural imaging studies have produced rather inconsistent results with regard to asymmetries related to handedness, although many studies point to a specific leftward asymmetry in the size of the M1 region in consistent right-handers, particularly in male subjects [2–4]. The left M1 region also displays more profuse intrinsic connectivity in consistent right-handers [5]. These anatomical findings did not, however, translate into corresponding asymmetries at the physiological level. For instance, only minor asymmetries were found when comparing sensorimotor activation levels elicited during movement of either the preferred hand or the less preferred hand [6,7]. When asymmetries are found they often go in the direction of a greater activation in the non dominant hemisphere [see 6 for a review]; a finding that could be explained by the fact that movements of the less preferred hand are more demanding and thus require greater control.

Transcranial magnetic stimulation (TMS) studies of hemispheric differences in cortical excitability have also produced rather conflicting results with regard to handedness. For example, while some reports found asymmetries in motor threshold (MT) with lower values for the dominant hemisphere [8], others reports did not find such asymmetries [9,10]. In the same vein, some reports pointed to an asymmetry in the strength of interhemispheric inhibition from the dominant over the non-dominant M1 in right-handers [11] but this finding could not be confirmed by others [12]. On the other hand, TMS studies of task-dependent changes in corticospinal excitability have provided more consistent results in line with those from neuroimaging studies in showing greater corticomotor facilitation in the non dominant M1 when the less

preferred hand was involved [13,14]. The overall picture that emerges from the TMS literature on handedness is one of difficulty establishing a strong link between the observed behavioural asymmetry in hand use and its neurophysiological correlates at the corticomotor level. As pointed out by Bernard et al. [15], beyond variations in experimental protocols, one major reason for the conflicting evidence is the fact that most authors have considered handedness only in terms of direction (i.e., either right or left preference) without consideration for the degree of lateralization (i.e., how strong is your preference). Interestingly, the relatively few studies that have considered the degree of handedness have produced much more consistent findings. For instance, Dassonville et al. [16] used the Edinburgh Handedness Inventory [17] to quantify the degree of hand preference in both right- and left-handers and showed that the greater the degree of handedness the greater the lateralized difference in motor cortex activation during use of the dominant hand. More recently, Martin et al. [18] made similar observations in showing that the parieto-cortical network activated during grasping was largely asymmetrical and determined by the degree of lateralization in either strongly right-handed or strongly left handed individuals. In a TMS study, Triggs et al. [8] used right-left differences in dexterity tests to quantify the degree of lateralization in groups of right-handers and left-handers. This index of handedness provided the best correlation with corresponding measures of right-left asymmetry in MT (i.e., the larger the manual asymmetry, the greater the threshold asymmetry). Thus, one way to tackle issues related to handedness and its neurophysiological correlates is to move beyond a simple right-left dichotomy to examine the whole spectrum of handedness using preference and performance measures to assess how strongly one is handed.

In the present study, we sought to further investigate the neurophysiological correlates of handedness using a set of TMS measures to characterize not only hemispheric differences in basic measures of corticospinal excitability but also differences in interhemispheric inhibition. To examine the influence of handedness, we used hand preference and performance measures to characterize the degree of handedness in our participants. Given recent evidence pointing to a relationship between degree of laterality and asymmetries at the cortical level, we hypothesized that hemispheric differences would emerge in individuals exhibiting strong preference for one hand (either right or left) and large manual asymmetries in performance when compared to individuals with no clear preference for one hand (i.e., mixed handedness) and lower degrees of manual asymmetries.

Materials and Methods

Ethics Statement

The study procedures were approved by the Research Ethics Board at the Bruyère Research Institute, Ottawa, Ontario, Canada. Written informed consent was obtained prior to participation from all participants in accordance with the *Declaration of Helsinki*. All assessments were performed in a controlled laboratory environment. Each participant received a small honorarium for his or her participation.

Participants

Thirty-four young healthy adults (18–30 years) were recruited for this study from the community in the Ottawa-Gatineau area. Participants were initially recruited on the basis of self-report of handedness. During the process, a special effort was made to recruit left-handed participants so that both hand preference groups would be adequately represented in our pool of participants. The final sample included 19 self-reported right-handers (8 females) and 15 self-reported left-handers (9 females). Before testing, all participants completed a medical questionnaire to assess their general health and to ensure that there were no contra-indications to TMS. The demographic characteristics of participants are described in Table 1.

Hand Preference Groups and Degrees of Handedness

To reflect differences in the degree of hand preference (HP), participants were divided into three HP groups on the basis of laterality quotients (LQs) computed from the Edinburgh Handedness Inventory as: (Right-Left)/(Right+Left)×100 [17]. We used the upper (LQ>+75) and lower quartiles (LQ<−75) respectively, to assign participants to either a strong right-handed group (RH, n = 17) or a strong left-handed group (LH, n = 10). The remaining were assigned to a mixed-handed group (MH, n = 7). Besides LQs derived from the Edinburgh Inventory, we computed two others laterality indices from measures of manual performance. The first index reflected right-left asymmetries in dexterity as measured with the Grooved Pegboard Test (GPT, Lafayette Instrument Co, IN 47903). The GPT consists of inserting 25 small pegs into keyhole-like grooves as fast as possible using a fine precision grip. Each hand was tested once and the timed performance in seconds (s) to complete the test (i.e. 25 peg insertions) was used to derive a dexterity laterality index ($Dext_{li}$) computed as: $(Right_{GPT}-Left_{GPT})/(Right_{GPT}+Left_{GPT})\times100$. Note that since the GPT reflects a timed performance, a positive $Dext_{li}$ corresponds to better performance with the left hand whereas a negative $Dext_{li}$ corresponds to better performance with the right hand. The second index assessed right-left asymmetries in the speed of execution with the Finger Tapping Test (FTT). The FTT was

Table 1. Characteristics of the participants with respect to hand preference and manual performance.

	Left Handed	Mixed handed	Right Handed
	(LQ≤−75)	(−75≤LQ≤+75)	(LQ[a]≥+75)
	(n = 10)	(n = 7)	(n = 17)
Age (years)	21.1±1.9	24.0±3.1	21.6±2.6
(range)	(18–25)	(22–30)	(19–29)
Gender	2 M, 8 F	5 M, 2 F	10 M, 7 F
Dexterity			
GPT (s)	RH: 60.0±6.3	RH: 56.6±4.4	RH: 54.6±6.3
	LH: 52.5±4.6	LH: 56.3±9.0	LH: 66.3±9.2
$Dext_{li}$[b]	6.6±5.8	0.7±6.4	−9.9± −4.4
Execution Speed			
FTT (#taps/15 s)	RH: 94.4±12.5	RH: 96.6±14.2	RH: 100.5±11.5
	LH: 98.7±9.5	LH: 101.3±14.9	LH: 90.4±8.3
$Speed_{li}$[c]	−2.4±4.0	−2.3±4.7	6.2±4.5

Values are given as mean and standard deviation.
[a]Laterality quotient (LQ = (Right-Left)/(Right+Left)×100) derived from self-report of hand preference with the Edinburgh Hand Inventory.
[b]Laterality index derived from performance in the Grooved Pegboard Test (GPT)) reflecting right-left asymmetries in dexterity ($Dext_{li}$ = Right-Left)/(Right+Left)×100).
[c]Laterality index derived from performance in the Finger Tapping Test (FTT) reflecting right-left asymmetries in speed of execution ($Speed_{li}$ = (Right-Left)/(Right+Left)×100).
Abbreviations: M: Male; F: Female; RH, Right Hand; LH, Left Hand.
doi:10.1371/journal.pone.0070286.t001

administered using a MoART panel with the accompanying PsymSoft II^{TM} software (Lafayette Instruments Co., IN 47903). The task consisted of tapping a circular target at the center of the board successively for 15 seconds with the index finger as rapidly as possible. Participants were instructed to focus on speed and not on accuracy. As for the GPT, each hand was tested once (order counterbalanced) and the performance in terms of number of valid taps (i.e., hitting the target) was used to derive a speed laterality index ($Speed_{li}$), which was calculated as: $(Right_{FFT}\text{-}Left_{FFT})/(Right_{FFT}+Left_{FFT})\times 100$.

Transcranial Magnetic Stimulation (TMS) and Motor Evoked Potentials (MEPs)

TMS was administered with participants comfortably seated in a recording chair. Magnetic stimulation was delivered with a Rapid^2 stimulator (Magstim Co. Dyfed, UK) connected to a figure-eight coil (90 mm outer loop diameter). MEPs were recorded using small auto-adhesive surface electrodes (Ag/AgCl, Kendall Medi-Trace^{TM} 130) placed over the first dorsal interosseous (FDI) muscles of the right and left hand. Electromyographic signals were amplified and filtered with a time constant of 10 ms and a low-pass filter of 1 kHz (AB-621G Bioelectric amplifier, Nihon-Kohden Corp., CA 92610). Signals were digitized at rate of 2 kHz (BNC-2090, National Instrument Corp.) and further relayed to a laboratory computer running custom software to control acquisition.

To determine the optimal site to evoke MEPs in the contralateral hand muscles, participants were fitted with a Waveguard TMS compatible cap (ANT North America Inc, WI 53719). A U-shaped neck cushion was also used to restrain head movements and prevent neck fatigue. With the coil held $\sim 45^{\circ}$ in the mid-sagittal plane, the approximate location of the hand motor area on the tested hemisphere was explored in 1-cm steps until reliable MEPs could be evoked in the target muscle. This site was then marked with a sticker to ensure consistent coil positioning. After determination of this stimulation "hotspot", the coil was held in place manually by one of the experimenters (FT) to derive specific measures of corticospinal excitability. The experimenter frequently reassessed the coil position to ensure that it remained over the optimal stimulation site throughout the experiment. All TMS testing sessions took place between 9am and 4pm to avoid diurnal variations in corticospinal excitability [19].

Measures of Corticospinal Excitability

In each participant, specific measures of corticospinal excitability were derived from each hemisphere, the order of testing between the two alternating between participants. The first measure consisted of the resting motor threshold (rMT), which reflects neuronal membrane excitability in a given motor representation [20]. For rMT determination, we used the procedure described by Mills and Nithi [21] which consisted of determining a upper (10/10 MEPs) and a lower threshold intensity (0/10 MEPs) following a series of single TMS pulses and taking the median as the rMT intensity. Such a method has been shown to lead to more reliable and accurate estimates of rMT than the conventional method of using the intensity at which 50% MEPs are elicited [22]. The second measure of excitability was the recruitment curve (RC) at rest, which describes the relationship between MEP amplitude and TMS intensity. The RC reflects the strength of corticospinal projections and the extent of a given motor representation [20]. Single TMS pulses at 90%, 100%, 110%, 120% and 130% of rMT were applied consecutively and 5–10 MEPs were recorded at each stimulation intensity. For both rMT and RC procedures, EMG activity was constantly monitored on a high gain oscilloscope to make sure that unwanted contractions did not interfere with the measurements. The third measure was obtained during active contraction and consisted in the contralateral silent period (cSP) with the associated MEP facilitation (MEP_{facil}). For the cSP, single TMS pulses at 120% rMT were delivered while participants exerted a constant static force (duration 5 s) at 25% of their maximal strength with a pinch gauge. Five trials were performed for each hand/hemisphere.

Measures of Transcallosal Inhibition

To examine hemispheric interactions, we used the ipsilateral silent period (iSP), to assess transcallosally-mediated inhibition between motor cortices [23]. To elicit the iSP, we used the approach described by Giovannelli et al. [24], whereby single TMS pulses (120% rMT) were delivered ipsilaterally to the maximally contracting hand (maximal force exerted on the pinch gauge), while the opposite hand exerted a light force by gently squeezing a soft ball between the thumb and index fingers ($\sim$15% of the maximal activation). This procedure was repeated five times for each hand/hemisphere.

TMS Data Analysis

TMS data were analysed off-line by the same investigator (TD) using numerically coded files to avoid any biasing with regard to HP groups. To assess RCs, MEPs evoked at each intensity were measured peak-to-peak to obtain mean MEP amplitudes. Then, the mean amplitude was plotted against TMS intensities to obtain the RC. As suggested by Ray et al. [25] we used linear regression analyses to characterize the relationship between MEP amplitude and TMS intensity. To improve the goodness of fit and to account for large inter-individual variations, we used squared root transformation of MEP values to assess the RC. Such transformation greatly improved the goodness of fit of the relationship (averaged r^2: untransformed, 0.89 ± 0.6; transformed, 0.94 ± 0.05). For statistical comparisons, we used the slope of the RC as a simple summary statistic for the RC relationship at rest [25]. For the cSP, we performed a trial-by-trial analysis to estimate its duration and to measure the amplitude of the associated MEP (MEP_{facil}). The duration of the cSP was determined in each trial in line with guidelines from previous studies [e.g., see 26,27] as the time interval from the onset of the MEP to the return of at least 50% of the mean pre-stimulus background EMG activity. From this analysis, mean values were computed for the cSP duration and the MEP_{facil} amplitude by averaging all trials for each hand/hemisphere. As for MEPs recorded at rest for the RC, the amplitude of facilitated MEP_s varied greatly between individuals (skewness$>$3.0, Shapiro-Wilk's normality test, $p<0.01$) and thus were subject to log-transformation to normalize the distribution [28]. For iSP recordings, we adopted the same trial-by-trial analysis as for the cSP to derive two specific measures of transcallosal inhibition. First, the iSP onset, which reflects the onset latency of transcallosal inhibition (LTI), was determined as the time from the stimulus onset until the 1st sign of significant decline ($>$25%) in the mean rectified EMG activity level. The second index come from determining the iSP duration by measuring the time in ms from the iSP onset until the 1st sign of recovery in the background EMG activity (i.e., iSP offset). The latter time point is relatively easy to determine, as the end of the myoelectric silence is generally followed by an abrupt return of EMG activity in the recovery period (see RESULTS, for examples of iSP recordings).

Statistical Analysis

To determine how handedness categories influence measures of intra-hemispheric excitability and interhemispheric inhibition and how these measures co-vary with laterality indices, we performed a series of repeated measures Analysis of Covariance (ANCOVA) using "hand/hemisphere" (right vs. left) as the repeated factor, HP group (RH, LH, MH) as the between-subjects factors and the two laterality indices ($Dext_{li}$, $Speed_{li}$) as co-variates. The significance level was set at $p<0.05$ for detection of main effects and interactions. Post-hoc comparisons were performed using Tukey's test. Planned comparisons were also performed to examine specific combinations using t-tests (paired and unpaired, Bonferroni-adjusted to reduce Type I errors). Linear regression analyses were used to examine the relationships between laterality scores derived from preference and performance measures. Most analyses were performed using SPSS® software version 17.0 for Windows® (Chicago, IL, USA). GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego California USA, www.graphpad.com) was used to prepare illustrations and to perform secondary analyses dealing with non-linear curve fitting.

Results

Manual Performance and Laterality Index

The performance in the behavioural tests to assess laterality, along with corresponding indices, is shown in Table 1 for the three HP groups. As expected, both RH and LH groups exhibited strong asymmetries on the two tests, which were reflected in the behavioural laterality indices. In contrast, the MH group showed correspondingly less asymmetry, particularly in the dexterity test. The relationship between participants' perceived degree of handedness, as indexed by LQs, and indices of laterality measured from behavioural tests can be further appreciated in Figure 1. As evident in the Figure (a and b), the strength of the association between LQs and actual asymmetries in manual performance was greater for the dexterity test than for the FTT. Indeed, LQs accounted for >70% of the variance in $Dext_{li}$, whereas they accounted for >50% of the variance in $Speed_{li}$. As for the relationship between the two behavioural indices (Figure 1c), they showed only a moderate degree of association, indicating that the two were somewhat divergent in reflecting manual asymmetries associated with handedness.

Hemispheric Differences in Corticospinal Excitability

In general, only small differences were observed between hemispheres in measures of corticospinal excitability, irrespective of HP. Typical examples of right-left variations in MEP amplitude at increasing TMS intensities are shown in Figure 2 (a) along with examples of cSP recordings (b). It can be seen that for both the RH and LH participants, the variations recorded were largely comparable between hemispheres. In fact, the only noticeable asymmetry found was in rMT, which tended to be higher on the right as compared to the left hemisphere in all participants. This threshold asymmetry is evident in Figure 3, where averaged variations in TMS measures computed for each hand/hemisphere are shown for each group. The ANCOVA confirmed the presence of a large effect of "hand/hemisphere" on rMT ($F_{1,\ 29}=30.0$, $p<0.001$), although no other interaction or main effects were detected ($F_{1,\ 29}<0.5$, $p>0.49$). Planned comparisons revealed a significant right-left difference ($t_9=6.8$, $p<0.001$) in rMT only in the LH group; the other two HP groups showing only trends for significance at the adjusted p-value (i.e., $p=0.016$; RH group, $t=2.1$, $p=0.052$; MH group, $t=2.7$, $p=0.04$). Besides this threshold asymmetry, no other main effect or interactions were detected for the other remaining TMS measures (RC, $F_{1,\ 29}<1.35$, $p>0.26$; MEP_{facil}, $F_{1,29}<2.5$, $p>0.11$; cSP, $F_{1,\ 29}<0.69$, $p>0.40$) (Figure 3).

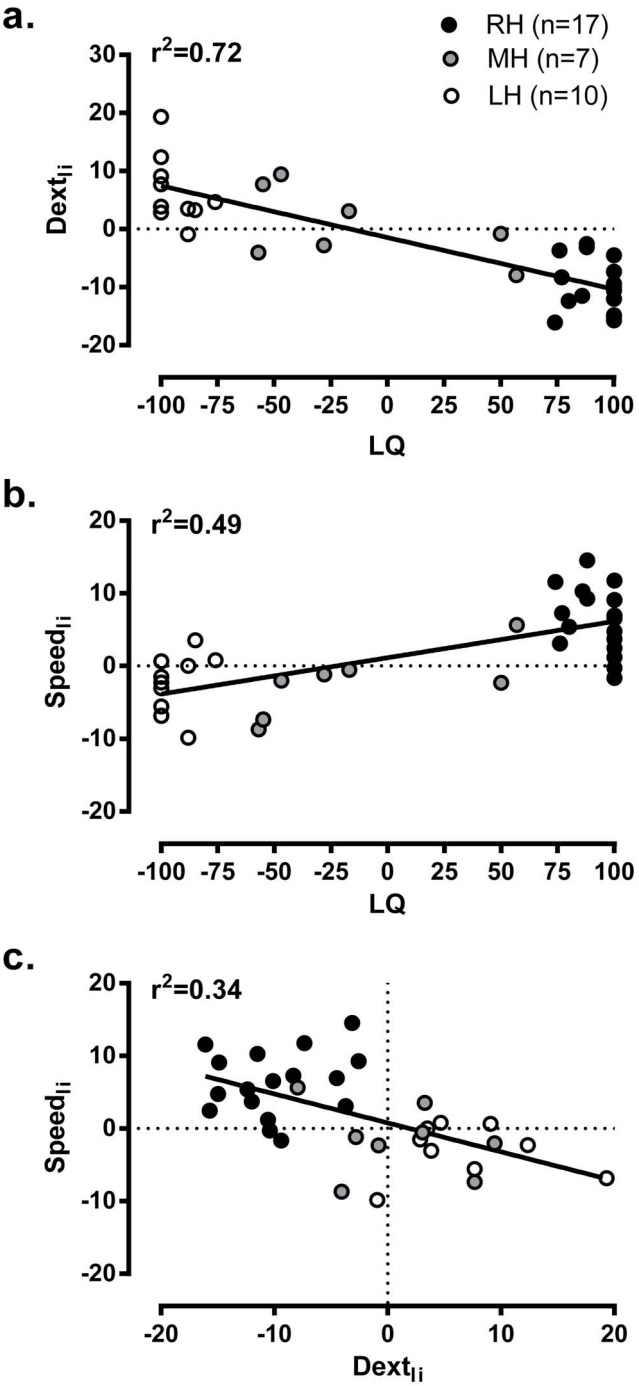

Figure 1. Relationships between preference and performance measures. a and b. Association between laterality quotients (LQ) computed from the Edinburgh Handedness Inventory and laterality indices computed from performance in the dexterity ($Dext_{li}$) and finger tapping tests ($Speed_{li}$), respectively. **c.** Association between the two laterality indices derived from performance tests is shown in c. All laterality indices were computed as: (Right-Left)/(Right+Left)×100.
doi:10.1371/journal.pone.0070286.g001

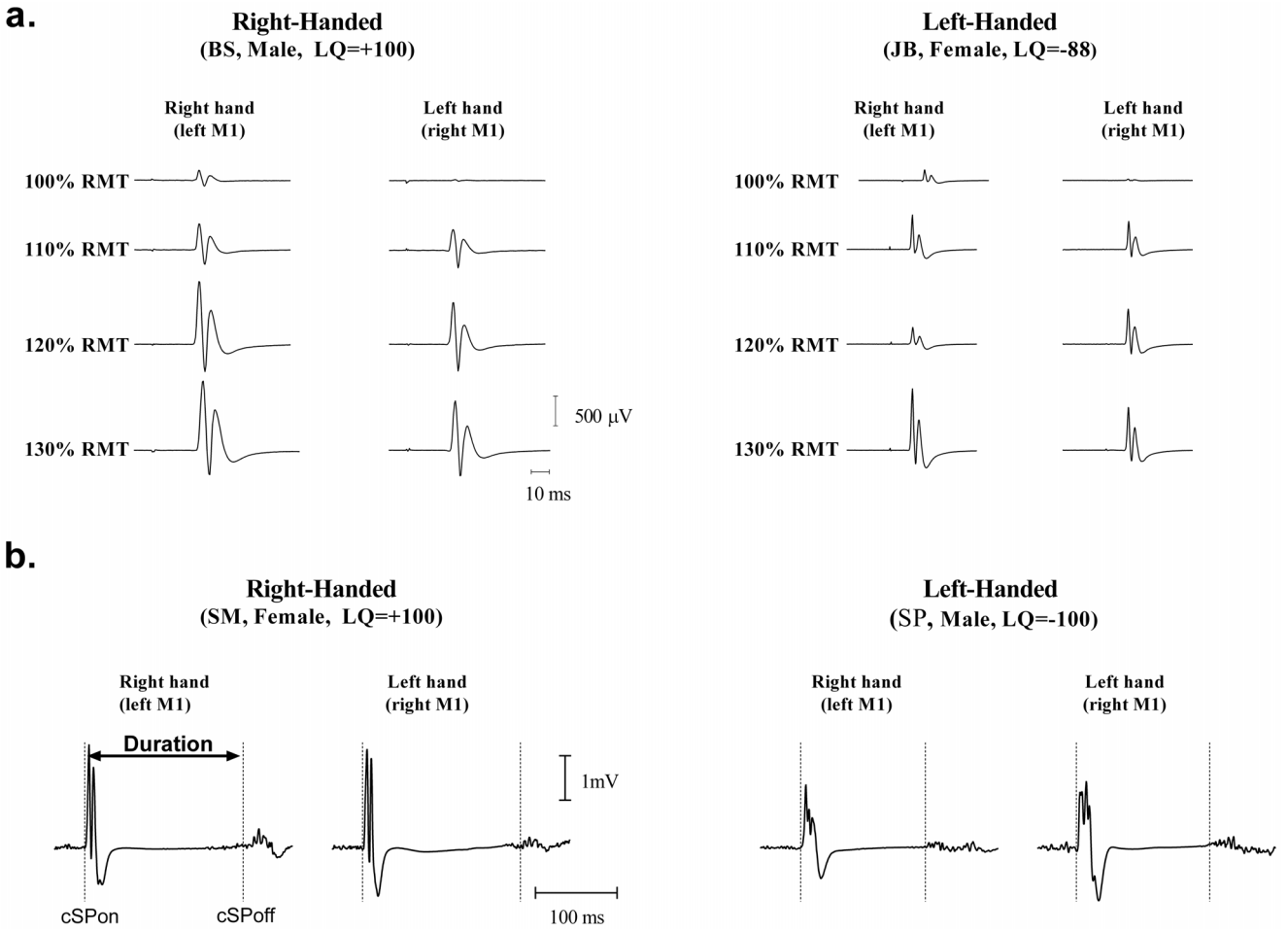

Figure 2. Hemispheric differences in corticospinal excitability in right-handed and left-handed participants. **a.** Examples of motor evoked potentials (MEP) amplitude recruitment in response to increasing stimulation intensity. **b**. Examples of recordings of contralateral silent period (cSP) obtained during active contraction with associated MEP facilitation. The two vertical dotted lines illustrate the approximate time for the onset and offset of the cSP. Note the relative symmetry in neurophysiological responses between the two hemispheres (**a** and **b)**. Abbreviations: RMT, resting motor threshold, LQ: laterality quotient.
doi:10.1371/journal.pone.0070286.g002

Hemispheric differences in Transcallosal Inhibition

As for measures of corticospinal excitability, measures of transcallosal inhibition derived from iSP recordings (i.e., LTI and DTI) were found to be largely symmetrical between hemispheres in all participants. However, some interesting differences emerged between groups. Such differences are illustrated in Figure 4 (a), showing examples of iSP recordings from two participants, one from the MH group and one from the LH group. In the MH participant, it can be seen that the onset of the ipsilateral inhibition (i.e., LTI) tended to be earlier and the period longer that than recorded in the LH participant. This difference in LTI was confirmed in the ANCOVA, where a highly significant group effect ($F_{2,29} = 10.3$, $p<0.001$) was detected; this factor alone accounting for >40% of the total variance. As shown in Figure 4 (b), post-hoc comparisons confirmed that LTI measures from the MH group were significantly shorter ($p<0.001$) than those derived from either the RH or LH group. In line with this, DTI measurements also tended to be longer in the MH group (Figure 4a), but this trend could not be confirmed in the ANCOVA ($F_{2,\ 29} = 1.61$, $p = 0.21$).

