THE EFFECT OF THERMAL STIMULATION ON CORTICOSPINAL EXCITABILITY

By

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DEDICATION

In memory of my dedicated loving father, Bahram Ansari.

He was an inspiration in life and in death.

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The highest praise belongs to God from whom all blessings flow.

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AUTHOR CONTRIBUTIONS

The work described in this thesis was carried out by the author (YA) under the supervision of Professor François Tremblay. The author and the supervisor conceived the experiments. Data collection was performed by the author with the assistance of Anthony Remaud and Professor Tremblay. The data were analyzed by the author. Both the author and supervisor were involved in the drafting, writing, and editing of the manuscripts included in the thesis. The Research Ethics Board at the Bruyère Hospital approved the procedures used in this study (Appendix A).

SUMMARY

This thesis describes a series of experiments to investigate the effect of thermal stimulation on corticospinal excitability using transcranial magnetic stimulation (TMS). Experiment I showed that innocuous cooling or warming of a single digit, produced short-lasting and mixed patterns of modulation only during actual thermal stimulation, with the inhibition being the most common pattern observed. In line with this finding, cooling stimulation applied to a larger area (i.e. multi-digits) produced variable but more sustained modulation in motor evoked potential (MEP) amplitude in the post-cooling phase (Exp II). Notably, the responses to cooling in terms of either suppressed or enhanced corticospinal excitability tended to be fairly consistent in a given individual with repeated applications. When examining possible sources of the observed variable MEP modulation, we found that individual characteristics such as age, sex and changes in skin temperature had no major influences. We hypothesized that the variability of responses might be related to individual differences in the excitability of intra-cortical circuits involved in sensorimotor integration. To test this hypothesis, we performed Experiment III using conditioning TMS paradigms. This experiment revealed that TMS markers of sensorimotor integration (SAI and SAF levels) were good predictors of individual variations in cooling-induced modulation in corticospinal excitability. This provided evidence supporting the role of SAI and SAF as markers to predict individual's response to focal thermal stimulation. The identification of such predictors could enhance the therapeutic applicability of this form of stimulation in neurorehabilitation. Collectively, these findings advance our understanding of the neurophysiological basis of thermal stimulation and shed light on the development of a more rational application of neurofacilitation techniques based on afferent stimulation in clinical populations, such as stroke survivors.

RÉSUMÉ

Cette thèse décrit une série d'expériences pour étudier l'effet de la stimulation thermique sur l'excitabilité corticospinale en utilisant de la stimulation magnétique transcrânienne (SMT). Expérience I a montré que le refroidissement ou le réchauffement distal d'un doigt produisait une modulation variable de courte durée pendant la stimulation thermique, l'inhibition étant la réponse plus fréquente. En accord avec ce résultat, l'application de froid sur plus grande surface (c.-à-d. étendue à plusieurs doigts) a produit une modulation aussi variable mais plus soutenue au niveau de l'amplitude des potentiels évoqués moteurs (PEM) dans la phase post-refroidissement (Expérience II). En outre, nos observations montraient que les réponses au refroidissement en termes d'inhibition ou de facilitation tendaient à être assez reproductibles chez un même individu avec des applications répétées, même si espacées de plusieurs semaines. Nous avons examiné les sources possibles de la modulation variable et nous avons trouvé que les caractéristiques individuelles comme l'âge et le sexe ainsi que les changements dans la température de la peau n'avaient pas d'effets majeurs. Nous avons fait l'hypothèse que la variabilité des réponses pouvait être liée à des différences individuelles dans l'excitabilité des circuits intra-corticaux, ainsi que ceux impliqués dans l'intégration sensori-motrice. Pour tester cette hypothèse, nous avons effectué l'expérience III en utilisant des paradigmes de SMT à impulsions appariées. Nos résultats ont révélé des associations significatives entre les marqueurs de l'intégration sensori-motrice (niveaux de SAI et SAF) et les modulations induites par le refroidissement. Ces données démontrent ainsi le rôle des SAI et SAF comme prédicteurs des réponses individuelles à la stimulation thermique. L'identification de tels prédicteurs éclaire l'applicabilité thérapeutique de cette modalité dans la réadaptation post-AVC. Dans l'ensemble, ces résultats font progresser notre compréhension des

bases neurophysiologiques de la stimulation thermique focale et contribuent à ouvre la voie à une application plus rationnelle des techniques de neurofacilitation basées sur la stimulation afférente pour les populations cliniques comme les survivants d'AVC.

LIST OF ABBREVIATIONS

ACC	Anterior Cingulate Cortex	EPSPs	Excitatory Postsynaptic Potentials
Ach	Acetylcholine	FDI	First Dorsal Interosseous
ADM	Abductor Digiti Minimi	fMRI	Functional Magnetic Resonance Imaging
A-δ	Mechanosensitive Aδ Afferent Fibers	GABA	G-aminobutyric Acid
ALS	Anterolateral System	H-reflex	Hoffman Reflex
αMN	Alpha Motoneuron	ICF	Intracortical Facilitation
AMYG	Amygdala	ISI	Inter-stimulus Intervals
ANOVA	Analyses of Variance	I-waves	Indirect Wave
BL	Baseline	LAI	Long-latency Afferent Inhibition
BOLD	Blood Oxygen Level–Dependent signal	LICI	Long-interval Intracortical Inhibition
CNS	Central Nervous System	MD	Multi Digit
CS	Cold Stimulation	MEG	Magnetiencephalography
cSP	Cortical Silent Period	MEP	Motor Evoked Potential
D-wave	Direct Wave	MI	Primary Motor Cortex
DRG	Dorsal Root Ganglion	MTAT	Motor Threshold Assessment Tool
EEG	Electroencephalography	NIBS	Non-invasive Brain Stimulation
EHI	Edinburgh Inventory Index	NMDA	N-Methyl-D-Aspartic Acid
EMG	Electromyography	NMES	Neuromuscular Electrical Stimulation

PAG	Periaqueductal Grey Matter	VMpo	Ventral Medial Nucleus
PES	Peripheral Electrical Stimulation	VPL	Ventro Posterolateral
PFC	Prefrontal Cortex	VPM	Ventro Posteromedial
POA	Preoptic Area	WS	Warm Stimulation
PPC	Posterior Parietal Cortex		
PS	Post Cooling		
PW	Post warming		
RMT	Resting Motor Threshold		
rCBF	Regional Cerebral Blood Flow		
SA	Slowly Adapting fibers		
SAF	Short-latency Afferent Facilitation		
SAI	Short-latency Afferent Inhibition		
SMA	Supplementary Motor Area		
TGI	Thermal Grill Illusion		
TMS	Transcranial Magnetic Stimulation		
TS	Thermal Stimulation		
UE	Upper Extremity		
VAS	Visual Analogue Scale		

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CHAPTER I. INTRODUCTION AND LITERATURE REVIEW

Introduction

Peripheral stimulation techniques are commonly used in stroke rehabilitation as potential interventions to facilitate functional recovery of paretic muscles (Chen, Liang et al. 2005, Takeuchi and Izumi 2015). Such facilitation techniques involve the use of tactile or proprioceptive stimuli (e.g. muscle stretch) to change motor excitability. Thermal stimulation (TS) in the form of either cooling or warming stimuli applied to superficial and deep tissues has been used as a therapeutic intervention to treat pain and injuries for centuries. In recent years, this modality has been advocated as an effective neurofacilitation method to improve motor function in stroke survivors (Chen, Liang et al. 2005, Chen and Shaw 2006, Chen, Lin et al. 2011, Chen and Shaw 2014, Tai, Lai et al. 2014). Such thermal stimulation provides a convenient, practicable and low-cost therapeutic intervention that can be used in both rehabilitation and home-care-based settings (Liang, Hsieh et al. 2012). The advantage of thermal stimulation over other forms of peripheral stimulation comes from neuroimaging studies showing extensive neural activations both at the subcortical (e.g. ventro-basal thalamus) and cortical levels in response to skin cooling or warming, i.e., extending beyond classical somatosensory areas (S1 and S2) to insular cortex and posterior cingulate cortex (Davis, Kwan et al. 1998, Gelnar, Krauss et al. 1999, Bokiniec, Zampieri et al. 2018). There is indeed clinical evidence that repeated applications of hot and cold stimuli combined with other neurofacilitation techniques can facilitate motor reorganization and recovery of the upper and lower limb function in acute and subacute stroke patients (Chen, Liang et al. 2005,

Wu, Lin et al. 2010, Chen, Lin et al. 2011, Liang, Hsieh et al. 2012, Hsu, Lee et al. 2013, Chen and Shaw 2014). In line with these findings, Tai et al. (2014) found a significant increase in the motor map size and MEP amplitude in the lesioned hemisphere in response to 30-minutes of alternating noxious warm and cold stimulation of the affected arm in stroke patients (Tai, Lai et al. 2014). Several lines of evidence from the TMS literature suggest that in addition to noxious thermal stimulation, peripheral afferent stimulation in the innocuous range can not only alter motor representation and modulate central excitability in normal individuals, but also can induce neuroplasticity and ultimately improve motor recovery in stroke survivors (Johansson, Lindgren et al. 1993, Hamdy and Rothwell 1998, Wong, Su et al. 1999, Ridding, McKay et al. 2001, Conforto, Kaelin-Lang et al. 2002, Kaelin-Lang, Luft et al. 2002, Chipchase, Schabrun et al. 2011). Such evidence; however, is based almost exclusively on the application of electrical nerve stimulation, as reported by most TMS studies, except for a few studies that reported modulation of corticospinal excitability in response to other modalities of sensory stimulation, such as vibrotactile afferent stimulation (Forner-Cordero, Steyvers et al. 2008). The mechanisms which underlie these changes are still not fully understood; however, GABAergic inhibitory circuits are thought to be involved in the dynamic process of map organization in the motor cortex in response to repetitive peripheral nerve stimulation (Jacobs and Donoghue 1991, Kaelin-Lang, Luft et al. 2002, Rosenkranz and Rothwell 2004). Interestingly, reduced short afferent inhibition (SAI), a marker of sensorimotor integration linked with cholinergic and GABA mediated intra-cortical inhibition, in the lesioned hemisphere of acute stroke patients has been associated with greater motor recovery at 6 months (Lazzaro, Profice et al. 2012). Taken together, these findings point to the importance of considering changes in the excitability of circuits mediating sensorimotor integration when assessing the effects of sensory stimulation on corticospinal excitability.

In summary, similar to other forms of somatosensory afferent inputs, thermal afferent

stimulation has the potential to change the excitability of cortical circuits either directly through thalamocortical projections or indirectly via projections from somatosensory areas (e.g. S1 and S2) to primary motor cortex (M1) (Hooks 2016). Given that thermal stimulation is particularly powerful in eliciting strong activations in the brain areas involved in somatosensory processing; the evidence for its clinical effectiveness is growing. However, there are still many key questions to be answered regarding the neurophysiological basis underlying the thermally-induced facilitatory effects. For instance, it is yet to be elucidated whether innocuous thermal stimulation can lead to sustained modulation in corticospinal excitability (and if so, is cooling or warming more effective?). Such information is essential for further development of thermal stimulation as an effective neuromodulation method in stroke rehabilitation.

In the following sections, the general characteristics of thermal sensation as well as the anatomy and physiology of the somatosensory system will be reviewed. The review will next focus on how thermal inputs are detected by peripheral receptors and then transmitted through afferent pathways in the spinal cord to reach cortical targets to produce thermal sensations and thermosensory responses. Then, the physiological basis of transcranial magnetic stimulation and measures of corticospinal excitability will be discussed. Finally, the relevant literature examining the effects of sensory afferent stimulation on corticospinal excitability and neuroplasticity will be reviewed.

1.1 General Characteristics of Thermal Sensation

Skin is the largest sensory organ that protects the body from potentially harmful environmental threats such as chemical, physical and noxious thermal stimuli (Schepers and Ringkamp 2009). In addition to its protective role, skin contains thermal afferents, which

contribute to the identification of materials and objects' properties by touch (e.g. metal feels colder than wood and thus can be readily distinguished from wood) (Schepers and Ringkamp 2009). This sensory organ also plays a crucial role in maintaining the optimum body temperature by providing the core temperature-sensing systems with thermoregulatory afferent signals to initiate homeostatic mechanisms (Schepers and Ringkamp 2009). In this process, the central nervous system (CNS) receives physiological information from the peripheral thermoreceptors to compare peripheral and core body temperatures and initiate various physiological responses that will be discussed in detail later (Mylius, Borckardt et al. 2012).

1.1.1 Temperature Sensitivity across the Body

Generally, skin temperatures around 33 °C are perceived as thermoneutral, whereas those below ~15 °C or above ~45 °C are associated with noxious cold or burning hot sensations (Basbaum, Bautista et al. 2009, Vriens, Nilius et al. 2014). Thermal sensitivity varies throughout the body depending on the region (e.g. proximal or distal), skin type (e.g. hairy or glabrous) and skin thickness, which affects encoding and transmission of thermal signals (Filingeri, Fournet et al. 2014, Filingeri and Ackerley 2017). The regional variations in skin thermosensitivity also depend on the density of thermally receptive fields on the skin and the central integration of peripheral thermal inputs (Filingeri and Ackerley 2017). For instance, cold sensitivity seems to be higher in torso and abdomen compared to the head and limbs (Stevens 1979), while warmth sensitivity is higher in the face than torso and the limbs (Gerrett, Ouzzahra et al. 2014). Interestingly, there is no cold-insensitive region in the body; while insensitivity to mild warming has been reported in regions of the forearm (several cm^2 in area) where increase in temperature is only detected at the nociceptive threshold (41°C) (Cruz 1998, Hollins 2010).

1.1.2 Inter-individual Variations in Thermosensitivity

Sensitivity to temperature significantly varies among individuals (Vriens, Nilius et al. 2014). This variation depends on the activity of the depolarizing and repolarizing ion channels as well as age, body-mass index, physiological mechanisms and prior temperature experiences (Hollins 2010, Vriens, Nilius et al. 2014). In clinical research, thermal stimuli provoke various sensations depending on the individual differences (i.e. sex, ethnicity and previous pain experiences), characteristic of the applied stimulus (i.e. stimulation history, duration and area of thermal stimulus, temperature ramp rate) and the experimental conditions (i.e. properties and condition of the skin, testing site and day time of measurement) (Chery-Croze 1983).

1.1.2.1 Age Differences

Both warm and cold sensations have been reported to decline with advancing age (Stevens and Choo 2009). The magnitude of this decline depends on the body regions, which have different temperature sensitivity levels as stated earlier (Stevens and Choo 2009). For instance, the greatest age-related decline in thermal sensitivity has been observed in the extremities, with the foot being mostly affected, while the central regions of the body show slower decline in thermal sensitivity with age (Stevens and Choo 2009). Warm sensitivity has been reported to decline in feet with advancing age; however, no significant age-related differences in threshold to warm and cold stimuli as well as heat pain have been observed (Kenshalo 1986).

1.1.2.2 Sex Differences

There are sex differences in the painful and affective aspects of thermal perception. Several studies have reported greater pain sensitivity and responsiveness (i.e. higher unpleasantness) in females compared to males (Berkley 1997, Riley Iii, Robinson et al. 1998, Sarlani, Farooq et al. 2003, Hollins 2010). Given similar thermal intensity ratings in females and males, this variation is not due to sex differences in the skin thermal properties or higher sensory excitability in females, but could be related to previous experiences, cultural influence, hormonal level, integration of nociceptive inputs in the central nervous system as well as modulation of afferent inputs by descending supraspinal pathways (Berkley 1997, Riley Iii, Robinson et al. 1998, Sarlani, Farooq et al. 2003, Hollins 2010). Several studies, on the other hand, reported no sex difference in thermal sensitivity (Bertelsmann, Heimans et al. 1985, Jamal, Hansen et al. 1985, Kenshalo 1986, Stevens and Choo 2009), except one study that reported higher thermal threshold in males than females (Meh & Denislic, 1995).

1.2 Neurophysiological Basis of Thermal Sensation from Skin to Spinal Cord

1.2.1 Peripheral Basis of Thermal Sensation

Peripheral thermosensory system consists of distinct neurons and receptors that selectively differentiate thermal stimuli and provides the body with information about environmental temperature (Solinski and Hoon 2019). The sensory neurons innervating the skin are pseudo-unipolar with cell bodies located in the trigeminal ganglion for the head, face and oral cavity, and in dorsal root ganglia (DRG) for the trunks and extremities (Vriens, Nilius et al. 2014, Bokiniec, Zampieri et al. 2018, Solinski and Hoon 2019). These sensory neurons send signals to higher centers by projecting their axons to the spinal cord and the spinal portion of the trigeminal nucleus

in the lower pons and medulla (Randall 2002, Solinski and Hoon 2019). According to electrophysiological studies, there are distinct classes of peripheral primary sensory neurons that selectively respond to heat, cold, or show paradoxical responses to both, heat and cold (Schepers and Ringkamp 2009, Solinski and Hoon 2019). The presence of these distinct classes of sensory neurons representing warm, hot and cold sensations has been confirmed by a calcium imaging study that analysed the thermal evoked responses in a large number of sensory neurons in the trigeminal ganglion (Yarmolinsky, Peng et al. 2016). The cold and heat responding neurons could be further classified according to their myelination level, rate of adaptation, stimulus threshold and reaction to other stimuli (Yarmolinsky, Peng et al. 2016), which will be described in greater details below.

Thermally Sensitive Afferent Neurons: A-δ and C Fibers

Cold and warm systems differ in several aspects such as higher number of cold receptors (Defrin, Petrini et al. 2009) and lower cold detection threshold (Hollins 2010). There are two major groups of thermally sensitive afferent neurons: unmyelinated C-fibres and thinly myelinated A-δ fibers that transduce, encode and transmit specific thermal information (Bautista, Siemens et al. 2007, Campero, Baumann et al. 2009, Schepers and Ringkamp 2009, Milenkovic, Zhao et al. 2014, Winter, Gruschwitz et al. 2017). Specifically, unmyelinated C-fibers transmit innocuous warm sensation, while thinly myelinated A-δ fibers convey cold sensation in addition to signalling heat pain (Chang, Arendt-Nielsen et al. 2005, Mylius, Borckardt et al. 2012, Kim, Anderson et al. 2016, Bokiniec, Zampieri et al. 2018).

1.2.1.1 Afferent Nerve Fibers mediating Innocuous Cold

Peripheral cold receptors are activated by varying temperature ranges (5 to 43 °C) (Hensel 1973). When repetitively stimulated at short intervals (<30–50 s), the activated cold receptors show transient excitation, maintain constant activity then quickly undergo fatigue and adapt to cold stimuli (Darian-Smith, Johnson et al. 1973, Schepers and Ringkamp 2009, Mylius, Borckardt et al. 2012). They demonstrate a bell-shaped stimulus response function at steady state temperature that is characterized by a maximum activity between 20 and 30 °C and a very low frequency discharge at lower (e.g. 17 °C) and higher (e.g. 40 °C) temperatures (Schepers and Ringkamp 2009). Interestingly, some cold receptors exhibit a paradoxical discharge between 40 °C and 45 °C when activated by noxious heat stimuli (Schepers and Ringkamp 2009, Filingeri and Ackerley 2017). Based on neurophysiological studies, there are two classes of cutaneous fibers encoding cool sensation; thinly myelinated A-δ fibers and unmyelinated C-fibers (Mylius, Borckardt et al. 2012, Filingeri and Ackerley 2017). Thinly myelinated A-δ fibers encode innocuous cooling of the skin in the range of 14 °C to 30 °C (Filingeri 2016) with maximum activity at temperatures around 27 °C (Hensel and Boman 1960). These cold sensitive fibers have conduction velocity of ~3–8 m/s and receptive field of 1 mm (Hensel 1981, Campero and Bostock 2010). They densely innervate the skin (1–19 spots per cm^2) with the highest and lowest innervation in the lips and palm, respectively (Hensel 1981). Compared to A-δ fibers, type 2 unmyelinated C cold thermoreceptors have slower conduction velocity (~1 m/s) and innervate the skin less densely (up to 1.7 per cm^2) with the highest innervation in the face and fingers and lowest density in the chest (Hensel 1981, Filingeri and Ackerley 2017). Besides their role in perceiving gentle touch and mechanical stimuli, low threshold C-fibers are involved in innocuous cold perception and thermoregulation (Campero, Serra et al. 2001, Schepers and Ringkamp 2009, Campero and

Bostock 2010). In addition to thinly myelinated A-δ fibers and unmyelinated C-fibers, large myelinated afferents are sensitive to cold and mechanical stimuli (Schepers and Ringkamp 2009). For instance, half of the slowly adapting mechanoreceptors (SA A-β fibers) such as those innervated by Merkel discs in the superficial layers and Ruffini endings in the deep skin layers are activated in response to decrease in the skin temperature from normal to 14.5 °C consistent with Weber's silver Thaler illusion in which cold objects appear heavier than warm objects (Tapper 1965, Stevens 1979, Cahusac and Noyce 2007).

1.2.1.2 Afferent Nerve Fibers mediating Noxious Cold

Temperatures of 10-15 °C and 18 °C have been reported to induce discomfort in glabrous and hairy skin, respectively (Davis, Kwan et al. 1998, Harrison and Davis 1999) provoking different sensations such as pricking, burning, aching and heat (Davis and Pope 2002). Indeed, noxious cold stimuli activate different populations of fibers including Aδ and C-fibers (Schepers and Ringkamp 2009). Specifically, noxious cold activates mechanosensitive Aδ afferent fibers (A-MSA) that are also sensitive to heat stimuli (Simone and Kajander 1996). Instead of signalling the intensity of a cold stimulus, these fibers mediate the pricking sensation provoked by noxious cooling (Schepers and Ringkamp 2009). Interestingly, blocking Aδ fibers has been reported to significantly reduce cold induced pricking sensation (Davis, Kwan et al. 1998). Indeed, during an Aδ fiber block, C afferents, which are normally blocked by Aδ fibers, are activated to provoke burning or heat sensation in response to noxious cold stimulus (Mackenzie, Burke et al. 1975, Yarnitsky and Ochoa 1990, Davis, Kwan et al. 1998, Schepers and Ringkamp 2009). In addition to Aδ fibers, C-nociceptors mediate pain sensation evoked by noxious cold (Bessou and Perl 1969, Simone and Kajander 1996). The majority of cold sensitive C-fibers are polymodal responding to both mechanical and heat stimuli similar to Aδ fibers (Campero, Serra et al. 1996, Simone and Kajander 1996).

1.2.1.3 Afferent Nerve Fibers mediating Innocuous Warm

C afferent fibers located at a depth of 20 to 600 μm in the epidermis and dermis respond to heat stimuli (Hensel and Iggo 1971, LaMotte and Campbell 1978, Tillman, Treede et al. 1995). Similar to cold fibers, warm fibers demonstrate a bell-shaped stimulus response curve characterized by continuous activity at static temperature of 30 °C, maximum discharge at 40-43 °C and minimum discharge at 50 °C (Hensel and Iggo 1971, LaMotte and Campbell 1978). C-fibers have small receptive fields and respond to heat stimuli between 37 and 49 °C with the conduction velocity of 1 m/s (LaMotte and Campbell 1978, Treede, Meyer et al. 1990, Schmidt, Schmelz et al. 1997). Some of the C-fibers are polymodal, responding to moderately intense mechanical stimuli and chemical irritants, but they are not responsive to gentle touch or dull pressure (Schepers and Ringkamp 2009).

1.2.1.4 Afferent Nerve Fibers mediating Noxious Warm

In addition to C-fibers, Aδ nociceptive fibers respond to hot stimuli. They have been classified into two types depending on their sensitivity to heat stimuli (Treede, Meyer et al. 1995, Treede, Meyer et al. 1998). Type I Aδ afferents with high heat thresholds (median threshold >53 °C) are present in the glabrous and hairy skin (Schepers and Ringkamp 2009). They have an average response latency of about 5 s when stimulated with intense heat stimuli (53 °C) and their responses increase during stimulation (Schepers and Ringkamp 2009). Burn injury can lead to hypersensitivity of type I afferents to heat stimuli, developing thermal hyperalgesia (Treede, Meyer et al. 1995). Compared to type I afferents, type II Aδ afferents are absent in the glabrous skin, have lower heat thresholds (47 °C), respond and adapt instantaneously (<1 s) to the intense heat stimuli (53 °C) (Schepers and Ringkamp 2009).