Further examination of overall variations in measures of transcallosal inhibition with respect to LQs revealed an interesting relationship. This relationship is illustrated in Figure 5, showing the distribution of individual LTI and DTI measures, after averaging right and left values, against corresponding LQs. It can be seen that participants with weaker degrees of handedness lateralization tended to show earlier LTI and longer DTI as compared to those with stronger degrees in either the rightward or leftward direction. Also evident in Figure 5 is the fact that each variable follows a non-linear distribution that matches to a large extent the distribution of LQs. In fact, curve-fitting analysis revealed a very good fit with a second order polynomial function for variations in LTI and a relatively good fit for variations in DTI (Figure 5). The same analysis performed with the two laterality indices revealed only a poor fit, however, for both LTI ($Dext_{li}$, $r^2 = 0.01$; $Speed_{li}$, $r^2 = 0.03$) and DTI ($Dext_{li}$, $r^2 = 0.02$; $Speed_{li}$, $r^2 = 0.10$).

Discussion

In this study, we examined hemispheric differences in selected measures of corticospinal excitability and interhemispheric inhibition to examine the influence of handedness in a group of participants who displayed different degrees of laterality as assessed by preference and performance measures. It was

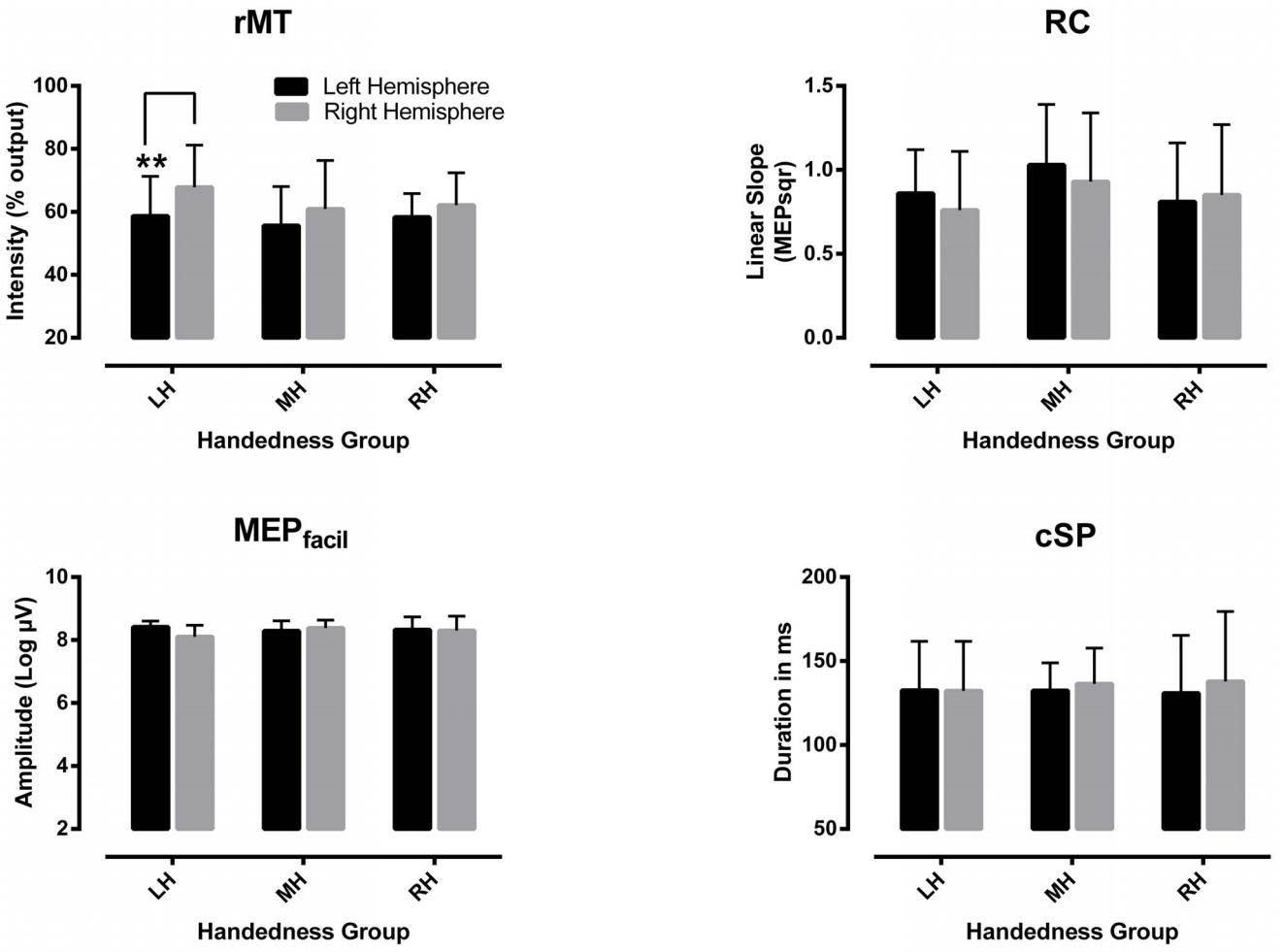

Figure 3. Mean variations (±1 SD) in measures of corticospinal excitability recorded between hemispheres in each group. In each graph, columns represent mean values computed from each hemisphere for all participants within each hand preference group (LH, left-handed group (n = 10), MH, mixed-handed group (n = 7); RH, right-handed group (n = 17). Abbreviations: rMT, resting motor threshold; MEP_{facil}, motor evoked potential facilitation during active contraction, RC, recruitment curve; cSP, contralateral silent period.** significant right-left difference as detected with a paired t-test.
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hypothesized that asymmetries would be revealed in individuals exhibiting higher degrees of lateralization either for the right or left hand when compared to those exhibiting lower degrees of lateralization. In general, our results did not support this hypothesis and revealed no major hemispheric asymmetries in relation to the degree of handedness for basic measures of corticospinal excitability, with the exception of rMT. Similarly, measures of transcallosal inhibition were found to be largely comparable between hemispheres and showed no asymmetry in relation to handedness. However, the same measures exhibited a distinct pattern of variations when compared across HP groups. In the next section, we will first examined issues related to measures and classifications of handedness. Then, we will interpret the significance of the present findings for the study of the neurophysiological correlates of handedness.

Measures of Handedness

In the present study, we attempted to address the issue of the neurophysiological correlates of handedness by moving from the simple right-left dichotomy to a three-way classification. However, one critical issue that arises when attempting to split handedness into more than two categories pertains to the definition of mixed handedness. In this study, we used the upper and lower quartiles of LQs as boundaries to sort out consistent handers (RH and LH groups) from less consistent handers (MH group). In this respect, our categorization is in line with the analysis performed by Dragovic [29] who demonstrated that a LQ of ±60 provided the best cut-off to separate mixed-handedness from right- and left handedness. In fact, in our MH group, LQs ranged from −55 to +57, which falls exactly within the optimal range described by Dragovic. According to the same author, such a cut-off point would allow extraction of ~20% of mixed-handed individuals in a given population, which represents the exact proportion extracted in this study (i.e., 7/34, 21%). Finally, the frequent observation that mixed handedness is closely associated with left handedness [30] was also confirmed in our MH group since the majority (5/7) displayed a leftward preference. Thus, our MH group exhibited the expected characteristics of individuals who show no clear preference for one hand, as reported in other studies using similar handedness categorization [29–31]. The fact that our classification represented valid categories along the handedness continuum was further confirmed by the high degree of correspondence found

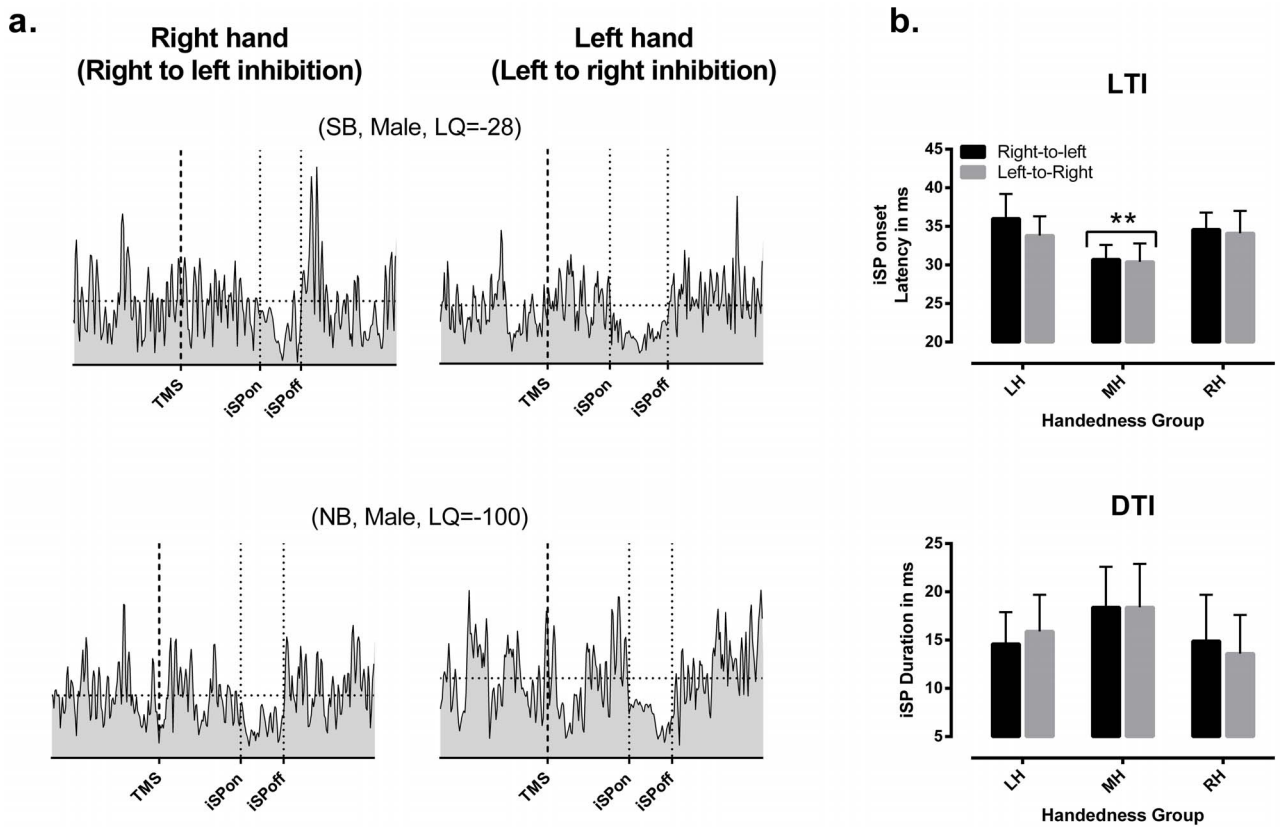

Figure 4. Hemispheric differences in transcallosal inhibition. a. Examples of ipsilateral silent period recordings (iSP) from a mixed-handed (MH) participant and a left-handed (LH) participants. In each recording, the averaged rectified electromyographic activity is shown to illustrate the approximate time points when the period ipsilateral inhibition was induced (i.e., iSP onset) and for how long it was maintained (i.e., iSP duration) after the stimulus delivery (1st thick dotted lines). The time delay between the 1st and 2nd dotted lines corresponds to the latency onset of transcallosally-mediated inhibition (LTI), whereas the time period delimited by 2nd and 3rd dotted lines corresponds to its duration (DTI). Note the earlier onset and the longer period of ipsilateral inhibition in the MH participant when compared to the LH participant. **b**. Mean variations (±1 SD) in measures of transcallosal inhibition recorded between hemispheres for each group. Note the significant difference (**$p<0.01$) observed between groups in LTI. A corresponding trend is also observed for longer DTI in the MH group, but this trend could not be confirmed statistically.
doi:10.1371/journal.pone.0070286.g004

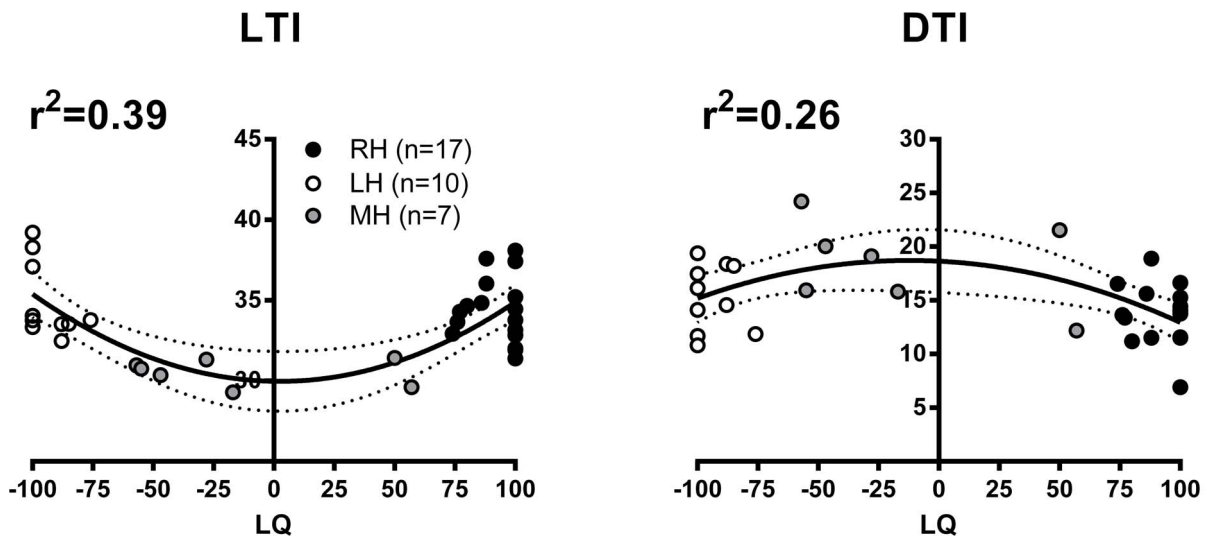

Figure 5. Variations in measures of transcallosal inhibition (onset latency and duration) as a function of laterality quotients (LQ) across all participants. Note the non-linear nature of the relationship for each variable, which fits with a 2nd order polynomial function. Note the relatively good fit, as judged from r^2 values, for variations in LTI measures and, to a lesser extent, with DTI measures. The dotted lines represent the 95% confidence interval.
doi:10.1371/journal.pone.0070286.g005

between preference measures (LQs) and actual asymmetries in performance revealed in behavioral tests. In this regard, the $Dext_{li}$ was clearly superior to the $Speed_{li}$ in reflecting the association between reported preference and actual performance. Similar observations with regard to the superiority of dexterity over tapping speed to assess differences in laterality have been made in other report where such associations have been examined [e.g., 30,32]. To summarize, our approach to categorize handedness using both preference and performance measures allowed us to identify a small subgroup of mixed-handed participants displaying the expected characteristics of individuals who are typically less lateralized than the majority. Interestingly, it is in this subgroup where significant differences were found at the neurophysiological level.

Handedness and Hemispheric Differences in Corticospinal Excitability

Contrasting with manual performance, basic measures of corticospinal excitability were largely comparable between hemispheres and were little influenced by the degree of handedness. In fact, the only asymmetry found was for a rightward elevation in rMTs in the LH group. In this regard our observations appear largely consistent with those of Bernard et al. [15], who performed similar comparisons of measures of corticomotor excitability (i.e., rMT, MEP size, motor mapping) and found no major asymmetries as a function of handedness using the same three-way classification as we used. As stated earlier, TMS studies have produced mainly conflicting evidence to support the existence of a strong link between hand dominance and laterality differences in corticospinal excitability [see 6 for a review]. For example, Livingston et al (2010) examined the influence of hand dominance, among other factors (e.g., gender), on interhemispheric differences using several TMS measures at rest, including rMT and MEP amplitude, in relatively large groups of right and left-handers and concluded that handedness had little influence. Bäumer et al. [33] reach a similar conclusion with regard to the influence of hand dominance on TMS measures of intra-cortical inhibition in right-handed and left-handed individuals. Interestingly, much like in the present study, these authors observed a similar rightward increase in rMTs in their participants regardless of handedness. The reason for this threshold asymmetry, particularly in left-handers, remains difficult to explain, but might be related to observations that left-handers tend to use their less preferred hand (i.e., right hand) more often than right-handers do, especially in tasks requiring fine visuo-motor control [e.g., 34]. In a recent study, Daligadu et al. [35] compared RCs in groups of right- and left-handers and found an asymmetry in excitability that favoured the non-dominant hemisphere over the dominant hemisphere. They reasoned that this asymmetry might represent a difference in motor representations whereby the non-dominant M1 may possess higher excitable elements confined to a smaller area whereas the dominant M1 would possess a larger representation composed of less excitable elements. While the observed threshold asymmetry in our LH group is consistent with this suggestion, we did not find, as reported by Daliglu et al, an asymmetry in RCs between hemispheres, which again highlights the difficulty in drawing any firm conclusion about lateralized differences in corticospinal excitability.

As for the other TMS measures obtained in the activate state, both MEP_{facil} and cSP were found to be of similar magnitude between hemispheres and neither showed evidence of asymmetry in relation to degree of handedness. Consistent with these observations, Priori et al. [36] found no difference between hemispheres in MEP facilitation elicited during tonic contraction in the FDI in both right- and left-handers, although they did report an asymmetry related to handedness for the SP, which was shorter in duration in the dominant hand/hemisphere. We did observe the same trend for shorter SP durations in the dominant hand/hemisphere in our groups, especially in the RH group, but the overall difference was not significant. This discrepancy between our results and those of Priori et al. [36] might be explained by the fact that they used different test intensities to assess SP durations (from 1 to 1.5×MT), whereas our measurements were based on a single test intensity (i.e., 1.2×MT). In fact, our observations are more in line with those of Braune and Fritz [37], who found a high degree of correspondence between hemispheres when measuring SP in small hand muscles in a large sample of healthy adults participants (n = 75). Thus, it seems that cortical circuits mediating inhibition during the SP are not subject to strong laterality effects in relation to handedness.

Handedness and Hemispheric differences in Transcallosal Inhibition

With regard to interactions between hemispheres, our observations revealed no asymmetries in relation to degrees of handedness in iSP measurements reflecting the onset (LTI) and depth (DTI) of transcallosal inhibition. Using bi-focal paired-pulse stimulation, De Gennaro et al. [12] made similar observations with regard to transcallosal inhibition and handedness, their results showing no differences between hemispheres in both right- and left-handers. The same report did find asymmetries, however, but these concerned only intrahemispheric measures (i.e., rMT and MEP amplitude), which led De Gennaro and colleagues to conclude that handedness was associated with asymmetries in corticospinal excitability but not in transcallosal inhibition. Interestingly, the Bäumer et al. [33] study, which we referred to earlier, reached the opposite conclusion, their findings pointing to a lateralized asymmetry in transcallosal inhibition in right- and left-handers, whereas intrahemispheric measures of excitability were not influenced by handedness. Again, such conflicting results illustrate the difficulties in trying to establish a link between handedness and lateralized differences in corticomotor excitability in TMS studies.

Although the current observations revealed no asymmetry in transcallosal inhibition between hemispheres, interesting differences still emerged when examining variations between groups. Indeed, one of the main findings of this study lies in the observation that participants in the MH group exhibited earlier LTI when compared to participants in either the RH or LH group. In line with this observation, MH participants also tended to show longer DTI, although this trend was not confirmed statistically. Still, both observations concur to suggest faster and deeper hemispheric interactions in the MH group than in the other two groups. Further support for this conclusion comes from the close association between the observed variations in both LTI and DTI measures and variability in LQs; the quadratic nature of the relationship highlighting the differences in the efficiency of transcallosal inhibition between less lateralized as opposed to strongly lateralized participants. In this respect, our observations appear entirely consistent with those of Bernard et al. [15], who observed that less lateralized individuals displayed features at the neurophysiological level that made them distinct from strongly lateralized individuals. For instance, Bernard et al observed that less lateralized individuals showed frequent occurrences of ipsilateral MEPs, a feature that suggests the existence of greater excitatory transcallosal connections in these individuals. Further to this, the increased occurrence of ipsilateral MEPs was associated with faster interhemispheric transfer time, as measured behaviourally with the Poffenberger task. Thus, both the present findings

and those of Bernard and colleagues (2011) support the notion that mixed-handedness is associated with faster and more efficient transcallosal communications, as detected with TMS measures. Enhanced transcallosal communications would allow for a greater degree of bi-hemispheric processing for action planning and execution in less lateralized individuals when compared to strongly handed individuals. Such a conclusion is further supported by anatomical evidence showing an inverse correlation between callosal thickness and degrees of handedness; individuals with weaker degrees of lateralization showing larger callosal dimensions in the anterior, mid-body and posterior regions [38].

Acknowledgments

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Author Contributions

Conceived and designed the experiments: TD FT. Performed the experiments: TD FT. Analyzed the data: TD. Contributed reagents/materials/analysis tools: TD FT. Wrote the paper: TD FT.

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Neurophysiological changes associated with history of concussions in sports in young adults

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ABSTRACT

The potential long term impacts of sport related concussions have been a growing concern among young athletes involved in contact sports. Recent studies suggest that even mild concussions can lead to chronic concussion related symptoms such as headache, dizziness and trouble remembering things or persons. However, this condition has often been associated with no significant long-term neuropsychological effects. Studies using transcranial magnetic stimulation (TMS) have found alterations in brain functions in athletes with a history of multiple concussions. In this study, we use TMS to investigate neurophysiological changes associated with concussions in young athletes. Behavioural and neuropsychological performance was also assessed. Participants consisted of a concussed group (n=16, >3 monthspost-concussion) and a control group (healthy controls matched for age and gender, n=16). Analysis of behavioural data revealed no difference between groups in dexterity, attention and memory task. No differences were also detected in basic measures of corticospinal excitability. However, further analysis did reveal significant between group differences (t-tests, $p<0.01$) in measures reflecting transcallosal inhibition derived from ipsilateral silent period (iSP). Participants in the concussion group showed delayed onset latency and shorter iSP duration (right to left inhibition only) when compared to the control group. These results, though still preliminary, suggest that concussion is associated in the long-term with neurophysiological changes affecting transcallosal inhibition between motor cortices.

Keywords: Transcranial magnetic stimulation, concussion, transcallosal inhibition, interhemispheric asymmetry, neuropsychology.

INTRODUCTION

In the last two decades, traumatic brain injuries (TBI) have been in the spotlight because of their high incidence in contact sports, notably in hockey and football players. Every year in North America, it is estimated that 1.6 to 3.8 million players will experience some form of TBI in the context of sport-related injuries [1]. In addition to a plethora of symptoms, TBI has been linked to an initial period of high-risk mortality and studies have found there is also an increased prevalence in late mortality [2,3]. The majority of sports-related head injuries (70-90%) are classified as mild TBI (mTBI), which is also designated as "concussions" [4]. Concussions or mTBIs are associated with various symptoms resulting from cerebral metabolic dysfunction and axonal shearing [5]. The most common include headaches, confusion, impaired working memory, dizziness and balance problems [5].

The detrimental effects of a concussion result primarily from the physical trauma and secondarily from the biochemical and physiological perturbations, both of which lead to neuronal loss and diffuse axonal injury [6]. The acute clinical symptoms of a concussion reflect a functional disturbance rather than a structural injury, thus, standard structural neuroimaging usually does not reveal any abnormalities in concussed individuals [7]. However, recent studies using diffusion tensor imaging have revealed that the corticospinal tract may be susceptible to white matter damage following a concussion [8,9]. Another recent review suggests that frontal associative areas as well as the commissural fibers of the corpus callosum (CC) are commonly damaged following an mTBI [10]. In line with this, a recent study revealed that callosal fibers projecting from M1 were particularly affected in formerly concussed female athletes [11].

The CC is a wide band of nerve fibers connecting the right and left hemispheres providing the primary pathway for the interhemispheric transfer of cognitive, somatosensory, motor, executive, and visual information [12,13]. Hence, damage to the callosal pathway following a concussion could partially explain a multitude of concussion symptoms. Additionally, a biomechanical analysis of concussions revealed that the CC alongside the thalamus had the greatest relative difference between strain levels for the concussion and no-injury cases [14].

The diagnosis of a concussion following a closed-head injury is difficult to establish for it occurs without leaving structural damages in most cases [15]. Therefore, a multimodal approach to concussion diagnosis using other investigative tools, such as transcranial magnetic stimulations (TMS), is required to properly assess the acute and long-term effects of concussions on the brain [16,17].

The majority of concussions (80% to 90%) resolve themselves in a relatively short period (i.e., 7 to 10 days) [18]. Nevertheless, there is growing evidence that sports-related concussions can lead to long-term neurological dysfunctions in the motor system with increasing concerns regarding their potential role in the development of chronic traumatic encephalopathy, as documented in professional athletes affected by multiple concussions over their career [19-23]. However, the current literature evaluating specific indices of changes in corticomotor excitability using transcranial magnetic stimulation (TMS) following a concussion remains quite limited. A study done by Livingston et al. (2010) revealed the presence of some alterations in the size and latency of motor evoked potentials (MEPs) in the acute stage after a concussion, but no change in central motor conduction time. Interestingly, these authors reported one case in whom TMS failed to evoke motor responses when tested 4 hours post-concussion, but whose excitability returned to normal 3 days later [24]. In another report, Chistyakov et al. 2001 described short-term

neurophysiological alterations following a TBI including elevated resting motor threshold and greater interhemispheric asymmetries in other measures of excitability. The presence of abnormal findings on the TMS measures was related to the severity of the brain injury [25].

While most of the reported alterations in the excitatory and inhibitory responses to TMS seem to normalize with time after the injury onset, persistent abnormalities associated with mTBIs have also been reported in some TMS studies. In this regard, the most consistent finding is the prolongation of the contralateral silent period [26-28]. The abnormally prolonged silent period, which reflects $GABA_B$-related intracortical inhibition, seems to be also correlated with the severity of symptoms and the number of concussions [27]. Subsequent investigations from the same group found that concussion is also associated with enhanced $GABA_B$-related long-interval intracortical inhibition (LICI) [29] and no effect on $GABA_A$-related short-interval intracortical inhibition (SICI) [18]. However, a recent study by Pearce and colleagues (2014) found that a history of concussion in Australian football players was related to a reduction in cSP duration, SICI and LICI. Additionally, these reductions in GABAergic inhibition were found to be related to impairments in dexterity and visuomotor reaction time associated [30]. Animal models have shown that an increase in GABAergic inhibition following a concussion may be beneficial in the acute stage; however, this may hinder recovery in the later stages [31], where GABAergic terminal loss was found to correlate with recovery following a TBI [32]. Thus, it could be surmised that alterations in GABAergic inhibition derived from TMS measures may be dependent on the time course of recovery experienced by each individual following a concussion.

In addition to alterations in the GABAergic system, there is also evidence for an alteration in cholinergic transmission in TBI, as suggested by a reduction in short latency afferent inhibition

(SAI) measured in severe TBI resulting in diffuse axonal injury [33,34]. These changes in SAI however were not found in concussed athletes [35]; although the evidence for this is still very limited.

Another persistent abnormality that has been noted in mTBI is an elevated resting motor threshold, which can persist long after the acute stage is resolved [36]; however, this finding was not found in other similar studies [27,37,38]. Such variability in findings can be expected given the heterogeneous patterns of non-specific alterations at the neurophysiological level following mTBI and the variable time course of recovery experienced by each individual. However, to our knowledge no TMS study has investigated the potential lasting effects of concussion on the integrity of motor transcallosal connections and how these potential alterations might be related to changes in functional performance.

Our primary objective in the present experiment was to determine whether there are lasting alterations in intra- and interhemispheric measures of cortical excitability associated with antecedents of concussion in young adults involved in contact sports. A secondary objective was to examine possible associations between the neurophysiological measures and functional measures of motor and cognitive performance.

MATERIALS AND METHODS

Ethics Statement

The Institutional Review Ethics Board approved the study procedure in accordance with the principles of the Declaration of Helsinki and informed consent was obtained before the experimental session. Participants also completed a questionnaire to ensure that there were no contra-indications to TMS. All assessments were performed in a controlled laboratory environment. Each participant received a small honorarium for his or her participation.

Participants

A total of 16 young adults with no history of concussions (NHC) and 16 young adults with a history of concussion (HC) were recruited from the population in the Ottawa, Ontario area. These two groups were matched according to age, height, weight, handedness, education level and gender (each group had 12 males and 4 females). All participants were right-handed, as determined by the Waterloo Handedness Questionnaire [39]. To be eligible in the HC group, the participant had to report at least one antecedent of concussion in a period of a least 6 months prior to testing. The participants were also screened to be cleared from post-concussion syndrome (self-reported). Typically, participants in the HC group had experienced multiple concussions (n=12) in the context of contact sports (e.g., ice hockey, football, soccer). The mean time elapsed between the testing session and the participant's latest concussion was 39.8 months with a range of 6 – 161 months. The relevant information regarding the antecedents of concussion in the HC groups along with demographic information from all participants is described in Table 1.

Neurocognitive and behavioural assessments

To assess the neurocognitive status, each participant was asked to complete an on-line version of the Immediate Post-Concussion Assessment and Cognitive Testing (ImPACT) battery (https://www.impacttest.com/). This test was done with the subject comfortably seated in a chair in front of a computer under the supervision of one of the researchers. ImPACT consists of six individual test modules that measure different neurocognitive abilities. ImPACT yields five composite scores, including verbal memory, visual memory, visuomotor speed, reaction time and impulse control.

To assess manual dexterity, participants completed the grooved pegboard test (GPT). The test was administered with each participant comfortably seated in front of a table. This test requires the participants to accurately place 25 grooved pegs in the corresponding holes as quickly as possible. The GPT was administered twice and then repeated for the other hand. Pinch strength was also assessed using a Baseline pinch dynamometer (Fabrication Enterprises Inc., Irvington, New York, USA). Mean individual performance for each hand was derived by averaging trials for the GPT (n=2 trials) and the pinch strength (n=3 trials).

Finally, motor speed tests were administered using the MOART™ panel with the accompanying PsymSoft II™ software (Lafayette Instruments., IN 47903) to measure simple reaction times (SRT) as well as a Go/NoGo reaction time task (GNG). For the SRT (n=20 trials/hand), participants were required to lift their index finger as quickly as possible in response to a green light. For the GNG task (n=25 trials/hand), the instructions were the same for the green light but, in addition, participants had to refrain from moving when a red light appeared instead of the green one (20% of the trial). Participants completed the tests with each hand with the order of

testing between the two counterbalanced across all participants. From all trials, individual mean values were computed for each test and each hand for further statistical comparisons.

EMG recording and TMS

Electromyographic (EMG) activity was recorded using small auto-adhesive surface electrodes (Ag/AgCl, Kendall Medi-Trace™ 130) placed over the first dorsal interosseous (FDI) muscles of the right and left hand. EMG signals were amplified (1000x) and filtered (bandwidth, 16 Hz to 1 kHz) with a polygraph amplifier (RMP-6004, Nihon-Kohden Corp.). Signals were digitized at 2 kHz sampling rate using custom software on a PC running under Microsoft Windows™ XP equipped with a digital/analogue acquisition card (PCI-6023, BNC-2090, National Instrument Corp.).

TMS was administered with participants comfortably seated in a recording chair. Magnetic stimulation was delivered with a Magstim 200 stimulator (Magstim Co. Dyfed, UK) connected to a figure-eight coil (90 mm outer loop diameter). To determine the optimal site to evoke MEP's in the contralateral hand muscles, participants were fitted with a Waveguard TMS compatible cap. A U-shaped neck cushion was used to restrain head movements and prevent neck fatigue. With the coil held ~45^{o} in the mid-sagittal plane, the approximate location of the hand motor area on the left hemisphere was explored in 1 cm steps until reliable MEP's could be evoked in the target muscle. This site was then marked with a red dot to ensure consistent coil positioning. After determination of this stimulation "hotspot", the coil was held in place manually by one of the experimenters. The experimenter frequently reassessed the coil position to ensure that it remained over the optimal stimulation site throughout the experiment. All testing took place

between 9am and 4pm to avoid any variability in corticospinal excitability which could be influenced by wakefulness [40].

From the TMS assessment, the following excitability measures were derived from each hemisphere: 1) Resting motor threshold (RMT), 2) MEP amplitude at rest, 3) short afferent inhibition (SAI), 4) contralateral silent period (cSP), and 5) ipsilateral silent period (iSP).

RMT. The RMT was determined using a software tool (TMS Motor Threshold Assessment Tool 2.0; Brain Stimulation Laboratory, Medical University of South Caroline, USA, http://www.clinicalresearcher.org/software.htm) developed to obtain fast and rapid estimates of the minimal intensity required to evoke reliable MEP's in the target muscle [41]. This technique is based on the maximum likelihood strategy for estimating motor thresholds and involves a pre-set stimulation pattern with the assumption of response failure (no MEP) for a subthreshold and a success for a suprathreshold stimulus intensity (MEP>50μV). In general, with this technique the RMT can be reliably estimated within 95% confidence limits with only 14 to 17 stimulations [42].

MEP Amplitude at rest. Resting MEPs (n=10) were obtained in the FDI at a stimulation intensity of 120% of RMT, while EMG was continuously monitored on an oscilloscope, at high gain, to ensure the absence of any muscle activity during the test. These trials were considered the "unconditioned" MEPs.

SAI. For the SAI, MEPs elicited at rest were conditioned by prior afferent stimulation 20 ms before the TMS pulse at 120% of RMT similar to the protocol described by Tokimura et al. [43]. The conditioning afferent stimulation consisted in the application of 200 μs pulses (S88 Stimulator, Grass Technologies, Astro-Med, Inc, West Warwick, RI 02893 U.S.A.) on the

median nerve at an intensity just above the motor threshold to evoke a minimal visible twitch of the thenar muscles. Similar to the unconditioned MEPs, 10 conditioned MEPs were elicited to assess SAI in each participant.

cSP and iSP. As in our previous studies [44,45], the cSP and iSP were measured concurrently using the protocol described by Giovannelli et al. [46], which involves bilateral contractions. On the hand ipsilateral to the stimulated hemisphere, participants were instructed to press as hard as they could on the pinch dynamometer using the thumb and index fingers. The iSP was elicited from this hand contracting maximally. Meanwhile, with the opposite hand contralateral to the stimulated hemisphere, participants were asked to lightly squeeze a soft exercise ball (~15% of the maximal voluntary contraction). From this hand, the cSP was elicited. Both hand contractions were maintained for 3 s (participants cued with a tone (550 Hz) generated by the computer) with the TMS pulse (130% RMT) being delivered at 2 s in the course of the trial (n=5/hand). The participants were told to focus on the maximally contracting hand ipsilateral to the stimulation and were given verbal feedback to maintain contraction. Short pauses (~30 s) were allowed between trials to prevent fatigue.

Analysis of MEP data and background EMG

All EMG data were stored for offline analysis. MEP amplitude (peak to peak) and latency data were obtained by averaging all trials (n=10) for each hand/participant. The level of SAI was assessed in terms of the percentage of the ratio obtained by comparing the conditioned MEPs with unconditioned MEPs (i.e., SAI= (avg. MEP_{cond}/avg. MEP_{uncond}) X100).

For the cSP and iSP, all trials were analyzed individually by visual inspection to determine their duration and derive other measures and then were averaged to get mean individual values for

each hand/participant. For the cSP, the duration was determined as the time interval between the onset of the facilitated MEP until a return in at least 50% of the background EMG. For the iSP, two parameters were measured for each individual trial. First, the onset latency of transcallosal inhibition (LTI) was determined as the time from the stimulus onset until the 1st sign of a sustained (>5 ms) decline in background EMG activity (>25% of prestimulus activity). The second parameters consisted of determining the duration of transcallosal inhibition (DTI) by measuring the time in ms from the onset of TI until the 1st sign of recovery in the background EMG, see Figure 1. From LTI measurements, we also computed the transcallosal conduction time (TCT) by subtracting the contralateral MEP latency (i.e. TCT in ms= LTI-MEP_{contra}lat), which provides a better estimate of central callosal transmission by eliminating the peripheral component involved in the LTI [47].

Statistical analysis

Right-left differences in the behavioural tests and TMS-derived measures were compared in each group with paired t-tests. When paired comparisons failed to reveal differences, right-left scores were averaged to perform between group comparisons using independent t-tests. Independent t-tests were also used to compare ImPACT composite scores. Linear regressions were performed on selected variables to examine the relationship between functional and neurophysiological measures. The presence of outliers was tested using the Grubb's test. Significance level for all tests was set at 0.05. All tests were performed using SPSS software version 21.0 for Windows™ (Chicago, IL, USA). Figures were prepared using GraphPad Prism version 5.00 for Windows™ (GraphPad Software, San Diego California USA, www.graphpad.com).

RESULTS

Comparison of neurocognitive performance

Performance in the ImPACT tests are shown in Table 2. Performance scores in the two groups were quite similar on the ImPACT battery; except for visual memory composite scores, where the HC group had significantly lower scores compared to the NHC group; $t(30) = 2.572$, $p = 0.015$.

Right-left differences in behavioural tests and association with HC

Right and left mean performances in the behavioural tests are shown in Table 3. As expected, both groups exhibited better performance in the GPT and strength tests ($p < 0.05$) in the right (dominant) hand when compared to the left hand. The HC group exhibited significantly faster SRT in the right hand of ($t(15) = 2.258$, $p = 0.039$), whereas this difference was not observed in the NHC group ($t(15) = 1.332$, $p = 0.203$). The GNG task did not reveal any significant difference between hands in the two groups. Between-group comparisons revealed no significant differences on any of the behavioural tests.

Right-left differences in TMS measures and association with HC

Table 4 shows the mean values of TMS measures computed in each group and for each hand. Paired comparisons revealed no significant right-left differences in either the NHC or the HC groups. Comparisons of averaged right-left values between groups also revealed no difference on any of the TMS measures of contralateral excitability, including the RMT, resting MEP, SAI or cSP duration. In contrast, comparisons of measures of transcallosal inhibition derived from the iSP revealed significant differences between groups. Figure 1 shows an example of differences

observed in iSP recordings between a HC and a NHC participant. As shown in Figure 2a, the DTI was found to be significantly shorter in the HC group when compared to the NHC group ($t(29) = 2.848$, $p = 0.008$). Similarly, both the LTI ($t(30) = -2.506$, $p = 0.018$) and TCT ($t(30) = -2.799$, $p = 0.009$ were found to be significantly delayed in the HC group compared to the NHC group (Figure 2 b and c). Note that the data from one participant in the NHC group had to be removed from the analysis as the Grubbs' test revealed he was a significant outlier ($p<0.01$) for DTI.

Association between iSP measures and performance in neurocognitive and behavioural tests

To further examine the impact of history of concussion on performance, Figure 3 shows the relationships observed between selected functional variables and measures of transcallosal inhibition derived from the iSP after averaging measures derived from both hands. As shown in Figure 2 (a and b), visual memory scores were significantly correlated with LTI, as well as with DTI values. Additionally, the TCT values were found to be significantly correlated to performance values obtained on the GNG task (2 c). LTI also tended to be related to values obtained on the GNG task (2 d).

DISCUSSION

This study investigated possible alterations in corticospinal excitability and transcallosal inhibition associated with a history of concussion in young athletes. Although the HC group performed similarly to the NHC group on most functional tasks and neurophysiological tests, a history of concussion was found to be associated with alterations in transcallosal inhibition

reflected in changes in iSP measures. Additionally, these changes in transcallosal inhibition were found to be related to changes in visual memory and the inhibitory control (i.e. GNG task).

Both NHC and HC groups had significantly better dexterity and strength in their dominant hand and the HC group also has quicker SRT with their dominant hand. However, this did not translate into asymmetries at the neurophysiological levels with all TMS-derived measures displaying similar values from either the left or right hemisphere.

The novel finding of this paper is the delayed LTI, prolonged TCT and shortened DTI found in the HC group relative to the NHC group, suggesting possible functional alterations in the transcallosal pathway. This observation is in line with results from Takeuchi et al. 2006 who found that the severity of a TBI was associated with a decrease in transcallosal inhibition derived from the iSP [6]. Additionally, these impairments in transcallosal function have been found to be correlated to memory and inhibitory control.

The lower scores of the HC group on the visual memory task indicates that experiencing a concussion can impair visual memory which has been documented as one of the cognitive symptoms associated with concussions [48]. Additionally, this memory impairment could be linked to alterations in the integrity of the transcallosal pathway as suggested by the correlation found between visual memory composite scores and iSP parameters. A recent study by McAllister et al 2014 using diffusion tensor imaging found that head impact was associated with damage to several brain regions including the CC, hippocampus, amygdala and thalamus [49]. In line with our correlation, the magnitude of change in mean diffusivity in the CC in that study was also associated with poorer verbal learning and memory [49].

Inhibitory control, as assessed with the GNG task, has been previously found to be significantly affected by concussions [50], yet no difference was found in our study although participants in the HC group tended to show longer response times (cf. Table 3). However, we did find that prolonged TCT was associated with longer response times on the GNG task. This suggests that delayed transcallosal transmission between motor cortices could contribute to reported alterations in executive inhibitory control in concussed athletes [9].

Our study has several limitations. For instance, we did not control for the age at which our HC group had their concussion. Many of our subjects had concussions at a relatively young age (average 21 years) making the CC more vulnerable to mechanical injury since young brains are believed to be more vulnerable to mTBIs [51,52]. In addition, the small sample size prevented further analysis to examine the impact of multiple concussions, which is a factor susceptible to lead to more permanent neurophysiological impairments and a higher symptom burden [53,54].

In conclusion, within the limitations of this study, our findings suggest that antecedents of concussion in young adults are not associated with major lasting changes at the neurocognitive or behavioural level. Similarly, basic measures of corticomotor excitability do not seem to be affected. However, our findings indicate possible alterations affecting the motor transcallosal pathway as a potential neurophysiological marker of lasting changes in cortical function secondary to concussion.

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Table 1. Demographic characteristics of the participants (mean ± sd)

Group	N	Age (years)	Height (cm)	Weight (kg)	Level of Education (years)	Time since last concussion (#months)	Number of concussions
No history of concussion (NHC)	16	24.4 ± 4.8	175.9 ± 11.1	75.6 ± 14.2	16.7 ± 2.2	NA	NA
History of concussion (HC)	16	24.3 ± 3.1	179.1 ± 11.1	79.4 ± 13.1	17.2 ± 2.0	39.8 ± 46.6	2.38 ± 1.1

Table 2. Composite neurocognitive scores derived from the ImPACT test (mean ± sd)

Group	Verbal memory	Visual memory	Visuomotor speed	Reaction time (ms)	Impulse control
NHC	89.1 ± 8.1	81.3 ± 7.1	43.9 ± 6.3	516.3 ± 52.9	3.5 ± 2.1
HC	83.5 ± 9.2	71.1 ± 14.3*	44.3 ± 6.9	535.0 ± 39.2	7.8 ± 9.7

NHC: No history of concussion; HC: History of concussion; *$p < 0.05$, independent t-test between groups

Table 3. Performance in behavioural tests (mean ± sd)

	NHC		HC	
Test	Right	Left	Right	Left
GPT (s)	53.6 ± 6.0 *	60.4 ± 6.7	54.6 ± 8.1 *	60.9 ± 9.6
SRT (ms)	204.1 ± 15.9	206.5 ± 17.0	209.5 ± 8.9 *	213.1 ± 9.6
Pinch Strength (kg)	22.7 ± 5.6 *	21.3 ± 5.1	23.4 ± 5.2 *	22.3 ± 4.3
GNG task (ms)	264.2 ± 20.6	266.8 ± 19.4	278.0 ± 29.7	284.4 ± 33.2

NHC: No history of concussion; HC: History of concussion; GPT: Grooved pegboard test; SRT: simple reaction time; GNG task: Go/NoGo task; *$p < 0.05$, paired t-test between hands

Table 4. Neurophysiological measures derived from TMS (mean ± sd)

	NHC		HC	
	Right	Left	Right	Left
RMT (% output)	40.4 ± 8.1	41.6 ± 8.7	36.8 ± 7.1	37.0 ± 7.6
Resting MEP (mV)	0.54 ± 0.32	0.50 ± 0.27	0.74 ± 0.61	0.77 ± 0.42
Resting MEP Latency (ms)	21.9 ± 1.7	22.0 ± 1.7	22.1 ± 2.2	21.9 ± 2.4
SAI (% MEP_{uncond})	0.37 ± 0.39	0.42 ± 0.31	0.46 ± 0.33	0.37 ± 0.22
cSP(ms)	147.7 ± 26.5	144.7 ± 28.6	138.2 ± 41.5	145.6 ± 34.6
DTI (ms)	17.1 ± 5.9	16.8 ± 5.1	11.2 ± 2.7	13.7 ± 5.6
LTI(MS)	34.5 ± 2.2	34.0 ± 2.0	35.9 ± 2.2	35.9 ± 1.9
TCT (ms)	12.5 ± 1.8	12.0 ± 1.8	14.0 ± 2.4	13.8± 1.8

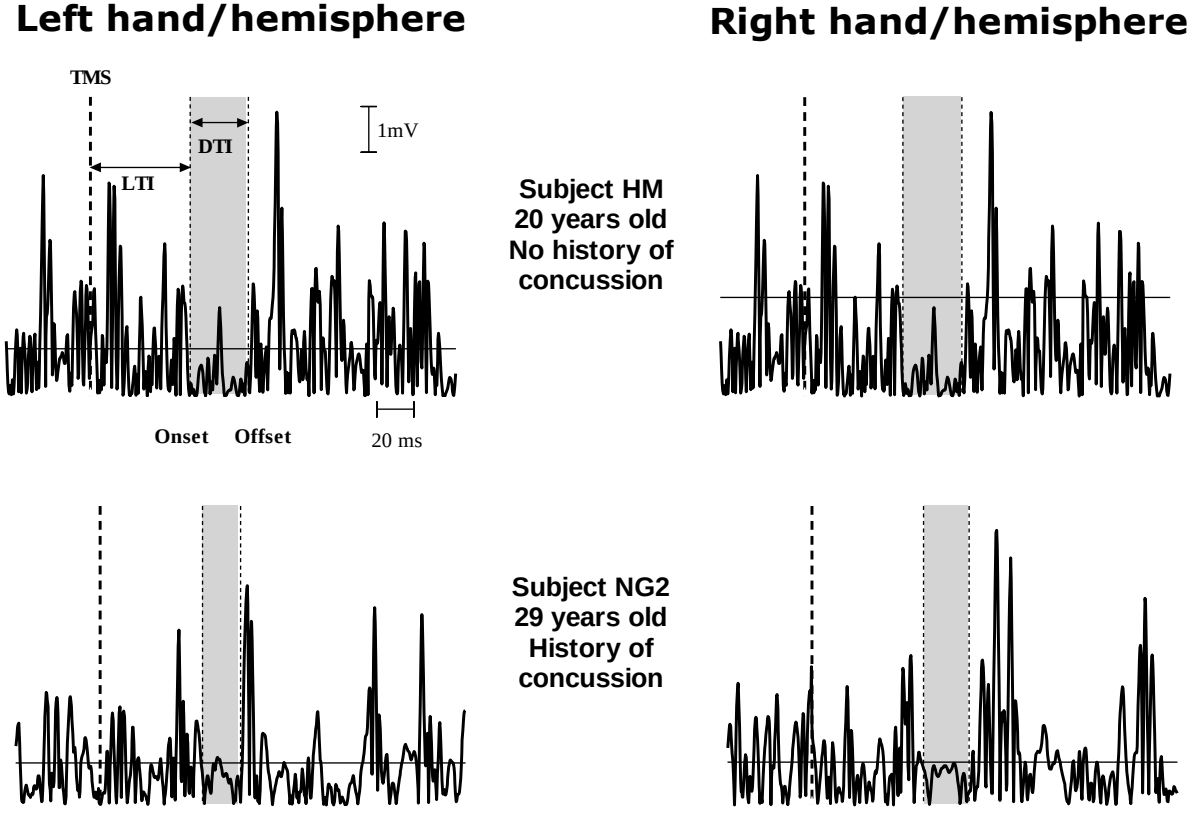
NHC: No history of concussion; HC: History of concussion; RMT: Resting motor threshold; MEP: Motor evoked potential; SAI: Short-latency afferent inhibition; cSP: Contralateral silent period; DTI: Duration of transcallosal inhibition; LTI: Latency of transcallosal inhibition; TCT: Transcallosal conduction time. Note that right and left refer to the hand tested and not the stimulated hemisphere (i.e., right RMT means that the left hemisphere was stimulated, while right DTI means that the left hemisphere was stimulated). Note also the absence of significant differences between measures from each hand (paired t-tests).