1.2.2 Molecular Basis of Thermal Sensation

The terminals of the free nerve endings of primary sensory afferent fibers in the skin contain transient receptor potential (TRP) ion channels that detect and transduce a broad range of temperatures, from noxious cold to noxious heat (Voets, Droogmans et al. 2004, Schepers and Ringkamp 2009, Bokiniec, Zampieri et al. 2018). In humans, these specialized thermal receptors are classified into six families (Pedersen, Owsianik et al. 2005), among which the melastatin or long TRP channels subfamily M member (TRPM), the vanilloid TRP channels (TRPV), and the ankyrin transmembrane protein channels (TRPA) are involved in thermoregulation (Schepers and Ringkamp 2009). More specifically, TRPM8 and TRPV3/4 encode innocuous cool and warm sensations, respectively, while TRPA1 and TRPV1/2 transduce noxious cold and heat, respectively (Figure 1) (Schepers and Ringkamp 2009, Bokiniec, Zampieri et al. 2018). Moreover, investigations on the basic mechanisms of temperature detection and the link between electrical signals and behavioural reactions led to the discovery of thermo-receptive proteins and receptors for the cold-temperature mimetic menthol and hot-mimetic capsaicin, which are activated by the temperatures below 25 °C and above 42 °C, respectively (Caterina, Schumacher et al. 1997, Bautista, Siemens et al. 2007, Solinski and Hoon 2019). Multiple lines of evidence suggest that the polymodal TRP thermal receptors are co-expressed in adult primary sensory neurons (Schepers and Ringkamp 2009, Mishra, Tisel et al. 2011, Usoskin, Furlan et al. 2014, Bokiniec, Zampieri et al. 2018). For instance, TRPM8 and TRPA1 function together to encode both innocuous and noxious cooling stimuli (Kwan, Allchorne et al. 2006, Bautista, Siemens et al. 2007, Colburn, Lubin et al. 2007, Dhaka, Murray et al. 2007, Karashima, Talavera et al. 2009, Winter, Gruschwitz et al. 2017, Bokiniec, Zampieri et al. 2018). Homeotherms have at least four classes of TRPM8-expressing cold-responsive neurons (Yarmolinsky, Peng et al. 2016) and multiple, molecularly distinct classes of TRPM8-cells in the trigeminal ganglion (Nguyen, Wu et al. 2017) that

collectively provide distinct information about the quality of cold to maintain a constant body temperature in a cold environment (Siemens and Kamm 2018, Tan and Knight 2018, Solinski and Hoon 2019). Indeed, thermal stimulation engages multiple and unknown mechanisms to transduce thermal perception (Schepers and Ringkamp 2009). According to gene knockout studies, the ability to respond appropriately to both external and internal thermal challenges are significantly reduced in animals lacking TRPV1- and TRPM8-neurons (Mishra, Tisel et al. 2011, Solinski and Hoon 2019). For instance, ablation of TRPM8-expressing DRG neurons significantly reduces behavioural cooling avoidance (Bautista, Siemens et al. 2007, Colburn, Lubin et al. 2007, Dhaka, Murray et al. 2007, Solinski and Hoon 2019) and the number of neurons activated by innocuous cooling in the range 26 to 29 °C (Milenkovic, Zhao et al. 2014, Bokiniec, Zampieri et al. 2018), while leaving the percentage of neurons activated by noxious cold unaltered (Ran, Hoon et al. 2016). This finding points to the involvement of additional cold receptors, a population of TRPV1-expressing DRG neurons and cold-sensitive nociceptors that selectively encode noxious cold stimuli (Vriens, Nilius et al. 2014, Ran, Hoon et al. 2016). Notably, the elimination of TRPV1 and capsaicin receptor had no major effect on acute withdrawal responses to heat stimuli, but significantly decreased injury-induced allodynia and hyperalgesia to heat stimuli (Caterina, Leffler et al. 2000, Pogorzala, Mishra et al. 2013). This indicates the presence of other receptors for heat or receptor redundancy as confirmed by the presence of a group of TRP protein ion-channels, TRPV1, TRPA1, and TRPM3, which are required for acute behavioural responses to heat (Vandewauw, De Clercq et al. 2018, Solinski and Hoon 2019).

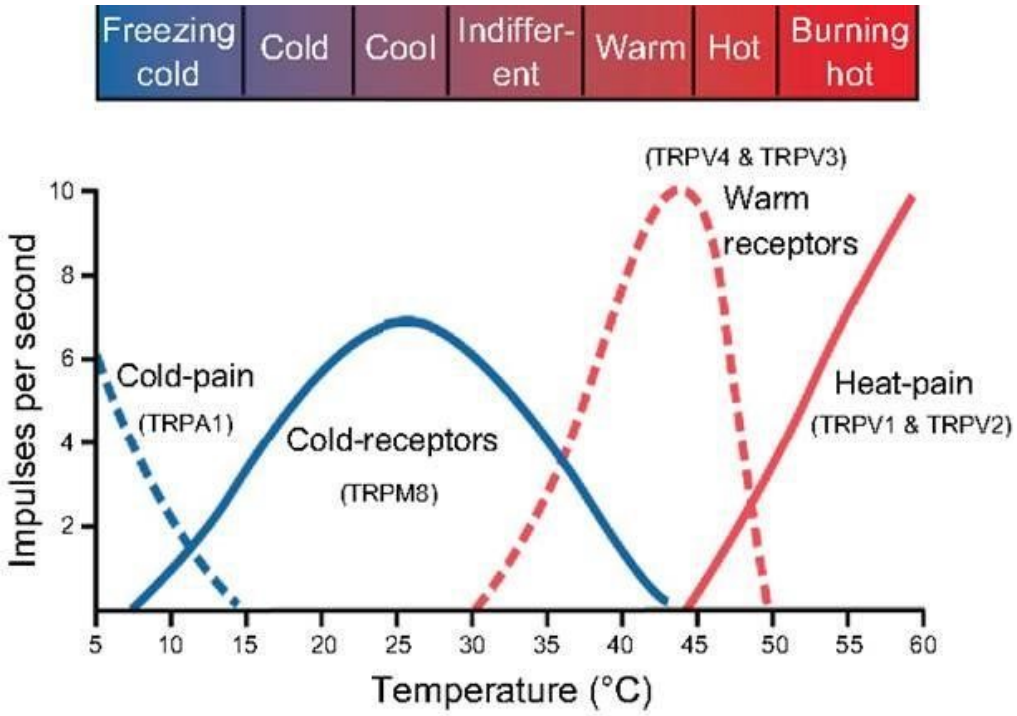

Figure 1. Discharge frequency of thermoreceptors and their associated TRP ion channels at different skin temperatures [with permission from (Tansey and Johnson 2015)].

As stated before, mammals are able to detect small fluctuations in core body temperature and respond to thermal challenges in the environment with several active compensatory mechanisms (Solinski and Hoon 2019). For instance, thermosensory detection systems and the organs that either dissipate heat or are involved in thermogenesis act concurrently to generate appropriate responses to cold or heat (Morrison, Nakamura et al. 2008, Solinski and Hoon 2019). Indeed, the nerve fibers in the skin and other organs in the body react to thermal challenges in the environment (e.g. reflex responses and organ withdrawal) by engaging specific neural circuits in the spinal cord and brain to maintain a steady body temperature (Solinski and Hoon 2019). Compared to reflex responses to thermal challenges, homeostasis involves potentially distinct transduction mechanisms. For instance, TRPV1, TRPA1 and TRPM3 are required for noxious temperature avoidance and heat seeking behaviours (Vandewauw, De Clercq et al. 2018), while

TRPM8 neurons are essential for long-term control of body temperature (Reimúndez, Fernández-Peña et al. 2018, Solinski and Hoon 2019). In addition to TRPV1- and TRPM8-neurons; thermosensory homeostasis centers in the CNS play crucial roles in the control of core body temperature (Siemens and Kamm 2018, Solinski and Hoon 2019). For instance, the preoptic area (POA) of the hypothalamus detects and controls small changes in core body temperature using the inputs from TRPM2-neurons (Song, Wang et al. 2016, Tan, Cooke et al. 2016, Solinski and Hoon 2019).

1.3 From Spinal Cord to Cortex

1.3.1 Spinal Processing of Temperature

Thermal information from periphery is integrated in the spinal neurons where robust calcium responses are triggered in response to cooling or heating (Ran, Hoon et al. 2016). According to histological studies, the minority of thermosensory neurons project to the deeper dorsal horn layers of the spinal cord, while the majority of them terminate in laminae I and II (LI/II) in the superficial dorsal horn of the spinal cord to directly synapse with LI/II neurons where the cross-talk between inputs are essential for shaping thermosensory responses (Christensen and Perl 1970, Light and Perl 1979, Craig and Bushnell 1994, Dostrovsky and Craig 1996, Bester, Chapman et al. 2000, Craig and Dostrovsky 2001, McCoy, Taylor-Blake et al. 2013, Solinski and Hoon 2019). In a study assessing thermal processing in the dorsal laminae of the mouse spinal cord using in vivo calcium imaging; the hind limb was immersed in a water bath and calcium responses in LI/II neurons of the lumbar spinal cord were monitored in response to alterations in bath temperature (Ran, Hoon et al. 2016). According to this study, there are significant differences in the representation of heat and cold in the dorsal horn where spinal neurons could be classified into cold, heat or broad responders. Most of the cold-sensitive LI/II neurons are preferentially activated by relative

decrease in temperature, while warm-sensitive neurons seem to encode absolute temperature in the heat range (Ran, Hoon et al. 2016). Moreover, 70% of cold-sensitive neurons in the spinal cord have high sensitivity to mild cooling and respond robustly to less than 6 °C cooling from skin temperature (Ran, Hoon et al. 2016). However, less than fifteen percent of heat-sensitive neurons respond to mild heating of the normal skin temperature (32 °C, $\Delta T = 5-8$ °C) with the majority (80 %) responding to more intense heating in the noxious range (> 42 °C and $\Delta T > 8$ °C) (Ran, Hoon et al. 2016). Heat and cold sensitive neurons also display distinct kinetics (Ran, Hoon et al. 2016). Warming responses remain at the plateau during stimulation with no adaptation regardless of the heating rate, while cooling responses reach maximum throughout the transient phase of the stimulation with rapid adaptation to cooling rate (Ran, Hoon et al. 2016). Finally, only 7% of all thermosensitive spinal neurons were found to respond to both innocuous warming and cooling, whereas 44% were activated by both noxious heat and cold (Ran, Hoon et al. 2016).

In addition to the heat and cold responsive neurons, there are broadly tuned neurons in the spinal cord that respond and slowly adapt to both cold and heat unlike cold sensitive neurons (Ran, Hoon et al. 2016). In these neurons, cooling to lower temperature has been associated with larger calcium response compared to cooling to milder temperature (Ran, Hoon et al. 2016). There are still open questions as to what the molecular identities and the interactions between heat, cold and broadly tuned spinal cord neurons are as well as which neurons convey thermosensory signals from the spinal cord to the brain (Solinski and Hoon 2019).

1.3.2 Ascending Somatosensory Pathways

Somatosensory system responds to four major classes of receptors including thermoreceptors, nociceptors, proprioceptors and mechanoreceptors (Goodwin 2001). Central somatosensory pathways consist of the dorsal column-medial lemniscus pathway conveying mechanical (i.e. tactile and proprioceptive) information and the anterolateral pathway (ALS) also known as spinothalamic tract conveying thermal and pain information (Goodwin 2001).

Anterolateral System (Spinothalamic Tract)

Aδ and C afferents convey thermal and noxious stimuli detected by thermoreceptors and nociceptors to the brain via anterolateral system (ALS) or spinothalamic pathway (Goodwin 2001, Dubin and Patapoutian 2010, Vriens, Nilius et al. 2014). There are two classes of projection neurons whose axons ascend in the anterolateral system in the dorsal horn of the spinal cord (Goodwin 2001). One class is nociceptive neurons that receive input from nociceptive primary afferent fibers and exclusively respond to noxious stimuli (Goodwin 2001). The other class, wide dynamic range neurons, receives input from both nociceptive and low-threshold mechanoreceptor fibers and respond to both noxious and innocuous stimuli (Goodwin 2001). In the spinothalamic pathway, first order somatosensory afferent neurons carry thermal and pain information to the dorsal horn of the spinal cord, where they synapse with the axons of the second order neurons, which cross over the midline to the other side of the spinal cord where they ascend in the white matter of the anterior lateral part of the spinal cord to the ventral posterolateral (VPL) nucleus of the thalamus where afferent information from the trunk and limb are represented (Goodwin 2001). In the VPL, the axons of the third order neurons convey the information in a somatotopic manner to S1 (Bokiniec, Zampieri et al. 2018). For the face, thermal and pain information is carried to the spinal trigeminal nucleus in the brainstem via trigeminal nerve and then is conveyed to the ventral

posteromedial (VPM) nucleus of the thalamus to be relayed to the face representation in S1 (Goodwin 2001, Vriens, Nilius et al. 2014, Hooks 2016). Most of the dorsal horn neurons and sensory neurons in the thalamus are polymodal and respond to warm, cold and tactile stimuli (Kenshalo, Leonard et al. 1982, Lee, Dougherty et al. 1999). Spinal, thalamic and cortical neurons also contain preserved labelled lines for warmth and cold specifically (Schepers and Ringkamp 2009). It is noteworthy that the neurons in the anterolateral system conveying pain and thermal information from the body cross at the level of spinal cord, while neurons in the dorsal column system conveying tactile and proprioceptive information cross in the lower medulla in the brainstem (Goodwin 2001). As a result, any lesion in the spinal cord causes deficit in tactile sensation on the same side of the body, whereas it leads to deficit in pain and temperature sensations on the opposite side of the body (Goodwin 2001).

1.3.3 Hypothalamus

The hypothalamus is the major thermoregulatory processing region that receives input from skin thermoreceptors, spinal cold and visceral afferents via spinothalamic tract (Mylius, Borckardt et al. 2012). Spinal thermo-sensory LI/II neurons project to the contralateral somatosensory nuclei of the thalamus (ventral medial nucleus (VMpo)) via the spinothalamic tract (Craig and Blomqvist 2002, Bokiniec, Zampieri et al. 2018). These neurons also travel to the lateral parabrachial nucleus of the brainstem and then project to the POA of the hypothalamus (Nakamura and Morrison 2007, Yahiro, Kataoka et al. 2017, Bokiniec, Zampieri et al. 2018). Lesions of this pathway resulted in the lack of cold avoidance indicating an important role for hypothalamic circuits in processing cold avoidance behavior (Nakamura 2011, Clapham 2012, Yahiro, Kataoka et al. 2017, Bokiniec, Zampieri et al. 2018). The hypothalamus contains cold and warm-sensitive and temperature-insensitive neurons (Mylius, Borckardt et al. 2012). When a change in core temperature is detected in the rostral part of the hypothalamus, the hypothalamic thermosensitive neurons fire (Cruccu,

Aziz et al. 2007) enabling the CNS to initiate various thermoregulatory behavioural and physiological responses to regulate core body temperature (Mylius, Borckardt et al. 2012). Moreover, as stated earlier, the POA within the anterior part of the hypothalamus contains temperature sensitive neurons that can detect slight changes in local brain temperature and mediates thermoregulatory processes such as cutaneous vasomotion, shivering and brown adipose tissue thermogenesis (Romanovsky, Almeida et al. 2009, Nakamura 2011, Mylius, Borckardt et al. 2012, Vriens, Nilius et al. 2014). Interestingly, certain chemical changes such as drop in plasma glucose level or high osmolarity can activate hypothalamic cold-sensitive neurons providing a partial explanation of why individuals feel cold when they are in fact hungry or dehydrated (De Tommaso, Brighina et al. 2010, de Andrade, Mhalla et al. 2011, Mylius, Borckardt et al. 2012). In brief, thermal information from cutaneous thermosensory neurons is first forwarded to thalamus via spinothalamic tract then through thalamocortical radiations, is further projected to the primary sensory cortex where temperature perception and voluntary behavioural responses are initiated (Romanovsky, Almeida et al. 2009, Vriens, Nilius et al. 2014).

1.4 Central Mechanisms of Thermal Perception

In primates, the CNS integrates inputs from populations of fibers to encode different thermal stimuli, discriminate their intensities and generate distinct affective responses accordingly (Johnson, Darian-Smith et al. 1979, Chang, Arendt-Nielsen et al. 2005, Filingeri and Ackerley 2017). Optimal peripheral integration of thermal stimuli is achieved when a minimum of ~15 peripheral fibers are concurrently activated (Johnson, Darian-Smith et al. 1979, Filingeri and Ackerley 2017). Indeed, a distributed network in the brain rather than a serial processing in somatic areas are involved in somatosensory processing of the quality of thermal stimulation (i.e. cold, burning cold, warm, hot, pleasant or unpleasant) (Berman, Kim et al. 1998, Chang, Arendt-Nielsen

et al. 2005). In other words, several cortical and subcortical regions such as the hippocampus, cerebellum, superior frontal gyrus, parabrachial nucleus, the POA, areas of the thalamus and superior parietal gyrus as well as anterior cingulate cortex (ACC) are coactivated in response to thermal stimuli to encode the quality and subjective intensity of thermal sensations (Figure 2) (Ploghaus, Tracey et al. 2000, Chang, Arendt-Nielsen et al. 2005, Nakamura and Morrison 2007, Solinski and Hoon 2019).

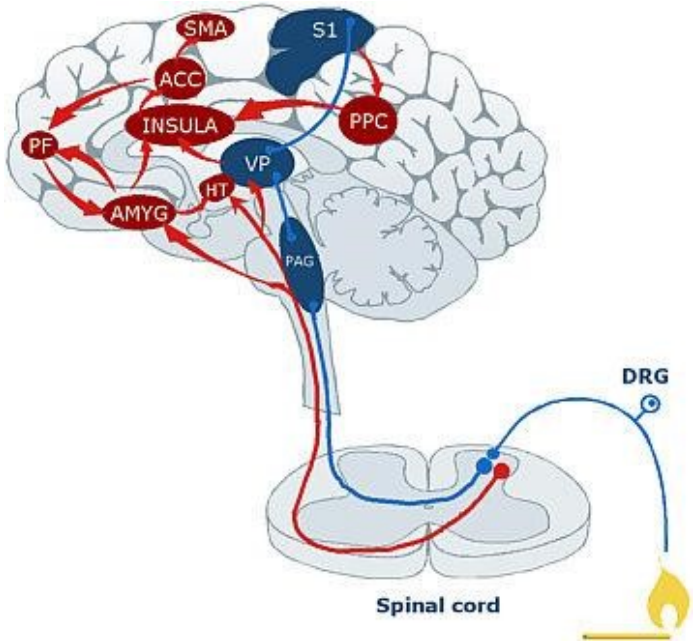

Figure 2. An illustration showing that thermal and pain perception share the same cortical representation in terms of activation to encode the sensory (areas in blue) and motivo-affectional (in red) components of noxious heat stimuli.

1.4.1 Cortical Activations evoked by Noxious and Innocuous Thermal Stimulation

Bilateral activations have been reported in most regions of cortex with a stronger activation contralateral to the stimulus (Chang, Arendt-Nielsen et al. 2005). The degree of these cortical activations is directly proportional to the stimulus intensity (Jones, Brown et al. 1991, Casey, Minoshima et al. 1996, Davis, Kwan et al. 1998, Derbyshire and Jones 1998). According to positron emission tomographic and fMRI studies, noxious thermal stimuli increase the regional

cerebral blood flow (rCBF) and the blood oxygen level–dependent (BOLD) signal predominantly in the S2, ACC and the insula rather than the S1, posterior parietal cortex (PPC), thalamus and prefrontal cortex (PFC) (Peyron, Laurent et al. 2000, Mylius, Borckardt et al. 2012). Despite common patterns of brain activation in response to both noxious hot and cold stimuli, an fMRI study reported that noxious cold stimuli activate the prefrontal area of the brain more intensely than noxious heat, which might explain why cold sensation (12-15 °C) is more unpleasant compared to warm sensation (40-43 °C) given that the right frontal area of the brain is associated with pain sensitivity and depressed mood (Tracey, Becerra et al. 2000, Chang, Arendt-Nielsen et al. 2005). Nevertheless, it remains a matter of debate which regions of the brain specifically react to warm or cold stimuli (Chang, Arendt-Nielsen et al. 2005). According to human imaging and electroencephalography (EEG) studies, both innocuous and noxious heat activate areas such as insular cortex (anterior and posterior), anterior cingulate cortex, contralateral frontal cortex, the bilateral primary sensorimotor areas (M1, S1), ipsilateral thalamus, cerebellum, PFC and lentiform nucleus (Chatt and Kenshalo 1977, Jones, Brown et al. 1991, Derbyshire and Jones 1998, Egan, Johnson et al. 2005, Rolls, Grabenhorst et al. 2008, Bokiniec, Zampieri et al. 2018). Several studies, on the other hand, have found distinct spatial patterns of activation in response to innocuous versus noxious thermal stimuli (Casey, Minoshima et al. 1996, Craig, Reiman et al. 1996, Davis, Kwan et al. 1998, Tracey, Becerra et al. 2000, Chang, Arendt-Nielsen et al. 2005). For instance, large changes in skin temperature has resulted in a more widespread activations in different regions within the lateral and medial thalamus, anterior insula and contralateral S2, while mild temperature changes were associated with the activations in the thalamus and posterior insula (Davis, Kwan et al. 1998, Gelnar, Krauss et al. 1999, Nadler, Weingand et al. 2004, Chen and Shaw 2014, Tai, Lai et al. 2014).

1.4.2 Role of the S1 in Thermal Perception

Primary somatosensory cortex is a very complex anatomical area in close proximity to M1 that plays an important role in processing somatosensory information (Frot, Magnin et al. 2013, Solinski and Hoon 2019). It consists of four cytoarchitectonically distinct subdivisions, known as areas 1 and 2 located on the crown of the postcentral gyrus, area 3a in the depth of the postcentral gyrus and area 3b in the posterior of the central sulcus (Tommerdahl, Delemos et al. 1996, Kenshalo, Iwata et al. 2000, Tommerdahl, Favorov et al. 2008, Frot, Magnin et al. 2013). According to intrinsic imaging study of the forepaw S1-cortex in mice, S1-cortex was not only responsive to cold stimulation but also required for appropriate behavioural responses to cold (Milenkovic, Zhao et al. 2014, Solinski and Hoon 2019). Importantly, S1 provides a spatial representation of both cold and touch as shown by the study of Bushnell et al. (2012) where cold and vibrotactile stimuli generated indistinguishable responses in S1 cortex, supporting the role of both cold and touch sensation in encoding the physical localization of objects (Bushnell, Seminowicz et al. 2012, Solinski and Hoon 2019).