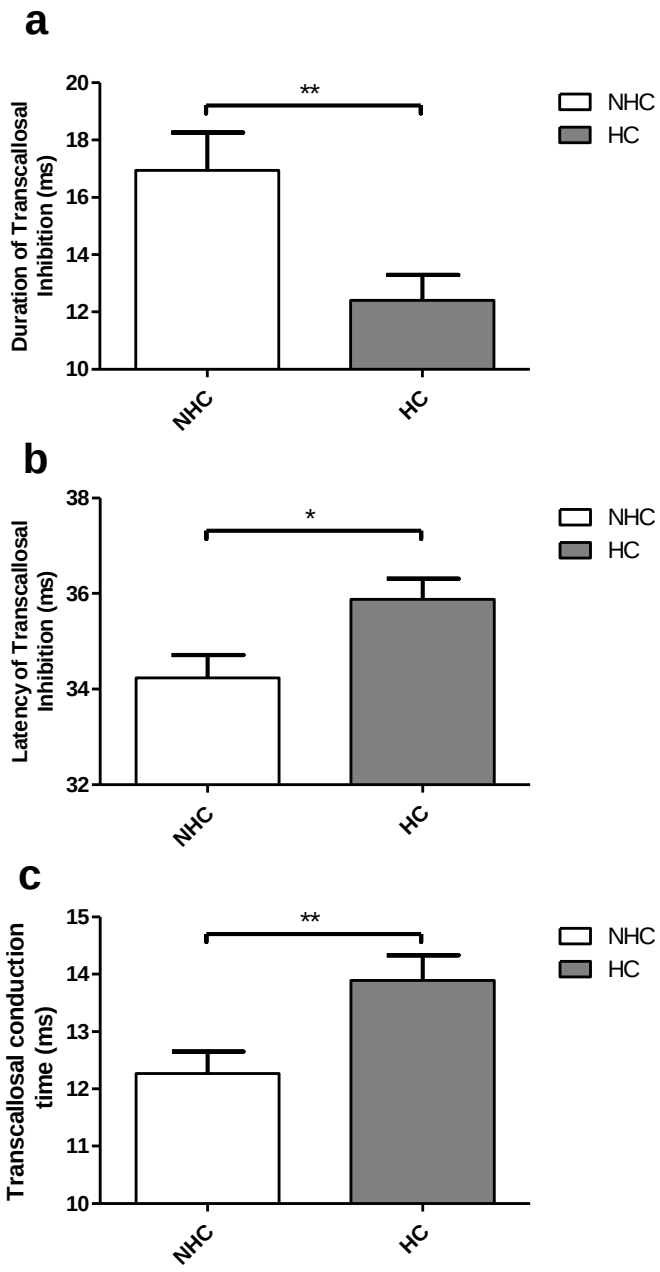
FIGURE LEGENDS

Figure 1. Individual examples of ipsilateral silent period measured in a NHC and a HC. Examples of ipsilateral silent period recordings (iSP) from a participant with no history of concussion (NHC) and a participant with history of concussion (HC). In each recording, the averaged rectified electromyographic activity is shown to illustrate the approximate time points when the period ipsilateral inhibition was induced (i.e., iSP onset) and for how long it was maintained (i.e., iSP duration) after the stimulus delivery (1^{st} thick dotted lines). The time delay between the 1^{st} and 2^{nd} dotted lines corresponds to the latency onset of transcallosally-mediated inhibition (LTI), whereas the time period delimited by 2^{nd} and 3^{rd} dotted lines corresponds to its duration (DTI). Note the lack of differences between hemisphere and the relative decrease in the duration of the iSP in the HC subject.

Figure 2. Between-group differences in measures of transcallosal inhibition. **a**.Difference in the duration of transcallosal inhibition (DTI) **b**. Difference of the latency of transcallosal inhibition (LTI). **c**. Difference of transcallosal conduction time (TCT=LTI-MEPlatency). Note that each column represent the mean computed in each group after averaging right-left values across participants. * $p<0.05$, ** $p<0.01$ independent t-tests.

Figure 3. Association between measures of transcallosal inhibition and selected functional measures. a. Association between visual memory composite scores and the duration of transcallosal inhibition (DTI). **b**. Association between the visual memory composite score and the latency of transcallosal inhibition (LTI). **c**. Association between inhibitory control, as assessed using the Go/NoGo task (GNG), and transcallosal conduction time (TCT). **d**. Assocaition between the GNG and LTI.


Left hand/hemisphere
Right hand/hemisphere
TMS
DTI
LTI
1mV
Onset
Offset
20 ms
Subject HM
20 years old
No history of
concussion
Subject NG2
29 years old
History of
concussion


a
Duration of Transcallosal Inhibition (ms)
20
18
16
14
12
10
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NHC
HC
NHC
HC
b
Latency of Transcallosal Inhibition (ms)
38
36
34
32
*
NHC
HC
NHC
HC
c
Transcallosal conduction time (ms)
15
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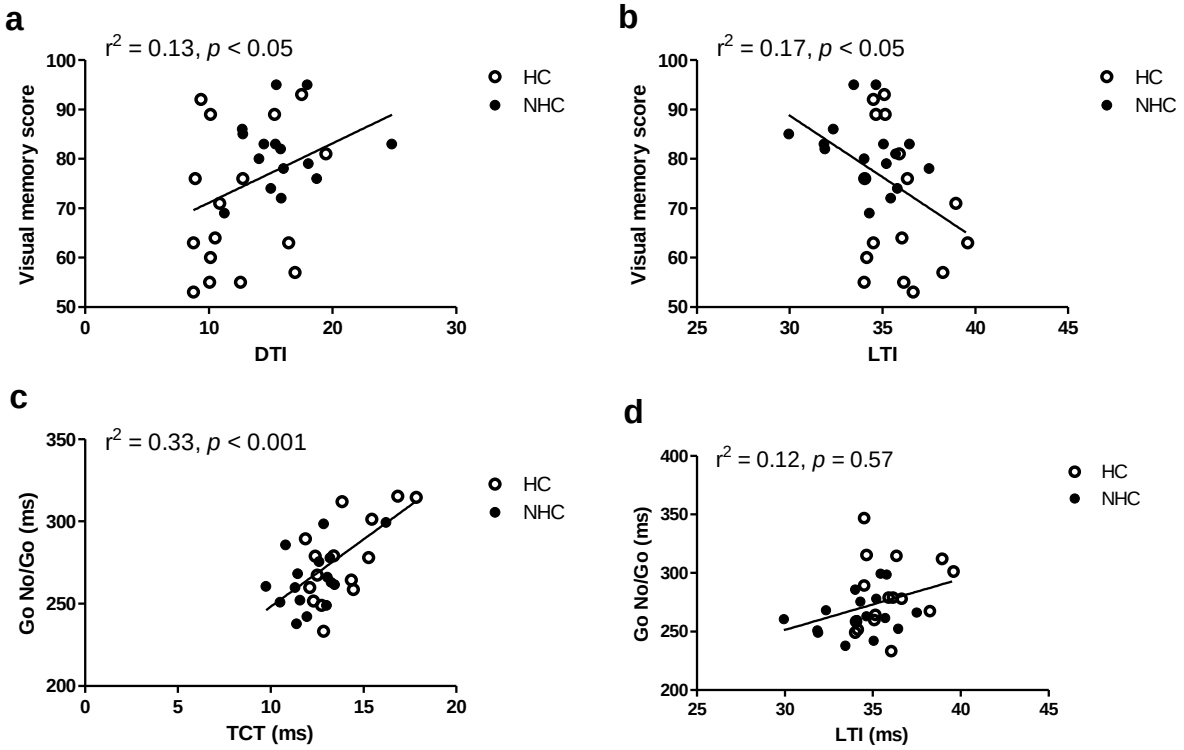
a
r2 = 0.13, p < 0.05
Visual memory score
DTI
HC
NHC
b
r2 = 0.17, p < 0.05
Visual memory score
LTI
HC
NHC
c
r2 = 0.33, p < 0.001
Go No/Go (ms)
TCT (ms)
HC
NHC
d
r2 = 0.12, p = 0.57
Go No/Go (ms)
LTI (ms)
HC
NHC

Relationship between transcranial magnetic stimulation intensity and ipsilateral inhibition induced in small hand muscles

To be submitted to Brain Stimulation

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ABSTRACT

Background: Transcranial magnetic stimulation (TMS) is an effective non-invasive method to assess the excitability of motor cortical circuits both within and between hemispheres via the contralateral and ipsilateral silent period (cSP- iSP), respectively. Stimulation intensity has been shown to influence the cSP duration, although the influence of this parameter on the iSP, and the extent of transcallosal inhibition, remains poorly characterized.

Objective/Hypothesis: To examine the relationship between stimulation intensity and the duration of contralateral and ipsilateral inhibition induced in small hand muscles to determine which intensity could be optimal when assessing transcallosal inhibition through the iSP.

Methods: Testing consisted of obtaining concurrent measures of the cSP and iSP at stimulation intensities ranging between 110% and 140% of the resting motor threshold (RMT) in fifteen healthy young adults. The duration of the cSP was computed in relation to intensity while both the durations and onset latency of transcallosal inhibition were derived from the iSP (DTI and LTI).

Results: Both the cSP duration and DTI linearly increased with stimulation intensity; however, the rate at which they increased were not correlated to one another. The probability of eliciting a measurable iSP also increased with increasing intensity up to 130% RMT before reaching a plateau at 140% RMT.

Conclusion(s): Although independent from one another, the cSP and iSP increase linearly in duration with increasing stimulation intensity. Our results indicate that a stimulation intensity of 130% of RMT seems to be optimal in healthy adults to reliably elicit iSP without overstimulation.

Keywords: transcranial magnetic stimulation, intensity, silent period, muscle contraction

INTRODUCTION

When applied to the primary motor cortex (M1), transcranial magnetic stimulation (TMS) is an effective non-invasive tool to assess corticospinal and transcallosal output. Corticospinal output can be assessed when a single TMS pulse is delivered to the contralateral M1 through recording of motor evoked potential (MEPs), which can be facilitated when the target muscle is actively contracting [1]. A facilitated MEP with contraction is usually followed by a period of decreased electromyographic activity (EMG) which is referred to as the contralateral silent period (cSP). The cSP is, for the most part, of cortical origin and is used as a measure to probe the excitability of intra-cortical inhibitory circuits [2]. The amplitude of the MEPs and duration of the cSP are dependent on TMS intensity both increasing with intensity until they eventually reach a plateau [3, 4]. Hence, sigmoid-shaped stimulus-response (S-R) curves can be constructed by plotting the relationship between MEP amplitude or cSP duration and stimulus intensity [3, 5]. The S-R curve for MEP and cSP provides an accurate, comprehensive, and clinically applicable method of evaluating corticospinal excitability and inhibition then the commonly used approach of measuring MEP amplitude or cSP duration at one stimulus intensity [5, 6].

As for transcallosal output, it can be also assessed with single pulse TMS via the ipsilateral silent period (iSP), which is an attenuation of EMG activity elicited when high intensity TMS is delivered to the MI ipsilateral to the maximally contracting limb muscles [7]. The absence or reduction of iSP in patients with callosal lesions or those with callosal agenesis provide evidence of the involvement of the transcallosal pathway in mediating ipsilateral inhibition [8, 9]. Indeed, the iSP is thought to be largely mediated by transcallosal fibres from the stimulated M1 to the non-stimulated MI, inhibiting the motor output of the ipsilaterally contracting muscles. Much

like the cSP, two studies found that higher absolute TMS stimulation intensity progressively increased the duration of transcallosal inhibition [8, 10]. These studies, however, used high stimulation and did not adjust the level according to individuals' threshold. Hence, there is still limited information with regard to the nature of this relationship, especially at lower near-threshold TMS intensities.

Since the iSP has a higher appearance threshold than the cSP [7, 11], it seems relevant to determine which stimulation intensity could be optimal to elicit iSP while avoiding an under or overstimulation minimum. Additionally, due to methodological differences between studies, results concerning the iSP are often difficult to compare from one study to the other. Beyond intensity, another factor known to influence iSP is the engagement of the contralateral MI in the task as shown by Giovannelli et al [12]. Indeed, these authors showed that voluntary MI activation associated with either real or imagined contraction of the contralateral hand muscles significantly enhanced the iSP elicited in the homologous muscles of the opposite hand in response to high intensity (fixed at 120% of the resting motor threshold) of the ipsilateral M1. They concluded that engaging the contralateral M1 during ipsilateral stimulation of the opposite MI was a critical factor to study interhemispheric inhibition through the iSP.

In the present study, we sought to further examine the relationship between stimulation intensity and the duration of contralateral and ipsilateral inhibition induced in small hand muscles to determine which intensity could be optimal when assessing transcallosal inhibition through the iSP.

MATERIAL AND METHODS

Ethics statement

The study procedures were approved by the Research Ethics Board at the Bruyère Research Institute, Ottawa, Ontario, Canada. Written informed consent was obtained from all participants in accordance with the Declaration of Helsinki prior to participation. All assessments were performed in a controlled laboratory environment. Each participant received a small honorarium for his or her participation.

Participants

Fifteen young healthy adults (19-33 years) participated in this study. All participants were right-handed as determined by the Edinburg Handedness Inventory [13]. Before testing, all participants completed a medical questionnaire to assess their general health and to ensure that there were no contra-indications to TMS. The demographic characteristics of participants are described in Table 1.

Electromyographic recordings and TMS procedures

TMS was administered with participants comfortably seated in a recording chair. Magnetic stimulation was delivered with a Magstim 200 stimulator (Magstim Co. Dyfed, UK) to the dominant hemisphere connected to a figure-eight coil (90 mm outer loop diameter). MEPs were recorded using small auto-adhesive surface electrodes (Ag/AgCl, Kendall Medi-Trace™ 130) placed over the first dorsal interosseous (FDI) muscles. FDI EMG activity and MEPs were monitored from both hands since cSP and iSP were elicited concurrently (see below). EMG

signals were amplified and filtered with a time constant of 0.03 s and a low-pass filter of 1 kHz (AB-621G Bioelectric amplifier, Nihon-Kohden Corp., CA 92610). Signals were digitized at a rate of 2 kHz (PCI6023, BNC-2090, National Instrument Corp.) and further relayed to a laboratory computer running custom software to control acquisition.

Each participant was fitted with a Waveguard TMS compatible cap (ANT North America Inc, WI 53719) to determine the FDI "hotspot". The participants rested their head in a U-shaped neck cushion was used to restrict head movements and prevent neck fatigue. With the coil held ~45^{o} in the mid-sagittal plane, the approximate location of the hand motor area was explored in 1-cm steps until reliable MEPs could be evoked in the target muscle. This site was then marked on the cap with a sticker to ensure consistent coil positioning. After determination of this stimulation "hotspot", the coil was held in place manually by one of the experimenters. The coil position was frequently reassessed to ensure that it remained over the optimal stimulation site throughout the experiment. To avoid diurnal variations in corticospinal excitability, TMS testing sessions took place between 9am and 4pm [14].

Measures of corticospinal excitability

The resting motor threshold (RMT) was determined on the left hemisphere using a software tool (TMS Motor Threshold Assessment Tool 2.0; Brain Stimulation Laboratory, Medical University of South Caroline, USA, http://www.clinicalresearcher.org/software.htm) developed to obtain fast and rapid estimates of the minimal intensity required to evoke reliable MEP's in the target muscle [14]. This technique is based on the maximum likelihood strategy for estimating motor thresholds and involves a pre-set stimulation pattern with the assumption of response failure (no MEP) for a subthreshold and a success for a suprathreshold stimulus intensity (MEP>50μV). In

general, with this technique the RMT can be reliably estimated within 95% confidence limits with only 14 to 17 stimulations [15].

TMS procedures for cSP and iSP recruitment

Prior to testing cSP and iSP, the maximal voluntary contraction (MVC) was estimated in the FDI muscle by asking participants to exert a maximal pinch force against a Baseline pinch dynamometer (Fabrication Enterprises Inc., Irvington, New York, USA) using a thumb-index lateral pinch. Participants performed three MVCs each lasting 3s, the average of the three trials was considered as their MVC. The cSP and iSP were measured concurrently using the approach described by Giovannelli et al. [12], which involved maximal contraction of the hand ipsilateral to the stimulated hemisphere coupled with light contraction of the contralateral hand. For cSP/iSP testing, participants were instructed to press as hard as they could on the pinch dynamometer using the thumb and index fingers with the left hand, while lightly squeezing another pinch dynamometer with their right hand. The latter contraction corresponded, on average, to ~15% of the MVC. Participants were trained to maintain the contractions for 3 s in sync with a tone (550 Hz) generated by the computer. The subjects were told to focus on the maximally contracting hand ipsilateral to the stimulation and were given verbal feedback to maintain contraction. In each trial, TMS was delivered on the left hemisphere ipsilateral to the maximally contracting hand at 2 s in the course of the trial. To test the influence of intensity, TMS pulses were delivered at four different intensities relative to the RMT: 110%, 120%, 130% and 140%. These stimulations were presented according to a predetermined random sequence in a block of 20 trials (5trials/intensity). The inter-trial interval was 10–15 s. Additional short 60s pauses of rest every 3 trials were allowed to prevent fatigue.

TMS data analysis

All analysis was performed off-line by the same investigator (TD) using numerically coded files. The methodological approach to analyze to determine the cSP and iSP duration has been described in detail elsewhere [17]. Briefly, we performed a trial-by-trial analysis to estimate the duration of cSP and to measure the amplitude of the associated MEP facilitation. The duration of the cSP was the time interval from the onset of the MEP to the return of at least 50% of the mean pre-stimulus background EMG activity. For iSP recordings, we adopted a trial-by-trial visual analysis to identify the prevalence of occurrence of the iSP and to derive the latency and duration of transcallosal inhibition (LTI and DTI). LTI is the time from the stimulus onset until the onset of the iSP defined as the 1st sign of sustained decline (>25% for at least 5 ms) the mean rectified EMG activity level. The DTI was the time between the iSP onset until the 1st sign of recovery in the background EMG activity (i.e., iSP offset). The latter time point is relatively easy to determine, as the end of the myoelectric silence is generally followed by an abrupt return of EMG activity in the recovery period (see Figure 2 for examples of iSP recordings). An iSP was deemed present if the EMG fell below the average EMG preceding the stimulus for a minimum of 5 ms [18] at a latency between 25 and 45 ms after the TMS pulse, the latter time point chosen to avoid the 2nd phase inhibition which is found relatively commonly in the FDI [19]. The pre-stimulus EMG amplitude was rectified and averaged for the 500ms prior to the stimulus. The probability of eliciting an iSP was calculated as the ratio of iSP present per attempt (# iSPs present/ #stimulations applied).

Statistical analysis

To determine how stimulation intensity (110% - 140% of RMT) influences measures of intra-hemispheric excitability and interhemispheric inhibition, we performed a series of repeated measures analysis of variance (ANOVA). The significance level was set at $p<0.05$ for detection of main effects and interactions. Post-hoc comparisons were performed using Tukey's test. Linear regression analyses were used to examine and establish the curve for the relationship between intensity of the stimulation and silent period duration. Most analyses were performed using SPSS software version 17.0 for Windows™ (Chicago, IL, USA). GraphPad Prism version 5.00 for Windows™ (GraphPad Software, San Diego California USA, www.graphpad.com) was used to prepare illustrations.

RESULTS

The average pre-stimulus EMG did not significantly differ between intensities ($F(3,42) = 0.568$, $p =0.64$) and was not related to the duration of either silent period ($p > 0.20$).

Effect of stimulation intensity on cSP

Stimulation intensity had no effect on the amplitude of MEP facilitated associated with the cSP ($F(3,42) = 2.19$, $p =.10$). However, as expected, there was a strong main effect of intensity on the duration of the cSP ($F(3,42) = 85.05$, $p <.001$). Post hoc analysis revealed that the duration of the cSP significantly increased ($p<.001$) with each 10% of RMT increase in stimulation intensity. A preliminary linear regression revealed that intensity was responsible for 46% of all variations

(r^2=.46) in the duration of the cSP with a slope of 1.96. Further analysis using only the average values derived from all participants at each intensity produced an almost perfect linear relationship (r^2=.99) as shown in Figure 1 a.

Effect of stimulation intensity on iSP parameters (DTI and LTI)

Stimulation intensity was found to have a large main effect ($F(3,42) = 13.11$, $p < 0.001$) on the probability of eliciting an iSP following a TMS pulse. As shown in Figure 1 b, post hoc analysis revealed that iSP's were elicited less frequently at 110% RMT (Mean, 57 % ± 37 %) than at all other intensities tested ($p<0.05$). The probability of obtaining an iSP at 120% RMT (79.3 % ± 25 %) was significantly less ($p<0.01$) than at either 130% (97.3 % ± 7 %) or 140% RMT (94.7 % ± 12 %), whereas no significant difference was detected between the two latter points ($p=.33$).

With regard to DTI and LTI, stimulation intensity had no effect on LTI ($F(3,42)=1.49$, $p =0.24$) but a large main effect was found on DTI ($F(3,33) = 37.19$, $p <0.001$). Post hoc analysis revealed that DTI increased significantly ($p<.05$) with each increase in stimulation intensity. Linear regressions revealed that stimulation intensity explained 38% of all variations (r^2=.38) in DTI with an average slope of 0.38. When a regression was performed on the overall mean values computed at each intensity, a near perfect linear relationship was found (r^2=.97), as shown in Figure 1 c. An example of the impact of stimulation intensity on the iSP duration is shown in Figure 2.

Relationships between stimulation intensity-related change in cSP and iSP

To further address the impact of intensity on ipsilateral and contralateral inhibition, additional correlations were performed to determine whether intensity-induced change in cSP duration were

related to those measured in the iSP. The slopes of the recruitment curve for the cSP and DTI were unrelated (r^2>0.1, p=0.75) denoting that the rate of increase in DTI with intensity were not associated with that measured in the cSP.

DISCUSSION

Our main findings are that both cSP and iSP (DTI) durations are independently and linearly affected by TMS increasing intensity from 110% to 140% RMT. Additionally, the probability of successfully eliciting an iSP does not seem to increase beyond an intensity equivalent to 130% of RMT.

The observation that facilitated MEPs associated with the cSP were unaffected by increasing stimulation intensity was expected, as MEPs are already under large facilitatory influences in the presence of active contraction. In fact, recent research has found that although resting MEPs may increase past 140% of RMT [20], MEPs elicited with contraction have been found to saturate around 140% of active motor threshold, which is equivalent roughly to 120% RMT [4].

As expected, both the cSP and iSP durations were significantly affected by stimulation intensity, as evidenced by the large portion of the variance accounted for by this factor in the present study (i.e., 46% and 38%, respectively). On the other hand, the two measures of central inhibition were affected independently by the intensity as revealed by the lack of correlation between their recruitment slopes. This reinforces the notion that they are mediated by distinct central mechanisms and different groups of cortical interneurons. This finding also highlights the importance of carefully examining stimulation parameters before concluding that differences in

iSP or cSP duration are due to alterations in intracortical or interhemispheric inhibition, given that such differences may reflect different sensitivity to stimulation intensity.

In the present study, the probability of eliciting an iSP increased with increasing intensity, but reached a plateau at 130% RMT. This indicates that an intensity of 130% RMT is sufficiently strong to reliably elicit an iSP on each stimulation in healthy individuals. In fact, increasing the stimulation intensity beyond this point did not significantly increase the probability of eliciting iSP, nor did it translate into longer iSP duration. On this basis, it would seem appropriate to recommend 130% RMT as an optimal intensity when the goal is to study interhemispheric inhibition through the iSP. The use of stronger intensities seems unjustified for it not only reduces the comfort for participants but also reduces the focality of the stimulation leading to activation of large extent of the motor representation [21-22].

While a stimulation intensity of 130% of RMT is recommended for healthy individuals, clinical populations that may have impairments in the transcallosal pathway, such as multiple sclerosis, stroke or traumatic brain injuries, may require higher levels of stimulation to consistently elicit iSPs. Still, on the basis of the present results, eliciting iSPs at 130% RMT would seem an appropriate intensity to begin even in clinical populations when exploring potential changes in interhemispheric inhibition relative to control groups.

Another caveat of the current research is that these results can only be generalized to studies using a monophasic TMS stimulator to obtain iSPs in the FDI. The presence and duration is dependent on the muscles being stimulated, with distal hand muscles demonstrating more transcallosal inhibition than proximal arm muscles [7]. Biphasic stimulation is associated with a longer cSP [23] and past studies have successfully obtained iSPs by stimulating at 120% of RMT

with a biphasic stimulator [24]; thus suggesting that a stimulation intensity lower than 130% of RMT may be optimal when using a biphasic stimulator. It is important to note, however, that studies examining changes in excitability in clinical populations rely almost exclusively on monophasic stimulation.

In conclusion, standardized stimulation techniques are needed in order to have consistency between studies utilizing the iSP to assess transcallosal inhibition in clinical populations. Our results suggest that a relative intensity of 130% of the participant's RMT seems optimal to elicit reliable iSP on each trial when the FDI is the target muscle and using a monophasic stimulator.

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Table 1.