1.4.3 Nociceptive- and thermally-evoked Responses in S1

The functional role of S1, and particularly the specific functions of its subregions in pain perception and processing of nociceptive and thermal inputs have been investigated in several studies (Chen, Dillenburger et al. 2011, Frot, Magnin et al. 2013). In humans, many electrophysiological and functional imaging studies have supported the contribution of S1 in the representation of sensory discriminative aspects of pain such as intensity, duration, location, and quality of pain sensation (Chen, Dillenburger et al. 2011). However, about half of the functional mapping studies have failed to demonstrate increased activity in S1 during painful stimulation (Peyron, Laurent et al. 2000, Apkarian, Bushnell et al. 2005, Chen, Dillenburger et al. 2011). For instance, no activity was found in area 3b in response to specific noxious heat laser stimuli using

intracranical electrode directly implanted in S1 (Valeriani, Barba et al. 2004, Frot, Magnin et al. 2013). Similarly, in primates, although nociceptive neurons have been identified in area 3a (Chen, Friedman et al. 2009), 3b, and 1 (Tommerdahl, Delemos et al. 1996, Kenshalo, Iwata et al. 2000, Frot, Magnin et al. 2013); inconsistent pattern of SI activation in response to noxious heat (i.e. either area 3a alone (Tommerdahl, Delemos et al. 1998) or areas 3a, 3b, and 1 (Chen, Friedman et al. 2009)) has been observed in several functional optical imaging studies (Chen, Dillenburger et al. 2011). Indeed, animal neurophysiological studies reported a limited number of neurons responding to mechanical and noxious thermal stimuli in area 3b of S1 (Kenshalo and Willis 1991, Kenshalo, Iwata et al. 2000, Frot, Magnin et al. 2013). On the other hand, a considerable body of electrophysiological research indicate some activities in S1 following stimulations (e.g. heat laser pulses) that selectively activate Aδ and C nociceptors or intraepidermal electrical stimuli (Ploner, Freund et al. 1999, Kanda, Nagamine et al. 2000, Timmermann, Ploner et al. 2001, Inui, Tran et al. 2002, Nakata, Inui et al. 2004, Ogino, Nemoto et al. 2005, Baumgärtner, Vogel et al. 2011, Chen, Dillenburger et al. 2011, Frot, Magnin et al. 2013). For instance, Frot et al. (2013) measured intracortical evoked potentials in S1 and M1 in response to noxious (Aδ-laser) and innocuous (Aβ-electrical) stimuli in epileptic patients and reported strong activation in area 3a in response to innocuous stimuli, but less activation to noxious heat (Frot, Magnin et al. 2013). In another study, functional representation of tactile and noxious heat stimuli in cortical areas along the central sulcus of anesthetized squirrel monkeys was mapped using high resolution BOLD fMRI (Chen, Dillenburger et al. 2011). They provided evidence supporting the involvement of not only areas 1, 3a and 3b but also, areas 2 and M1 cortices in the processing of noxious heat stimuli. More specifically, they found consistent and stronger activations in areas 3b, and 2 (possible role in early processing of nociception), but less frequent activations in areas 3a, 1, and M1 in response to noxious heat (47.5 ^{0}C) stimulation of digits supporting their earlier observation showing the

stronger activation of area 3b to mechanical nociceptive stimulation than area 3a and 1 (Chen, Friedman et al. 2009, Chen, Dillenburger et al. 2011). They also found noxious heat activations to be larger in spatial extent than tactile activations. Indeed, differential BOLD nociceptive heat responses in signal magnitude and temporal profile suggest different roles for each of these areas (3a, 3b, 1, 2) in the processing and encoding of nociceptive inputs (Chen, Dillenburger et al. 2011). It has been hypothesized that the perceived quality of a nociceptive stimulus is probably characterized by activation patterns between these areas within S1 cortex (Chen, Dillenburger et al. 2011). Together with the lack of colocalization of noxious heat activations and tactile responses within area 3b; this data suggests that noxious heat and innocuous mechanical stimuli may be processed by different clusters of neurons in different areas (Chen, Dillenburger et al. 2011). Despite strong evidence pointing to the pain-related activity in S1, it remains a topic of substantial debate why focal S1 injury does not cause a significant deficit in pain sensation or why direct S1 electrical stimulation does not induce pain sensations (Mazzola, Isnard et al. 2011) while inducing thermal sensations in awake humans (Penfield, Boldrey et al. 1937, Frot, Magnin et al. 2013).

1.4.4 Nociceptive- and thermally-evoked Responses in M1

The role of the primary motor cortex in thermal and pain perception has been reported in human imaging studies (Peyron, Laurent et al. 2000, Apkarian, Bushnell et al. 2005); however, the specific function of M1 in pain modulation remains to be identified (Chen, Dillenburger et al. 2011). Nociceptive stimulation has been reported to influence motor cortical activity as reflected by increase in cerebral blood flow in M1 area (Casey, Minoshima et al. 1996) and increase in M1 BOLD response to noxious heat stimulus (Chen, Dillenburger et al. 2011). Nociceptive heat laser stimuli, for instance, has been reported to induce local activations in M1 (Frot, Magnin et al. 2013). Moreover, heat pain activates premotor and motor cortical areas as shown by functional imaging

studies (Gelnar, Krauss et al. 1999, Frot, Magnin et al. 2013). These activations; however, have been associated with pain-evoked movement or suppression of movement (Apkarian, Bushnell et al. 2005, Frot, Magnin et al. 2013). Evidence of inhibitory interactions between pain and motor cortices in both normal and chronic pain patients comes from functional imaging, electroencephalography (EEG), magnetoencephalography (MEG), and TMS studies (Restuccia, Valeriani et al. 1999, Lefaucheur, Drouot et al. 2006, Chen, Dillenburger et al. 2011). Of note, painful stimulation has been reported to inhibit motor cortex excitability (Restuccia, Valeriani et al. 1999, Chen, Dillenburger et al. 2011). This inhibitory interaction between sensory and motor areas are supported by a handful of studies where electrical and transcranial magnetic stimulation of motor cortex have been reported to reduce neuropathic pain (Lima and Fregni 2008, Lefaucheur, Drouot et al. 2009, Leung, Donohue et al. 2009, Thomas, Bledsoe et al. 2009, Chen, Dillenburger et al. 2011), persistent central pain (Son, Lee et al. 2006, Defrin, Grunhaus et al. 2007), complex regional pain syndrome (Velasco, Romo et al. 1984), experimental pain (Restuccia, Valeriani et al. 1999), and thalamic pain (Tsubokawa, Katayama et al. 1993). It is noteworthy that M1 receives thalamic afferent projections form the caudal portion of the ventral lateral nucleus, the anterior segment of the VPL nucleus (Jones, Wise et al. 1979, Matelli, Luppino et al. 1989, Shindo, Shima et al. 1995, Craig 2008) and to a lesser degree from the intralaminar (central lateral and central medial) nuclei (Darian-Smith, Darian-Smith et al. 1990, Huffman and Krubitzer 2001, Kultas-Ilinsky, Sivan-Loukianova et al. 2003). These spinothalamic inputs to M1 have been speculated to quickly and effectively interrupt nociceptive stimulations by engaging motor reactions (Frot, Magnin et al. 2013).

1.4.5 Conclusions

In summary, thermal stimulation has the potential to influence corticospinal excitability and motor responses in healthy adults by inducing cortical activation in the premotor, M1, posterior

region of the ACC and the supplementary motor area (SMA), which are involved in motor planning (Davis, Kwan et al. 1998, Kwan, Crawley et al. 2000, Tracey, Becerra et al. 2000, Brooks, Nurmikko et al. 2002, Chang, Arendt-Nielsen et al. 2005, Chen and Shaw 2006, Wu, Lin et al. 2010). In healthy adults, innocuous thermal stimuli activate the somatosensory thalamus, hippocampus and insula via the lateral-thalamus pathway (Gelnar, Krauss et al. 1999). In stroke patients, however, this activation has been observed in response to noxious stimuli involving both lateral and medial pain pathways (Gelnar, Krauss et al. 1999, Treede, Kenshalo et al. 1999). The simultaneous activations of these multiple brain regions in response to thermal stimulation may enhance neuroplasticity and sensorimotor reorganization, which is essential to facilitate recovery of motor function after stroke (Davis, Kwan et al. 1998, Gelnar, Krauss et al. 1999). Indeed, noxious thermal stimulation is thought to exerts its beneficial effects on motor recovery by stimulating peripheral somatosensory receptors and inducing cortical excitation (Chen, Tang et al. 2019). These signals then project to parietal somatosensory areas where they can interact with frontal motor areas through neural pathways promoting neuroplasticity (Sheffler and Chae 2007, Chipchase, Schabrun et al. 2011, Chen, Tang et al. 2019). In the next section, the non-invasive brain stimulation (NIBS) techniques and their applications in investigating the neurophysiological basis of changes in brain excitability resulting from afferent stimulation in human participants will be examined.

1.5 Neurophysiological and Physical Basis of NIBS Techniques

TMS and transcranial direct current stimulation (tDCS) are the two most common NIBS techniques with physiological and therapeutic applications in humans (Mylius, Borckardt et al. 2012). TMS is a non-invasive powerful stimulation technique with a well-established safety profile in both animal and human models to explore brain physiology and function (Tamura, Okabe et al. 2004, Rossi, Hallett et al. 2009). In this technique, a magnetic coil coupled to a high voltage capacitor is placed on the scalp to induce a brief current (100 μs) over a given cortical target. The magnetic field then flows through the surrounding tissues and induces an electrical field, which excites cortical neural circuits by inducing propagated actions potentials (Figure 3 and 5A) (Mills 1999, Mylius, Borckardt et al. 2012). TDCS, on the other hand is a modulation technique to influence excitability of cortical neurones (Mylius, Borckardt et al. 2012). With this technique, a weak direct current is delivered over a given cortical target via electrodes placed on the scalp to alter the resting membrane potential of axons (Mylius, Borckardt et al. 2012).

1.5.1 Basic Measures of Excitability in response to Single Pulse TMS

1.5.1.1 Motor Evoked Potentials (MEPs)

In the TMS technique, an electromagnetic coil is placed on the scalp over the motor cortex hot spot, which is defined as the scalp position with the lowest threshold and high density of corticospinal cells and spinal motor neurons for a given target muscle (Wassermann, McShane et al. 1992, Abbruzzese and Trompetto 2002, Mercier, Gagné et al. 2016). A single TMS pulse of adequate intensity applied over the motor area provokes action potentials that will travel along the corticospinal tract and peripheral motor nerve to induce contraction in the contralateral target muscles, which is recorded as motor evoked potentials (MEPs) using surface electromyography (EMG) (Figure 3 and 5 A) (Abbruzzese and Trompetto 2002, Bestmann and Krakauer 2015,

Chang, Fried et al. 2016). The MEP is a compound signal representing different descending cortico-spinal volleys, which contribute to MEP amplitude depending on a variety of processes such as temporal dispersion and spinal mechanisms (Bestmann and Krakauer 2015). The MEP latency indicates the conduction time required for neuronal impulses to travel from the cortex to peripheral muscles (Bestmann and Krakauer 2015). The amplitude and latency of MEPs provide a simple and reliable index of corticospinal excitability and integrity; therefore, can be used to evaluate the extent of damage to motor pathways and predict motor recovery after stroke (Tai, Lai et al. 2014).

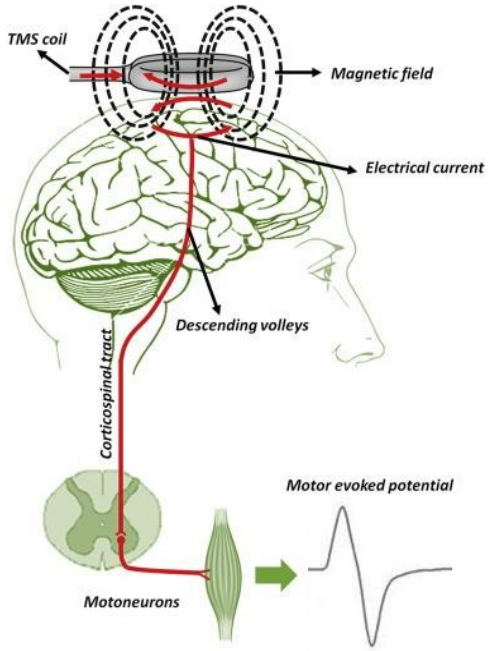

Figure 3. Schematic representation of TMS mechanism of action [with permission from (Rodríguez-Labrada, Velázquez-Pérez et al. 2018)].

1.5.1.2 Descending Waves

Single pulse magnetic stimulation of the motor cortex generates indirect (I) waves, which are defined as high frequency (approx. 670 Hz) repetitive discharge of corticospinal fibers (Di

Lazzaro, Profice et al. 2012). The I-waves originate in the motor cortex by indirect activation of trans-synaptic cortico-cortical and intra-cortical afferents projecting to pyramidal tract neurons within M1 (Abbruzzese and Trompetto 2002, Perez and Cohen 2009, Bestmann and Krakauer 2015). They are classified into I1, I2, I3 and I4 waves (Abbruzzese and Trompetto 2002, Perez and Cohen 2009). Direct synaptic input onto cortico-spinal pyramidal tract neurons generates first I-wave (I1) following a D-wave (Fisher, Nakamura et al. 2002, Perez and Cohen 2009), while activation of cortico-cortical circuits generates later I-waves at latencies between 2.4 and 7 ms following the D-wave (Perez and Cohen 2009, Groppa, Oliviero et al. 2012). Various TMS protocols such as short-and long-interval intracortical inhibition (SICI, LICI), inter-hemispheric inhibition or short afferent inhibition (SAI) inhibit the motor cortex by suppressing later I waves, particularly the I3 wave (Lazzaro, Ziemann et al. 2008, Di Lazzaro, Profice et al. 2012). Similarly, TMS protocols of motor cortex facilitation such as intra cortical facilitation (ICF) tend to increase the magnitude of late I waves, particularly the I2 and I3 waves (Hanajima, Ugawa et al. 2002, Ilić, Meintzschel et al. 2002, Di Lazzaro, Profice et al. 2012). On the other hand, continuous theta burst stimulation, a repetitive TMS protocol that induces long lasting inhibition of MEP amplitude, preferentially decreases the I1 wave rather than later I waves, in contrast to other rTMS protocols, which modify late I waves (Di Lazzaro, Profice et al. 2012, Lazzaro, Profice et al. 2012).

1.5.1.3 Resting Motor Threshold

Resting motor threshold (rMT) is defined as the minimum TMS intensity required to provoke reliable MEPs (e.g. 50 μV) in a given target muscle. It is lower for activation of intrinsic hand muscles compared to more proximal and lower limb muscles indicating differences in the strength of corticospinal projections (Chen 2000). The rMT is also influenced by neuronal membrane excitability as reflected by increase in rMT in response to certain drugs (e.g. antiepileptic drugs) that block voltage gated sodium channels (Ziemann, Lönnecker et al. 1996, Chen 2000). One of

the methods to determine rMT is the method of relative frequency, where the rMT corresponds to the intensity at which the MEPs of 50 µV are evoked in 5 of 10 trials (Rossini, Barker et al. 1994, Abbruzzese and Trompetto 2002). Other methods, such as those based on adaptative threshold hunting algorithms, have been proposed as better alternative to the relative frequency method (Awiszus 2003).

1.5.1.4 Cortical Silent Period

The cortical silent period (cSP) refers to an interruption in EMG activity when TMS is applied during voluntary muscle contraction (Figure 4) (Abbruzzese and Trompetto 2002). This inhibition is thought to be cortical in origin for the major part reflecting GABAergic inhibition and the loss of excitatory drive to the spinal motoneurons (Abbruzzese and Trompetto 2002). The duration of the cSP tends to increase following cortical lesions in stroke, while decreases in certain pathological conditions, such as Parkinson's diseases (Abbruzzese and Trompetto 2002).

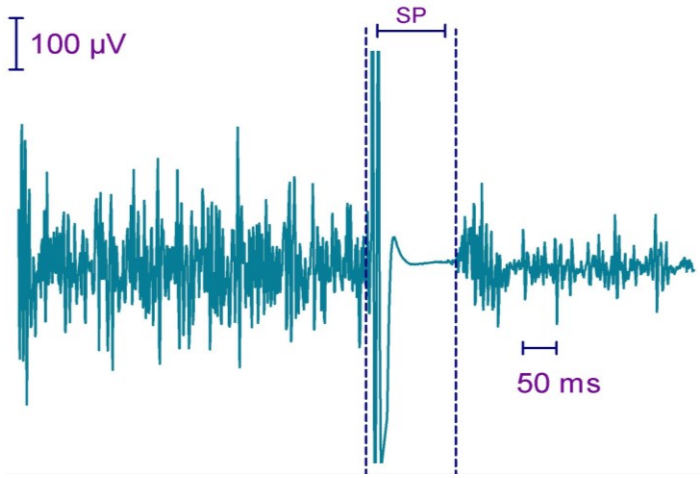

Figure 4. MEP produced in the FDI hand muscle during active contraction. It is followed by a silent period (SP) defined by the absence of electrical activity. The SP is measured from the onset of MEP to the return of EMG activity (Image from Clinical Neuroscience Lab, BRI, Ottawa).

1.5.2 Measures of Intra-cortical Excitability with Conditioning TMS Paradigms

Paired-pulse TMS also known as conditioning-test paradigm is used to measure cortical excitability (Abbruzzese and Trompetto 2002). The effect of the conditioning stimulus on the motor response evoked by the test stimulus varies depending on the presence of voluntary muscle contraction, the intensity of both conditioning and test stimuli as well as their interval (Abbruzzese and Trompetto 2002). To investigate intracortical inhibitory and excitatory circuits, both conditioning and test stimuli are applied to the same region (typically M1) using different inter stimulus intervals (ISI) (Dayan, Censor et al. 2013).

1.5.2.1 Short- and Long-interval Intracortical Inhibition

Short-interval intracortical inhibition (SICI) can be tested using the paired-pulse paradigm in which a subthreshold conditioning stimulus precedes a suprathreshold test stimulus by a short ISI (2-5 ms) to activate GABA A receptors in the motor cortex mediating short-lasting inhibitory intraneuronal pathways (Figure 5B) (Di Lazzaro, Oliviero et al. 2000, Ilić, Meintzschel et al. 2002, Rosenkranz and Rothwell 2003, Roy and Gorassini 2008, Schabrun, Jones et al. 2013). SICI seems to contribute to the fractionation of muscle activity (i.e. focus muscle activation inputs) at the beginning of a movement or during motor tasks, which is essential for fine motor control in distal muscles (Rosenkranz and Rothwell 2003, Ridding, Pearce et al. 2005). Long-interval intracortical inhibition (LICI), on the other hand, is tested when a suprathreshold conditioning stimulus is delivered 50-200 ms before the suprathreshold test stimulus (Fuhr, Agostino et al. 1991, Rosenkranz and Rothwell 2003). In contrast to SICI, the resulting long-lasting inhibition mediated by GABA B receptors is essential during later phases of movement (Rosenkranz and Rothwell 2003). SICI and LICI are modulated by a different sets of cortical interneurons (Sanger, Garg et

al. 2001, Rosenkranz and Rothwell 2003), despite being mediated by cortical GABAergic circuits (Fuhr, Agostino et al. 1991, Inghilleri, Berardelli et al. 1993). Besides, SICI and LICI are effective in suppressing corticospinal neurons (i.e. MEPs) induced by high and low TMS intensities, respectively (Sanger, Garg et al. 2001, Rosenkranz and Rothwell 2003).

1.5.2.2 Intracortical Facilitation

Intracortical facilitation (ICF) is an excitatory phenomenon that can be studied using paired pulse TMS paradigm in which two near- or supra-threshold stimuli at very short ISIs (ranging between 1 and 4.5 ms) are paired (Figure 5B) (Abbruzzese and Trompetto 2002, Roy and Gorassini 2008, Dayan, Censor et al. 2013). The resulting facilitation reflects the interaction between N-Methyl-D-aspartic acid (NMDA) receptors and glutamatergic interneurons to induce I-waves (Schabrun, Jones et al. 2013, Rocchi, Erro et al. 2017).

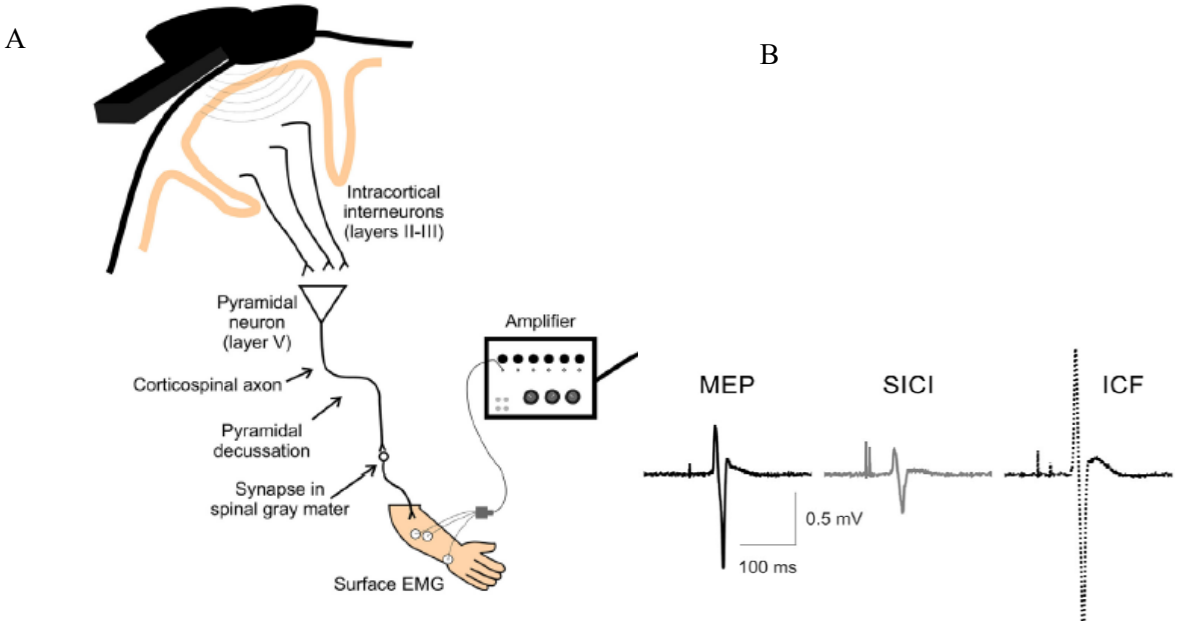

Figure 5. A schematic of TMS-evoked measures of single and paired-pulse corticospinal excitability [with permission from (Auriat, Neva et al. 2015)]. A) Transcranial magnetic stimulation (TMS) generates an electric field, which can activate intra-cortical, cortico-spinal and trans-cortical projections (Bestmann and Krakauer 2015). B) Examples of motor-evoked potential (MEP), short-interval intracortical inhibition (SICI), and intracortical facilitation (ICF) recorded by surface electrode.

1.5.2.3 Markers of Sensorimotor Integration

The influence of sensory afferent inputs from hand on both excitatory and inhibitory cortical networks of the primary motor cortex can be assessed using afferent nerve conditioning with TMS (Turco, El-Sayes et al. 2018). In this paradigm, brief electrical nerve stimulation is delivered to the median or ulnar nerve before a single TMS pulse over the primary motor cortex (Tokimura, Di Lazzaro et al. 2000, Mercier, Gagné et al. 2016). For instance, short-latency afferent inhibition (SAI) is used to probe sensorimotor integration by delivering the electrical nerve stimulation 18-24 ms prior to the TMS pulse (Figure 6) (Mercier, Gagné et al. 2016). Another period of long-latency afferent inhibition (LAI) occurs when longer ISIs (180-220 ms) are used between nerve stimulation and the TMS pulse (Devanne, Degardin et al. 2009). Both SAI and LAI reflect inhibition at the cortical level and depression of late I-waves activity via cholinergic and GABAergic pathways (Di Lazzaro, Oliviero et al. 2000, Tokimura, Di Lazzaro et al. 2000, Roy and Gorassini 2008). Afferent facilitation (SAF), on the other hand, occurs when conditioning stimulation of median or ulnar nerve precedes TMS pulse by 40-80 ms in the resting hand muscles (Mariorenzi, Zarola et al. 1991, Deletis, Schild et al. 1992). This facilitation likely occurs through cortical mechanisms involving the activation of large proprioceptive afferent fibers originating from muscle spindles (Devanne, Degardin et al. 2009).