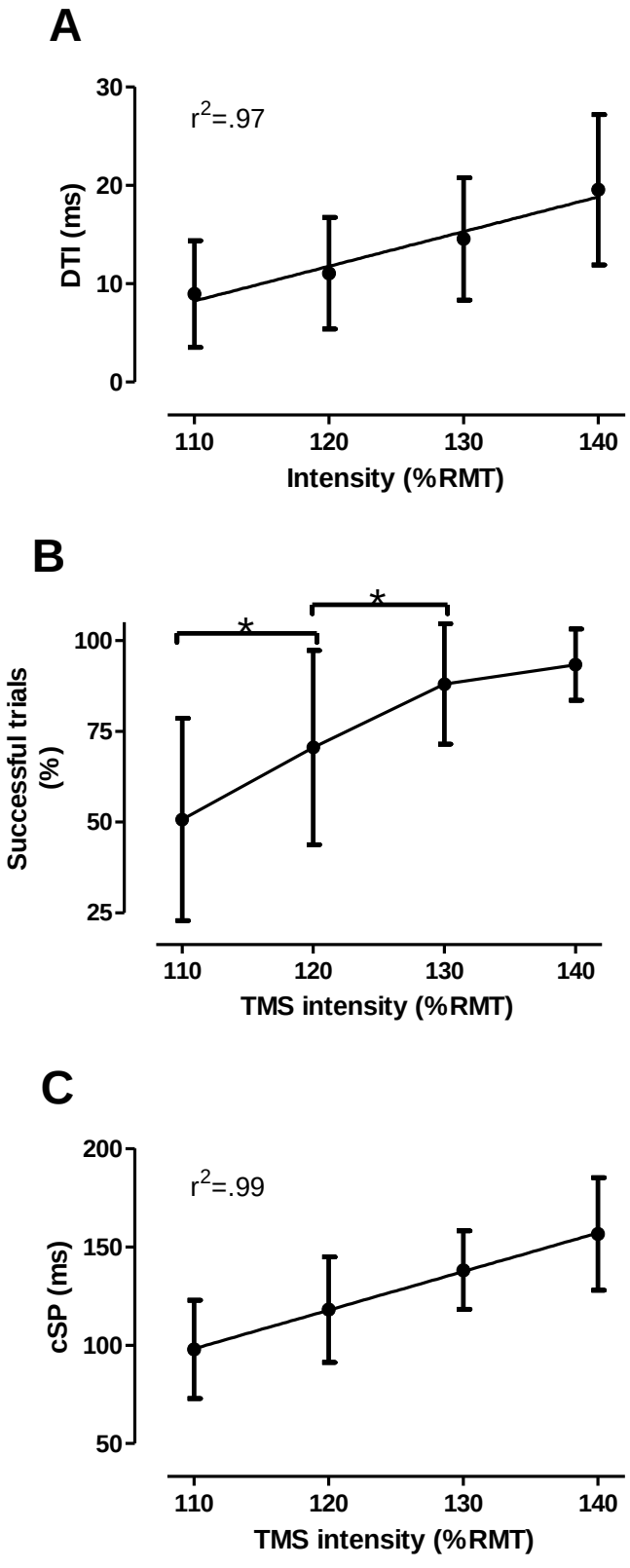
Demographic data of the participants (mean ± standard deviation)

	Sex	Age (years)	Pinch Strength (kg)		Resting motor threshold (% stimulator output)	
			Right	Left	Right	Left
Participants	9 ♂ 6 ♀	23.9 ± 4.1	9.5 ± 2.1	9.0 ± 2.3	36.3 ±5.2	37.3 ±5.5

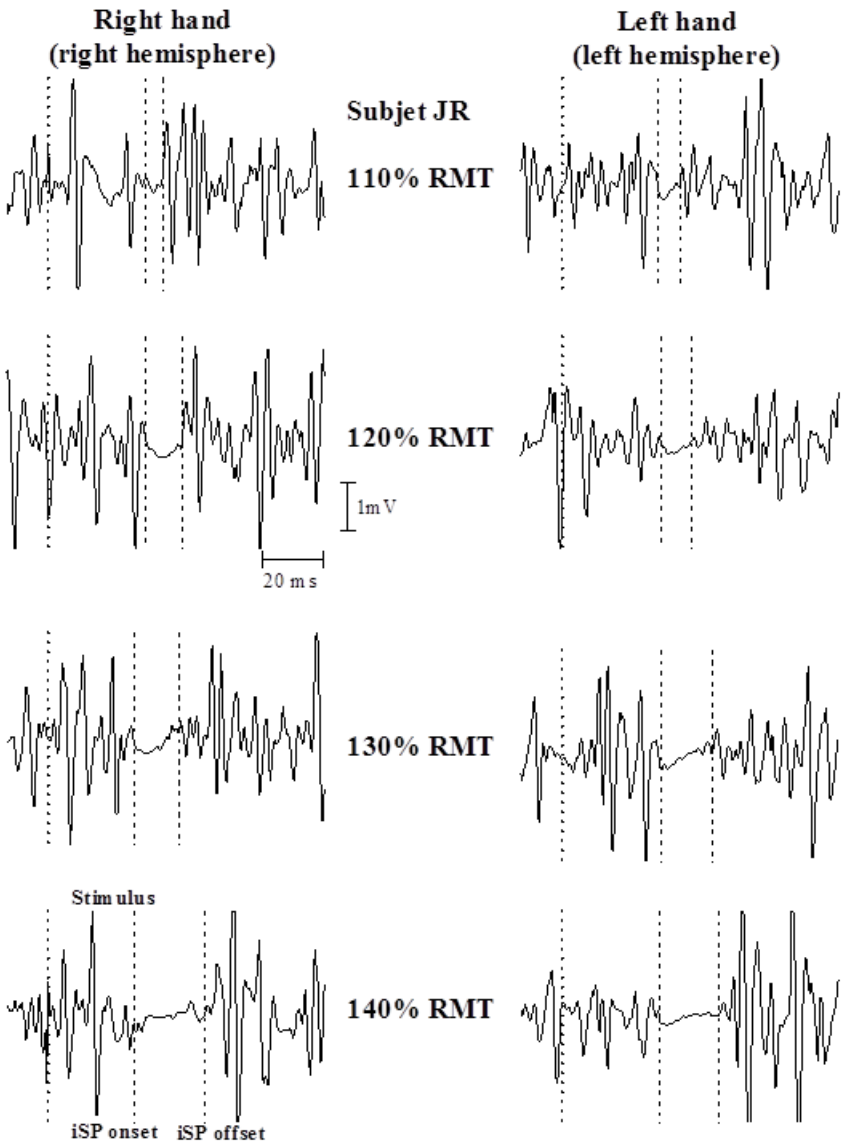
FIGURE LEGENDS

Figure 1. Influence of stimulation intensity on the cSP duration (A), probability of eliciting iSP (B) and iSP duration (DTI) (C). Linear regressions were performed using the four combined (averaged right and left) mean values and standard deviations obtained at each of the stimulation intensities. In b, * denotes significant difference (p<0.05) between intensities in chances of successfully eliciting an iSP following a TMS stimulus.

Figure 2. Examples of iSP calculation and prolongation with intensity from single raw EMG tracing at different intensities obtained from stimulating the left or right hemisphere from a single participant (JR)


A
30
20
10
0
DTI (ms)
r2=.97
110
120
130
140
Intensity (%RMT)
B
100
75
50
25
Successful trials (%)
*
*
110
120
130
140
TMS intensity (%RMT)
C
200
150
100
50
cSP (ms)
r2=.99
110
120
130
140
TMS intensity (%RMT)

Ipsilateral silent period



Reliability of TMS measures of motor cortical inhibition: A pilot study

To be submitted to Brain Stimulation

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ABSTRACT

Background: Transcranial magnetic stimulation (TMS) is a non-invasive method to assess cortical excitability of motor intra-cortical circuits within and between hemispheres. The reliability of TMS measures has been questioned, however. There is a need to establish how reliable TMS-derived measures are when repeated across sessions

Objective/Hypothesis: To examine the intersession reliability of selected TMS measures reflecting intra-cortical inhibition and transcallosal inhibition.

Methods: Intra-cortical excitability was assessed with short-latency afferent inhibition (SAI). Unconditioned resting MEP amplitude was first measured in response to single TMS pulse (120% RMT) and then with conditioning produced by median nerve stimulation at the wrist. Interhemispheric inhibition (IHI) was assessed using paired-pulse short and long interval IHI (SIHI and LIHI respectively). The contralateral and ipsilateral silent periods (cSP and iSP) were also measured. Inter-session reliability for all measures was assessed using intraclass correlation coefficient (ICC) computed from two identical sessions separated by one-week interval. Reliability of between raters for measures derived from visual inspection of cSP and iSP was also assessed.

Results: The intraclass correlation revealed that the SAI and cSP duration had good intersession reliability (ICC: 0.70 and 0.75, respectively). SIHI and LIHI only had fair reliability (ICC: 0.409 - 0.474). The iSP duration and latency of transcallosal inhibition obtained had good to excellent reliability (ICC: 0.71 and 0.927, respectively).

Conclusion(s): Measures reflecting motor intra-cortical inhibition within a hemisphere like the SAI and the cSP showed good reliability between sessions. On the other hand, measures of IHI derived from paired pulsed stimulation with double coils at rest had poor between sessions reliability. This was not the case with the iSP, which showed good reliability in terms of duration and onset latency between sessions. In this respect, the iSP is the preferable method to assess the integrity of the transcallosal pathway.

Keywords: transcranial magnetic stimulation, reliability, transcallosal inhibition

INTRODUCTION

Transcranial magnetic stimulation (TMS) is a non-invasive brain stimulation technique that can be used to assess the excitability of motor cortical circuits both within and between hemispheres. Hemispheric interactions between the two motor cortices can be assessed via the ipsilateral silent period (iSP). The iSP is characterized by a short attenuation or interruption of ongoing EMG activity in the contracting muscle ipsilateral to the stimulated hemisphere [1]. The iSP is absent or delayed in individuals with callosal abnormalities suggesting that this interhemispheric inhibition is mediated via the transcallosal pathways [2-4]. Transcallosal interactions can also be evaluated using a two-coil stimulation paradigm, where a conditioning pulse is applied on one hemisphere a few milliseconds before a test pulse is applied on the opposite hemisphere. The net effect being a reduced motor evoked potential (MEP) amplitude elicited by the test stimulus compared to when the conditioning stimulus is absent. At rest, two periods of inhibition can be found: one at a short inter-stimulus intervals (6-10 ms, i.e. short-interval interhemispheric inhibition: SIHI) and one at a long intervals (40-50 ms, i.e. long-interval interhemispheric inhibition: LIHI) [8]. Much like the iSP, this inhibition is thought to be mediated by the transcallosal pathway because of its cortical origin [5, 6] and its absence in a patient with callosal agenesis [7]. However, recent evidence has demonstrated that subcortical connections also have a role in mediating IHI [8]. Furthermore, Chen et al. [8] reported that iSP duration was strongly correlated with LIHI but not with SIHI, which indicated that that LIHI and iSP may be mediated by similar populations of callosal neurones [9].

In terms of intrahemispheric processes, several TMS-derived measures have been introduced in the last two decades to assess excitability within a hemisphere. One such common measure is

short-latency afferent inhibition (SAI), which is obtained when peripheral nerve stimulation precedes (18-24 ms interval) the TMS cortical pulse. This conditioning produces an inhibition of the MEP response and is thought to reflect sensory and motor interactions at the cortical level [10]. SAI is likely of cortical origin owing to the reduction in corticospinal output as demonstrated by a decreased in amplitude of late I-waves elicited by the TMS [10]. SAI is thought to be mediated by central cholinergic activity since the inhibition can be blocked when acetylcholine antagonists are administered [10]. Although SAI is easily elicited, the reliability of this measure remains poorly characterized and still requires further investigation.

The question of the reliability remains a problematic issue in TMS research, especially considering the inherent variability of TMS-derived measures of cortical excitability and inhibition. Approximately 40–50% of this variability appears to originate from within-subject variations [11]. While certain measures of corticospinal excitability such as MEP latency possess good reliability, multiple trials are required to reliably estimate MEP amplitude and may still lack consistency between testing sessions [12, 13]. This variability in MEP amplitude at rest could partially explain why both SIHI and LIHI display poor reproducibility between sessions [14]. While the contralateral silent period (cSP) has been found to have good reliability [15], there is still very limited information with regard to reliability of transcallosal inhibition measures derived from iSP measures. Establishing reliability is critical to assure that any changes observed in repeated measure designs are genuine and are due to physiological changes rather than errors arising from methodological variability or experimental error [16].

The objective of the present study was to determine the inter-session reliability of selected measures reflecting excitability of motor cortical circuits both within (SAI and cSP) and between

hemispheres (IHI, iSP). The goal was to provide further information regarding reliability of these measures to validate their use as index of motor cortical excitability, which is pivotal for the interpretation of changes measured in experimental and clinical research.

METHODS

The Research Ethics Boards of the University of Ottawa and the Bruyère Research Institute approved the study procedure in accordance with the principles of the Declaration of Helsinki. Participants completed a questionnaire to assure that they did not possess any contra-indications to TMS and gave informed consent before the experimental session. All assessments were performed in a controlled laboratory environment. All participants received a small honorarium for their participation in the two testing sessions, separated by one week.

Participants

Participants consisted of 10 healthy young adults (7 men, 3 women) between the ages of 21-27 years old from the Ottawa-Gatineau area. The participants were all right handed, as determined by the modified Edinburg Handedness Inventory [17]. The group of men (mean age: 21.8 ± 1.6 years) had a mean height of 178.7 ± 5.3 cm, whereas the women (mean age: 22.5 ± 3.0 years) had a mean height of 166.0 ± 9.2 cm.

Electromyographic recordings and Maximum voluntary contraction (MVC) procedures

Electromyographic (EMG) activity was recorded using small 10 mm auto-adhesive surface electrodes (Ag/AgCl, Kendall Medi-Trace™ 130) placed over the first dorsal interosseous (FDI) muscles of the right and left hand. EMG signals were amplified and filtered with a time constant

of 0.03 s and a low-pass filter of 1 kHz (AB-621G Bioelectric amplifier, Nihon-Kohden Corp., CA 92610). Signals were digitized at a rate of 2 kHz (PCI-6023, BNC-2090, National Instrument Corp.) and further relayed to a laboratory computer running custom software to control acquisition. MVCs of the dominant and non-dominant FDI muscles were assessed by asking participants to pinch as hard as they could on a Baseline pinch dynamometer (Fabrication Enterprises Inc., Irvington, New York, USA). EMG traces were analyzed through custom software. Three trials were recorded in each hand.

TMS procedures

TMS was administered with participants comfortably seated in a recording chair. Magnetic stimulation of the left hemisphere was delivered using a Magstim 200 (Magstim Co. Dyfed, UK). A Rapid^2 stimulator (Magstim Co. Dyfed, UK) was also used to stimulate the non-dominant right hemisphere in order to assess IHI with the dual coil technique. Each stimulator was connected to their own figure-eight coil. To determine the optimal site to evoke MEPs, participants were fitted with a Waveguard TMS compatible cap (ANT North America Inc, WI 53719). A U-shaped neck cushion was provided to restrain head movements and prevent neck fatigue. With the coil held ~45 degrees in the mid-sagittal plane for the dominant hemisphere and parallel to the mid-sagittal plane for the non-dominant hemisphere, the approximate location of the hand motor area on each of the tested hemisphere was explored in 1-cm steps until reliable MEPs could be evoked in the target muscle. After determination of this stimulation "hotspot", the site was marked with a sticker to ensure reliable coil positioning. The experimenter held the coil in place manually and frequently reassessed the coil position to ensure that it remained over the optimal stimulation site throughout the experiment. Two testing sessions took place with a

one week interval between. The two sessions were performed between 9am and 4pm to avoid diurnal variations in corticospinal excitability [18].

Resting motor threshold (RMT) and MEP at rest

Measurements of the RMT and of MEPs at rest were performed in the left hemisphere using the Magstim 200 stimulator (Magstim Co. Dyfed, UK) while the right hemisphere was tested using the Rapid^2 stimulator (Magstim Co. Dyfed, UK). As in our previous studies, the RMT was determined using a software tool (TMS Motor Threshold Assessment Tool 2.0; Brain Stimulation Laboratory, Medical University of South Caroline, USA, http://www.clinicalresearch.org/sorfware.htm) developed to obtain fast and rapid estimates of the minimal intensity required to evoke reliable MEP's in the target muscle [19, 20]. Once the RMT was determined, 10 MEPs were elicited at rest sequentially from each hemisphere at an intensity of 120% RMT to establish baseline MEPs. EMG activity was constantly monitored on a high gain oscilloscope to ensure the absence of any unwanted contractions that would interfere with the measurements.

Short-latency afferent inhibition (SAI)

SAI was assessed only for the right hand/left hemisphere by applying conditioning afferent stimulation 20 ms before the TMS pulse (120% of RMT) in line with the protocol described by Tokimura et al. 2000 [10]. The conditioning afferent stimulation consisted in 200 μs electrical pulses (S88 Stimulator, Grass Technologies, Astro-Med, Inc, West Warwick, RI 02893 U.S.A.) delivered on the right median nerve at an intensity just above the motor threshold to evoke a minimal visible twitch of the thenar muscles. 10 trials were recorded.

Contralateral Silent Period (cSP)

The cSP was assessed from the right hand/left hemisphere. To elicit the cSP, a TMS pulse at 130% RMT was delivered while the participants maintained a contraction by pinching a dynamometer at ~ 25% of their MVC [21]. 10 trials were recorded.

Paired-pulse interhemispheric inhibition (IHI)

IHI was evaluated using double coil stimulation using the right FDI as the target muscle. Test pulses (120% RMT) were delivered to the left hemisphere with a Magstim 200 (Magstim Co. Dyfed, UK) while conditioning pulses (120% RMT) were delivered to the right hemisphere with a Rapid^2 stimulator (Magstim Co. Dyfed, UK), each connected to their own figure-eight coil (90 mm outer loop diameter). For SIHI, the interval between test and the preceding conditioning pulse was 10 ms, whereas it was fixed at 40 ms for LIHI. A total of 10 trials were recorded at each interval (SIHI and LIHI).

Ipsilateral Silent period (iSP)

The iSP was assessed using the left FDI as the target muscle. The iSP was elicited by applying a TMS pulse (Magstim 200) at 130% RMT to the left primary motor cortex ipsilateral to near-maximally contracting left hand (~95% MVC) and contralateral to a mildly contracting right hand (~25% MVC) each holding a pinch dynamometer. Participants were trained to maintain the contractions for 3 s in sync with a tone (550 Hz) generated by the computer. A minute of rest was given every 3 trials to prevent a drop in EMG in the maximally contracting ipsilateral (left) hand until ten trials were collected.

TMS data analysis

TMS data were analyzed off-line using alphanumerically coded files. MEP trials were overlaid and analyzed according to MEP latency and peak-to-peak amplitude. MEP latency was the time interval between the stimulus and first deflection of EMG caused by the MEP. Baseline MEP values were taken as the average of the unconditioned MEPs. The level of inhibition for SAI, SIHI and LIHI was determined in terms of percentage of baseline MEP amplitude (MEP_{cond} / MEP_{uncond} X 100).

Silent period data was analyzed offline on a trial-by-trial basis by visual inspection by two separate evaluators. This approach has previously been determined reliable between raters [21, 22] and demonstrated excellent inter-rater reliability in this study as shown by an intra-class correlation coefficient of 0.99, 0.97 and 0.97 for the cSP, DTI and LTI, respectively. The duration of the cSP was defined as the duration of EMG silence from the onset of the MEP to the recovery of 50% of background EMG activity [23]. Mean values were computed by averaging the individual trials. Two specific measures of transcallosal inhibition were derived from the iSP recordings. First, latency of transcallosal inhibition (LTI) was determined as the time from the stimulus onset until the the iSP onset, iSP onset being 1st sign of significant decline (>25%) in the mean rectified EMG activity level. The second index comes from determining the iSP duration by measuring the time interval between the iSP onset until the 1st sign of recovery in the background EMG activity (i.e., iSP offset). The latter time point is relatively easy to determine, as the end of the myoelectric silence is generally followed by an abrupt return of EMG activity in the recovery period. An iSP was deemed present if the EMG fell below the average EMG preceding the stimulus for a minimum of 5 ms at a latency between 25 and 45 ms.

Statistical analysis

Sample means and coefficient of variation (CV) were used to estimate the variability of the true mean. The CV is used to compare the degree of variation from data series to another. CV was calculated as a percentage of the sample mean (standard deviation/sample mean x 100). Intra-class correlation coefficients (ICC) were used to evaluate intersession reliability of each TMS-derived measure. According to published guidelines, values of less than 0.4 were classified as poor; 0.40 – 0.59 as fair; 0.60 – 0.74 as good; and 0.75 – 1.0 as excellent [25]. ICC was also used to determine inter-rater reliability of the cSP, LTI and DTI. All statistical analyses were performed using SPSS software version 17.0 for Windows ™ (Chicago, Il, USA).

RESULTS

Table 1 and Table 2 provide a description of the overall results for all the selected variables. The variability between the two sessions can be also examined from inspection of Table 1 and Table 2. ICC values for all measurements are summarized in Table 3.

Reliability of RMT and MEP at rest

As shown in Table 1, RMT values obtained from each hemisphere demonstrated excellent intersession reliability as shown by ICC values over 0.95 and a relatively small CV values ranging between 15% and 21.1%. Resting MEP amplitude measured in each hand demonstrated fair to good reliability while taking into account both their ICC values (0.56 and 0.69) as seen in

Table 3. Resting MEP amplitudes also had a large amount of inter-individual variation as shown by their large CV values (Table 1).

Reliability of SAI and cSP measures

The two measures of intrahemispheric inhibition, SAI and cSP, demonstrated good intersession reliability as shown by their ICC values between 0.72 and 0.74, respectively. There was a relatively low degree of inter-individual variation in cSPs as indicated by the relatively small CVs (14.6% and 17.8%). The SAI estimates varied significantly due to high inter-individual variability with the CV ranging from 65.7% and 98.6%.

Reliability of IHI and iSP measures

SIHI and LIHI had fair reliability according to ICC values of 0.41 and 0.47, respectively. Additionally, these measures had a relatively large CV values ranging between 83.4% and 103.9%, indicating large inter-individual variability. In comparison, measures of transcallosal inhibition derived from the iSP were found to have good to excellent reliability according to the ICC values and relatively small CV values. LTI was found to be the most reliable with an ICC value of 0.927 and a CV ranging between 9.9% and 10.4%. DTI had good reliability with its ICC value of 0.71 and CV values ranging between 15.6% and 16.7%. Further analysis of the DTI data revealed that only two participants exhibited differences > 5 ms between the two sessions. These two participants were re-assessed for a third session to repeat measurements of the iSP. This second re-test provided values very close to the second session, and, thus greatly improved the ICC values (from 0.71 to 0.977).

DISCUSSION

The present study examined the inter-session reliability of selected TMS measures of corticospinal excitability and of interhemispheric inhibition. Our results confirm good reliability of some measurements, like RMT and cSP duration and the poor reliability of other measures such as resting MEP amplitude. More specifically, our results contrast the good reliability of measures of transcallosal inhibition derived from the iSP when compared to that found from IHI measures derived from paired-pulse stimulation.

Intersession corticospinal excitability

RMT was determined to be very reliable given its low CV values and ICC over 0.95 for both hemispheres which were stimulated with different orientation and stimulators. This is in agreement with many studies that have reported good short and long-term reliability of RMT at rest in healthy subjects [26-28]. The same cannot be said for the MEP amplitude which had fair to good reproducibility similar to other studies [26, 29, 30]. This variability in MEP amplitude is believed to be caused by the rapid and spontaneous fluctuations in excitability at the cortical and spinal levels [31]. Additionally, there exists a considerable amount of inter-individual variability in MEP amplitudes according to the relatively large CV values. This variability needs to be controlled in order to compare MEP results between subjects and studies.

Intersession reliability of SAI

To our knowledge, this is the first study to examine the reliability of the SAI as a measure of intra-cortical inhibition. Our results show that SAI can be reliability assessed between sessions and, similar to the MEP amplitudes from which it is derived, there exists considerable inter-

individual variability in the SAI, as seen by the relatively large CV values. Nevertheless, our finding of good SAI reliability between sessions for our young, healthy participants may promote its use for further insight into how age related changes in neuromodulation affect motor system functioning [32]. Cholinergic function has been found as a strong predictor of dexterity and speed of processing with aged populations [33]; a decrease in SAI therefore, is associated with normal aging as it correlates to declines in central cholinergic neuromodulation [37].

Intersession reliability of the cSP

In the cSP, we found good intersession reliability (ICC of 0.74) and low inter-subject variability. This is in accordance with other studies where low intra-subject cSP variability has been reported in healthy subjects [36, 37]. The analysis of cSP is used regularly amongst neurologic populations to further examine pathophysiology [38, 39] and evaluate intervention effects [40, 41]. Consistent with other reports, we show that intersession reproducibility of the cSP is reliable and provide a measure that can be confidently utilized as a means for examining changes corticospinal excitability.

Intersession reliability of SIHI and LIHI

Both SIHI and LIHI were found to have a fairly low amount of intersession reliability (ICC of 0.41 and 0.47, respectively) and a large amount of inter-individual variability as reflected by the relatively large CV. Paired-pulse IHI measures has been traditionally elicited using two monophasic Magstim 200 stimulators [5]; however, in this study we utilized a Magstim 200 (monophasic stimulator) to deliver the test pulse and a Rapid^2 (biphasics stimulator) to deliver the conditioning pulse. This study demonstrates that this is an effective technique to elicit either

the SIHI or LIHI; however, our results that this technique does not have good inter-session reliability which is in agreement with a similar study using two monophasic Magstim 200 stimulators that found that there was a high between- and within-subject variability in IHI between two separate sessions [14]. High within-subject and inter-session variability of SIHI and LIHI does not allow researchers to determine if changes in transcallosal inhibition over time can be attributed to structural and functional modifications or if they are a result of random fluctuations. Similar to SAI, it is possible that the variability of IHI measures between sessions is rooted in the relative high variability of baseline MEP amplitude. Thus, while they are still widely used, IHI measures derived from dual coil stimulation are to be considered with caution when it comes to interpret changes over repeated sessions.