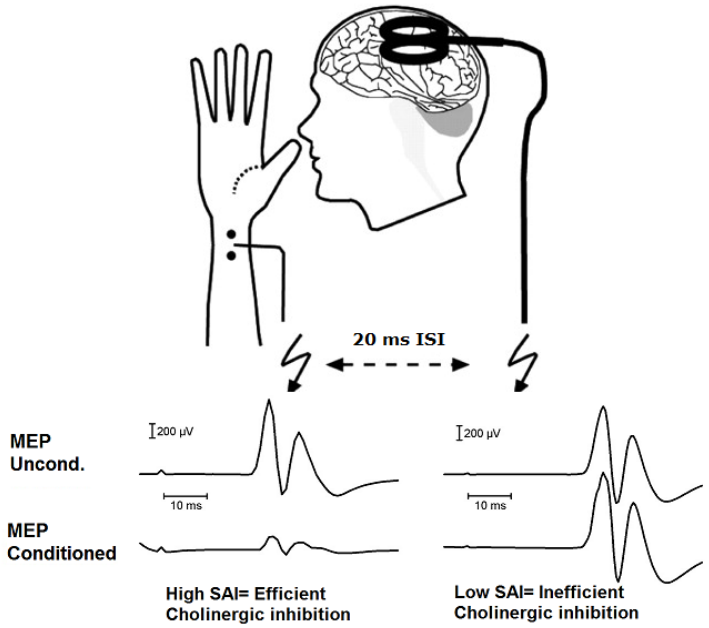

Figure 6. An image illustrating short afferent inhibition (SAI) and inter-individual variations in SAI level depending on the efficacy of cholinergic inhibition. (Image from Clinical Neuroscience Lab, BRI, Ottawa).

1.5.3 Measure of Spinal Excitability: the Hoffman Reflex

The Hoffman reflex (H-reflex) is an electrically induced reflex consisting of afferent and efferent pathways to measure the efficacy of synaptic transmission and alpha motoneuron (αMN) excitability (Abbruzzese and Trompetto 2002, Palmieri, Ingersoll et al. 2004). Following percutaneous short-duration and low-intensity electrical stimulation of a mixed nerve, action potentials travel along afferent (Ia sensory) fibers toward the spinal cord, where they synapse with aMN to induce excitatory postsynaptic potentials (EPSPs) (Palmieri, Ingersoll et al. 2004). The generated action potentials in depolarized αMNs travel down along efferent fibers to reach the neuromuscular junction where acetylcholine is released to initiate muscle twitch and H-reflex (Palmieri, Ingersoll et al. 2004). In addition to the afferent pathway in the H-reflex, electric stimulation of the peripheral nerve directly activates the efferent fibers and triggers action

potentials travelling toward the neuromuscular junction to evoke muscle response (M-wave) (Palmieri, Ingersoll et al. 2004).

1.6 Modulation of Corticospinal Excitability in response to Sensory Stimulation Protocols

Afferent stimulation has been reported to modify the excitability and organization of the sensorimotor cortex and neurons of the CNS, which have the ability to alter their connections and functional organization, a phenomenon known as plasticity (Duffau 2006). In the following section, I will provide a review of TMS studies investigating the neurophysiological effects of peripheral (i.e. electrical, vibrotactile and thermal) stimulation on corticospinal excitability.

1.6.1 Effects of Repeated Electrical Nerve Stimulation

Peripheral electrical stimulation (PES) can induce transient changes in cortical representation and elicit neuroplasticity in the adult motor cortex (Uy and Ridding 2003, Chipchase, Schabrun et al. 2011, Léonard, Mercier et al. 2013). For instance, repeated electrical nerve stimuli delivered to the ulnar or radial nerve at the wrist has been reported to induce modulation in the excitability of the corticospinal projections to small hand muscles (Ridding, Brouwer et al. 2000, Ridding, McKay et al. 2001). The extent of this effect depends on the nerve involved (i.e. mixed (Bertolasi, Priori et al. 1998, Tokimura, Di Lazzaro et al. 2000) or purely cutaneous nerves (Palmer and Ashby 1992, Ridding and Rothwell 1999) as well as the topography of the stimulation area (i.e. MEP amplitude is affected only in muscles near or adjacent to the stimulation site) (Tamburin, Manganotti et al. 2001, Rosenkranz and Rothwell 2003). Interestingly, repetitive peripheral stimulation has been reported to only facilitate TMS-induced MEPs, but not the MEPs evoked by cervicomedullary stimulation, indicating that facilitation

appears likely to have a cortical origin (Ridding, McKay et al. 2001, Rosenkranz and Rothwell 2003). This evidence is further supported by the lack of the peripheral nerve stimulation effect on the occurrence and size of F-waves (Ridding, Brouwer et al. 2000) or on the responses evoked by transcranial electrical stimulation (Tamburin, Manganotti et al. 2001, Rosenkranz and Rothwell 2003). Notably, the direction of the changes in excitability induced by PES seems to be highly dependent on the stimulus amplitude (intensity), the nature of motor stimulation (muscle flicker vs muscle contraction) and pulse frequency (number of pulses per second) of PES (Robertson, Reed et al. 2006, Chipchase, Schabrun et al. 2011). For instance, low-frequency electrical stimulation protocols have been reported to increase corticospinal excitability (Hamdy, Rothwell et al. 1998, Ridding, Brouwer et al. 2000), while inconsistent results have been obtained for high-frequency stimulation protocols, with some studies reporting increased (Heroux, Tremblay et al. 2003), decreased (Mima, Oga et al. 2004) or unchanged corticospinal responsiveness (Ridding, Brouwer et al. 2000, Léonard, Mercier et al. 2013). Stimulation site seems also to be an important parameter that influences corticospinal excitability in response to electrical stimulation, as shown by the inhibitory effect of prolonged high frequency afferent electrical stimulation on the corticospinal excitability of lower limb muscles compared to upper limb muscles (Léonard, Mercier et al. 2013). In addition, there are opposite intensity-dependent changes in corticospinal excitability in response to PES (Chipchase, Schabrun et al. 2011, Schabrun, Ridding et al. 2012). For instance, peripheral electric stimulation at stimulus amplitude sufficient to induce a sensory perception below motor threshold at 10 Hz and 100 Hz decreases corticospinal responsiveness of the stimulated muscle and its antagonist regardless of the stimulus frequency (Hamdy, Rothwell et al. 1998, Mima, Oga et al. 2004, Tinazzi, Zarattini et al. 2005, Chipchase, Schabrun et al. 2011). In addition, when the 10 Hz stimulus amplitude is increased beyond motor contraction to induce noxious response, the corticospinal responsiveness of both muscles decreases (Chipchase,

Schabrun et al. 2011). However, stimulus amplitude sufficient to evoke a visible muscle twitch (30 Hz) increases the responsiveness of the corticospinal pathway (Ridding, Brouwer et al. 2000, Ridding, McKay et al. 2001, Schabrun and Ridding 2007, Chipchase, Schabrun et al. 2011). Consistent with this finding, neuromuscular electrical stimulation (NMES) or functional electric stimulation has been reported to increase the excitability of M1 and corticospinal pathways as measured by increase in amplitude of MEPs (Mang, Bergquist et al. 2012). Such facilitatory effect on corticospinal pathways start within ∼10–20 min of the beginning of an NMES session and can last up to hours after a single session (Hamdy, Rothwell et al. 1998, Ridding, Brouwer et al. 2000, Mang, Clair et al. 2011) or days after repeated sessions (McKay, Ridding et al. 2002, Mang, Bergquist et al. 2012). This enhanced excitability induced by NMES has been associated with increased activity of facilitatory pathways and reduced activity in inhibitory pathways involved in SAI and ultimately improved motor performance (McDonnell and Ridding 2006, Mang, Bergquist et al. 2012). In addition, PES has been reported to co-modulate the excitability of primary sensory and motor cortical areas irrespective of stimulus intensity and frequency (Schabrun, Ridding et al. 2012). As a result, modulation of S1 excitability with PES may lead to altered M1 excitability possibly through cortico-cortical projections between S1 and M1 that is involved in motor learning (Schabrun, Ridding et al. 2012). These findings are coherent with the results of randomized clinical trials reporting intensity dependent improved motor performance following electrical stimulation (Price and Pandyan 2000, Ada and Foongchomcheay 2002, Ragnarsson 2007, Sujith 2008, Chipchase, Schabrun et al. 2011).

1.6.2 Effects of Vibrotactile Stimulation

Vibration activates mechanoreceptive nerve afferents at the receptor level and thus provides a more natural stimulation in changing the excitability of intracortical GABAergic circuits

compared to the electrical stimulation (Rosenkranz and Rothwell 2003). In addition, when applied to tendon, vibration can preferentially activate muscle spindle receptors, whose inputs have a stronger and more selective effects on the excitability of motor cortex compared to cutaneous inputs (Rosenkranz and Rothwell 2003). Interestingly, there is a differential pattern of effects on the excitability of cortical inhibitory neurons projecting to different output zones of the motor cortex in response to vibratory stimulation (Rosenkranz and Rothwell 2003). For instance, low amplitude muscle vibration (0.5 ms; 80 Hz; duration 1.5 s) has been reported to increase the MEP amplitude and LICI, but decrease SICI in the vibrated intrinsic hand muscles, while it has an opposite effect on the MEP amplitude and LICI in the non-vibrated muscles (Claus, Mills et al. 1988, Kossev, Siggelkow et al. 1999, Rosenkranz and Rothwell 2003).

1.6.3 Effect of Thermal Stimulation on Spinal Excitability

The effect of thermal stimulation on spinal excitability can be assessed in the lower limb muscles using the H-reflex (Abbruzzese and Trompetto 2002, Palmieri, Ingersoll et al. 2004). Several studies have reported lasting increase in spinal excitability in response to cold stimulation (Krause, Hopkins et al. 2000, Hopkins and Stencil 2002, Palmieri, Ingersoll et al. 2004). For instance, Hopkins and Stencil (2002) reported increase in the soleus H-reflex both during application of crushed ice over the calf muscles and up to 90 min post-application (Hopkins and Stencil 2002). Similarly, Palmieri-Smith and colleagues (2007) reported an increase in spinal excitability (H:M ratio) lasting up to 20 min post-ice application at the ankle (Palmieri-Smith, Leonard-Frye et al. 2007). The lack of correlation between increase in H: M ratio and the elevation of circulating plasma norepinephrine levels indicates that alterations in spinal excitability were independent of peripheral sympathetic responses (Palmieri-Smith, Leonard-Frye et al. 2007). As a result, cooling seems to increase spinal excitability by possibly decreasing the descending inhibition.

1.6.4 Effects of Thermal Stimulation on Corticospinal Excitability in Stroke Patients

The therapeutic effects of noxious thermal stimulation was first examined by Chen et al. (2005) who used alternate cycles of heating and cooling stimulation to improve motor recovery in acute stroke patients (Chen, Liang et al. 2005, Chen, Tang et al. 2019). To provide constant thermal stimulation, Wu et al. (2010) used a custom-made water circulation system and reported a significant improvement in motor recovery of the paretic arm in subacute stroke patients (Wu, Lin et al. 2010, Chen, Tang et al. 2019). Tai et al. (2014) used TMS to compare changes in corticospinal excitability in response to noxious and innocuous heat and cold stimuli applied to the affected arm in stroke patients (Tai, Lai et al. 2014, Chen, Tang et al. 2019). Based on the MEP amplitude and map size, their results showed that only the group exposed to noxious heat and cold stimuli showed significant changes in corticospinal excitability (Tai, Lai et al. 2014). However, their claim regarding the greater efficacy of noxious thermal stimulation over innocuous stimulation is questionable considering the small number of patients tested in each group (n=8), and the large inter-individual variability observed in both groups (>100% coefficient of variation for MEP post-thermal stimulation). Besides, the use of noxious temperatures raises question as to whether the reported modulations could simply reflect pain–motor interactions rather than thermally-induced effects per se. Recently, a randomized controlled trial study investigated the effects of a hybrid protocol combining NMES with noxious thermal stimulation on the recovery of the paretic arm in chronic stroke patients (Chen, Tang et al. 2019). Their results showed no difference with the hybrid protocol when compared to each protocol applied independently in most outcome measures (e.g. Ashworth scale, Motricity index, Barthel index) (Chen, Tang et al. 2019). However, authors noted that the participants receiving the hybrid protocol exhibited greater recovery of arm function as reflected in the Fugl-Meyer assessment than participants treated with NMES or thermal stimulation alone (Chen, Tang et al. 2019). They concluded that, despite inducing pain during

intervention, noxious thermal stimulation can reduce muscle tension and thus seems to be a safe intervention for stroke patients (Chen, Tang et al. 2019). Nevertheless, innocuous thermal stimulation also seems to be effective in changing corticospinal excitability and cortical activation as shown in a study performed in our lab (Tremblay, Remaud et al. 2015), where innocuous cooling applied to the scalp induced lasting changes in corticospinal excitability as reflected in MEP amplitude of the FDI muscle. In fact, a 10 °C decrease in scalp temperature via cold compression reduced MEP amplitude up to 20 min post-cooling (Tremblay, Remaud et al. 2015). In another study, thermal-tactile oral stimulation with an ice stick induced rapid and short-lasting changes in the activation of cortical sensory swallowing areas in healthy participants (Teismann, Steinsträter et al. 2009).

In summary, there is now strong evidence that application of peripheral afferent stimulation can modulate corticospinal excitability and induce neuroplastic changes in the human M1. Most of this evidence comes from studies reporting the beneficial effect of peripheral electrical stimulation in improving and re-educating voluntary contraction in patients with stroke and spinal cord injury (Price and Pandyan 2000, Ada and Foongchomcheay 2002, Conforto, Kaelin-Lang et al. 2002, Ragnarsson 2007, Sujith 2008, Chipchase, Schabrun et al. 2011), and a few studies reporting changes in corticospinal excitability in response to other forms of afferent stimulation, such as vibrotactile afferent stimulation (Forner-Cordero, Steyvers et al. 2008). In addition, thermal stimulation has been used as an effective neurofacilitation method to improve motor recovery following stroke (Chen, Liang et al. 2005, Chen and Shaw 2006, Chen, Lin et al. 2011, Chen and Shaw 2014, Tai, Lai et al. 2014). Although such thermal stimulation appears to be a promising rehabilitative intervention, there are still many unanswered key questions regarding the neurophysiological mechanisms for the reported beneficial effects. For instance, it is not clear which mode of thermal stimulation, warming or cooling, is more effective in inducing lasting

modulation in corticospinal excitability. In the same vein, and given the widespread cortical activation elicited by thermal stimuli, it is still unknown what are the effects of the extent of stimulation on corticospinal excitability and whether thermal stimulation can alter other markers of cortical excitability reflecting intra-cortical inhibition (i.e. SICI) and sensori- motor integrations (i.e. SAI and SAF). Finally, psychophysical studies have shown that sensitivity to temperature is not greatly affected by sex, but tends to decline with age, especially in the extremities, the foot being the area where the largest decrease in sensitivity is found (Kenshalo 1986, Stevens and Choo 2009). Nonetheless, we are lacking critical observations with regards to the effect of individual factors, such as age and sex on the thermally induced modulations at the neural level. It also remains to be seen whether simultaneous application of innocuous warm and cold stimulation leads to a stronger and more consistent effect in corticospinal excitability.

Objectives of the Thesis

The overall goal of this thesis was to gain a better understanding of the neurophysiological basis of focal thermal stimulation in influencing corticospinal excitability in healthy participants using TMS. The long-term goal was to eventually help design better physical therapy interventions for clinical populations such as stroke survivors. To this end, we designed and performed three series of experiments.

In Experiment I, our primary goal was to determine which modality of innocuous thermal stimulation, cooling or warming, was more effective in eliciting lasting modulation in corticospinal excitability (as reflected in MEP amplitude). The secondary objective was to determine whether individual characteristics such as age and sex influence thermally-induced changes in corticospinal excitability. Finally, we also examined the role of individual changes in skin temperature.

In Experiment II, our goal was to extend and complement Experiment I to determine whether the variability we observed in response to thermal stimulation could be reduced by extending the depth and extent of the focal thermal effects at the peripheral level. We hypothesized that extending the area of cooling distally could lead to more consistent modulation at the individual level, thereby reducing the variability of responses.

In the light of results of Experiment, I and II, we designed another experiment (Experiment III) as an attempt to directly address the source of the variability of thermally-induced modulation in corticospinal excitability. We hypothesized that the variability observed in terms of MEP facilitation/inhibition might be related to individual differences in the central processing of thermal sensory inputs at the sensorimotor level. Indeed, variability in response to plasticity-inducing TMS protocols have been associated with inter-individual variations in markers of intra-cortical excitability, such as SAI and SICI (Guerra, Lopez-Alonso et al. 2017). In Experiment III, we

sought to determine whether inter-individual differences in SAI and SAF at baseline, as markers of sensorimotor integration within the M1, could predict whom will show suppression or enhancement of MEPs in response to focal cooling stimulation. Our design also investigated SICI, as another marker of intra-cortical excitability.

While Experiment I, II and III represent the core of this thesis, we also present in the general discussion the results from a preliminary experiment (Experiment IV) designed to explore the effects of combining focal cold and warm stimulation on perceived intensity of thermal sensation and on corticospinal excitability.

CHAPTER II. RESEARCH PAPERS

List of published research papers

I. Variations in Corticomotor Excitability in response to Distal Focal Thermal Stimulation

Paper published in Somatosensory & Motor Research:

Ansari, Y., Remaud, A., and Tremblay, F. (2018). Variations in corticomotor excitability in response to distal focal thermal stimulation. *Somatosens. Mot. Res.* 35**,** 69-79. doi: 10.1080/08990220.2018.1460263.

II. Modulation of Corticomotor Excitability in response to Distal Focal Cooling

Paper published in PeerJ:

Ansari, Y., Remaud, A., and Tremblay, F. (2018). Modulation of corticomotor excitability in response to distal focal cooling. *PeerJ* 6**,** e6163. doi: 10.7717/peerj.6163.

III. Short-latency Afferent-induced Facilitation and Inhibition as Predictors of Thermally-induced Variations in Corticomotor Excitability

Paper published in Experimental Brain Research:

Ansari, Y. & Tremblay, F. (2019). Short-latency Afferent-induced Facilitation and Inhibition as Predictors of Thermally-induced Variations in Corticomotor Excitability. *Exp Brain Res*. 237: 1445. https://doi.org/10.1007/s00221-019-05522-1.

Variations in Corticomotor Excitability in Response to Distal Focal Thermal Stimulation

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Running title: Thermal stimulation and corticomotor excitability

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Variations in Corticomotor excitability in Response to Thermal Stimulation

Abstract

In this study, we investigated the effects of thermal stimulation on corticomotor excitability with TMS. Participants consisted of healthy young adults (n=20) and seniors (n=15). Each experimental session consisted of a baseline (BL) assessment, followed by a warming and a cooling protocol. At BL, recordings of motor evoked potentials (MEPs) and skin temperature were performed with the index finger covered with a "neutral" gel pack (24°C). For warming, the same measurements were performed but with the index covered with a warmed gel pack (45°C). The gel pack was kept for 5 min, and the measurements were performed at 1 min during warming and 5 and 10 min post. After a break, participants were tested with the cooling protocol (gel pack 10°C) by repeating the same sequence as in the warming. The two thermal protocols induced the desired range of skin temperatures (warming, 35-45°; cooling, 13-24°). For MEP modulation, the primary analysis revealed no main effects or interactions, owing to the variability of responses to either warming or cooling stimulation. Further analysis of individual responses revealed that modulation, when present, was short-lasting and was characterized by a depression in about half of the participants. Facilitation was also observed but only in smaller clusters, especially with cooling (13/35). Modulation in MEP amplitude did not correlate with changes in skin temperature. These results are consistent with previous reports regarding variability in response to sensory stimulation protocols. In the case of thermal stimulation, such variability likely reflects individual differences in the influences exerted by thermal afferents centrally.

Keywords

Motor evoked potentials, peripheral stimulation, thermal afferents

Introduction

Peripheral sensory stimulation has been advocated for years as a rehabilitation strategy to influence motor responses and induce neuroplastic changes following brain injuries (Chipchase, Schabrun et al. 2011). In this regard, the use of thermal stimulation has drawn attention recently as an adjuvant method to facilitate motor responses in patients recovering from a stroke (Chen, Lin et al. 2011, Liang, Hsieh et al. 2012, Hsu, Lee et al. 2013). Compared to other forms of sensory stimulation (e.g., vibro-tactile, electrical) thermal stimuli readily evoke strong sensations, which are associated at the neural level with a large cortical and subcortical network involving the parietal operculum, anterior/posterior insula and thalamic nuclei (Casey, Minoshima et al. 1996, Craig, Reiman et al. 1996, Davis, Kwan et al. 1998). In a series of clinical studies, Chen, Tai and colleagues (Chen, Liang et al. 2005, Chen, Lin et al. 2011, Liang, Hsieh et al. 2012, Hsu, Lee et al. 2013) investigated the effects of thermal stimulation in post-stroke patients. In their first report, Chen, Liang et al. (2005) showed that a thermal intervention consisting of repeated daily applications of hot and cold packs targeting the affected arm and hand was effective in reducing impairment and improving function in patients when compared to usual cares. Using a similar intervention for the lower extremity, the same group (Chen, Lin et al. 2011) showed that thermal stimulation was also effective in improving balance and mobility functions for up to 3 months post-intervention. In a subsequent report, Hsu, Lee et al. (2013) compared the therapeutic efficacy of noxious and innocuous temperatures stimulation in the lower limb and concluded that temperatures in the noxious range were most effective.

While these reports support the use of thermal stimuli as a neurofacilitation method, the underlying mechanisms to account for the reported motor improvements remain poorly understood. For Hsu, Lee et al. (2013), the motor improvement was likely a reflection of the cortical activation elicited by the thermal stimuli, leading to motor reorganization. In a preliminary

study, Tai, Lai et al. (2014) attempted to address the question using transcranial magnetic stimulation (TMS) to probe changes in the hand motor representation in response to thermal stimuli in stroke patient. Patients were assigned to receive either noxious (heat 46°C, cold 7°C) or innocuous thermal stimulation (heat 41°C, cold, 20°C) targeting the affected upper extremity for 30 min. By comparing motor maps in the lesioned hemisphere before and after stimulation, the authors found a significant increase in map size in the group exposed to noxious stimuli but not in those exposed to innocuous temperatures. While these findings were consistent with a thermally-induced motor reorganization, their impact was somewhat mitigated by the small number of patients tested (n=8 per group) and the important variability observed between participants. Besides, the use of noxious temperatures and this report and other reports by the same group raises questions as to whether the reported changes could simply reflect pain-motor interactions rather than pure thermally-induced modulation.

Currently, there is ample evidence in the TMS literature that peripheral afferent stimulation in the non-noxious range can modulate corticomotor excitability and induce plasticity (for a review see Chipchase, Schabrun et al. (2011). One important caveat, however, is the fact that this evidence is based almost exclusively on applications of electrical stimulation. In fact, apart from a few isolated studies, most TMS reports investigating the effects of afferent stimulation have focused on electrical nerve stimulation. Still, there is also evidence that other modalities of sensory stimulation, such as vibrotactile afferent stimulation (Heroux, Tremblay et al. 2003, Forner-Cordero, Steyvers et al. 2008), have the potential to alter motor representations and modulate central excitability. In the case of thermal stimuli, as mentioned earlier, there is very little information as to whether this form of sensory stimulation can lead to rapid and sustained changes in corticomotor excitability. Such information appears critical to foster any further development regarding applications of thermal stimuli in the context of neurorehabilitation interventions.

In the present study, we sought to investigate the neurophysiological basis underlying thermally-induced afferent modulation at the corticomotor level with TMS. We were primarily interested in determining whether innocuous focal thermal stimulation targeting the hand could lead to immediate and lasting changes in corticomotor excitability. Secondarily, we asked whether individual factors related to age and gender could influence thermally-induced modulation. To address these questions, we performed a series of measurements in the same groups of participants composed of young adults and healthy seniors to assess modulation in corticomotor excitability in response to either focal warming or cooling of a single finger. Through this series of measurements, we were able to show that thermally-induced modulation was very short-lasting and variable and, this, regardless of the participants age or gender.

Material and Methods

The Institutional Review Ethics Board (Bruyère Hospital Ottawa, Protocol M16-17-001) approved the study procedure in accordance with the principles of the Declaration of Helsinki, and informed consent was obtained from all participants before the experimental session. All experiments were performed in a controlled laboratory environment. Participants received a small honorarium for their participation.

Participants

Thirty-five healthy participants were recruited for this study with a young group (28 ± 5 years) composed of 20 individuals (10 men, 10 women) and a senior group (67 ± 4 years) composed of 15 individuals (six men, nine women). The sample size was estimated using the standard mean difference of 0.79 mV reported by Chipchase, Schabrun et al. (2011) regarding changes in MEP amplitude in response to repeated afferent stimulation. A power analysis showed that a sample size of 15 in each group had a 95% to detect such a difference with a power of 95%. Most participants were recruited from the community and, except for two young adults, were

completely naïve with regards to the purpose of the study. At the time of testing, all participants were considered healthy and presented with no contra-indications to TMS. Senior participants were further screened to ensure that they had no recent or actual history of complaints regarding sensibility of their hands. All but two participants (young group) were right-handed as determined by the Edinburg Hand Inventory (online version http://www.brainmapping.org/shared/Edinburgh.php).

General procedure for TMS and recordings of motor evoked potentials

All TMS assessment were performed with participants comfortably seated in a custom-made chair specifically designed for neurophysiological testing equipped with armrests and footrests. Given that the thermal stimulation was directed at the index finger, the *first dorsal interosseous* (FDI) was considered the primary target muscle to assess modulation in excitability. MEPs elicited in the FDI were recorded using surface sensors (DE-2.1, Delsys Inc., Boston, MA, USA) placed in a belly-tendon montage. Note that MEPs were also monitored from the *abductor digiti minini* but these responses could not be reliably obtained in all participants and are not reported here. After amplification and filtering (Bagnoli™ 4 System, Delsys Inc., bandwidth=6–450 Hz, gain=1,000), electromyographic signals were digitized at a rate of 2 kHz (PCI-63203, National Instrument Corp. Austin, TX) and further relayed to a laboratory computer running custom software to control acquisition and saved for later off-line analysis. TMS pulses were applied on the hemisphere contralateral to the preferred hand over the motor hot spot for the FDI (marked with a sticker) using a focal coil (70 mm, P/N 3190) connected to a Magstim 200 (Magstim Co. Ltd, Whitland UK). Participants were fitted with a Waveguard™ TMS compatible EEG cap (ANT Neuro, Madison, WI, USA) with markers to facilitate coil placement and ensure consistent positioning. Also, a U-shaped neck cushion was used to maintain head position and prevent neck fatigue. The resting motor threshold (rMT) was determined using the Motor Threshold Assessment

Tool software (MTAT 2.0; Clinical Researcher, Knoxville, TN, USA). 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, Molnar et al. 2004). All subsequent testing was performed at 130% of the rMT. During TMS, participants were instructed to stay relaxed, while staying alert and, to this end, they were asked to count the number of stimuli delivered to avoid shift of attention or sleepiness. All tests were performed between 9 am, and 4 pm to avoid diurnal variations (Doeltgen and Ridding 2010) in a temperature-controlled room maintained at 22 °C.

Protocols for thermal stimulation

Figure 1 illustrates the experimental protocol to assess the effects of thermal stimulation on corticomotor excitability. Each testing session, which lasted about 2 hours, began with baseline (BL) measurements and then proceeded sequentially with the warming and cooling protocols, with a 20-min break between the two. As shown in Figure 1, all measurements were performed with the index finger wrapped in a Torex® gel pack sleeve specifically designed for finger application (TXRT-2540, Torex Health Products, Tallmadge, OH, USA). The gel pack sleeve covered the index finger from the tip down to the base of the metacarpophalangeal joint, sparing the area overlying the FDI muscle. The gel's temperature was manipulated depending on the experimental conditions (i.e., neutral, warm or cold temperature). Two thermocouple sensors were affixed with tape at the level of the proximal interphalangeal joint to monitor skin temperature, leaving the bare ends exposed in direct contact with the skin. The thermocouple sensors were connected to a K-type digital thermometer (Model# TC41FBA, Perfect-Prime, Dayton, NJ, USA) with a resolution ± 0.1°C. As stated earlier, all TMS measures were performed using a test intensity of 130% rMT with 20 MEPs being recorded (3-5 sec between pulses) at each time point. These parameters (intensity and number of trials) were selected on the basis of recent reliability studies regarding

measures of resting corticomotor excitability (Cuypers, Thijs et al. 2014, Brown, Lohse et al. 2017).

For BL measurements, the index finger was covered with a gel pack that was kept at room temperature (~24° C). This "neutral" gel pack was used to account for the tactile feedback associated with the finger wrapping in the other conditions. With the neutral gel pack in place, readings of skin temperature were performed and MEPs were recorded. Following BL measurements, we proceeded with the warming protocol. The neutral gel pack was removed and replaced by another one that had been pre-heated to ~ 45°C by prior immersion in a warm water. With the warmed gel pack in place, both skin temperature and MEPs were recorded at 1-min (W1). After collecting these data, which took about 3 min to complete, the gel pack was kept in place until 5 min had elapsed. Then, the gel pack was removed, and participants were instructed to remain immobile. At 5 min and 10 min post-warming (PW5 and PW10), both skin temperature and MEPs were measured again with a neutral gel pack in place, here again to maintain recording conditions regarding tactile feedback. With the warming protocol completed, participants were asked to rest for another 20 min to wash out any lingering temperature effects and allow motor excitability to return to BL. Indeed, superficial heat applications are known to have only limited effects in terms of duration and penetration (e.g., see Lohman, Bains et al. (2011)) and a 35 minutes period (i.e., 15 min post-warming + 20 min pause) was considered sufficient to dissipate any effects induced by a 5-min application restricted to a single finger. In fact, skin temperature quickly returned to BL in the post-warming period (see below, Results). For cooling, the same protocol as the one described for warming was used, except that the warmed pack was replaced by a cooled pack (~10°C) that had been stored in a mini-refrigerator before testing. The procedure to measure

MEPs and skin temperature at the different time points both during (C1) and post-cooling (i.e., PC5 and PC10) was identical to that used for the warming protocol (Figure 1).

Data analysis and statistical procedures

For skin temperature, readings from the two thermocouple sensors were averaged in each experiment to get mean individual values at BL and at each time point. The data were then entered into a 4 × 2 × 2 repeated measures analysis of variance (ANOVA) with *Time* (BL, C1/W1, PC5/PW5, PC10/PW10) as the within-subject factor and *Age* and Gender as the between-subjects factors. The Sidak test was used for post-test comparisons. For MEP data, the amplitude (peak-to-peak) and latency in each trial was analyzed off-line using a MATLAB script (The Mathworks, Natick, MA, USA) to derive mean individual values at each time point. Since amplitude data were not normally distributed (D'Agostino & Pearson normality test, $p<0.05$), individual mean values were log-transformed to normalize the distribution (Nielsen 1996). Latency measures did not need such transformation. Variations in MEP amplitude and latency were analysed using the same approach as the temperature data using 4 × 2 × 2 repeated measures ANOVAs and the Sidak's test for *post-hoc* comparisons. Pearson's moment correlation was used to examine relationship between variables. The significance level was set at $p<0.05$ for all tests. Statistical analyses were performed using SPSS 17.0 software package (SPSS Inc., Chicago, IL, USA) and GraphPad Prism version 7.00 for Windows (GraphPad Software, San Diego, CA, USA, www.graphpad.com), respectively. All data are reported as mean ± standard deviation (SD).

Results

General observations

All participants completed the testing without issues. The test stimulation intensity for TMS (130% rMT) corresponded on average to 52.6 ±14.1% of the maximal stimulator output in the young group, whereas it corresponded to 69.3±15.6% in the senior group. This difference reflected

the fact that rMTs were lower, on average, in the young group than in the senior group (40.5 ±10.9% vs. 54.2 ± 12.9%). Participants well tolerated the thermal protocols, all reporting the expected sensations of either warming or cooling and, more importantly, none reported pain or major discomfort attributable to the thermal stimuli.

Variations in response to local warming

Figure 2 A shows the mean skin temperature, MEP amplitude, and latency computed in the two groups at BL and at the different time points during the warming protocol. As expected, the temperature increased markedly during warming and then tended to return to BL in the post-warming phase. The ANOVA confirmed the large effect of *Time* on skin temperature ($F_{3,29}$=105.1, p<0.001) but no effect of either *Age* or *Gender* ($F_{1,31}$<1.2, p>0.30) nor interactions. Post-hoc tests confirmed that skin temperatures recorded at W1 and PW5 were significantly different from BL (p<0.001, mean difference of respectively + 7.4° and+1.6°) whereas skin temperature measured at PW10 (+0.9°) was not. For variations in MEP amplitude, it can be seen that the pattern of modulation was very similar in the two age groups with a tendency for depression at W1 followed by a return towards BL at PW5 and PW10. Also, inter-individual variability was relatively large at each time point, as evident in the error bars. This variability was reflected in the ANOVA results, where no main effect was detected for neither the factor *Time* ($F_{3,29}$=1.6, p=0.21) nor the *Gender* and *Age* ($F_{3,29}$<2.2, p>0.11) factors. No interaction was detected either. For MEP Latency, variations between time points were small when compared to the large effect of age (Figure 2 A). The *Age* effect was confirmed by the ANOVA along with that of *Gender* ($F_{1,31}$>32.0, p<0.001), while, as expected, *Time* did not affect variability ($F_{3,29}$=0.16, p=0.92). No interaction between factors was detected for MEP latency. *Post-hoc* comparisons confirmed that younger participants had, on average, shorter latencies than seniors (p<0.001, 22.4 vs. 25.0 ms, respectively), while the

gender effect was explained by the fact that women had shorter latencies than men ($p<0.001$, 22.0 vs. 25.4 ms).

While the primary analysis revealed that warming stimulation had only a marginal influence on MEPs, a closer inspection of individual variations when expressed relative to BL (i.e., % change from BL) revealed that many participants exhibited similar patterns. As shown in Figure 2 B, the largest cluster (18/35) consisted of individuals who exhibited MEP depression (i.e., >10% reduction from BL) during warming (W1) followed by either a facilitation or return to BL post-warming. Another small group (n=5) exhibited an opposite pattern characterized by MEP facilitation (>10% increase from BL) both during warming (W1) and also in the post-warming phases. Finally, about one-third (12/35) of the participants showed no clear modulation (i.e., MEP size ±10% from BL) during warming followed by some variable modulation in the post-warming phase. Given the presence of a large cluster of participants exhibiting depression (n=18), we applied a secondary analysis on this subset using a repeated measures ANOVA. This analysis revealed a main effect of "Time" ($F_{3,12}=17.2$, $p<0.001$) on variations in amplitude but no interaction with either *Age* or *Gender* ($F_{3,12}<2.7$, $p>0.10$). Post-hoc comparisons confirmed that MEPs recorded at W1 were significantly smaller than BL ($p<0.001$), whereas such a difference was not found for those recorded at PW5 or PW10. For MEP latency, no effect of *Time* ($F_{3,12}<1.8$, $p>0.20$) nor interaction was detected in the ANOVA.

Variations in response to local cooling

Mean skin temperature and MEP characteristics measured at BL and during cooling are compared in Figure 3 A. Much like the warming protocol, variations in temperature were mainly affected by the factor *Time* with a marked decrease at C1 in both age groups, followed by a gradual return towards BL at PC5 and PC10. The ANOVA confirmed the main effect of *Time* ($F_{3,29}=218.9$, $p<0.001$) but no interaction with *Age* or *Gende*r ($F_{3,29}<1.8$, $p>0.17$). The post-test comparisons

confirmed that skin temperature remained significantly lower than BL both during cooling (C1, $p<0.001$) and in the post-cooling phase (PC5, $p<0.001$; PC10; $p=0.003$). For MEP amplitude, it can be seen that variations observed in the two groups were little influenced by cooling and that a substantial variability was present at each time point (Figure 3 A). The ANOVA confirmed the fact that *Time* ($F_{3,29}=1.6$, $p=0.21$) had no significant effect and the lack of interactions with either *Age or Gender* ($F_{3,29}<1.6$, $p>0.20$). Similar to warming, variations in latency observed with cooling were mainly affected by *Age* and *Gender* ($F_{1,31}>30.3$, $p<0.001$) with no significant influence of *Time* ($F_{3,29}<2.0$, $p>0.12$).

Like reported earlier for warming, examination of individual variations in responses to cooling, when expressed relative to BL, revealed commonalities amongst subsets of participants. As shown in Figure 3 B, 16/35 participants exhibited MEPs depression during cooling (C1) with a return towards BL or even facilitation in the post-cooling phase. For another 13 participants, MEPs were facilitated both during and post-cooling, while the remaining participants (n=6) showed no clear modulation. When also applied a secondary analysis with ANOVAs to assess the impact of the factors in the two largest clusters of participants, i.e., those with MEP depression and those with facilitation. In the cluster exhibiting depression (16/35), a main effect of *Time* ($F_{3,10}=18.5$, $p<0.001$) was detected but no interaction with *Age* or *Gender* ($F_{3,10}<0.12$, $p>0.94$). Post-test comparisons confirmed that MEPs at C1 were significantly different from those at BL ($p<0.001$). Such a difference was not found for MEPs at either PC5 or PC10. For the cluster with MEP facilitation (n=13), a main effect of *Time* ($F_{3,7}=16.9$, $p=0.001$) was detected, which was attributable again to the difference in MEP size measured at C1 when compared to BL (post-test comparison, $p=0.003$). Significant interactions were also detected with *Age* and *Gender* ($F_{3,7}=7.6$, $p=0.013$), which reflected the larger MEP size measured at PC10 between young and senior (Mean difference, 0.40 log-μV) and between male and female (Mean difference, 0.39 log-μV)

participants. In both clusters (depression and facilitation), analysis of variations in MEP latency only reiterated the mains effects of *Age* and *Gender* ($F>8.1$, $p<0.02$) on this variable with no effect of *Time*.

Correlation analysis

To further characterize the influence of thermal stimulation on corticomotor excitability, we performed a series of correlations to examine specific relationships between variables. First, we examined how variations in MEP amplitude (% relative to BL) and in latency (Difference with BL) observed during actual warming (W1) or cooling (C1) stimulation were related to changes in skin temperature at the individual level (Difference with BL). As shown in Figures 4, variations in amplitude were largely independent of individual changes in skin temperature observed either during warming (A) or during cooling (B). Second, we were also interested in examining how modulation elicited in one thermal modality was related to that seen in the other modality. To this end, variations in MEP characteristics observed with warming were plotted against those observed with cooling at each time point. As shown in Figure 5, for variations in amplitude, changes observed at W1 were only poorly correlated with those observed at C1, whereas those observed later in the post-stimulation phase were correlated. For latency, it can be seen that differences computed for warming were highly correlated with those computed for cooling at all time points.

Control experiment for changes in peripheral nerve conduction

As described earlier, in the present study, we used a specially designed gel pack sleeve to induce a localized change in skin temperature. While the application was restricted to the index finger, there is still a possibility that the thermal changes could have spread to more proximal areas and notably to the FDI muscle. To address this possibility, we assessed peripheral conduction time and motor excitability using the maximal M wave. This assessment was performed in a separate testing session a few weeks after the original one and involved a subset of young participants

(n=10, four females). To this end, the ulnar nerve was electrically stimulated at the wrist using supramaximal pulses (200 μs pulses, DS7A, Digitimer Ltd, Hertfordshire, UK) to evoke maximal M waves in the FDI muscle. The protocol was identical to the one described for the main experiment, except that the measures were performed only during application of the gel pack (BL, gel at 24°; Warming, gel at 45°; Cooling, gel at 10°) and not post-application. Data recordings started 1 min after application of the gel pack and lasted until five M waves had been recorded with a 10 s interval between pulses (total duration ~3 min). Amplitude (peak-to-peak) and onset latency data were then analyzed using separate one-way- repeated measures ANOVA with the Dunnett's test for post-hoc comparisons. This analysis revealed no significant difference ($F=0.31$, $p=0.66$) between conditions when comparing maximal M wave amplitude at BL (12.5±2.4 mV) with that measured either during warming (12.04 ± 2.7 mV) or during cooling (12.1±2.8 mV). Likewise, comparison of distal latency measurements showed no significant difference ($F=2.5$, $p=0.12$) between conditions (BL, 4.1±0.56 ms; Warming, 4.4 ±0.60 ms; Cooling, 4.2 ±0.59 ms). Changes in skin temperature measured under the different thermal conditions were similar to those reported in the main experiment (BL, 29.5±2.3°; Warming, 39.1 ± 3.0°; Cooling, 20.7, ± 4.4°).

Discussion

This study investigated the effects of thermal stimulation when applied locally and distally to a single digit on corticomotor excitability. With the warming protocol, our results showed that participants exhibited variable modulation in MEP amplitude with no clear influence of either age or gender. Further examination of individual response revealed that about half of the participants exhibited MEP depression during warming, while another third showed no modulation (12/35) with a minority showing facilitation (535). Similar observations were made with the cooling protocol with about half of the participants (16/35) showing MEP depression. In contrast to warming, however, a larger proportion (13/35) exhibited facilitation, while only a small subset

(n=6) showed no clear modulation. Variations in latency measures were mainly affected by age and gender and were little influenced by the thermal protocols. In the following discussion, we will attempt to interpret these findings regarding their neurophysiological significance for the use of thermal stimuli as a means to influence motor excitability and induce plasticity in clinical populations.

Effects of warming and cooling protocols on skin temperature

Before considering the neurophysiological effects, it is important first to address whether our thermal protocols were effective in inducing the desired changes in skin temperature. In this study, we elected to use a gel pack specifically designed for finger applications to induce focal cooling and warming. Indeed, gel packs provided a safe and practical means to deliver thermotherapy in the extremities (Nadler, Weingand et al. 2004). In particular, the sleeve design of the gel pack allowed us to deliver very focal stimulation targeting the digital nerve of the index finger while largely sparing other adjacent fingers and other more proximal areas from temperature changes. The latter aspect was indeed critical in our study protocol to avoid issues related to temperature-dependent changes in muscle properties and nerve conduction (Rutkove 2001). In this regard, the control experiment we performed using the M wave supports our contention that our thermal protocols largely spared the FDI and did not interfere with its neuromuscular function. This contention is further supported by the fact that MEP latency, which mostly reflect peripheral conduction, showed no systematic variations in relation to changes in skin temperature elicited both with cooling and warming protocols. With regards to the efficacy of the thermal stimulation, both the observed range of skin temperatures and subjective reports from participants suggest that the gel pack was effective in eliciting the desired sensations of cooling and warming, without causing overt pain. As stated earlier, in the context of this study, we deliberately tried to avoid noxious temperatures by limiting both the extent and intensity of the thermal stimuli to address the

more specific role of thermal afferents in modulating corticomotor excitability. In fact, neuronal recordings (reviewed in Vriens, Nilius et al. (2014)) have shown that cutaneous thermal afferents, either warm- or cold-sensitive, are most active in response to transient changes in skin temperature corresponding to moderate cooling or warming, while nociceptive neurons are readily recruited when temperatures reach the noxious range (i.e., <15°C for noxious cold and >45°C for noxious heat). In this study, a closer examination of the range of skin temperatures observed when the gel pack was applied under each thermal protocol (Warming: 35-45°C; Cooling: 13-24°C) confirms that noxious temperatures were largely avoided. From all these considerations, we can conclude that our thermal protocols were effective in eliciting activation of cold and warm-sensitive skin afferents in the digital nerve with little involvement of nociceptive afferents.

Corticomotor excitability in response to warming and cooling

Given that the warming and cooling protocols produced very similar results, the two will be discussed conjointly. In fact, in both protocols, MEP modulation was variable between participants and, when present, was short-lasting and occurred only during actual thermal stimulation (i.e., at C1 and W1). Also, in both protocols, age and gender had little influence on individual responses to either cooling or warming, although both factors did influence MEP latency. The latter influence is not surprising since both of these factors can affect nerve conduction, aging through a direct effect on nerves and gender through mainly differences in height (Rivner, Swift et al. 2001, Matamala, Nunez et al. 2013). Also common was the pattern of variability which was characterized by the presence of a relatively large cluster showing depressed MEPs during stimulation, which co-existed with other smaller clusters who either showed MEP facilitation or no modulation. Such a variability is reminiscent to that seen in previous TMS protocols examining modulation in response to repeated afferent stimulation. As reviewed by Chipchase, Schabrun et al. (2011) such protocols, largely based on repeated electrical stimulation, have produced variable results both

within- and between studies. For instance, when comparing responses to four different stimulation protocols aiming at increasing corticomotor excitability, Charlton, Ridding et al. (2003) reported unexpected MEP depression in about 25% of their cases. In this connexion, it is worth noting that similar observations have been made with regards to change in spinal excitability in response to repeated electrical nerve stimulation, participants showing highly variable modulation in the Hoffman reflex (H-reflex) at the ankle (Goulet, Arsenault et al. 1994). Thus, finding large inter-individual variability in response to afferent stimulation protocols is not uncommon in neurophysiological studies investigating measures of central excitability. In their review, Chipchase, Schabrun et al. (2011) pointed to variations in stimulation parameters (e.g., intensity and duration) as a potential factor to explain the variability observed both between participants and between studies. Interestingly, a subsequent study by the same group (Chipchase, Schabrun et al. 2011) showed that controlling for intensity produced opposite sign of modulation in MEP amplitude, stimulation at the sensory level (i.e., cutaneous afferents) leading to depression, while stimulation at the motor level (cutaneous and proprioceptive) led to facilitation. It could be tempting to relate these observations to the pattern of variability we observed in this study and invoke possible differences in the degree of individual skin cooling or skin warming to account for variability in MEP modulation. However, as mentioned earlier, our correlations showed that variations in MEP characteristics had no systematic relationship with changes in skin temperature with both thermal protocols. Still, the observation that MEP modulation, when present, was short-lasting and coincided with application of the thermal agent (i.e., C1 and W1 time points), suggests that sensory signals arising from thermal afferents were likely critical in influencing corticomotor excitability. In this regard, the fact that a substantial proportion of participants showed depressed MEPs in response to thermal stimulation would be consistent with Chipchase, Schabrun et al.

(2011) observation that cutaneous sensory stimuli, either mechanical or thermal, tends to reduce corticomotor responsiveness.

The neural mechanisms as to why many participants exhibited depression, while others showed facilitation, remain unclear, but it appears to be mainly central in origin. As discussed earlier, a peripheral origin seems quite unlikely, given our observations regarding the lack of any systematic associations between either skin cooling or warming and changes in MEP characteristics and M wave measurements. With regards to central contributions, we need to consider a possible role of spinal mechanisms given reports of sustained alterations in spinal excitability in response to thermal applications in the extremities. In general, these reports have focused on cooling effects in the lower limb, and their conclusions converge for an increase in spinal reflex excitability both during and post-intervention (Hopkins and Stencil 2002, Dewhurst, Riches et al. 2005, Palmieri-Smith, Leonard-Frye et al. 2007). While interesting, a spinal contribution could not be directly tested in the present study because of time constraints and also considering the inherent difficulty in eliciting the H-reflex in the upper extremity. The F-wave could provide an alternative, but its validity as an index of motoneuronal excitability has been seriously questioned (McNeil, Butler et al. 2013). While an increase in spinal excitability seems appealing to explain facilitation (see below), such an increase is hardly compatible with the high prevalence of MEP depression exhibited by many participants in response to either cooling or warming; which points to a contribution at the cortical level. Given that innocuous temperatures, whether warm or cold, readily activate somatosensory regions, including S1 and S2 (Craig, Reiman et al. 1996, Davis, Kwan et al. 1998), this activation could have contributed to depress motor excitability through thermally-induced interactions between somatosensory cortices and motor cortex. Such a mechanism would be consistent with observations that thermal pain induced by focal laser pulses tends to depress motor excitability when delivered at intervals reflecting

sensorimotor interactions at the cortical level (Valeriani, Restuccia et al. 1999, Suppa, Biasiotta et al. 2013). It is true that the latter sensorimotor interactions were not seen when the laser was set to evoke non-noxious warm sensation (Valeriani, Restuccia et al. 1999), but the lack of modulation could be explained by the extreme focality of the laser stimulation. In the present study, the fact that our stimulation covered a larger area (whole finger) and for a longer duration might have contributed to enhance thermally-induced effects at the cortical level. For the group of participants, who showed facilitation during cooling (13/35) or warming (5/35), the increased responsiveness might have reflected individual differences in the central processing of thermal stimuli. For instance, in these individuals, the reported increase in spinal excitability discussed earlier, notably in response to cooling, might have outweighed the inhibitory effects exerted at the cortical level. In line with this, we observed that facilitation was more frequent with cooling than with warming (13 vs. 5 participants), which is congruent with reports showing that cooling has more consistent effects in increasing spinal excitability than warming (Dewhurst, Riches et al. 2005). Finally, for participants who showed no apparent modulation in response to cooling (n=5) or warming (n=12), it may reflect, as stated before, individual differences in the processing and integration of thermal afferents at the spinal and cortical level. For example, it is possible that, in these individuals, facilitation exerted at the spinal level might have been counteracted by the inhibition exerted at higher levels, resulting in little or no apparent MEP modulation. Another possibility is that these individuals exhibited poor susceptibility to afferent-mediated modulation, although this seems unlikely given that only one participant showed no modulation for both cooling and warming stimulation. Summarizing, much like studies examining modulation in response to electrical nerve stimulation, individual responses to innocuous thermal stimulation, either cool or warm, were quite variable between participants, likely reflecting the complex interplay between inhibitory and facilitatory influences exerted at the spinal and cortical level in the processing of thermal inputs.