Intersession reliability of the iSP

To our knowledge, the present study is the first to report that the iSP onset and duration can be reliably reproduced between testing sessions (ICC of 0.93 for LTI and 0.71 for DTI). Using the iSP to track changes in transcallosal inhibition in a homogenous group over time is validated by the current research. Better reliability implies that single measures are more precise, and changes are able to be tracked with more certainty [42]. Addtionally, LTI and DTI were found to have a low amount of inter-subject variability. The LTI was both more reliable and less variable than the DTI. This could be explained by the fact that LTI is a measure of signal conduction while the DTI is influenced by multiple factors that can influence the excitability of the transcallosal interneurons.

Secondary analysis revealed that retesting the two subjects with a larger intersession difference in DTI yielded a much stronger ICC. These two subjects did not have any characteristics that

distinguished them from the other subjects or each other; therefore, future studies are required to help identify factors that could influence the iSP, such anxiety towards TMS [43]. Applying additional testing sessions over a longer time period could help further establish the inter-session reliability of iSP measures. When compared to the paired-pulse IHI measure, using the iSP has been shown to be the more a reliable measure at analyzing transcallosal inhibition in a specific population across time.

CONCLUSION

This study is the first to establish the reliability of SAI and iSP in a group of young healthy individuals over a relatively short time period of one week. Both these measures demonstrated good to excellent reproducibility in our small sample. The iSP could also be determined as the more reliable method to assess transcallosal inhibition using TMS because of the poor reproducibility of the LIHI and SIHI techniques.

Further research on the reliability TMS-derived measures of cortical inhibition should expand participant demographics as we only studied young healthy adults, and therefore cannot comment on the use of our conclusions with respect to participants beyond this demographic. Furthermore, as our SAI and iSP findings are novel in nature, it is especially crucial that the study population be expanded during further investigations.

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Table 1.

Variability between sessions in selected measures of corticospinal excitability.

		Session 1		**Session 2**	
		Mean ± SD	CV	Mean ± SD	CV
RMT (% output)	Right hand	40.8 ± 8.6	21.1%	39.6 ± 7.6	19.2%
	Left Hand	62.2 ± 9.3	15%	63.7 ± 10	15.8%
MEP (mV)	Right Hand	0.88 ± 0.37	41.2%	0.81 ± 0.48	59.3%
	Left Hand	1.08 ± 0.77	71.2%	0.88 ± 0.48	55.2%

RMT= Resting motor threshold; **MEP** = Baseline motor evoked potential amplitude; **SD** = Standard deviaton; **CV** = Coefficient of variation

Table 2.

Variability between sessions in select measures of intrahemispheric and interhemispheric inhibition.

Measures	Session 1		Session 2	
	Mean ± SD	CV	Mean ± SD	CV
cSP (ms)	141.4 ± 21.2	14.6%	133.2 ± 21.2	17.8%
SAI (% Baseline)	0.31 ± 0.20	65.7%	0.31 ± 0.30	98.6%
SIHI (% Baseline)	0.51 ± 0.53	103.9%	0.35 ± 0.30	85.1%
LIHI (% Baseline)	0.75 ± 0.62	83.4%	0.64 ± 0.54	84.1%
LTI (ms)	35.2 ± 3.6	10.4%	34.9 ± 3.4	9.9%
DTI (ms)	15.6 ± 3.2	20.2%	16.7 ± 4.4	25.4%

SAI = Short afferent inhibition; **cSP** = contralateral silent period duration; **SIHI and LIHI**= Paired-pulse interhemispheric inhibition at 10 and 40 ms interstimulus interval, respectively; **LTI**= Latency of transcallosal inhibition; **DTI**= Duration of transcallosal inhibition

Table 3.

Intraclass correlation coefficients (ICC) for a variety of TMS measures between two sessions and raters.

ICC	RMT Right hand	Left Hand	MEP Right hand	Left Hand	SAI	cSP	SIHI	LIHI	LTI	DTI	DTI (retest)✝	TCT
Inter-session	0.98	0.96	0.56	0.69	0.72	0.74	0.41	0.47	0.93	0.71	0.98	0.93

ICC= Intraclass correlation coefficient; Abbreviations are the same as Table 1 and Table 2.

✝ Two participants were tested a 3rd time due major differences between 1st and 2nd session in DTI measurements. The 2nd and 3rd session measurements were much closer and aligned with the rest of the group. These measure were incorporated in new ICC computations labeled DTI (retest).

Chapter III - General discussion and conclusion

The projects in the present thesis provide original evidence that there are a number of individual factors that can significantly alter motor transcallosal inhibition, as assessed with TMS. As a whole, the current thesis demonstrates that age, degree of laterality and concussion each have their own independent effect on transcallosal inhibition. The final methodological papers provide evidence that iSP measures are reliably obtained and repeatable to assess motor transcallosal inhibition, limiting the possibility of random fluctuations confounding the interpretation of results. Each article presented in this thesis provides important insights as to how individual and methodological factors can influence the duration and latency of motor transcallosal inhibition. Collectively, these experiments provide strong neurophysiological evidence that the iSP is a reliable means to assess hemispheric interactions between motor cortices mediated by transcallosal pathway.

The first and third projects demonstrate that neural alterations in the form of neurodegeneration associated with healthy aging or axonal shearing following a concussion in young persons can significantly alter transcallosal inhibition as reflected by changes in iSP measures. Additionally, the age-related changes in callosal inhibition seem to have behavioural consequences, age-related alterations in iSP being correlated with a decline in manual performance. While behavioural performance in terms of hand function and motor speed seems to be largely unaffected in individuals having recovered from a concussion, alterations in transcallosal inhibition were found to be associated with lower performances in a task assessing inhibitory control and also in visual memory scores. Altogether, these correlations in aged participants and concussed athletes provide suggestive evidence linking physiological alterations in motor transcallosal inhibition and behavioural changes in performance in tasks assessing motor and cognitive functions.

With regard to handedness, the second project provides corroborating evidence of an association between the degree of hand preference and motor callosal functions, less lateralized individuals (i.e., mixed handed) showing evidence of more efficient interhemispheric communication when compared to either strong right- or left-handed counterparts. This study provides further evidence that handedness does not follow a simple right-left dichotomy and that greater ambilaterality is somehow reflected in the ability of the transcallosal pathway to exchange information either from right M1 toleft M1or left M1 to-right M1.

One additional important finding arising from our first set of experiment is the absence of clear asymmetry in measures of contralateral and ipsilateratal inhibition derived from each hemisphere, irrespective of age effects or changes associated with hand preference or history of concussion. This indicates that valid measure of either the cSP or the iSP can be derived from stimulation of just one hemisphere, whithout worrying too much about possible hemispheric differences. Such information may prove to be critical in clinical studies investigating changes in the integrity of the transcallosal pathway by gathering iSP data from a single hemisphere instead of both hemispheres.

Our methological studies first highlighted the role of stimulation intensity in influencing ipsilateral inhibition elicited in small hand muscles. Indeed, our experiment (Project IV) demonstrates the strong influence of stimulation intensity on the iSP duration, as reflected in the DTI; leaving the LTI unaffected. Our observations thus stress the importance of selecting an appropriate level of stimulation to measure transcallosal inhibition from the iSP. In this respect, we show that, when adjusted relative to individual's RMT, 130% appears to be an optimal intensity to reliably elicit iSPs without over-stimulating participants. In the last project (Project V), we show that measures of interhemispheric inhibition derived from the iSP have a relatively good inter-

session reliably when compared to those derived from resting paired-pulse stimulation. Such observations on the reliability of iSP measures strengthen our previous conclusions with regards to the respective effects of age, handedness and history of concussion on transcallosal inhibition; i.e. these reported effects were likely true reflections of changes in motor callosal integrity and not just random fluctuations in our sampling.

One of the major limitations in our findings is the relatively small sample size in each study, which is a common limitation in TMS studies. Having large sample size could have exposed additional interactions and trends in the data that did not reach significance because of the small sample size, such as the trend for longer DTI in the mixed-handed group found in the second project. The fact that we did not strictly control for the number of males and females in all our studies is another limitation that could have also affected our ability to detect differences in transcallosal inhibition linked to sex. In fact, we did not find any sex differences in our studies in spite of evidence for an influence of sex in the macro- and microstructural composition of the CC [181, 182] and that the iSP may vary during the menstrual cycle [183]. However, other studies examining the iSP have failed to find sex differences [106]. Finally, another potential limitation pertaining to our methodological studies is the fact that our observations were collected only from healthy young adults. Therefore, we cannot infer that observations regarding intensity and reliability could be applied to older populations such as middle-aged adults and seniors. Testing intensity effects and reliability issues will be material left for future studies.

It needs to be mentioned also that TMS is not appropriate for everyone as demonstrated by the necessity of the questionnaire for transcranial magnetic stimulation. Despite TMS being a safe non-invasive means to stimulate the brain [184], minor adverse events can occur such as vasovagal syncope which we experienced in our lab in some participants. This led us to revise our TMS

questionnaire (Appendix A) to include a history of fainting or strong reactions to unusual circumstances (e.g. needles).

Our studies examining the impact of age and history of concussion were cross-sectional in nature owing to time and cost contraints. Future studies using longitudinal design might be necessary to determine how physiological alteraction in transcallosal inhibition evolve as a function of age or in the different phases after a concussion.

While our experiments focused on factors influencing the iSP, one possible direction for future studies is to examine the impact of interventions to modulate or improve callosal interactions and interhemispheric communications. These interventions may consist in strength training [185], bimanual tasks training [186] or neuromodulating techniques such as transcranial direct current stimulation (tDCS). In this respect, in parallel work in our lab, we have conducted a recent study to examine, among other factors, the effects of anodal tDCS on transcallosal inhibition. This study, which shows that a-tDCS can effectively modulate transcallosal inhibition, is provided as an appendix to this thesis (Appendix B) to illustrate future directions where the iSP can used to probe changes in transcallosal inhibition.

In conclusion, the present work provides a series of new experimental observations regarding the iSP elicited by TMS. We show that the iSP is sensitive in detecting subtle changes in motor transcallosal inhibition associated with age, hand preference and following antecedents of concussion. We provide new evidence to support its reliability between sessions and ways to improve its sensitivity by adjusting intensity of stimulation relative to an individual's motor threshold. Based on this thesis, we can assert that the iSP as a valid and reliable non-invasive method to assess transcallosal inhibition and callosal integrity between motor cortices.

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Appendix A - Revised TMS Questionnaire

INSTITUT DE RECHERCHE BRUYÈRE RESEARCH INSTITUTE
Affilié à l'Université d'Ottawa
Affiliated with the University of Ottawa

uOttawa
L'Université canadienne
Canada's university

QUESTIONNAIRE FOR TRANSCRANIAL MAGNETIC STIMULATION

Participant # _____

IMPORTANT NOTICE

The present study involves the use of a magnetic stimulator to induce micro-current in the brain through a coil applied on the scalp. Although the technique is known to be safe and virtually painless, we need to screen our subjects to exclude those who might be at risk. This is why we ask you to fill this questionnaire, as a potential participant.

As a reminder, you cannot receive TMS if you present with one of the following conditions:

- **You have epilepsy or someone in your immediate family has epilepsy;**
- **You have metallic implants (plates, clips, including cochlear implants) inside your brain, your hairs or the scalp;**
- **You have a cardiac pace-maker;**
- **You are pregnant or you think you might be.**

1. Questions about your health.

To assess your health status and make sure that you do not present with conditions (or are taking medications associated with conditions) that may affect your responses to TMS, we need to ask questions about your health and the use of medications.

a. In the last 12 months, have you consulted a health professional or have you been investigated for health-related issues

☐ No

☐ Yes

If yes, was the consultation or the investigation about any of the following conditions (check appropriate cases):

☐ Migraine

☐ Traumatic brain injury

☐ Depression

☐ Stroke or TIA (Transient Ischemic Attack)

☐ Heart Conditions (angina, heart failure)

☐ Hypertension (high blood pressure)

☐ Sciatica or disc problems

☐ Arthritis

☐ Diabetes

☐ Parkinson's disease, multiple sclerosis or other neurologic conditions:

b. Are you presently taking or have you taken during the last month any of the following medications?

□ Cold medicine containing cough suppressant with DM (e.g., Balminil DM™, Benylin DM™, Triaminic DM™)

□ Medications against stress, anxiety or sleep disorders (e.g., Valium™, Ativan™, Serax™, Xanax™)

□ Medications to treat depression or mood disorders (e.g.: Élavil™, Anafranil™, Prozac™, Zoloft™)

□ No, I have not taken and I am not currently taking any of these medications

c. Do you tend to faint or have strong reactions in response to unusual situations (e.g., seeing blood, on the dentist's chair)?

□ No,

□ Yes

If this is the case, then you must know that in susceptible individuals magnetic stimulation can sometimes provoke strong emotional responses when experienced for the 1st time. It is not a contra-indication necessarily, but we prefer to warn you in advance. You will have the chance to experience the effect of the stimulation before we begin and if you do not like it you can refuse to continue without any other obligations on your part.

2. Questions about your hearing capacity.

The TMS machine produces a sudden clicking noise when it discharges. This is why it is recommended to wear hearing protections during the procedures (those will be provided). In addition, we have to take further precautions in persons with hearing conditions. This is why we are asking the next questions.

a. Do you have cochlear implants for hearing or do you use hearing aids?

□ No,

□ Yes

If you use hearing aids (audio-prosthesis), we will ask you to remove them so they will not get damaged during the procedure.

b. Do you have hearing problems or suffer from tinnitus (i.e., whistling or ringing noise in the head)?

□ No,

□ Yes

If you have tinnitus, we will require that you wear hearing protections during the procedure.

I acknowledge that I have answered this questionnaire to the best of my knowledge and that my answers truly reflect my health status.

Mark your initials here: _____ Date: _____

Appendix B - Continuation project with transcranial direct current stimulation

frontiers in Human Neuroscience

ORIGINAL RESEARCH
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Predicting Modulation in Corticomotor Excitability and in Transcallosal Inhibition in Response to Anodal Transcranial Direct Current Stimulation

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Introduction: Responses to neuromodulatory protocols based either on transcranial direct current stimulation (tDCS) or transcranial magnetic stimulation (TMS) are known to be highly variable between individuals. In this study, we examined whether variability of responses to anodal tDCS (a-tDCS) could be predicted from individual differences in the ability to recruit early or late indirect waves (I-waves), as reflected in latency differences of motor evoked potentials (MEPs) evoked by TMS of different coil orientation.

Methods: Participants (n = 20) first underwent TMS to measure latency of MEPs elicited at different coil orientations (i.e., PA, posterior-anterior; AP, anterior-posterior; LM, latero-medial). Then, participants underwent a-tDCS (20 min @ 2 mA) targeting the primary motor cortex of the contralateral preferred hand (right, n = 18). Individual responses to a-tDCS were determined by monitoring changes in MEP amplitude at rest and in the duration of the contralateral silent period (cSP) and ipsilateral silent period (iSP) during contraction; the latter providing an index of the latency and duration of transcallosal inhibition (LTI and DTI).

Results: Consistent with previous reports, individual responses to a-tDCS were highly variable when expressed in terms of changes in MEP amplitude or in cSP duration with ∼50% of the participants showing either little or no modulation. In contrast, individual variations in measures of transcallosal inhibition were less variable, allowing detection of significant after-effects. The reduced LTI and prolonged DTI observed post-tDCS were indicative of an enhanced excitability of the transcallosal pathway in the stimulated hemisphere. In terms of predictions, AP-LM latency differences proved to be good predictors of responses to a-tDCS when considering MEP modulation.

Conclusion: The present results corroborate the predictive value of latency differences derived from TMS to determine who is likely to express "canonical" responses to a-tDCS in terms of MEP modulation. The results also provide novel suggestive evidencethat a-tDCS can modulate the excitability of the transcallosal pathway of the stimulated hemisphere.

Keywords: non-invasive brain stimulation, motor evoked potential, motor cortex, transcranial magnetic stimulation, transcranial direct current stimulation (tDCS)

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INTRODUCTION

Transcranial direct current stimulation (tDCS) has gained much credentials in recent years as a neuromodulatory intervention to induce lasting changes in cortical excitability. By passing a very weak current (e.g., 1–2 mA) through surface electrodes applied on the scalp, one can attempt to modulate the excitability of the underlying cortical region presumably by acting of resting membrane potential (Dayan et al., 2013). Anodal tDCS (a-tDCS) tends to increase the spontaneous firing rate and the excitability of cortical neurons by depolarizing the membrane, whereas cathodal stimulation leads to hyperpolarization of the neuronal membrane and thus tends to decrease excitability (Stagg and Nitsche, 2011; Dayan et al., 2013). One of the major drawbacks in this simplistic model is that stimulation-induced behavioral and physiological effects tend to vary substantially from one individual to another (for a review, see Horvath et al., 2014). In the case of tDCS, this variability has been highlighted in two recent reports (López-Alonso et al., 2014; Wiethoff et al., 2014) where after-effects on cortical excitability were monitored using motor evoked potentials (MEPs) derived from transcranial magnetic stimulation (TMS). In both reports, only ∼half of the participants responded in the canonical manner to tDCS applications and exhibited the expected modulation in terms of either MEP facilitation (anodal) or depression (cathodal). As stressed by the authors of these reports, such a large inter-individual variability has implications not only for studies examining behavioral and physiological effects of neuromodulatory protocols but also for studies interested in the therapeutic potential of tDCS in clinical populations.

While differences in experimental protocols (e.g., electrode types, stimulator settings) might account for some of the variability, individual differences in anatomical factors such as the thicknesses of the cerebrospinal and the skull can also contribute significantly, as they are known to have a direct influence on the current flow that reaches the cortex (Datta et al., 2009, 2012; Laakso et al., 2015). Another potential and important source of variability highlighted in a recent study by Hamada et al. (2013) when examining MEP modulation in response to theta burst stimulation (TBS) was individual susceptibility to activate certain population of cortical interneurons by TMS. This conclusion was based on observations regarding differences in MEP latency evoked by monophasic TMS pulses of different coil orientations to assess how easily direct waves (D-wave) or indirect waves (I-waves) can be recruited in a given individual. Indeed, previous *in vivo* recordings in humans have shown that, depending on the coil orientation and intensity, monophasic TMS pulses can evoke different combinations of descending waves, which reflect different cortical generators (Di Lazzaro et al., 2004). For instance, high intensity latero-medial (LM) induced currents tend to evoke D-wave and early I-waves (Sakai et al., 1997; Di Lazzaro et al., 2004). The D-waves are termed "direct" because they are thought to originate from direct activation of axons of layer V pyramidal tract neuron (Di Lazzaro et al., 2004). Correspondingly, I-waves are considered "indirect" because they are thought to result from indirect, transynaptic activation of pyramidal tract neurons (Di Lazzaro et al., 2012). With conventional posterior-anterior (PA) currents, early I-waves (I1-I2) can be easily elicited even with low intensity stimulation, probably reflecting activation of low-threshold cortical elements in layers II and III making mono and oligo-synaptic contacts with pyramidal tract neurons (Di Lazzaro et al., 2012). In contrast, anterior-posterior (AP) currents tend to evoke only later I-waves (I3, I4) owing to their presumed polysynaptic origin in link with activation of a network composed of cortical elements in upper layers (II and III) and local interneurons acting on pyramidal tract neurons through reciprocal excitatory and inhibitory connections (Di Lazzaro et al., 2012). Following this rationale, Hamada et al. (2013) used differences in MEP latency evoked at the AP and LM orientation (i.e., AP-LM differences) as a surrogate measure of individual susceptibility to recruit early or late I-waves in response to TMS and by inference, activate different population of cortical interneurons. With this approach, Hamada et al. (2013) were able to predict with great accuracy who was likely to express either lasting facilitation or lasting depression in response to neuromodulatory TBS applications based on how readily early or late I-waves could be recruited, as reflected in AP-LM latency differences. In a subsequent study, the same group (Wiethoff et al., 2014) used a similar approach to examine inter-individual variability in response to tDCS. As noted before, MEP modulation in response to either anodal or cathodal stimulation was quite variable but, interestingly, here again AP-LM latency differences proved to be a good predictor of whom was likely to exhibit "canonical" responses to tDCS. The prediction was particularly compelling for individuals showing small AP-LM differences and thus, D-wave or early I-waves recruitment, in whom MEP facilitation was large and consistent after anodal stimulation.

Thus, one way to tackle inter-individual variability in response to neuromodulatory protocols may consist in identifying in a given individual and prior to application which populations of cortical interneurons are likely to be modulated. In the present report, we sought to further investigate this question along the path opened by Wiethoff et al. (2014) to examine whether variability of responses to a-tDCS could be predicted by individual susceptibility to recruit early or late I-waves, as reflected in MEP latency differences. To this end, we monitored changes in corticomotor excitability before and after tDCS using MEP at rest since they provide the most reliable outcomes of neuromodulatory protocols (Horvath et al., 2015). In addition to MEPs, we also monitored changes in the contralateral and ipsilateral silent period (cSP and iSP, respectively) during active contractions. The cSP provides another index of corticomotor excitability reflecting modulation of central inhibition via γ-amino butyric acid (GABA) B receptors (Ziemann et al., 2015). The iSP was recorded to assess changes in transcallosal inhibition (Meyer et al., 1995) from the stimulated hemisphere towards the non-stimulated hemisphere.

MATERIALS AND METHODS

Participants

Twenty healthy adults (mean age 24.3 ± 7.3 years, 15 men) were recruited from the local community, most being university students in the Ottawa area. Before testing, all participants were screened with a safety questionnaire (adapted from Keel et al., 2001) to ensure that there were no contra-indications to TMS. The majority (18/20) were right handed based on the Edinburg Handedness Inventory. Prior to participation, written informed consent was obtained from all participants in accordance with the *Declaration of Helsinki* and the study procedures were approved by the local institutional Research Ethics Board (Bruyère Research Institute, Ottawa, ON, Canada). All assessments were performed in a controlled laboratory environment and participants received a small honorarium for their participation.

Experimental Paradigm

The experimental paradigm is illustrated in **Figure 1**. Participants first underwent TMS testing to measure MEP latency with three different coil orientations, i.e., PA, LM and AP. Then, baseline measures of corticomotor excitability were performed to determine MEP characteristics at rest using the conventional PA coil orientation. The cSP and iSP were also measured concurrently during active contractions. Following baseline measurements, participants underwent a-tDCS for 20 min. Then, MEPs were measured again at 10 min (T10) and 20 min (T20) post-application. At T20, the cSP and iSP were also measured and then again at 40 min post-application (T40). The delayed testing till the 20th min for the cSP and iSP was introduced to avoid possible interferences with measurements of MEPs at rest associated with active contractions (i.e., post-contraction changes in excitability, see Goldsworthy et al., 2014).

Pinch Strength Measurements During Maximum Voluntary Contraction (MVC)

Prior to neurophysiological testing, participants were tested with a mechanical pinch gauge (Model, 12-0201, Fabrication Enterprises Inc., Irvington, NY, USA) during maximal efforts to measure strength and obtain an index of muscle activation in the first dorsal interosseous (FDI) muscle. For pinch strength, participants were instructed to press as hard as they could against the dynamometer using a lateral "key" pinch with the thumb and index fingers. Participants were instructed to synchronize their effort with an auditory tone lasting 3 s generated by the computer. Three trials were recorded for each hand with at least 30 s rest between contractions. The order of testing with the right and left hand alternated between participants.

General Procedure for TMS and MEP Recordings

TMS was administered with participants comfortably seated in a recording chair. Participants were fitted with a Waveguard TMS compatible cap (ANT North America Inc., WI, USA) to allow localization and to ensure consistent coil positioning. A U-shaped neck cushion was also used to restrain head movements and prevent neck fatigue. Magnetic stimulation was delivered via Magstim 200 monophasic stimulator (Magstim Co., Dyfed, UK) connected to a figure-eight coil (90 mm outer loop diameter). MEPs were recorded using small auto-adhesive surface electrodes (Ag/AgCl, Kendall Medi-Trace^{TM} 130) placed in a belly-tendon montage on the FDI muscles of each hand. Electromyographic (EMG) signals were amplified and filtered with a time constant of 0.03 s and a low-pass filter of 1 kHz (AB-621G Bioelectric amplifier, Nihon-Kohden Corp., CA, USA). Signals were digitized at rate of 2 kHz (PCI-6023E, BNC-2090, National Instruments Corp) and further relayed to a laboratory computer running custom software to control acquisition and saved for offline analysis.

Each TMS testing session proceeded with the same sequence for all participants. First, the motor hot spot for the FDI was determined on each hemisphere, starting with the hemisphere contralateral to the preferred hand. With the coil held ∼45° in the mid-sagittal plane, the approximate location of the hand motor area was explored in 1 cm steps until reliable MEPs could be evoked in the FDI. This site was then marked with a circular sticker to ensure consistent coil positioning throughout the testing session. After determination of the "hotspot", the resting and and active motor thresholds (rMT and aMT) were determined using the Motor Threshold Assessment Tool software (MTAT 2.0[1]). The software allows for fast estimation of motor threshold through the maximum-likelihood strategy based on the PEST (Parameter Estimation by Sequential Testing) algorithm (Mishory et al., 2004). For the rMT, participants were instructed to relax while EMG was monitored on an oscilloscope to ensure that no unwanted contraction was present. For the aMT, TMS pulses were delivered while the FDI was lightly activated by asking participants to press against the pinch dynamometer with a low force corresponding to ∼10% of their maximum. For the sequential testing algorithm, the minimal acceptable MEP amplitude was set at 50 μV for the rMT and at 200 μV for the aMT. Once completed for one hemisphere, the same procedure for determination of the hotspot, rMT and aMT was repeated on the opposite hemisphere.