Comparison of modulation between thermal modalities

When comparing MEP modulation observed in the two thermal protocols, two major observations can be made. First, as noted earlier, although the overall pattern of results was similar between protocols, the warming was associated with a larger proportion of participants showing no clear modulation than cooling (12 vs. 6, respectively). This difference might have reflected the stronger influence of cooling in term of afferent stimulation when compared to warming. Indeed, while there is evidence that the same neural network is involved in processing thermal sensations (Tracey, Becerra et al. 2000, Chang, Arendt-Nielsen et al. 2005), differences have also been reported between the two thermal modalities. For instance, Chang, Arendt-Nielsen et al. (2005) reported specific alterations in brain theta activity over the contralateral hemisphere in response to cooling, which was attributed to differences in the emotional valence associated with cold stimuli as opposed to warm stimuli (i.e., unpleasant vs. pleasant). Along the same line, Tracey, Becerra et al. (2000) showed that noxious cold elicited stronger activation in prefrontal areas than noxious heat. Further evidence that cold stimuli tend to elicit stronger activation comes from recent animal experiments showing that mild cooling of the skin recruited a large population (~70%) of cold-responding neurons in the dorsal horn, whereas mild skin warming recruited only about 15% of heat-responding neurons (Ran, Hoon et al. 2016). Thus, the present observation that cooling elicited more frequent modulation than warming can be attributed in part to the increased neural activation at the spinal and cortical level associated with cold stimulation, as reported in human and animal experiments. Second, our correlations showed that, during thermal stimulation, cooling-induced variations in MEP amplitude were only poorly related with those induced by warming (i.e., at C1 and W1), whereas variations observed later in the post-stimulation phase were correlated. The lack of correlation found during the stimulation phase likely reflect the larger influence of cooling over warming in eliciting amplitude modulation, which is in line with the

stronger effects attributed to cold stimulation at the neural level, as discussed above. Such a correlation (i.e., absence of) also reinforces our earlier interpretation that activation of thermal afferents when the gel pack was in contact with the skin was indeed the most critical factor in modulating excitability. With a reduction in thermal afferent activity after removal of the thermal agent in the post-stimulation phase, corticomotor excitability simply returned towards BL, which explains the higher degree of correlations observed post-5 min and post-10 min. The latter observation, along with those regarding MEP latency at the different time points, also attest to the reliability of our recordings conditions.

Study limitations and conclusion

As mentioned earlier, this study investigated the effect of thermal stimulation on corticomotor excitability using a simple method (gel pack) to induce localized change in skin temperature. Such method did not allow for a strict control over the thermal stimulation delivery since it cannot be assumed that temperatures were uniform across the gel (although the fact that we used a small gel pack for the finger might have mitigated temperature differences). The use of other methods, such as water immersion, might have been a better choice to deliver uniform thermal stimulation, but such methods, admittedly, are not very practical for TMS studies. Also, the fact that we limited the thermal stimulation to 5 min might be seen as a limitation; however, given the location of the application and that we aimed at investigating innocuous temperatures, limiting the duration was necessary. It remains to be seen whether longer applications, targeting larger areas could produce less variable and lasting modulation. Another possible limitation is the lack of concurrent measures of spinal excitability to interpret changes in MEP amplitude. As discussed above, such measures could have provided more insights into the neural mechanisms

involved in MEP depression/facilitation but, at present, there is no simple and valid method to assess spinal contribution in upper limb muscles.

In conclusion, the present report shows that focal thermal stimulation, in the form of either innocuous cooling or warming of a single digit, has a variable and short-lasting, influence on corticomotor excitability in the hand motor representation. The fact that a large subset of participants showing depressed corticomotor excitability co-existed with other smaller subsets showing either facilitation or no modulation seems to reflect individual differences in way thermal sensory inputs are processed at the spinal and cortical level. Such observations have implications for applications of thermal stimuli in the context of rehabilitation interventions in neurological populations. In particular, our results highlight the need to consider individual variability when applying thermal stimuli and the fact that thermal influences can lead to either corticomotor facilitation or inhibition and are short-lasting.

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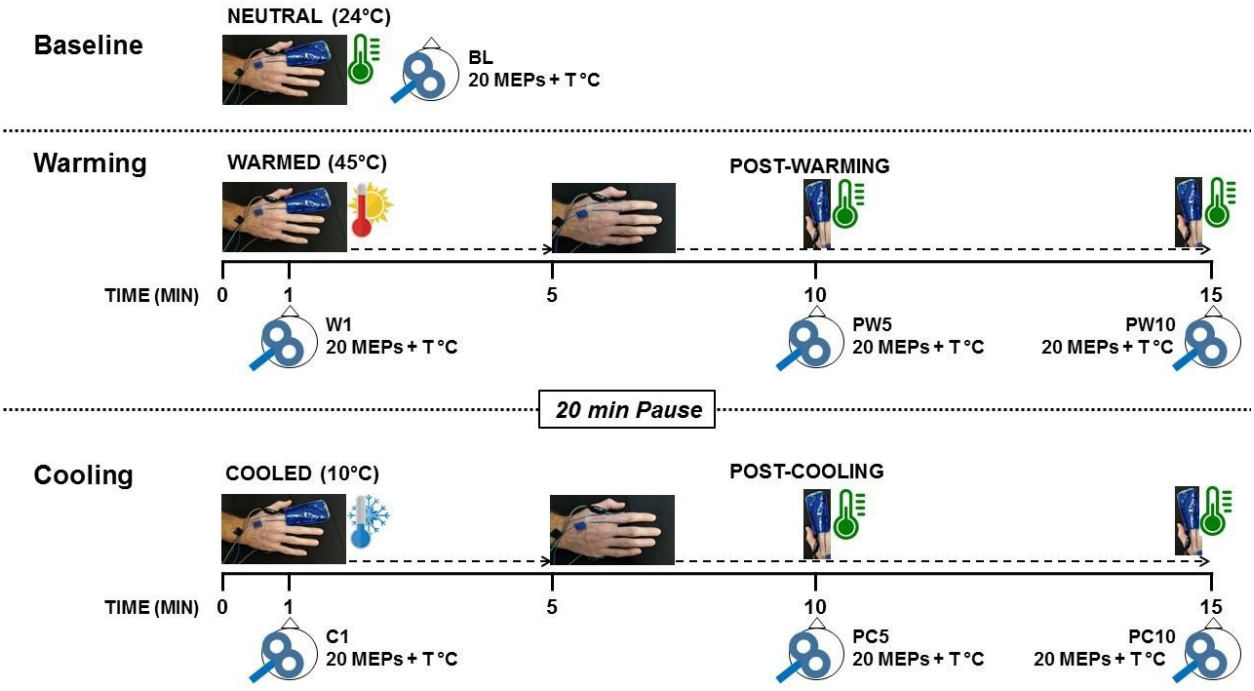
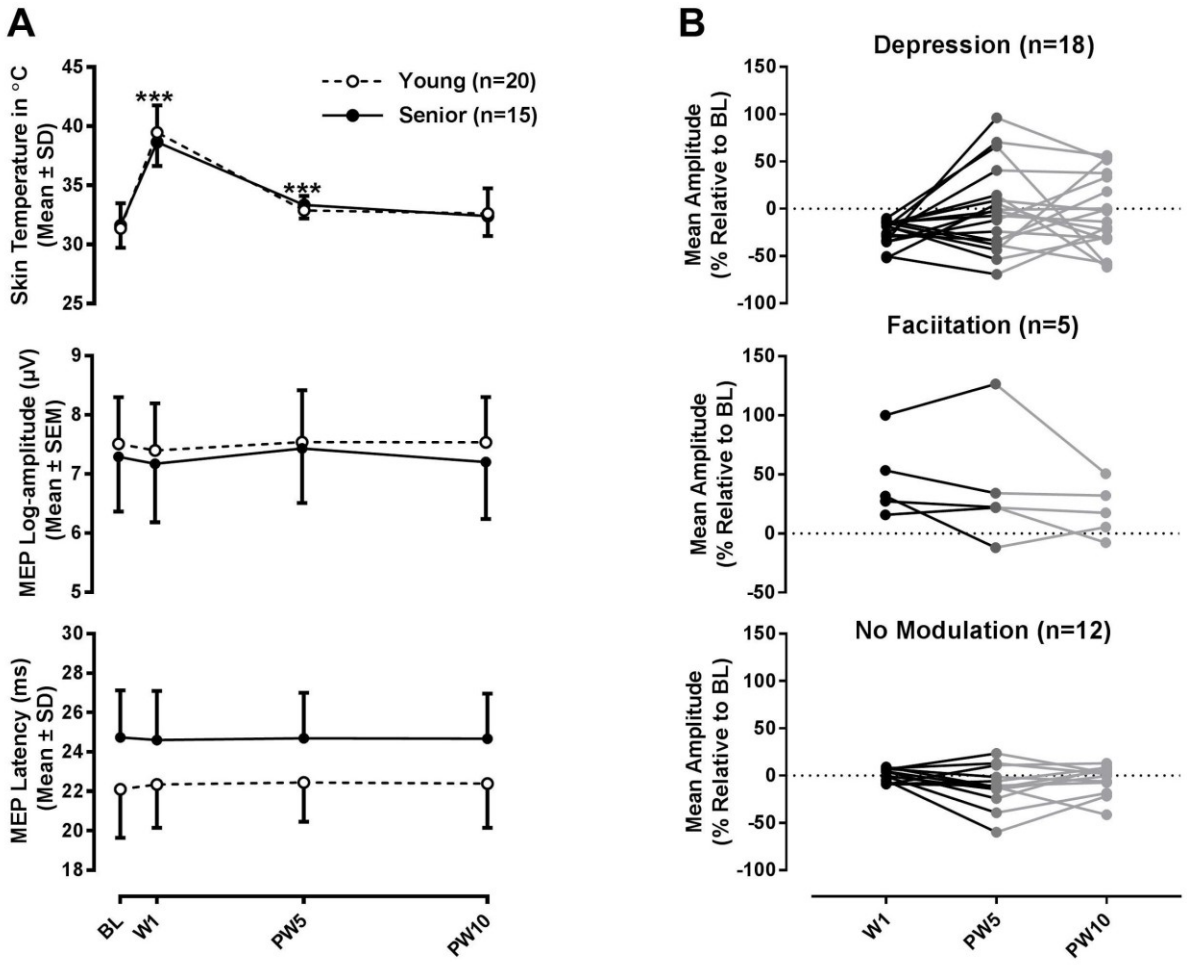
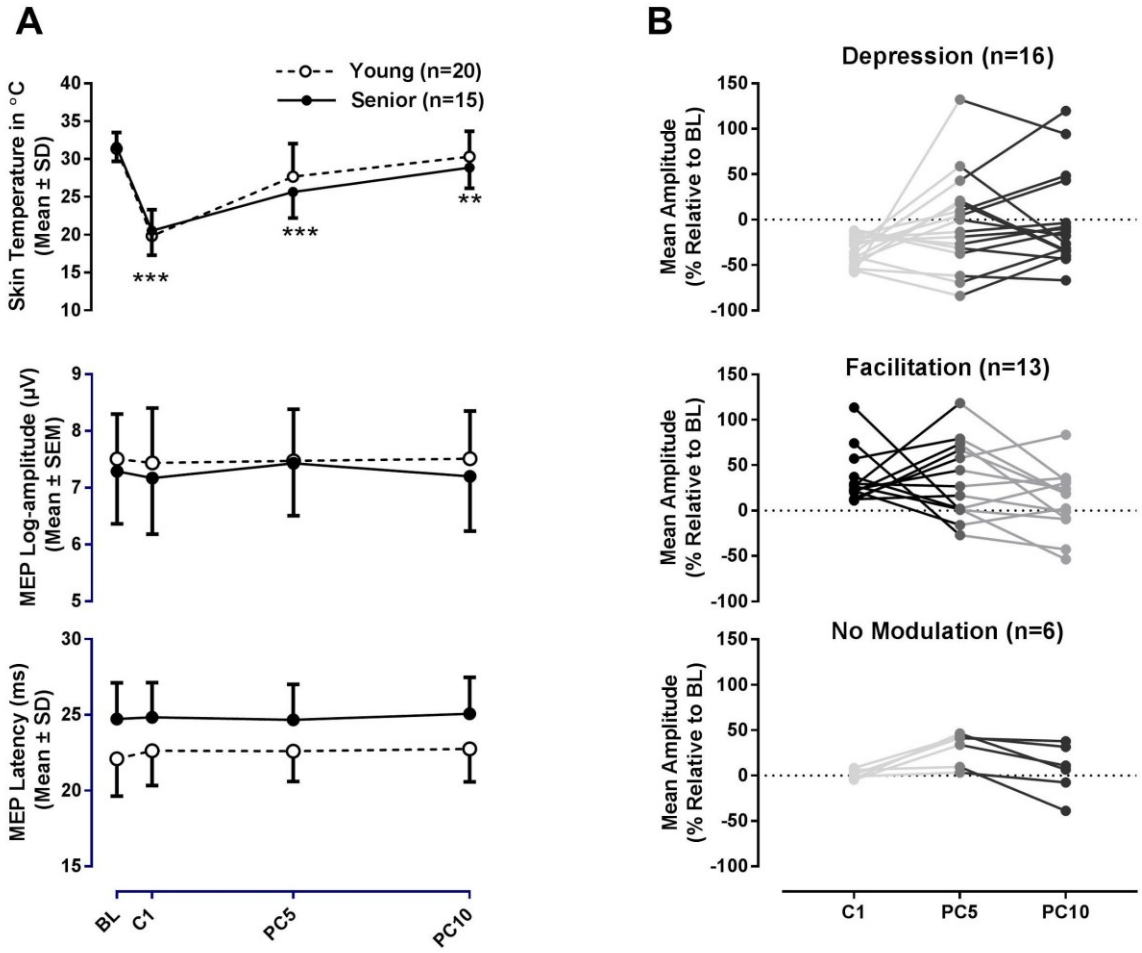


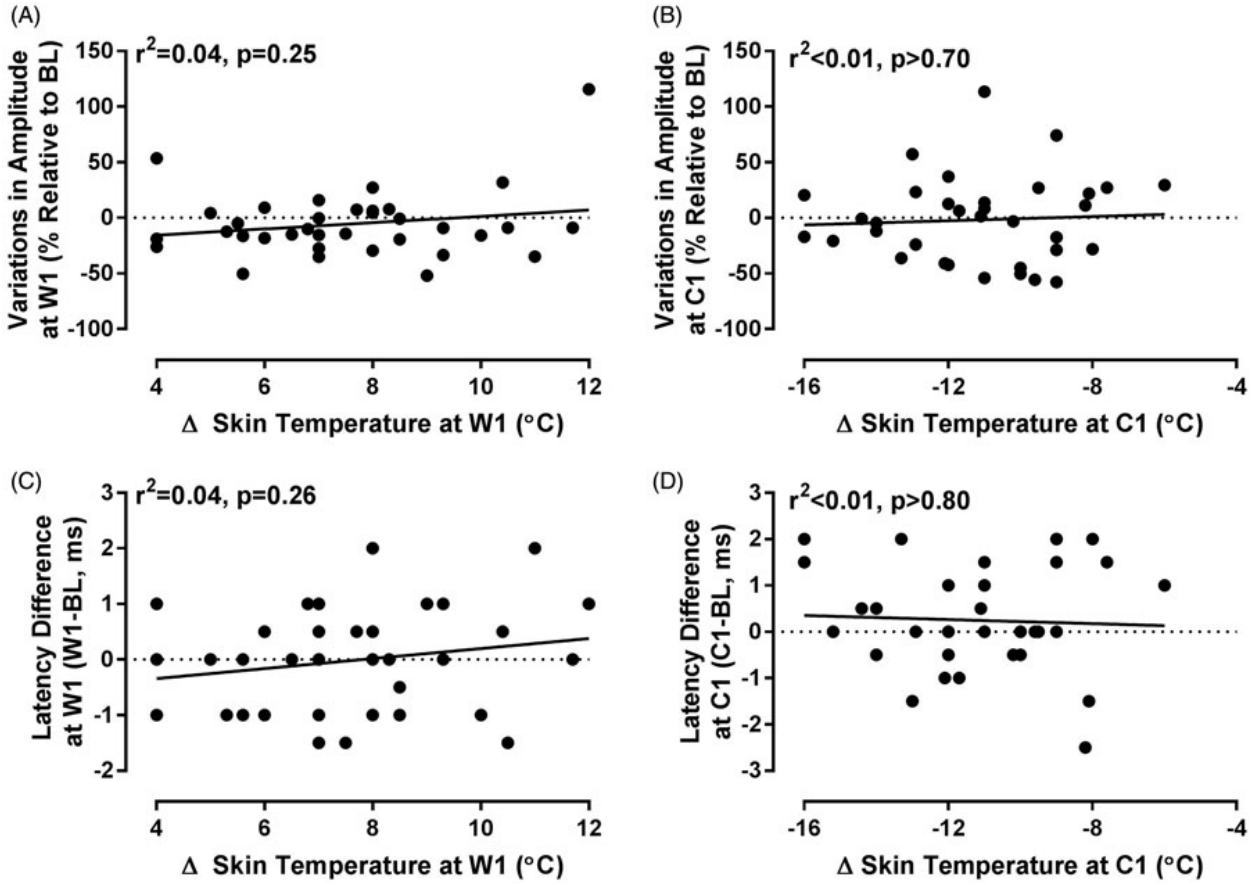
Paper 1: Figure 1. Schematic representation of the experimental protocol to assess the effects of thermal stimulation on corticomotor excitability, as reflected in motor evoked potentials (MEPs). Baseline (BL) measures of excitability and skin temperature (T°) were first established with the index finger of the preferred hand covered in a "neutral" gel pack kept at room temperature. Then, the effects of skin warming were assessed using a gel pack that had been pre-heated to ~45 °C before application. The gel pack was applied for 5 minutes and measures were performed both during warming (W1) and 5 and 10 min post-warming (PW5, PW10). After an additional 20 min pause, the effects of skin cooling were assessed using a gel pack that had been pre-cooled to ~10 °C. The protocol was identical to that used for warming with measures performed both during cooling (C1) and after cooling (PC5, PC10). Note that the "neutral" gel pack was reapplied when measurements were performed in the post-warming (PW5, PW10) or post-cooling (PC5, PC10) phases to control for tactile feedback associated with the index finger wrapping.



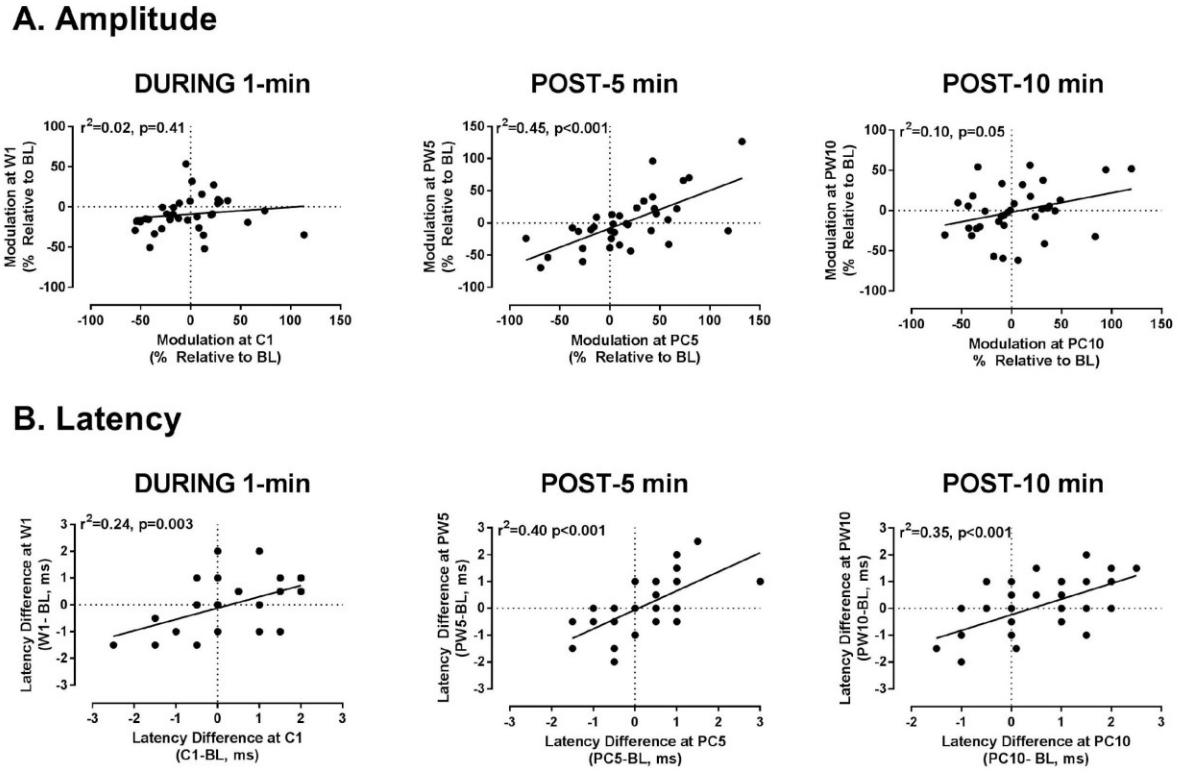
Paper 1: Figure 2. Effects of local warming on skin temperature and MEP characteristics (Amplitude and Latency). A) Mean skin temperature, MEP amplitude and latency measured in the two age groups at BL, during and post-warming (Asterisks denote significant differences from BL, $p<0.001$). B) Individual variations in response to warming after regrouping participants into clusters depending on the sign of modulation when expressed relative to percent change from BL. During warming (W1), a large cluster exhibited MEP depression during (>10% reduction from BL), while other smaller clusters exhibited either facilitation (>10% increase from BL) or no clear modulation (±10% of BL).



Paper 1: Figure 3. Effects of local cooling on skin temperature and MEP characteristics. A) Mean skin temperature, MEP amplitude and latency measured in the two groups at BL, during and post-cooling (Asterisks denote significant differences from BL, ***$p<0.001$, **$p<0.01$). B) Individual variations in response to cooling after regrouping participants according to the sign of modulation, as described in Figure 2. As for warming, a large cluster of participants exhibited MEP depression during cooling (C1) while others showed facilitation. A small number showed no clear modulation.



Paper 1: Figure 4. Results of the correlational analysis of individual variations in MEP measures in relation to changes in skin temperature for the warming (A) and cooling (B) protocol. For variations in MEP amplitude the relative change with respect to BL at C1/W1 was computed for each participant, whereas for latency measures the difference in ms from baseline was computed. For skin temperature, the delta (Δ) reflects the difference between the skin temperature measured either during warming or cooling at C1/W1 with that measured at BL. Note the lack of any systematic relationship between MEP characteristics and changes in skin temperature at the individual level.



Paper 1: Figure 5. Results of the correlational analysis of individual variations in MEP Amplitude (A) and Latency (B) observed during warming in relation to those observed during cooling at each time point. Note that variations in amplitude and latency were computed as described in Figure 4. Also, note that the apparent reduction in the number of scattering points in B is simply due to overlaps between participants exhibiting identical latency differences.

Modulation of corticomotor excitability in response to distal focal cooling stimulation

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ABSTRACT

Background: Thermal stimulation has been proposed as a modality to facilitate motor recovery in neurological populations, such as stroke. Recently (Ansari, Remaud et al. 2018), we showed that application of cold or warm stimuli distally to a single digit produced a variable and short-lasting modulation in corticomotor excitability. Here, our goal was to extend these observations to determine whether an increase in stimulation area could elicit more consistent modulation.

Methods: Participants (n=22) consisted of a subset who participated in our initial study. Participants were asked to come for a second testing session where the thermal protocol was repeated but with extending the stimulation area from single-digit (SD) to multi-digit (MD, four fingers, no thumb). As in the first session, skin temperature and motor evoked potentials (MEPs) elicited with transcranial magnetic stimulation were measured at baseline (BL, neutral gel pack at 22 ˚C), at 1-min during the cooling application (pre-cooled 10˚C gel pack) and 5- and 10-min post-cooling (PC5 and PC10). The analysis combined the data obtained previously with single-SD cooling (Ansari, Remaud et al. 2018) with those obtained here for MD cooling.

Results: At BL, participants exhibited comparable measures of resting corticomotor excitability between testing sessions. MD cooling induced similar reductions in skin temperature as those recorded with SD cooling with a peak decline at C1 of respectively, -11.0 and -10.3 °C. For MEPs, the primary analysis revealed no main effect attributable to the stimulation area. A secondary analysis of individual responses to MD cooling revealed that half of the participants exhibited delayed MEP facilitation (11/22), while the other half showed delayed inhibition (10/22); which was sustained in the post-cooling phase. More importantly, a correlation between variations in MEP amplitude recorded during the SD cooling session with those recorded in the second session with MD cooling revealed a very good degree of correspondence between the two at the individual level.

Conclusion: These results indicate that increasing the cooling area in the distal hand, while still eliciting variable responses, did produce a more sustained modulation in MEP amplitude in the post-cooling phase. Our results also highlight that responses to cooling in terms of either depression or facilitation of corticomotor excitability tend to be fairly consistent in a given individual with repeated applications.

INTRODUCTION

In recent years, the use of peripheral stimulation has gained a renewed attention as a potential adjuvant intervention in stroke rehabilitation. The use of thermal stimuli, in particular, has been proposed as a simple intervention to provide sensory stimulation to elicit motor facilitation in the affected arm or leg (Chen, Liang et al. 2005, Chen, Lin et al. 2011). While there is clinical evidence that thermal stimulation (TS) through repeated applications of either cold or hot stimuli to the skin

can assist in facilitating motor recovery in patients (Liang, Hsieh et al. 2012, Hsu, Lee et al. 2013), there is still very limited information regarding the neural basis underlying such facilitatory effects. For the proponents of this approach (Hsu, Lee et al. 2013, Tai, Lai et al. 2014), the facilitation was likely a reflection of the ability of thermal stimuli to elicit activation in both somatosensory and motor areas at the cortical level (Davis, Kwan et al. 1998, Gelnar, Krauss et al. 1999); leading to motor reorganization. To test this hypothesis, Tai, Lai et al. (2014) used transcranial magnetic stimulation (TMS) to probe changes in the motor maps in chronic stroke patients in response to either noxious (heat 46°C, cold 7°C) or innocuous (heat 41°C, cold, 20°C) temperature stimuli targeting the affected upper extremity. Their results revealed a larger expansion of the motor maps in the lesioned hemisphere with noxious temperatures than with innocuous temperatures, although the magnitude of the effects was quite variable between patients. To further address the issue, we recently showed (Ansari, Remaud et al. 2018), also using TMS, that distal focal TS in the form of either innocuous cooling (10 °C) or warming (45 °C) produced a variable and short-lasting modulation in motor evoked potential (MEP) amplitude, irrespective of the age (young and old) and sex (men and women) of healthy participants. In fact, participants exhibited mixed patterns of modulation characterized by either depression or facilitation and only during actual cooling or warming stimulation. Our results also revealed that cooling was more likely to elicit modulation than warming; a finding consistent with the greater sensitivity to cold stimulation in human observers (Jones and Ho 2008) and the larger effects reported for local cooling stimuli at the neurophysiological level (e.g., Chang, Arendt-Nielsen et al. 2005, Dewhurst, Riches et al. 2005).

One possibility to explain the variability we observed in response to TS is related to the depth and extent of the focal thermal effects at the peripheral level. Given the critical role of spatial summation in thermal sensibility (Jones and Ho 2008, Stevens 2013), it is possible that our

stimulation, which was restricted to a single digit, might have been suboptimal to elicit modulation in corticomotor excitability, as reflected in MEP amplitude. Indeed, considerable spatial summation has been reported for both heat and cold modalities with increased area of stimulation leading to higher magnitude of sensation and lower detection threshold (reviewed in Stevens 2013). Spatial summation appears to be particularly important for cold sensations given the higher density of innervation of cold receptors in the skin (Stevens 2013). As demonstrated by Stevens and Marks (1979), for a given degree of skin cooling, one can double the magnitude of cold sensation just by doubling the stimulated area. Thus, the area of stimulation in relation to spatial summation seems to be a critical factor when considering the central effects of thermal stimuli at the periphery.

In the present report, our goal was to extend our previous observations regarding the influence of distal focal TS on corticomotor excitability to determine whether extending the area of stimulation would lead to more consistent effects and help reduce inter-individual variability. To this end, we recruited a subset of participants from our initial study to reassess their responses to TS using the same protocol when the area of stimulation is extended from one single digit (SD) to multi-digits (MD). Also, for this study, we elected to use only cooling stimulation, given our previous observations regarding cooling and warming effects (see above). We hypothesized that MD cooling would elicit more consistent modulation in our participants than what we observed previously with SD cooling, particularly in the form of depression, as this pattern was the most predominant in our previous report (Ansari, Remaud et al. 2018).

MATERIALS & METHODS

The study was approved by the Institutional Research Ethics Board (REB, Bruyère Hospital Ottawa, Protocol# M16-17-001) in accordance with the principles of the Declaration of Helsinki.

All participants gave written informed consent before the experimental session. All experiments were performed in a controlled laboratory environment. Participants received a small honorarium for their participation.

Participants

As stated earlier, participants for this study were recruited from the pool (n=35) who participated in our initial study (Ansari, Remaud et al. 2018). Since age did not affect our previous conclusions regarding TS effects, both young and senior participants were approached to participate in a second session. The final sample (n=22) consisted of 13 young adults (30 ± 4 years, eight men, five women) and nine seniors (68 ± 4 years, two men, seven women). The other participants (n=13) either refused or were unavailable to participate. The second experimental session took place between 2 and 9 months after the initial participation. At the times of testing, all participants were considered healthy and were free of conditions that may have interfered with the study procedures (i.e., no reports of acute or chronic musculoskeletal or neurological conditions or recent trauma to the upper extremity). In addition, they were screened to ensure that they presented no contra-indications to TMS, notably for pregnancy for young women, metallic implants in the skull and antecedents of seizures. Senior participants were also screened to ensure they were able to discriminate temperature reliably in the distal hand using tubes filled with cold (15 °C) and warm (42 °C) tap water. All but three participants (two young, one senior) were right-handed as determined by the Edinburg Hand Inventory (online version http://www.brainmapping.org/shared/Edinburgh.php).

General procedure for TMS and recordings of motor evoked potentials

All the procedures for TMS and recordings have been described previously (Ansari, Remaud et al. 2018). Briefly, TMS assessments were performed in a temperature-controlled room (22 ±

2°C) with participants comfortably seated in a chair with armrests. MEPs elicited in the *first dorsal interosseous* (FDI, preferred hand) were recorded using surface sensors (DE-2.1, Delsys Inc., Boston, MA, USA) placed in a belly-tendon montage. After amplification and filtering (Bagnoli™ 4 System, Delsys Inc., bandwidth=6–450 Hz, gain=1,000), electromyographic (EMG) signals were digitized at a rate of 2 kHz (PCI-63203, National Instrument Corp. Austin, TX) and saved for later off-line analysis. TMS pulses were applied on the hemisphere contralateral to the preferred hand over the motor hot spot for the FDI (marked with a sticker) using a focal coil (70 mm, P/N 3190) connected to a Magstim 200 (Magstim Co. Ltd, Whitland UK). Participants were fitted with a Waveguard™ TMS compatible EEG cap (ANT Neuro, Madison, WI, USA) with markers to ensure consistent coil placement. The resting motor threshold (rMT) was determined using the Motor Threshold Assessment Tool software (MTAT 2.0; Clinical Researcher, Knoxville, TN, USA) (Mishory, Molnar et al. 2004). All subsequent testing was performed at 130% of the rMT with 20 trials recorded at each block. During TMS, participants were instructed to count the number of stimuli delivered to prevent shift of attention or sleepiness.

Thermal stimulation protocol

As alluded earlier, the TS protocol included two major changes from our previous protocol. First, the extent of stimulation in the distal hand was increased to include all fingers but the thumb. Second, for this experiment, the TS consisted only of cooling stimulation for the reasons explained earlier (see Introduction). As shown in Figure 1, the MD stimulation was obtained by applying a gel pack sleeve designed for wrist and ankle applications (TXRT-4060, 4" x 6", Torex® Health Products, Tallmadge, OH, USA) that covered the four fingers up to the metacarpophalangeal joint. The gel pack sleeve was of the same conductive material as the one we used for SD cooling (TXRT-2540, Torex Health Products) in our previous experiment. Asides from these two changes, the protocol was identical to that described in Ansari, Remaud et al. (2018). Briefly, both skin

temperature and MEPs (n=20) were first measured at baseline (BL) with the fingers covered with a neutral gel pack kept at room temperature (~24 °C). Then, the cooling stimulation was applied for 5 min using a pre-cooled gel pack at ~10 °C. Such an application is in line with clinical practice guidelines for cold applications in the extremities (Knight and Draper 2012) and, in our previous experiment, was effective in producing skin temperature changes in the innocuous cold range (i.e., from 15 to 25 °C). At 1-min during cooling (C1 block), both skin temperature and MEPs were measured again, which took about 1½ min to complete (i.e., 20 TMS pulses delivered with a 5-sec interval between each pulse). The gel pack was kept in place until the 5 min had elapsed. Then, the cooled gel pack was removed. After 5-min post-cooling (PC5 block), skin temperature and MEPs were measured again with the hand covered with the neutral gel pack. After an additional 5-min (PC10), the same measures were repeated. Monitoring of skin temperature was achieved through to a K-type digital thermometer (Model# TC41FBA, Perfect-Prime, Dayton, NJ, USA, ± 0.1°C) connected to two thermocouple sensors affixed on the dorsal aspect of the proximal phalanx of the index and pinky fingers.

Data analysis and statistical procedures

Individual means were obtained for BL and at the different time points in the cooling protocol by averaging recordings (2 records/bock) for skin temperature and trials (20/block) for MEP amplitude (peak-to-peak) and latency. To determine the effect of stimulation area, the data obtained in our companion study with SD cooling was combined with that obtained in the current study for MD cooling in the analysis. Temperature and MEP data were checked for the normality of distribution (D'Agostino & Pearsons 'test) before proceeding with an analysis of variance (ANOVA). All data were normally distributed. Two-way repeated measures ANOVA's using "Area" (SD vs MD) and "Time" (BL, C1, PC5, PC10) as the repeated factors were performed on temperature and MEP data. Only age was considered as a between-subjects factor for the present

analysis, given that our previous report showed no effect of gender. Post-hoc analysis was performed using the Sidak's test. Pearson's moment correlation was also used to examine relationship between variables. Additional analyses are described below. The level of significance was set at $p<0.05$ for all tests. Statistical analyses were performed using SPSS 17.0 software package (SPSS Inc., Chicago, IL, USA) and GraphPad Prism version 7.00 for Windows (GraphPad Software, San Diego, CA, USA, www.graphpad.com), respectively. All data are reported as mean values and standard deviation (SD).

RESULTS

Comparison of baseline measures of corticomotor excitability

Since the present report is based on TMS measures performed in the same group of participants at two different intervals spread over several weeks, it was critical to establish first whether BL measures of corticomotor excitability corresponded between sessions. To this end, we applied paired t-tests to compare rMTs and MEP characteristics. These comparisons revealed no significant difference between sessions for any of the measures. In fact, rMTs measured at session 1 (SD cooling, Mean stimulator output, 45.7± 12.2%) were very comparable (t_{21}=1.7, p=0.11) to those measured at session 2 (MD cooling, 44.1 ±12.8%). Similarly, MEP characteristics both in terms of amplitude (Session 1, 1.8±1.5 mV; Session 2, 1.9 ± 1.4 mV; t_{21}=0.23, p=0.82) and latency (Session 1, 23.2 ±1.9 ms; Session 2, 23.1 ±2.2 ms; t_{21}=0.64, p=0.53) showed a high degree of correspondence between sessions. Thus, BL measures of corticomotor excitability were highly comparable between SD and MD cooling testing sessions at the individual level.

Variations in skin temperature and in MEPs in response to distal cooling: SD vs MD stimulation

Mean skin temperature and MEP characteristics measured at BL and during the cooling protocol are compared in Figure 2 between SD and MD stimulation. It can be seen (Figure 2 A) that skin temperature tended to be lower with MD cooling than SD cooling, although both applications exhibited a similar time course peaking at C1 (MD, -11.0 ˚C; SD, -10.3 ˚C) with a slow return towards BL in the post-cooling phase. The ANOVA revealed a large main effect of Time ($F_{3,18}$=476.7, p< 0.001), but no effect or interaction with Area ($F_{1,20}$=1.83, p=0.19); indicating similar decline in temperature for both SD and MD applications. Age had no effect nor interaction (F<1.2, p>0.36). Post-test comparisons confirmed that skin temperature remained significantly lower than BL both during cooling (C1, p < 0.001) and in the post-cooling phase (PC5, p < 0.001; PC10; p < 0.003). For MEPs, it can be seen in Figure 2 B that variations in amplitude were little influenced by cooling for both SD and MD stimulation and that a substantial inter-subject variability was present at each time point. The ANOVA confirmed that neither Time ($F_{3, 18}$=0.84, p <=0.50) or Area ($F_{1, 20}$=0.48, p=0.50) had an effect on MEP amplitude and also the lack of interaction ($F_{3, 60}$=1.1, p= 0.41). Age had no effect or interaction either (F< 1.0, p>0.54). For MEP latency (Figure 2 C), no main effect of Time was found ($F_{3,18}$=2.3, p=0.11), although a trend was detected for Area ($F_{1,20}$=3.8, p=0.06). As shown in Figure 2 C, the latter trend reflected the difference in latency measured at PC5 for SD versus MD cooling (23.3 vs. 24.1 ms, respectively, p=0.003, Sidak's post-test). No other main effect or interaction was detected for MEP latency.

Analysis of individual patterns of response to MD cooling

Like in our first study with SD cooling (Ansari, Remaud et al. 2018), closer inspection of

individual responses to MD cooling revealed differences in the way participants responded to the stimulation. In fact, when individual responses were classified in terms of either inhibition or facilitation using a cut-off value of 10% from BL to characterize clinically-relevant changes in cortical excitability (Hinder, Goss et al. 2014, Perellon-Alfonso, Kralik et al. 2018), two distinct patterns emerged. As shown in Figure 3 A, for half of the participants (11/22), the pattern was characterized by predominant facilitation (MEPs>10% BL), which was particularly evident in the post-cooling phase (i.e., PC5 and PC10). In contrast, for the other half (10/22)[1], MEPs were depressed in amplitude (MEPs<10% BL) both during (with two exceptions) and after the cooling application. Examples of the two patterns of modulation are shown in Figure 3 B. The ratio of young/seniors and male/female was comparable in the two subgroups (Age: Facilitation, 6/5; Inhibition, 6/4; Gender: Facilitation: 5/6; Inhibition: 5/5), indicating that age and sex were not influential factors. We performed a secondary analysis on MEP amplitude data with the two subgroups of participants using one-way ANOVA with "Time" as the repeated factor. For those showing facilitation (n=11), a significant main effect ($F_{3,10}$=3.9, p=0.04) was found with post-test comparisons (Dunnett's test) pointing to significant differences from BL at PC5 (p=0.01) and PC10 (p=0.03), but not at C1 (p=0.27). Similar results were obtained for those showing inhibition (n=10) with a main effect being detected for Time ($F_{3,9}$=6.7, p=0.009) and significant differences from BL being found at PC5 (p=0.02) and PC10 (p=0.04) in post-test comparisons. The ANOVA for variations in MEP latency showed no significant effect for the subgroup with facilitation (Time, $F_{3,10}$=1.8, p=0.19), whereas a significant effect was found for the subgroup with inhibition (Time, $F_{3,9}$=6.3, p=0.006). In this latter subgroup, post-test comparisons indicated significant differences from BL for latency measured at C1 (mean difference, +1.15 ms, p=0.008) and at PC5 (mean

[1] One participant showed inconsistent modulation that could not be classified as either inhibition or facilitation.

difference, +1.25 ms, p=0.01), but not at PC10 (p=0.10). To summarize, participants exhibited two opposite patterns in response to MD cooling, which was characterized by a delayed effect in the form of either sustained facilitation or inhibition in the post-cooling phase.

Correlations between modulation for SD and MD cooling application

The observation that participants exhibited variable modulation in response to cooling stimulation raised the interesting question as to whether a given individual would display a consistent response on repeated applications. To address this question, we examined the relationship between MEP modulation reported previously for SD cooling with that seen in the present report with MD cooling at the different time points. The results of this analysis are shown in Figure 4. As evident in the Figure 4 A, during the cooling phase (C1), there was a relatively good correspondence between the modulation elicited in response to SD cooling with that observed with MD cooling. At PC5 (Figure 4 B), the correspondence was even stronger with >40% of the variance observed with MD cooling accounted for by the variance with SD cooling. At PC10 (Figure 4 C), however, the association was weaker and no longer significant.

DISCUSSION

In the present study, we sought to extend our observations regarding thermally-induced modulation in corticomotor excitability to examine the impact of stimulation area. To this end, we combined our previous observations regarding the impact of SD cooling with new ones regarding the impact of MD cooling in the same subset of participants. While our primary analysis revealed no main effect attributable to cooling area on MEP modulation, a secondary analysis indicated that participants exhibited variable responses to MD cooling, much like what we observed with SD cooling. In fact, half of the participants exhibited a predominant MEP facilitation in response to

MD cooling, while the other half exhibited inhibition. This modulation in response to MD cooling was delayed and more sustained than that previously reported for SD cooling. Our results also showed that modulation in response to cooling stimulation tended to be fairly consistent at the individual level when repeated over time.

Measures or resting corticomotor excitability at BL

As stated earlier, one critical aspect of the present report was to ensure that participants exhibited comparable excitability measures at BL. This was indeed critical, given the longitudinal design of the study, where previous measurements derived from a subset of participants were compared to new measurements obtained several weeks after the initial experiment. Our design was justified given the evidence that basic measures of excitability, such as rMT and MEPs at suprathreshold intensity, are relatively stable over time for a given individual (Brown, Lohse et al. 2017). In line with this, our basic measures of resting corticomotor excitability (rMT and MEP) data showed very good reproducibility over time in our group of participants; which strengthens our contention that MEP modulation observed in the present report reflected physiological responses to cooling stimulation and not just random fluctuations in excitability.

Variations in skin temperature and in MEP amplitude in response to cooling

Regarding skin temperature, we observed a similar profile of declining temperature with MD cooling as the one we observed with SD cooling. This was expected given that thermal agent was made of the same conductive material and was applied at the same temperature to restrict cooling to the innocuous range. The observation that the range of temperatures recorded at C1 was comparable for both SD and MD cooling (range, 13.0-26.6 and 13.3-26.0, respectively) confirms that both applications produced the desired decrease in skin temperature. Although we did not

record subjective ratings, most participants reported that their sensory experience with the larger gel pack (MD cooling) was more intense (i.e., colder) than that felt previously for the smaller gel pack (SD cooling); an observation consistent with the effect of spatial summation on perceived cold sensation (Stevens and Marks 1979).

With regards to MEP modulation, our primary analysis revealed no effect attributable to area, a finding that goes against our prediction that increasing stimulation area would produce more consistent effects and help reduce variability. Such a conclusion, however, would obscure the fact that almost all participants exhibited a significant modulation in response to MD cooling, and only that this modulation was of opposite signs for half of them. In fact, similar to our previous observations with SD cooling, the presence of large subsets of participants showing either inhibition or facilitation contributed to blur any effects attributable to increased stimulation area. We have discussed previously the possible reasons as to why corticomotor excitability could be depressed in one individual and enhanced in another in response to the same cooling stimulation applied peripherally (see Ansari, Remaud et al. 2018). Besides the inherent variability of individual responses to sensory stimulation reported in studies examining modulation of excitability (Chipchase, Schabrun et al. 2011), we can reiterate the possible role of spinal mechanisms to explain the presence of facilitation in many participants, as local cooling in the extremities is known to increase motoneuronal excitability (Dewhurst, Riches et al. 2005, Palmieri-Smith, Leonard-Frye et al. 2007). For those showing inhibition, as we have argued before (Ansari, Remaud et al. 2018), spinal facilitation may have been overrun by inhibition exerted at the motor cortical level via activation of somatosensory areas (primary and secondary) and insular cortex resulting from cold afferent stimulation (Casey, Minoshima et al. 1996, Craig, Reiman et al. 1996). The fact that the MEP latency was significantly delayed in the subset with inhibition, but not with

those with facilitation, would be compatible with a depressed excitability at the cortical level since prolonged latency could reflect a reduced excitatory drive to corticospinal neurons leading to greater temporal dispersion of descending impulses. Alternatively, a temperature-dependent decrease in peripheral nerve conduction is also possible to account for the prolonged MEP latency, although this explanation is hardly compatible with the observation that latency was changed in only one subset (those with inhibition) and only for certain time points (i.e., C1 and PC5). Besides, we have provided evidence previously (Ansari, Remaud et al. 2018) that cooling restricted to the distal finger did not affect proximal nerve conduction. Ultimately, the presence of differential responses to cooling may reflect individual differences in the way thermal afferent information is processed from the periphery (e.g., differences in skin properties, and in receptors density) up to the cortex (e.g., degree and extent of cortical activation).

Whatever the reasons for the presence of facilitation and inhibition in response to cooling, one noticeable difference between SD and MD stimulation was in the time course of the modulation. With SD cooling, the modulation was restricted mainly to the cooling phase (i.e., C1), whereas with MD cooling, the modulation was delayed to the post-cooling phase (i.e., PC5-PC10) for both facilitation and inhibition. Such a difference in modulation could be related to factors linked with spatial summation of cold afferent and the way the cooling agents interacted with the skin locally. The observation that MEP modulation was delayed with MD cooling might have reflected differences in the efficiency of the cooling application, which could not be detected with our temperature sensors. For instance, the large gel pack covered only the dorsal and palmar aspect of the index finger and not its lateral aspects, unlike the small gel sleeve we used for SD cooling. Thus, initially the cooling effects might have been more efficient with the small gel sleeve than the large gel sleeve, as far as the index finger (and FDI muscle) is concerned; hence the lack of

clear modulation at C1. However, as time passed, the rapid increase in the number of active cold afferents and their activity level, as the cooling extended spatially to adjacent fingers, might have contributed to sustain the afferent-induced modulation for minutes, even after the gel pack had been removed. Summarizing, while our main analysis failed to confirm our predictions regarding the influence of stimulation area, a secondary analysis of individual responses provided evidence that increasing cooling area was associated with a more sustained modulation in the form of either inhibition or facilitation.