Assessment of MEP Latency with Different Coil Orientation

Following Hamada et al. (2013), the relative efficiency in recruiting early vs. late I-waves in a given individual was estimated by eliciting MEPs at three different coil orientations. As described earlier, current induced with the PA direction tends to recruit early I-waves, while current induced with the AP direction tends to recruit later I-waves (Ni et al., 2011). In the LM direction, strong TMS pulses produced MEPs with a latency close to those elicited by transcranial electrical stimulation and thus, are thought to reflect D-waves recruitment (Di Lazzaro et al., 2004). For the PA current, the coil was held tangentially to the scalp in the usual orientation, i.e., with the handle pointing backward and the coil oriented ∼45° in the mid-sagittal plane. For the LM orientation, the coil was applied on the hot spot with the handle pointing downward

[1]http://clinicalresearcher.org/software.htm

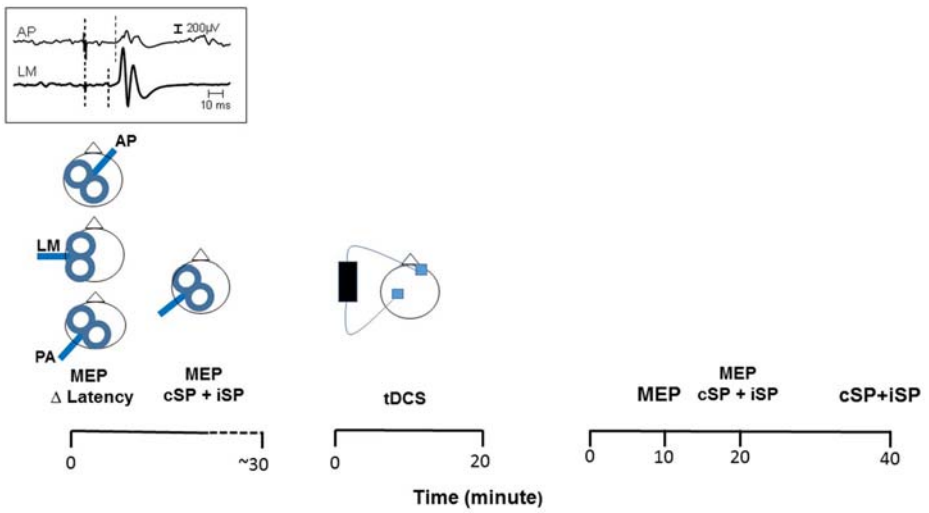

FIGURE 1 | Schematic representation of the experimental paradigm. Participants (n = 20) first underwent transcranial magnetic stimulation (TMS) to determine latency of motor evoked potentials (MEPs) using three different coil orientations: anterior-posterior (AP), latero-medial (LM) and posterior-anterior (PA). An example of differences in MEP latency evoked in AP and LM orientation is shown in the inset. Then, participants underwent TMS again to determine baseline value for the MEP amplitude at rest and the contralateral and ipsilateral silent period (cSP and iSP) during contraction. Then, anodal transcranial direct current stimulation (a-tDCS) was applied (20 min, 2 mA) with the anode placed on the motor cortex contralateral to the preferred hand (right, n = 18) and the cathode placed on the opposite supra-orbital region. Finally, to assess the after-effect of tDCS, changes in the MEPs at rest were measured at 10 and 20 min post-tDCS. At the 20th min, the cSP and iSP were also measured and then again at 40th min post.

in line with the inter-aural line. For the AP current, the coil was simply rotated ∼180° form the usual PA orientation with the handle pointing anteriorly. At each orientation, 10 MEPs were elicited by stimulating the hemisphere contralateral to the preferred hand (right, n = 18) and while the FDI was actively contracting using the pinching task described above (∼10% MVC). The stimulation intensities at each orientation were based on the aMT previously determined (see preceding section). For the PA orientation the intensity was set at 110% of aMT, whereas for the LM and AP orientation the intensity was set at 140% aMT. The order of testing with each coil orientation was counterbalanced across participants. Examples of MEP latency evoked at the different coil orientations can be seen in **Figure 1**.

Baseline MEP Amplitude and cSP/iSP Assessment

After testing with the different coil orientations, baseline MEP amplitude at rest was determined by recordings 10 MEPs in response to single TMS pulses delivers at 120% rMT on the hemisphere (left, n = 18) contralateral to the preferred hand. Then, the cSP and iSP were assessed concurrently as described previously (Davidson and Tremblay, 2013a). Briefly, participants were instructed to press as hard as they could on a pinch dynamometer with their preferred hand (right, n = 18), while exerting a light constant pinching force (∼25% of the maximal strength) with their opposite hand on a second dynamometer. Participants were trained to maintain the contractions for 3 s in synchronization with a tone (550 Hz) generated by the computer. In each trial, a supra-threshold (130% rMT) TMS pulse was delivered on the hemisphere ipsilateral to the maximally contracting hand at 2 s in the course of the 3 s trial. From this hemispheric stimulation, an iSP could be elicited in the ipsilateral hand (maximal contraction) while, at the same time, a cSP with the accompanying MEP could be recorded in the opposite hand (light contraction). Such procedure was repeated five times to get a sample of iSP and cSP recordings with an interval of at least 60 s between trials to prevent fatigue.

Anodal tDCS (a-tDCS) Intervention

The a-tDCS intervention was performed using the typical montage to induce changes in corticomotor excitability (Nitsche and Paulus, 2000) with the anode (35 cm^2) positioned over the FDI motor hotspot (left, n = 18) and the cathode (100 cm^2) in the contralateral supra-orbital area. Prior to application, the electrodes were placed in sponges previously soaked with saline solution (0.9% sodium chloride, Baxter, Corp., Toronto, ON, Canada). The a-tDCS was produced using a SmartStim Model 200[2] (NorDocs Technologies Inc., Sudbury, ON, Canada) and consisted of 2 mA current applied for 20 min with a 30 s ramp-up and ramp-down. Participants were asked to fill a brief questionnaire both during (5 min) and after the stimulation to monitor for possible side effects.

Post-tDCS Assessment

Once the stimulation completed, participants underwent TMS at T10 and T20 to assess change in resting corticomotor excitability by recording 10 MEPs @ 120% rMT. At T20, and once MEPs

[2]Investigation Testing Authorization No. 213954, Health Canada.

had been recorded, the cSP and iSP were assessed during active contractions. These measures were again repeated at T40. As indicated before, the joint assessment of the cSP and iSP was delayed until the 20th min to avoid possible confound with assessment of excitability at rest owing to post-contraction after-effects (Goldsworthy et al., 2014).

Data Analysis

All the analyses were performed offline by the same investigator (TD). The analysis was carried out in three steps. First, MEPs trials recorded with the different coil orientation (i.e., PA, LM, AP) were superimposed in each participant to determine their onset latency using visual inspection. From this data set, the AP-LM and PA-LM latency differences were computed in each participant to estimate the relative ease of recruiting early and late I-waves, respectively. Second, MEPs recorded at baseline and at each interval post-tDCS were averaged to derive mean amplitude values for each participant. Finally, cSP and iSP trials were analyzed to measure duration and other parameters. For this analysis, a trial-by-trial approach was performed. For trials with the contralateral hand (light contraction), the cSP duration was determined as the time interval from the onset of the MEP to the return of at least 50% of the mean pre-stimulus background EMG activity. Note that the size of the facilitated MEP (peak-to-peak) in each trial was also measured but was not considered as an outcome in this study. For trials with the ipsilateral hand, the iSP onset and offset were determined to derive two measures of transcallosal inhibition. The iSP onset was determined as the time from the stimulus onset until the 1st sign of significant decline (i.e., $\geq$25%) in the mean rectified EMG activity from pre-stimulus level for at least a 5 ms duration (i.e., 10 consecutive sampling points at 2 kHz), whereas the iSP offset was determined as the 1st sign of sustained recovery (>5 ms in duration) in the background EMG activity. The latter time point is usually easy to determine, as the end of the myoelectric silence is generally followed by an abrupt return of EMG activity in the recovery period (Davidson and Tremblay, 2013a,b). From these two time points, we used the iSP onset as an index of the latency of transcallosal inhibition (LTI) and the difference in ms between the offset and onset as an index of the duration of transcallosal inhibition (DTI).

Statistical Analysis

To examine the variability of inter-individual responses, variations measured at each time point post-tDCS were averaged for all dependent variables (i.e., MEP amplitude, cSP duration, LTI and DTI) to get a grand average. The grand average was then expressed as a ratio relative to baseline for each participant. To assess the overall effect of a-tDCS, one-way repeated-measures analyses of variance (ANOVA) was performed on each dependent variable to detect main effect. Upon detection of main effect, the Dunnett's multiple tests was performed to locate significant differences from baseline. MEP amplitude data were not normally distributed (Shapiro-Wilk test $p < 0.05$) and had to be log-transformed before entering the ANOVA. Finally, linear regression analyses were performed to examine the relationship between MEP latency differences and individual responses to a-tDCS expressed as ratios for all dependent variables. In addition, following the observations of Wiethoff et al. (2014), the predictive value of baseline MEP amplitude was also examined using regression analysis. The level of significance was set at $p < 0.05$ for all tests. All the analyses and graphical illustrations were performed using GraphPad Prism version 6.00 for Windows (GraphPad Software, La Jolla, CA, USA[3]).

RESULTS

General Observations, Motor Threshold and MEP Latency

In general, both the TMS and tDCS were well tolerated. During the tDCS application, most participants reported tingling (60%) and itching 40%, but these effects were rated as mild to moderate on a 6-point scale. The average threshold for the rMT and aMT was respectively 37.5% ($\pm$6.0) and 32.1% ($\pm$2.5). As expected, the MEP onset latency measured with the LM orientation (mean 19.4 $\pm$ 1.5 ms) was, on average, 1.4 and 2.6 ms shorter than that measured with either the PA (20.8 $\pm$ 1.6 ms) or AP orientation (22.0 $\pm$ 1.5 ms).

Effect of tDCS on MEP Modulation and cSP in the Contralateral Hand

Individual responses to tDCS were quite variable when considering changes in MEP amplitude. This variability is evident by inspecting **Figure 2A**, where individual variations in MEP amplitude relative to baseline are shown for all participants. Note that MEP data from one participants (#5) had to be removed for her facilitation post-tDCS far exceeded the range observed in other participants (significant outlier, Grubb's test, $Z = 2.7$, $p < 0.05$). Only ~40% of the participants (8/19) exhibited the expected pattern of MEP facilitation, while the remaining either showed a depression ($n = 6$) or minor changes in amplitude relative to baseline ($n = 5$). In view of this variability, no significant main effect ($F_{(2,18)} = 0.13$, $p = 0.81$) was detected when comparing MEP log-amplitude before and after a-tDCS (**Figure 2C**). With regards to changes in the cSP duration, the pattern of responses was characterized by a large proportion of "non-responders" (12/20) who showed little or no modulation relative to baseline, as can be seen in **Figure 2B**. Accordingly, the ANOVA failed to detect main effect ($F_{(2,19)} = 2.2$, $p = 0.14$) in the cSP duration in response to a-tDCS (**Figure 2D**).

Effect of tDCS on Measures of Transcallosal Inhibition

As shown in **Figure 3A**, individual variations measured in LTI in response to a-tDCS showed less variability between participants than those seen for either MEPs or cSP. In fact, while the magnitude of changes tended to be small relative to baseline, the direction of change was highly consistent with 75% (15/20) of participants showing reduced LTI. The latter after-effect was

[3]www.graphpad.com

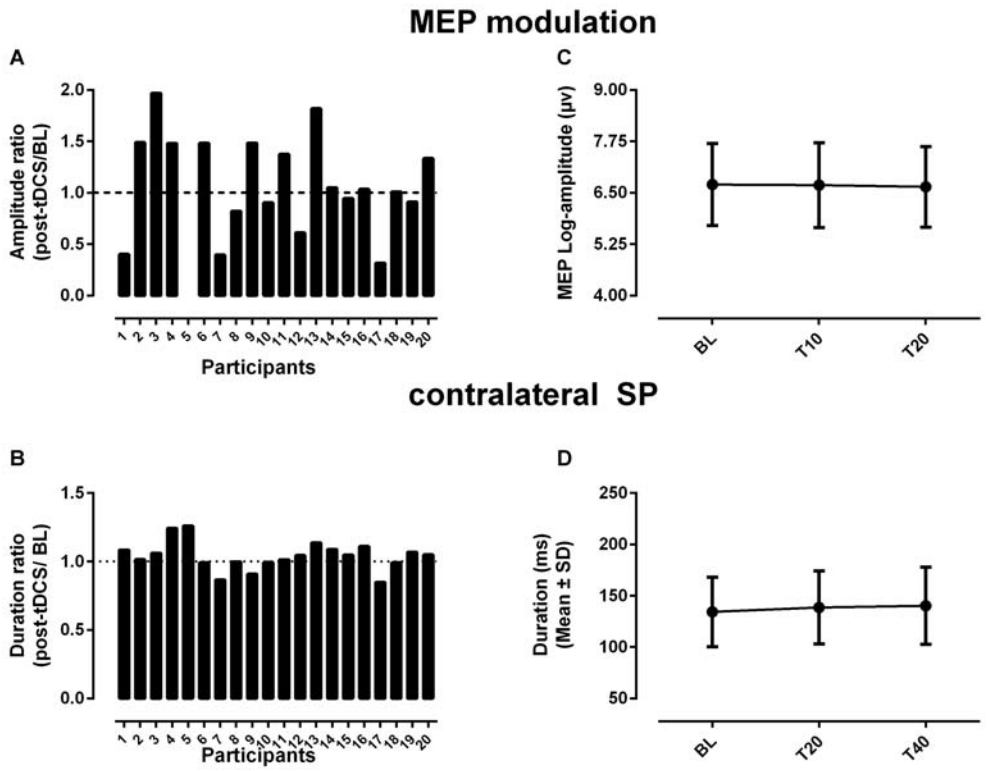

FIGURE 2 | Changes in MEP amplitude (A,C) and in the contralateral silent period (SP) duration (B,D) in response to a-tDCS. In **(A,C)** changes are shown for each individual as a ratio of the grand average computed for all time points post-tDCS divided by baseline (i.e., post-tDCS/BL). The dotted lines in each graph indicate no change relative to baseline. Note that the missing data **(A)** refers to an outlier (participants #5) whose MEP data had to be removed from the analysis. In **(B,D)** the overall changes are shown as means and standard deviations computed across all participants and for each time point before (BL) and after a-tDCS. Note the relatively large variability observed between individuals for modulation in MEP amplitude **(A)** and in cSP duration **(C)**.

confirmed by the ANOVA ($F_{(2,19)}$ = 10.5, p = 0.001) and significant differences from baseline were detected for both T20 and T40 (**Figure 3C**). Although the direction of changes in DTI was less consistent than that seen for LTI, a majority of participants (13/20) exhibited an increase in duration, as shown in **Figure 3B**. The ANOVA confirmed that DTI was significantly changed post-tDCS ($F_{(2,19)}$ = 8.97, p = 0.009) with significant differences from baseline being detected both at T20 and T40 (**Figure 3D**).

Relationship Between MEP Latency Differences and Responses to tDCS

The results of the regression analysis to examine predictors of individual responses to a-tDCS are shown in **Table 1**. It can be seen that among the candidate variables only latency differences exhibited some degrees of association with variations observed in corticomotor excitability and in transcallosal inhibition following a-tDCS. AP-LM latency differences proved to be particularly strong predictors of MEP modulation, this factor accounting for >40% of the variance seen after tDCS. PA-LM latency differences were also good predictors of MEP modulation. Finally, AP-LM latency differences also tended to predict variations observed in DTI, but the relationship was only marginally significant (p = 0.06). In **Figure 4**, the association between latency differences and individual responses to tDCS can be further appreciated for both MEP (**Figures 4A,B**) and DTI (**Figure 4C**) variations.

DISCUSSION

In this study, we examined whether variability in response to a-tDCS in terms of changes in corticomotor excitability and in transcallosal inhibition could be predicted from individual susceptibility to activate early of late I-waves, as reflected in latency differences arising from TMS with different coil orientation. Consistent with other reports (López-Alonso et al., 2014; Wiethoff et al., 2014), individual responses in terms of changes in MEP amplitude were quite variable among participants leading to overall marginal after-effects. In comparison, changes in transcallosal inhibition appeared more consistent, leading to detection of significant changes in both LTI and DTI in the stimulated hemisphere post-tDCS. In addition, in agreement with Wiethoff et al. (2014), we showed that latency differences derived from TMS are indeed good predictors of individual changes in MEP amplitude observed after a-tDCS. In the following discussion, we will address the significance of

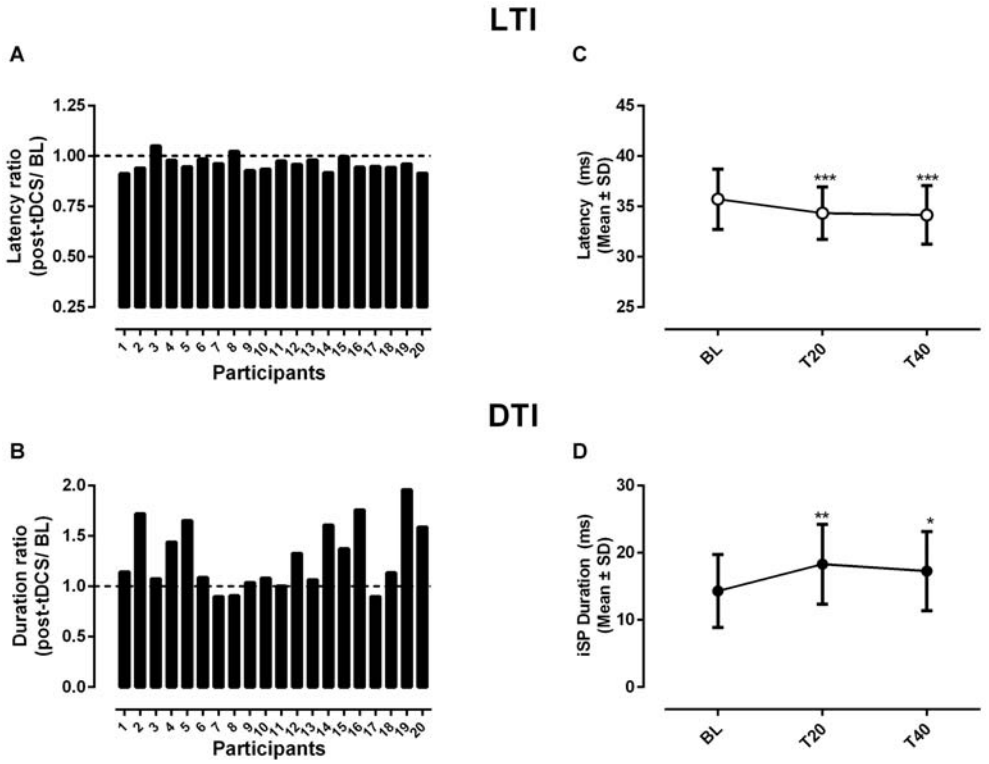

FIGURE 3 | Changes in the onset latency (A,C) and in the duration (B,D) of transcallosal inhibition (respectively, LTI and DTI) in response to a-tDCS. In **(A,C)** changes are shown for each individual as a ratio of the grand average computed for all time points post-tDCS divided by baseline (i.e., post-tDCS/BL). The dotted lines in each graph indicate no change relative to baseline. In **(B,D)** the overall changes are shown as means and standard deviations computed across all participants and for each time point before (BL) and after tDCS. Note the significant changes in both LTI and DTI relative to baseline detected in post-test comparisons as indicated by the asterisks (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

these results for studies on neuromodulation and discuss the relationships observed between tDCS after-effects and latency differences.

Variability in Measures of Corticomotor Excitability

As stated above, the after-effects of a-tDCS were characterized by a high degree of inter-individual variability when expressed in terms of changes in MEP amplitude and in cSP duration. In both measures, this variability arose from the presence of a substantial proportion (40–50%) of "non-responders" whose response deviated from to the expected "canonical" response, i.e., they either showed no modulation or a modulation in the opposite direction (e.g., inhibition instead of facilitation). In terms of MEP modulation, our observations on inter-individual variability closely match with those in other recent reports (López-Alonso et al., 2014; Wiethoff et al., 2014), which provides further corroboration that individual responses to a-tDCS are indeed inherently variable when using MEP amplitude as an outcome. As stressed by Wiethoff et al. (2014), this variability has implications for planning experiments or interventions in clinical populations for the expected presence of a substantial proportion of "non-responders" needs to be accounted in designing the intervention. As for changes in the cSP, only a handful of tDCS studies have examined this question and while some reports did find after-effects with tDCS (Hasan et al., 2012; Tremblay et al., 2013), others found no after-effects (Suzuki et al., 2012; Hendy and Kidgell, 2013). In the present report, we did not detect changes in the cSP in our group of participants owing to the presence of large number of non-responders. Our observation on the cSP are consistent with the report of Suzuki et al. (2012) who also failed to detect modulation in cSP after either cathodal or anodal a-tDCS. Thus, the effects of a-tDCS in modulating GABAergic inhibition, as reflected in the cSP duration, remain equivocal given the pattern of inconsistent responses observed within and between studies. To summarize, in agreement with recent reports, a great deal of variability seems to characterize individual responses to a-tDCS, especially when considering variations in MEP amplitude or in cSP duration, leading to marginal after-effects when changes are averaged across all participants.

Variability in Measures of Transcallosal Inhibition

Contrasting with the large variability seen for MEPs, changes in LTI and DTI were correspondingly far more consistent both

TABLE 1 | Coefficient of determination (r^2) computed from regression analysis for candidate predictors of individual variations in measures of corticomotor excitability and in transcallosal inhibition following anodal tDCS.

	Measures of excitability derived from the contralateral hand		Measures of transcallosal inhibition derived from the ipsilateral hand	
Predictor	**MEP (n = 19)**	**cSP**	**LTI**	**DTI**
BL MEP[1]	0.13	0.12	<0.01	0.11
AP-LM LD	**0.44****	0.13	<0.01	0.18
PA-LM LD	**0.23***	0.01	0.02	0.01
AP-PA LD	0.09	0.11	0.02	0.17

*Note that all dependent variables correspond to ratio values of the grand average post-tDCS/baseline. Also note that, except for baseline MEP (n = 19, 1 outlier), all correlations are based on 20 participants. Abbreviations: MEP, Motor evoked potential, cSP, contralateral silent period; LTI, latency of transcallosal inhibition; DTI, duration of transcallosal inhibition; BL, Baseline, AP-LM LD, Anterior-posterior-Latero-medial latency differences; PA-LM LD, Posterior-anterior-Latero-medial latency differences; AP-PA LD, Anterior-posterior-Posterior-anterior latency differences. Significant correlations are denoted in bold characters and asterisks (*p < 0.05), **p < 0.01.*

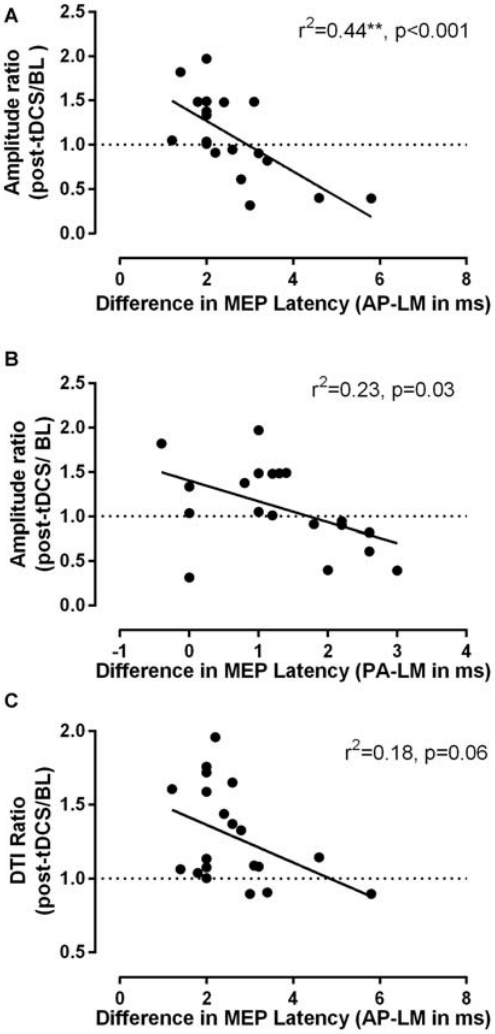

FIGURE 4 | Relationships between MEP latency differences measured with different TMS coil orientations (AP, LM, PA) and individual responses to a-tDCS. In **(A,B)** the respective value of AP-LM and PA-LM latency differences in predicting variations in MEP amplitude is shown, while in **(C)**, the predictive value of AP-LM differences to explain variations in DTI is shown. Note that small differences in both AP-LM and PA-LM orientations were strongly predictive of the probability of exhibiting MEP facilitation (i.e., ratio > 1) in response to a-tDCS.

in terms of magnitude and direction. This smaller variability allowed for detection of significant after-effects post-tDCS, which were indicative of an increased excitability of the transcallosal pathway in the stimulated hemisphere. To our knowledge, this study is the first to report a lasting modulation in the transcallosal inhibitory drive from the stimulated hemisphere towards the non-stimulated hemisphere, as reflected in the shorter LTI and prolonged DTI observed post-tDCS. In their 2004 report, Lang et al. (2004) also observed an increased transcallosal inhibitory drive in association with anodal stimulation but only from the non-stimulated hemisphere towards the stimulated hemisphere. To account for this observation, the authors speculated on the possibility of a remote influence of t-DCS on the inhibitory interneurons of the opposite hemisphere receiving transcallosal excitatory projections from the stimulated hemisphere. In the present study, both DTI and LTI changes suggest a more direct action of a-tDCS in increasing the excitability of transcallosal projections from the stimulated hemisphere. The reason as to why Lang et al. (2004) failed to detect a direct effect on the transcallosal pathway of the stimulated hemisphere might be linked with the fact that their tDCS intervention was only applied for 10 min at 1 mA. In this study, we used twice the time and intensity, which may explain the difference given that a higher intensity of tDCS may be required to modulate transcallosal excitability as interhemispheric connections have higher thresholds than corticospinal neurons (Wassermann et al., 1991). Another reason for the discrepancy might be related to methodological differences in eliciting the iSP. Lang et al. (2004) used very high TMS intensity (150% rMT) during mild bilateral contraction of the FDI (50% max), whereas we used the procedure advocated by Giovannelli et al. (2009), see "Materials and Methods" Section, which involves mild contraction of the contralateral hand with maximal contraction of the ipsilateral hand. In spite of these differences, both our observations and those of Lang et al. (2004) converge to indicate that tDCS can potentially induce lasting modulation in the excitability of the transcallosal pathway between motor cortices. One important caveat, however, is the fact that in the absence of a sham condition, we cannot exclude the possibility that other factors (e.g., changes in alertness, tiredness) might have contributed to the observed modulation in transcallosal inhibition.

Predicting Modulation in Corticomotor Excitability and in Transcallosal Inhibition

Consistent with the work of Hamada et al. (2013) and those of Wiethoff et al. (2014), we found that latency differences derived

from TMS proved to be good predictors of individual responses to a neuromodulatory intervention. These predictions concerned changes in MEP amplitude, in particular, and not those affecting the cSP duration, which is somewhat expected given that the two measures reflect different underlying mechanism at the cortical level (i.e., modulation of glutaminergic excitatory transmission vs. modulation by GABA B receptors; Ziemann et al., 2015). Contrary to Wiethoff et al. (2014) we did not find an association between smaller baseline MEP amplitude and larger facilitation post-tDCS, although the authors emphasized that the association was only "borderline". Thus, the potential role of baseline amplitude in predicting MEP modulation in response to a-tDCS remains equivocal. On the other hand, our observations on the role of latency differences in predicting MEP modulation after a-tDCS resonate strongly with those of Wiethoff et al. (2014), since in both their study and ours, AP-LM latency differences proved to be the best predictor (i.e., compared to the other latency differences) of whether a given individual would show facilitation or inhibition in response to stimulation. In fact, in close correspondence with their results (Wiethoff et al., 2014), our analysis revealed that AP-LM latency differences <2.5 ms were highly predictive of the probability of exhibiting the canonical response to anodal stimulation. Such finding provides corroborating evidence that individual susceptibility to recruit early I-waves, as reflected in small AP-LM differences, is indeed a good predictor of whom is likely to respond favorably to a-tDCS. As discussed by Wiethoff et al. (2014) and others (for a review, see Stagg and Nitsche, 2011), the fact that tDCS is thought to exert its influence on the cell body of pyramidal neurons, where early I-waves are likely generated, might account for the link between AP-LM latency differences and the probability of showing either inhibition or facilitation after a-tDCS. The fact that PA-LM latency differences were also predictive of MEP modulation, though to a lesser degree than AP-LM differences, further point to the interplay between early I-waves and a-tDCS after-effects. As pointed out by Hamada et al. (2013), small differences in PA-LM latency (i.e., 1–2 ms) is suggestive of individuals in whom D-wave or early I-waves can be easily recruited even when the stimulation is delivered in the conventional PA orientation. Consistent with this interpretation, only participants with PA-LM latency differences <2 ms exhibited MEP facilitation (see **Figure 3B**).

With regards to changes in transcallosal inhibition, although a trend was seen for an inverse relationship between AP-LM differences and DTI changes observed post-tDCS, more observations will be needed to confirm the nature of this relationship, i.e., whether a higher susceptibility to recruit D-waves in response to TMS also reflects a higher probability to show modulation in the transcallosal pathway. There is neurophysiological evidence that corticospinal and transcallosal pyramidal neurons share common neuronal circuitry at the intra-cortical level (Trompetto et al., 2004; Avanzino et al., 2007), and thus, it is possible that individuals who are more likely to express corticospinal facilitation in response to a-tDCS, i.e., owing to easy recruitment of D-wave as evidenced by small AP-ML differences, may also exhibit parallel changes in the transcallosal pathway arising from the stimulated hemisphere. Although still highly speculative this possibility certainly deserves more attention in future studies.

In conclusion, the present study adds further observations with regards to the importance of considering inter-subject variability when planning experiments based on neuromodulatory protocols design to induce lasting changes in corticomotor excitability, such as a-tDCS. Such consideration appears particularly important for tDCS studies when the aim is to modulate motor excitability to enhance motor responses such as in patients with stroke or with Parkinson's disease. Our report also adds further evidence to corroborate the value of latency differences, as surrogate measures of early and late I-waves recruitment, to predict individual changes in MEP modulation in response to a-tDCS. Finally, our report provides new observations suggesting that a-tDCS can potentially exert modulatory influence on the excitability of the transcallosal pathway originating from the stimulated hemisphere.

AUTHOR CONTRIBUTIONS

TWD participated in the design of the study, carried out the data collection, analyzed the data, and drafted the earlier version of the manuscript. MB participated in the design of the study. FT conceived the study, aided with data collection and in drafting and editing the final version of the manuscript. All authors read and approved the final manuscript.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Appendix C – Handedness Questionnaires

Handedness Questionnaire

Edinburgh Handedness Inventory

Please indicate your preferences in the use of hands in the following activities by putting a check in the appropriate column. Where the preference is so strong that you would never try to use the other hand, unless absolutely forced to, put 2 checks. If in any case you are really indifferent put and a check in both columns.

Some of the activities listed below require the use of both hands. In these cases the part of the task, or object, for which hand preference is wanted is indicated in brackets.

Please try and answer all of the questions, and only leave a blank if you have no experience at all with the object or task.

	Left	Right
1. Writing	☐ ☐	☐ ☐
2. Drawing	☐ ☐	☐ ☐
3. Throwing	☐ ☐	☐ ☐
4. Scissors	☐ ☐	☐ ☐
5. Toothbrush	☐ ☐	☐ ☐
6. Knife (without fork)	☐ ☐	☐ ☐
7. Spoon	☐ ☐	☐ ☐
8. Broom (upper hand)	☐ ☐	☐ ☐
9. Striking Match (match)	☐ ☐	☐ ☐
10. Opening box (lid)	☐ ☐	☐ ☐
TOTAL(count X's in both columns)		

Scoring:
Add up the checks in both left and right columns.
Whichever number is greater, would be considered your handedness.

Test de Latéralité d'Edinburgh

Le test de latéralité d'Edinburgh est un questionnaire qui permet de déterminer votre latéralité dominante, en d'autres termes de savoir si vous êtes droitier, gaucher, ou ambidextre.

Vous devez simplement indiquer votre préférence pour l'utilisation de la main (ou du pied ou de l'oeil) au cours des activités suivantes, en indiquant D (droitier) ou G (gaucher) dans la case correspondante. Si votre préférence est tellement forte que vous êtes certain(e) que vous n'utiliserez jamais votre autre main à moins d'y être forcé(e), seulement en cas de fracture du bras par exemple, alors vous devez écrire DD ou GG. Si vous n'avez réellement aucune préférence de latéralité pour une activité donnée, vous pouvez indiquer A (ambidextre).

Certaines des activités proposées demandent l'utilisation des deux mains. Dans ce cas, la partie de l'activité ou de l'objet pour lequel la préférence manuelle est demandée est indiquée entre parenthèses. Essayez de répondre à ***toutes*** les questions, et ne laissez un blanc que si vous n'avez réellement aucune expérience de cet objet ou activité.

écrire	
dessiner	
lancer	
utiliser une paire de ciseaux	
se brosser les dents	
utiliser un couteau (sans la fourchette)	
utiliser une cuillère	
balayer (la main qui est au-dessus sur le manche)	
allumer une allumette (main qui tient l'allumette)	
ouvrir une boîte (main qui s'occupe du couvercle)	
shooter dans une balle (avec le pied)	
vue monoculaire (quel oeil reste ouvert, par exemple pour regarder dans le viseur d'un appareil photo)	

Waterloo Handedness Questionnaire Revised (English)

Instructions: Please indicate your hand preference for the following activities by circling the appropriate response. If you always (i.e. 95 % or more of the time) use one hand to perform the described activity, circle Ra or La (for right always or left always). If you **usually** (i.e. about 75% of the time) use one hand circle Ru or Lu as appropriate. If you use both hands equally often (i.e. you use each hand about 50% of the time), circle Eq.

1. Which hand would you use to adjust the volume knob on a radio?	La	Lu	Eq	Ru	Ra
2. With which hand would you use a paintbrush to paint a wall?	La	Lu	Eq	Ru	Ra
3. With which hand would you use a spoon to eat soup?	La	Lu	Eq	Ru	Ra
4. Which hand would you use to point to something in the distance?	La	Lu	Eq	Ru	Ra
5. Which hand would you use to throw a dart?	La	Lu	Eq	Ru	Ra
6. With which hand would you use the eraser on the end of a pencil?	La	Lu	Eq	Ru	Ra
7. In which hand would you hold a walking stick?	La	Lu	Eq	Ru	Ra
8. With which hand would you use an iron to iron a shirt?	La	Lu	Eq	Ru	Ra
9. Which hand would you use to draw a picture?	La	Lu	Eq	Ru	Ra
10. In which hand would you hold a mug full of coffee?	La	Lu	Eq	Ru	Ra
11. Which hand would you use to hammer a nail?	La	Lu	Eq	Ru	Ra
12. With which hand would you use the remote control for a TV?	La	Lu	Eq	Ru	Ra
13. With which hand would you use a knife to cut bread?	La	Lu	Eq	Ru	Ra
14. With which hand would you use to turn the pages of a book?	La	Lu	Eq	Ru	Ra
15. With which hand would you use a pair of scissors to cut paper?	La	Lu	Eq	Ru	Ra
16. Which hand would you use to erase a blackboard?	La	Lu	Eq	Ru	Ra
17. With which hand would you use a pair of tweezers?	La	Lu	Eq	Ru	Ra
18. Which hand would you use to pick up a book?	La	Lu	Eq	Ru	Ra
19. Which hand would you use to carry a suitcase?	La	Lu	Eq	Ru	Ra
20. Which hand would you use to pour a cup of coffee?	La	Lu	Eq	Ru	Ra
21. With which hand would you use a computer mouse?	La	Lu	Eq	Ru	Ra
22. Which hand would you use to insert a plug into an outlet?	La	Lu	Eq	Ru	Ra
23. Which hand would you use to flip a coin?	La	Lu	Eq	Ru	Ra
24. With which hand would you use a toothbrush to brush your teeth?	La	Lu	Eq	Ru	Ra
25. Which hand would you use to throw a baseball?	La	Lu	Eq	Ru	Ra
26. Which hand would you use to turn a doorknob?	La	Lu	Eq	Ru	Ra
27. Which hand would you use for writing?	La	Lu	Eq	Ru	Ra
28. Which hand would you use to pick up a piece of paper?	La	Lu	Eq	Ru	Ra
29. Which hand would you use a hand saw?	La	Lu	Eq	Ru	Ra
30. Which hand would you use to stir a liquid with a spoon?	La	Lu	Eq	Ru	Ra
31. In which hand would you hold an open umbrella?	La	Lu	Eq	Ru	Ra
32. In which hand would you hold a needle while sewing?	La	Lu	Eq	Ru	Ra
33. Which hand would you use to strike a match?	La	Lu	Eq	Ru	Ra
34. Which hand would you use to turn on a light switch?	La	Lu	Eq	Ru	Ra
35. Which hand would you use to open a drawer?	La	Lu	Eq	Ru	Ra
36. Which hand would you use to press buttons on a calculator?	La	Lu	Eq	Ru	Ra
37. Is there any reason (i.e. injury) why you have changed your hand preference YES/NO for any of the above activities'? (circle one)					**YES/NO**
38. Have you been given special training or encouragement to use a particular hand for certain activities? (circle one)					**YES/NO**
39. If you have answered YES for either Questions 37 or 38, please explain:					

Waterloo Handedness Questionnaire Revised (French)

Instructions: S'il vous plait indiquez votre main préférée lors des activités décrites sous-dessous en encerclant la réponse approprié. Si vous utilise toujours une main pour l'activité (95% ou plus du temps), encercle Gt ou Dt (pour Gauche toujours ou Droite toujours). Si vous utilisez généralement une main (75% du temps), encerclez Gg ou Dg. Si vous utilisez les deux mains également (utilise chaque main 50% du temps), encerclez Eg.

1. Quelle main utilisez-vous lorsque vous utilisez le bouton de volume sur le radio?	Gt	Gg	Eg	Dg	Dt
2. Quelle main utilisez-vous lorsque vous peignez un mur avec un pinceau?	Gt	Gg	Eg	Dg	Dt
3. Avec quelle main utiliserez-vous une cuillère lorsque vous mangez de la soupe?	Gt	Gg	Eg	Dg	Dt
4. Quelle main utilisez-vous lorsque vous pointez du doigt?	Gt	Gg	Eg	Dg	Dt
5. Quelle main utilisez-vous pour lancer une fléchette?	Gt	Gg	Eg	Dg	Dt
6. Avec quelle main utiliserez-vous l'efface sur le bout de votre crayon?	Gt	Gg	Eg	Dg	Dt
7. Dans quelle main que vous tiendrez une canne?	Gt	Gg	Eg	Dg	Dt
8. Quelle main utilisez-vous pour manipuler un fer à repasser?	Gt	Gg	Eg	Dg	Dt
9. Quelle main utilisez-vous pour dessiner un portrait?	Gt	Gg	Eg	Dg	Dt
10. Dans quelle main tenez-vous votre verre de café?	Gt	Gg	Eg	Dg	Dt
11. Avec quelle main utilisez-vous afin d'enfoncer un clou avec un marteau?	Gt	Gg	Eg	Dg	Dt
12. Quelle main utilisez-vous lorsque vous utilisez la télécommande pour le TV?	Gt	Gg	Eg	Dg	Dt
13. Quelle main utilisez-vous pour couper du pain avec un couteau?	Gt	Gg	Eg	Dg	Dt
14. Quelle main utilisez-vous pour tourner les pages dans un livre?	Gt	Gg	Eg	Dg	Dt
15. Quelle main utilisez-vous pour coupeau du papier avec des ciseaux?	Gt	Gg	Eg	Dg	Dt
16. Quelle main utilisez-vous pour effacer un tableau?	Gt	Gg	Eg	Dg	Dt
17. Avec quelle main utiliserez-vous des pinces à épiler?	Gt	Gg	Eg	Dg	Dt
18. Quelle main utilisez-vous pour ramasser une livre?	Gt	Gg	Eg	Dg	Dt
19. Quelle main utilisez-vous pour transporter une valise?	Gt	Gg	Eg	Dg	Dt
20. Quelle main utilisez-vous pour verser un verre de café?	Gt	Gg	Eg	Dg	Dt
21. Avec quelle main utiliserez-vous une souris?	Gt	Gg	Eg	Dg	Dt
22. Quelle main utilisez-vous pour insérer une fiche dans une prise?	Gt	Gg	Eg	Dg	Dt
23. Quelle main utilisez-vous afin d'envoyer une pièce de monnaie?	Gt	Gg	Eg	Dg	Dt
24. Avec quelle main utiliserez-vous votre brosse à dent?	Gt	Gg	Eg	Dg	Dt
25. Quelle main utilisez-vous afin de lancer un baseball?	Gt	Gg	Eg	Dg	Dt
26. Quelle main utilisez-vous pour tourner une poignée de porter?	Gt	Gg	Eg	Dg	Dt
27. Quelle main utilisez-vous pour écrire?	Gt	Gg	Eg	Dg	Dt
28. Quelle main utilisez-vous afin de ramasser un morceau de papier?	Gt	Gg	Eg	Dg	Dt
29. Avec quelle main utiliserez-vous une scie à main?	Gt	Gg	Eg	Dg	Dt
30. Quelle main utilisez-vous pour mélange une liquide avec une cuillère?	Gt	Gg	Eg	Dg	Dt
31. Avec quelle main tenez-vous un parapluie?	Gt	Gg	Eg	Dg	Dt
32. Dans quelle main tenez-vous une aiguille lorsque vous coudrez?	Gt	Gg	Eg	Dg	Dt
33. Quelle main utilisez-vous pour allumer une allumette?	Gt	Gg	Eg	Dg	Dt
34. Quelle main utilisez-vous pour pousser un levier afin d'allumer la lumière?	Gt	Gg	Eg	Dg	Dt
35. Avec quelle main ouvrez-vous un tiroir?	Gt	Gg	Eg	Dg	Dt
36. Quelle main utilisez-vous pour peser des boutons sur une calculatrice?	Gt	Gg	Eg	Dg	Dt
37. Est ce qu'il y a une raison (blessures) pour laquelle vous avez changé votre préférence de main pour n'importe des activités ci-dessus OUI/NON? (encerclez une)					**OUI/NON**
38. Avez-vous reçus de l'entrainement spécial ou de l'encouragement pour utiliser une main en particulier pour certain activité? (encerclez une)					**OUI/NON**
39. Si vous avez répondu OUI à question 37 ou 38, s'il vous plait expliqué:					

Modified Edinburgh Handedness Questionnaire

Which hand do you prefer to use when:

	Left	No Preference	Right	Do you ever use the other hand?
Writing:	❑	❑	❑	❑ Yes
Drawing:	❑	❑	❑	❑ Yes
Throwing:	❑	❑	❑	❑ Yes
Using Scissors:	❑	❑	❑	❑ Yes
Using a Toothbrush:	❑	❑	❑	❑ Yes
Using a Knife (without a fork):	❑	❑	❑	❑ Yes
Using a Spoon:	❑	❑	❑	❑ Yes
Using a Broom (upper hand):	❑	❑	❑	❑ Yes
Striking a Match:	❑	❑	❑	❑ Yes
Opening a box (holding the lid):	❑	❑	❑	❑ Yes
	Additional Items			
Holding a Computer Mouse:	❑	❑	❑	❑ Yes
Using a Key to Unlock a Door:	❑	❑	❑	❑ Yes
Holding a Hammer:	❑	❑	❑	❑ Yes
Holding a Brush or Comb:	❑	❑	❑	❑ Yes
Holding a cup while Drinking:	❑	❑	❑	❑ Yes

This handedness questionnaire was adapted by Cohen 2008 from:

Oldfield, R.C. *"The assessment and analysis of handedness: the Edinburgh inventory."* Neuropsychologia. **9**(1):97-113. 1971.

Modified Edinburgh Handedness Questionnaire (French)

Quelle main est-ce que vous préférez lorsque vous :

	Gauche	Pas de Préférence	Droit	Est ce que vous utilisé l'autre main?
Écrire:	❑	❑	❑	❑ Oui
Dessiner:	❑	❑	❑	❑ Oui
Lancer:	❑	❑	❑	❑ Oui
Utiliser des ciseaux:	❑	❑	❑	❑ Oui
Brosser les dents:	❑	❑	❑	❑ Oui
Utiliser un couteau (sans fourchette):	❑	❑	❑	❑ Oui
Utiliser une cuillère:	❑	❑	❑	❑ Oui
Balayer (Main du haut):	❑	❑	❑	❑ Oui
Allumer une allumette:	❑	❑	❑	❑ Oui
Ouvrir une boîte (tenir le couvercle):	❑	❑	❑	❑ Oui
Items additionnels				
Tenir une souris d'ordinateur:	❑	❑	❑	❑ Oui
Débarrer une porte avec une clé:	❑	❑	❑	❑ Oui
Utiliser un marteau:	❑	❑	❑	❑ Oui
Tenir un peigne:	❑	❑	❑	❑ Oui
Tenir un verre pour boire:	❑	❑	❑	❑ Oui

This handedness questionnaire was adapted by Cohen 2008 from:

Oldfield, R.C. *"The assessment and analysis of handedness: the Edinburgh inventory."* Neuropsychologia. **9**(1):97-113. 1971.

Appendix D – Ethics Approvals

SOINS CONTINUS
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April 7, 2010

Mr. Travis Davidson
M.Sc. Student
School of Human Kinetics
University of Ottawa
EBRI/Health of the Elderly

Dr. Francois Tremblay
Scientist,
EBRI

RE: Functional and Neurophysiological Correlates of Corticospinal Function in Human Aging.
(Bruyère REB Protocol # M16-10-009)

Final Approval

Dear Dr. Tremblay and Mr. Davidson,

Thank you for your response to our conditional approval, dated March 4, 2010. The revised documents were received on April 1, 2010. This study has satisfied all of the ethical requirements.

We are pleased to give ethical approval for one year (April 7, 2010 to April 7, 2011) to proceed with the above titled study.

As you know, Pledges of Confidentiality will need to be submitted by all research staff prior to initiation of recruitment.

As per our new continuing review process for studies having undergone full REB review, we now require that an Interim Status report be submitted six months after every annual approval. Your first Interim Status report will be due on October 7, 2010.

We wish you the best of luck with this study.

Dr. Lisa Sweet, C. Psych.
Chair of the Research Ethics Board
Bruyère Continuing Care
(613) 562-6262 ext 1368

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June 25, 2012

Mr. Travis Davidson
Graduate Student
School of Human Kinetics
University of Ottawa
Bruyère Research Institute

Dr. Francois Tremblay
Professor
School of Health Sciences
University of Ottawa
Scientist, Bruyère Research Institute

RE: Neurophysiological and functional assessment of hemispheric motor functions in Asymptomatic Concussed Athletes.
(Bruyère REB Protocol # M16-12-023)

Final Approval

Dear Mr. Davidson and Dr. Tremblay,

Thank you for your response to our conditional approval letter. With the revisions, the application for full review has satisfied all ethical requirements.

As such, the Bruyère Continuing Care Research Ethics Board (REB) is pleased to give ethical approval for the period June 22, 2012 to June 22, 2013.

Please be advised that any complaints made by participants must be reported to the REB.

All changes to the approved protocol must be approved by the REB.

Please complete an Annual Project Update/Notification of Termination form by the approval end date as noted above.

We wish you the best of luck with your research endeavors.

Sincerely,

Dorothy Kessler, M.Sc., O.T. Reg (Ont), PhD Candidate
Chair
Research Ethics Board
Bruyère Continuing Care
(613) 562-6262 ext 1420

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October 15, 2013

Mr. Travis Davidson
Graduate Student
School of Human Kinetics
Faculty of Health Science
University of Ottawa
Care of the Elderly
Bruyère Research Institute

Re: Hemispheric inhibition between motor cortices: a reliability study of TMS-derived measures of interhemispheric inhibition.
(Bruyère REB Protocol # M16-13-040)

Final Approval

Dear Mr. Davidson,

Thank you for your response to our conditional approval letter. With the revisions, the application has satisfied all ethical requirements.

As such, the Bruyère Continuing Care Research Ethics Board (REB) is pleased to give you ethical approval for the period October 15, 2013 to October 15, 2014.

Please be advised that any complaints made by participants must be reported to the REB.

All changes to the approved protocol must be approved by the REB.

Please complete an Annual Project Update/Notification of Termination form by the approval end date as noted above.

We wish you the best of luck with your research endeavors.

Sincerely,

Dorothy Kessler, M.Sc., O.T. Reg. (Ont), PhD Candidate
Chair, Research Ethics Board
Bruyère Continuing Care
(613) 562-6262 ext 1420
dkessler@bruyere.org

Cc : Dr. François Tremblay

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August 29, 2013

Dr. Francois Tremblay
Full Professor, Physiotherapy Program
Rehab Sciences/Health Sciences
University of Ottawa
Scientist
Bruyère Research Institute

Re: Variations in motor evoked potentials (MEP) latency as predictors of responses to anodal transcranial direct current stimulation (tDCS) treatment: a pilot study.
(Bruyère REB Protocol # M16-13-025)

Final Approval

Dear Dr. Tremblay,

Thank you for your response to our conditional approval letter. With the revisions, the application has satisfied all ethical requirements.

As such, the Bruyère Continuing Care Research Ethics Board (REB) is pleased to give you ethical approval for the period August 29, 2013 to August 29, 2014.

Please be advised that any complaints made by participants must be reported to the REB.

All changes to the approved protocol must be approved by the REB.

Please complete an Annual Project Update/Notification of Termination form by the approval end date as noted above.

We wish you the best of luck with your research endeavors.

Sincerely,

Dorothy Kessler, M.Sc., O.T. Reg. (Ont), PhD Candidate
Chair, Research Ethics Board
Bruyère Continuing Care
(613) 562-6262 ext 1420

cc: Travis Davidson