Correlations between SD vs. MD cooling

Considering the variability of individual responses to cooling stimulation, it was important in the present report to address the issue of repeatability, i.e. whether a given individual would show consistent responses on repeated applications. In this regard, our correlative analysis of MEP modulation elicited with either SD or MD cooling provides some interesting insights on this important question. In particular, our correlations showed that modulation elicited in a previous session with SD cooling were significantly associated with those elicited with MD cooling in the current study so that individuals that showed inhibition with SD cooling also tended to show inhibition with MD cooling (same for those with facilitation). This association was particularly strong in the post-cooling phase at PC5, where >40% of the variance with MD cooling was explained by SD cooling; an observation that can be linked with the delayed modulation associated with the larger gel pack, as discussed in the preceding section. Along the same line, the lack of significant association at PC10 could also be explained by the observation that modulation was more sustained with MD, as compared to, SD cooling. As stressed earlier, while the reasons as to why someone would show facilitation and another one inhibition remain unclear, our results nevertheless show that there at least a fairly good probability that an individual showing depressed

(or enhanced) excitability in response to cooling would also show a depression (or facilitation) over time with repeated applications.

CONCLUSIONS

The present results extend our previous observations regarding the influence of distal local cooling stimulation on corticomotor excitability. In particular, our observations show that increasing the cooling area in the distal hand, while still eliciting variable responses in terms of facilitation and inhibition, is associated with more sustained modulation in the post-cooling phase. In addition, our correlative analysis of MEP modulation between sessions for SD and MD cooling provides evidence of a fairly good within-subject repeatability over time on repeated applications. While our observations were obtained from healthy participants, they nevertheless point to critical aspects regarding the physiological effects of cooling stimulation as a means to modulate corticomotor excitability for rehabilitation purposes.

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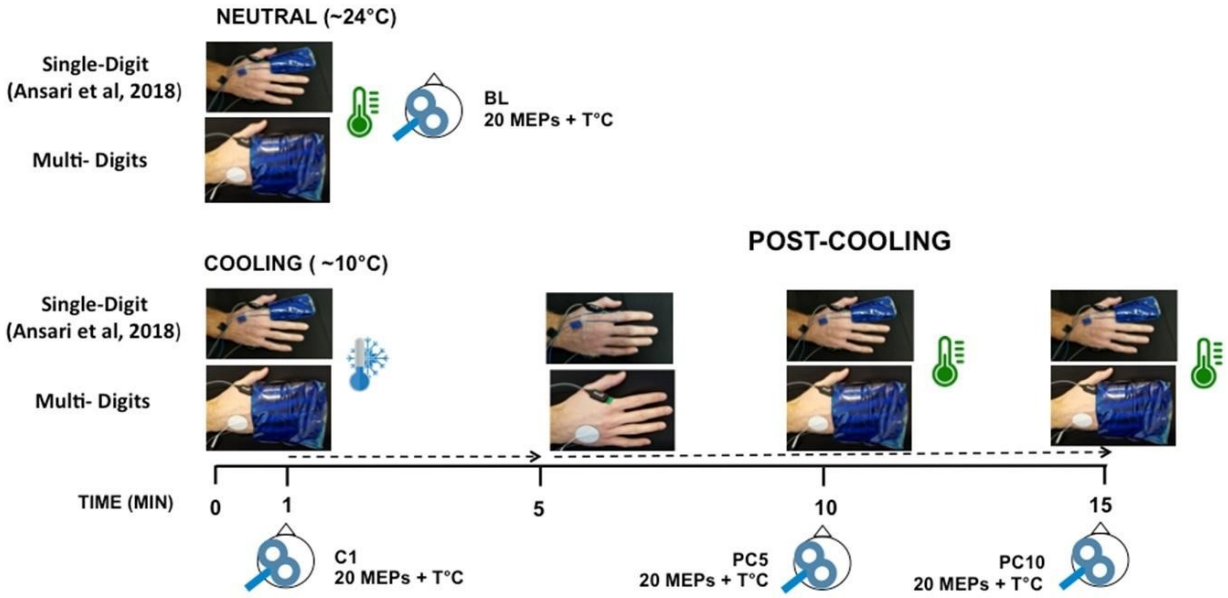
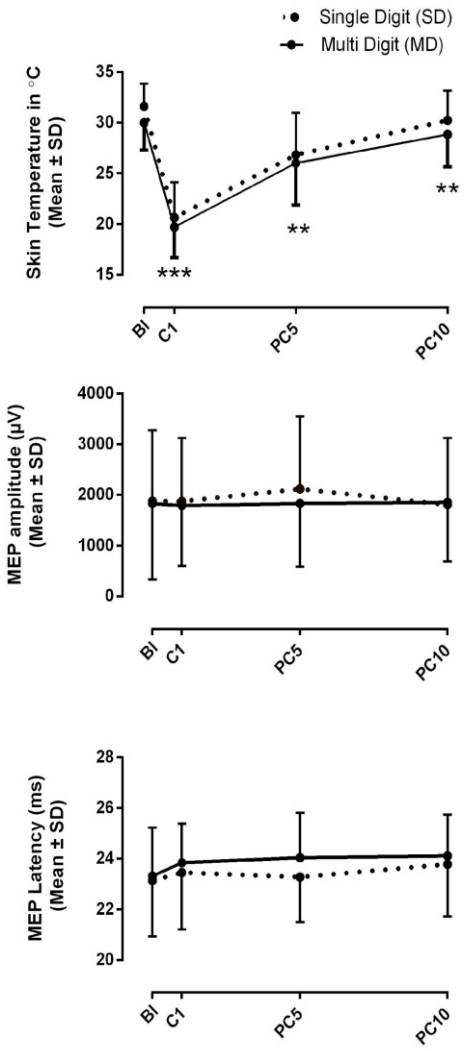
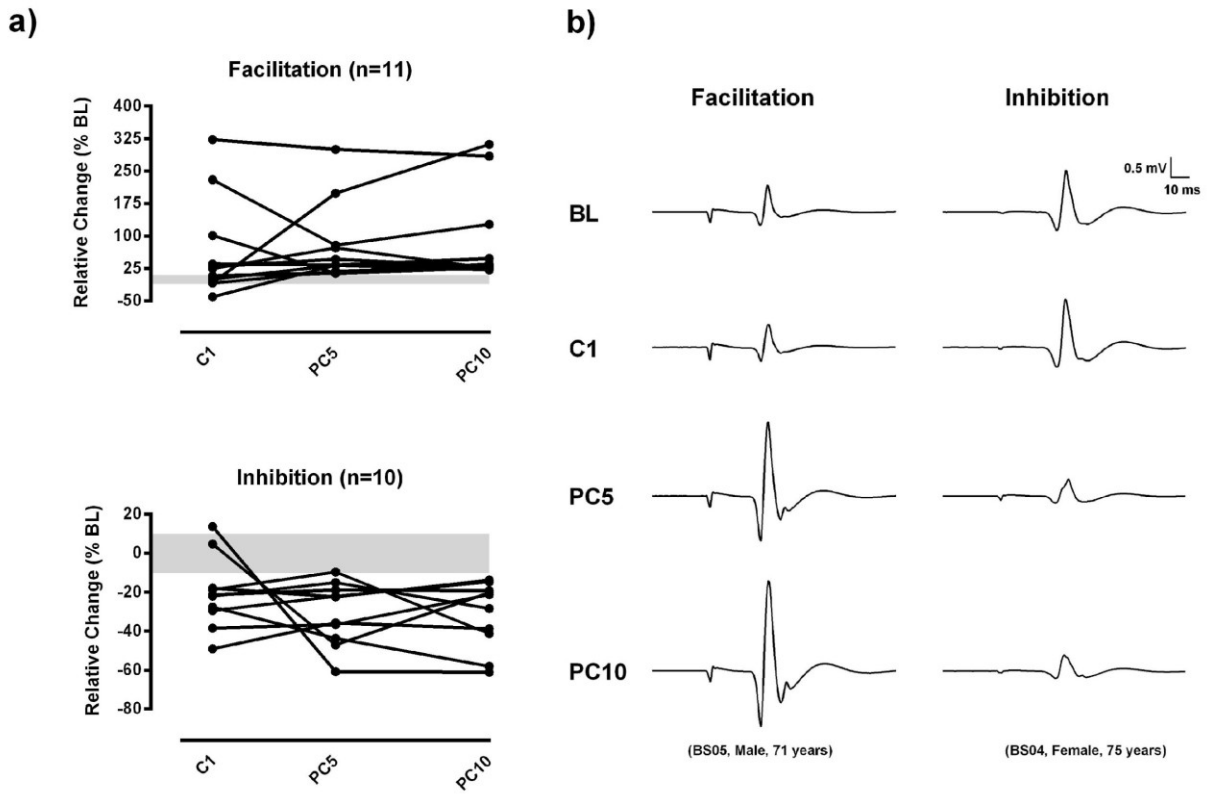


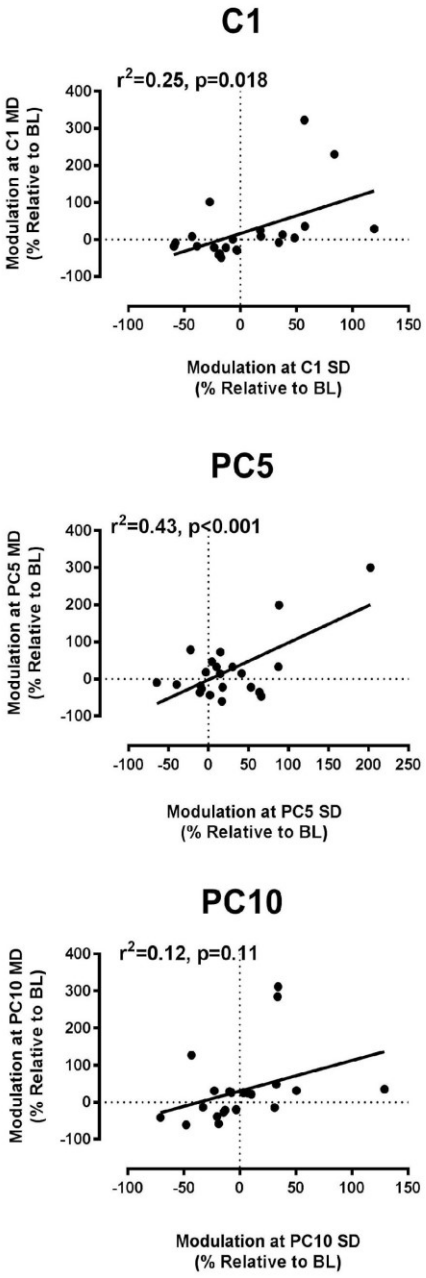
Paper 2: Figure 1. Schematic representation of the experimental protocol to assess modulation in corticomotor excitability in response to distal cooling. In our initial study, the cooling targeted a single digit (index finger) using a small gel pack sleeve. Skin temperature (T) and motor evoked potentials (MEPs) were recorded at baseline (BL, neutral gel pack), during cooling at 1 min (C1, cooled gel pack) and at 5 min (PC5) and 10 min (PC10) post-cooling with the neutral gel pack put back in place. In the current report, the thermal protocol was repeated in the same group of participants in a second testing session but this time with multi-digits cooling using a larger gel pack to cover the four fingers (no thumb) both at BL and during cooling.



Paper 2: Figure 2. Mean skin temperature recordings and MEP data measured at BL and in response to single digit (SD) and multi-digits (MD) cooling. (A) Note the similar time course of temperature decline with SD and MD cooling with no marked difference in terms of reductions between the two. Significant differences from BL temperature were found at all time points (**$p < 0.01$, ***$p < 0.01$). Also, note the large inter-individual variability for variations in MEP amplitude (B) and in latency (C). No effect of Area (i.e., SD vs. MD) was found for either amplitude or latency data. Abbreviations as in Fig. 1.



Paper 2: Figure 3. (A) Individual variations in MEP amplitude in response to MD cooling after regrouping participants according to the sign of modulation when expressed relative to percent change from BL. In each graph, the gray area represents plus or minus 10% change from BL. In half of the participants (11/22), MEPs were predominantly facilitated (i.e., MEPs > 10% BL), particularly in the post-cooling phase (PC5 and PC10). In contrast for the other half (10/22), MEPs were inhibited (i.e., MEPs < 10% BL) both during (with two exceptions) and in the post-cooling phase. One participant exhibited an inconsistent modulation. (B) Individual examples of MEP facilitation and inhibition in response to MD cooling. Note that both participants initially showed little modulation during actual cooling (C1), it is only in the post-cooling phase (PC5 and PC10) that facilitation or inhibition became evident. Abbreviations as in Figs. 1 and 2.



Paper 2: Figure 4. Correlations between MEP modulation observed in the second session for MD application with that observed in the first session for SD application at each time point relative to cooling stimulation. Note the relatively good of correspondence between sessions in (A) and (B) for MEP modulation elicited at C1 and PC5, but not at PC10 (C). Abbreviations as in Figs. 1 and 2.

Short-latency Afferent-induced Facilitation and Inhibition as Predictors of Thermally-induced Variations in Corticomotor Excitability

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Abstract

Recently (Ansari, Remaud et al. 2018, Ansari, Remaud et al. 2018), we showed using transcranial magnetic stimulation (TMS) that focal application of innocuous thermal stimuli to the distal hand produced variable responses in terms of motor evoked potentials (MEP) suppression or enhancement. Here, we sought to investigate possible causes of this variability by examining circuits mediating sensorimotor integration and intra-cortical inhibition. Participants (n=21) first underwent TMS to assess baseline corticomotor excitability by measuring MEPs at rest with the index finger wrapped in a gel pack at room temperature (24 °C). Then, conditioned protocols were applied to assess short-latency afferent inhibition (SAI), short-latency afferent facilitation (SAF) and short interval intra-cortical inhibition (SICI). Following baseline measures, MEP modulation in response to distal cooling was recorded with the index finger wrapped in gel pack at ~10 °C. At baseline, participants exhibited variable levels of SAI, SAF and SICI. Participant also exhibited variable responses to cooling with about half of them (11/21) showing suppressed excitability and one-third showing enhanced excitability (7/21). A linear regression analysis revealed that SAI and SAF proved to be good predictors of cooling-induced variations in corticomotor excitability but not SICI. These results provide novel evidence linking variations in SAI and SAF with those in corticomotor excitability elicited in response to focal thermal stimulation, suggesting that these markers could be used to predict responses to sensory stimulation protocols.

Keywords

Thermal stimulation, Motor evoked potentials, short-latency afferent inhibition, Sensorimotor integration, Peripheral stimulation, Transcranial magnetic stimulation

Introduction

In recent years, the use thermal stimulation in the form of either cooling or warming stimuli applied to superficial and deep tissues have been proposed as an effective facilitation method, notably for patients recovering from stroke (Chen, Liang et al. 2005, Chen, Lin et al. 2011). The rationale for using thermal stimulation has been based largely on indirect evidence from neuroimaging studies showing extensive neural activation both at the subcortical (e.g., ventrobasal thalamus) and cortical level (e.g., insular cortex, primary (S1) and secondary (S2) somatosensory areas) in response to skin cooling or warming (Bokiniec, Zampieri et al. 2018). On this basis, it was proposed (Hsu, Lee et al. 2013) that repeated applications of thermal stimuli could lead to neuroplastic changes in the injured brain and promote motor reorganization. In line with this, Tai, Lai et al. (2014) showed that alternating noxious warm and cold stimulation to the affected arm of stroke patients for 30-min increased the motor map size area and motor evoked potential (MEP) amplitude in the lesioned hemisphere.

In recent investigations (Ansari, Remaud et al. 2018, Ansari, Remaud et al. 2018), we used transcranial magnetic stimulation (TMS) to examine the neurophysiological basis of thermal stimulation on corticomotor excitability. In the first study (Ansari, Remaud et al. 2018), we showed that innocuous cooling or warming restricted to a single digit elicited short-lasting and variable

responses in our participants characterized by either MEP suppression or enhancement. When examining possible sources of this variability, we found that individual characteristics related to age (young vs. old) or sex had no influence. Likewise, individual changes in skin temperature had no effect. In a follow-up study (Ansari, Remaud et al. 2018), we showed that approximately tripling the cooling surface area on the distal hand still elicited mixed responses amongst participants with half showing suppression and the other half showing MEP enhancement, although these effects were more sustained in the post-stimulation phase. Our results also revealed that thermally-induced variations in corticomotor excitability were fairly consistent in a given individual with repeated applications, even with many weeks between sessions.

While our findings on the variability of thermally-induced modulation aligned with previous observations regarding the outcomes of stimulation protocols targeting cortical plasticity (Chipchase, Schabrun et al. 2011), they still raised the question as to why a simple intervention could produce such mixed responses at the individual level. We propose that this variability could be related to differences in the way thermal afferents are processed centrally through sensorimotor integration. Like other forms of somatosensory afferent inputs, thermal afferents have the potential to modulate corticomotor excitability either directly via thalamocortical projections or indirectly through projections from somatosensory areas (e.g., S1 and S2) to primary motor cortex (M1) (Hooks 2016). In humans, the excitability of cortical circuits thought to be involved in sensorimotor integration can be assessed through short-latency afferent inhibition (SAI) (Turco,

El-Sayes et al. 2018). SAI is obtained when MEPs evoked by cortical stimulation are conditioned by prior afferent nerve stimulation at short intervals (i.e., 18-24 ms). When the interval between the afferent nerve conditioning and the TMS pulse is increased between 40-70 ms, the afferent modulation becomes facilitatory, a phenomenon that has been referred to as short-latency afferent facilitation (SAF) (Devanne, Degardin et al. 2009). Both SAI and SAF magnitudes are influenced by the nature of the afferent volley (e.g., cutaneous vs. proprioceptive) and its amplitude (Devanne, Degardin et al. 2009, Bailey, Asmussen et al. 2016). Several studies have established links between variations in SAI level and excitability of circuits mediating sensorimotor integration For instance, in patients in the acute stage after stroke, Di Lazzaro, Profice et al. (2012) reported a significant reduction in SAI level in the lesioned hemisphere, likely reflecting an adaptation to enhance responsiveness to sensory inputs. In healthy participants, Mang, Bergquist et al. (2012) investigated how SAI and SAF were modulated in response to an increase in corticomotor excitability elicited by repeated neuromuscular electrical stimulation. Their results showed that both markers exhibited a parallel change (i.e., decreased SAI coupled with increased SAF) after the intervention, both pointing to a net increase in the excitatory drive exerted by sensory inputs onto corticospinal neurones in response to enhanced corticomotor excitability.

The above results, collectively, point to the importance of considering changes in the circuits mediating sensorimotor integration when examining the impact of sensory stimulation protocols on corticomotor plasticity. Interestingly, several investigations (Yarnall, Ho et al. 2016, Koizume,

Hirano et al. 2017) including our own (Young-Bernier, Tanguay et al. 2014), have shown that SAI level can vary substantially from one individual to another, even in healthy participants of the same age group. Such variations in SAI suggest that the state of M1 intra-cortical excitability at baseline may vary substantially between individuals, which may, in turn, influence how individuals will respond to sensory stimulation. Indeed, there is evidence that inter-individual variations in SAI level, or with other markers of intra-cortical excitability, such as short-interval-intra-cortical inhibition (SICI), can be predictors of whom will exhibit suppression or enhancement in response to plasticity-inducing TMS protocols (Guerra, Lopez-Alonso et al. 2017). Along the same reasoning, one can ask whether inter-individual differences in the excitability of circuits mediating sensorimotor integration could provide some insights for the variability observed in response to thermal stimulation.

In the present report, we sought to address the above question using TMS to examine the relationship between inter-individual variations in SAI and SAF level and MEP modulation elicited in response to distal focal cooling. SICI was also assessed as an additional marker to probe individual differences in the excitability of circuits mediating intra-cortical inhibition. Based on the reviewed evidence, we anticipated that levels of SAI and SAF, as markers of sensorimotor integration, would be good predictors of cooling-induced variations in corticomotor excitability in contrast to SICI, which would show no relationship.

Material and Methods

This study was approved by the Institutional Review Ethics Board (Bruyère Hospital Ottawa, Protocol M16-18-015) in accordance with the principles of the Declaration of Helsinki. All participants gave written informed consent before the experimental session, which was performed in a controlled laboratory environment. Participants received a small honorarium for their participation.

Participants

Twenty-one healthy young adults were recruited for this study (28 ± 7 yr, 11 females). Before testing, all participants were screened for conditions likely to affect their participation (e.g., nerve diseases, sensory problems or pain in the upper extremities, recent trauma and aversion to cold). Participants were also screened with a questionnaire (adapted from Keel, Smith et al. 2001) for contra-indications to TMS. All tests were performed on the preferred hand (right, n=19), as determined by the Edinburg Hand Inventory (online version http://www.brainmapping.org/shared/Edinburgh.php).

Electromyography and Transcranial Magnetic Stimulation

Recording and stimulation procedures have been described in detail previously (Ansari, Remaud et al. 2018). Briefly, electromyographic (EMG) activity was recorded using surface sensors (DE-2.1, Delsys Inc., Boston, MA, USA) placed over the first dorsal interosseous (FDI)

of the dominant hand. After amplification and filtering (Bagnoli™ 4 System, Delsys Inc., bandwidth=6–450 Hz, gain=1,000), EMG signals were digitized at a rate of 2 kHz (PCI-63203, National Instrument Corp. Austin, TX) and were relayed to a laboratory computer and saved for later off-line analysis.

For TMS, participants were comfortably seated in a custom-made chair equipped with armrests and footrests. Participants were fitted with a Waveguard™ TMS compatible EEG cap (ANT Neuro, Madison, WI, USA) with markers to facilitate coil placement and ensure consistent positioning. Also, a U-shaped neck cushion was used to maintain head position and prevent neck fatigue. TMS pulses were applied on the hemisphere contralateral to the preferred hand over the motor hot spot for the FDI (marked with a sticker) using a focal coil (70 mm, inner loops) connected to a BiStim^2 stimulator (Magstim Co. Ltd, Whitland UK). Stimulation was performed with the coil held in the conventional orientation (i.e., ~45° from the sagittal plane). The resting motor threshold (rMT) was determined using the Motor Threshold Assessment Tool software (MTAT 2.0; Clinical Researcher, Knoxville, TN, USA). During TMS, participants were asked to stay relaxed and count the number of stimuli delivered to prevent shift of attention or sleepiness. All tests were performed during regular working hours (9 am to 5 pm) to avoid diurnal variations (Doeltgen and Ridding 2010).

Experimental Protocol

The current experiment was conducted in three steps. As shown in Figure 1, the first step consisted of establishing baseline values for corticomotor excitability as reflected in unconditioned MEPs at rest. As in our previous experiments, this assessment was performed with the index finger wrapped in a gel pack sleeve designed for finger application (TXRT-2540, Torex® Health Products, Tallmadge, OH) that was kept at room temperature (i.e., neutral ~24 °C) to account for the tactile feedback associated with the finger wrapping. As reported before, skin temperature was monitored through thermocouple sensors attached at the level of the proximal interphalangeal joint and connected to a K-type digital thermometer (Model# TC41FBA, Perfect-Prime, Dayton, NJ, USA, resolution ±0.1 °C). With the neutral gel pack in place, skin temperature was recorded and MEPs were elicited (n=20) at a suprathreshold intensity equivalent to 130% rMT to derive a reliable estimate of resting corticomotor excitability (Brown, Lohse et al. 2017). The second step consisted of assessing SAI, SAF and SICI using paired-pulse protocols. To this end, blocks of trials were performed sequentially to record conditioned and unconditioned MEPs (15-20 /block) for SAI, SAF and SICI; the order of testing with each block being counterbalanced across participants. As indicated in Figure 1, all conditioned MEPs were recorded with the neutral gel pack sleeve in place. The final step consisted of assessing MEP modulation in response to cooling. As in our previous reports, the cooling was induced by replacing the neutral gel pack with a pre-cooled one (~10 °C). After 1-min application, skin temperature was recorded, and 20 MEPs were

elicited (130% rMT). This final block took 2-4 min to complete, so the cooling stimulation was limited to 5 min max. At the end of the last block, participants were asked to rate the perceive intensity and comfort associated with the cooling stimulation using a 5-point numerical rating scale for Intensity/Comfort level (Geurts, Sleivert et al. 2005): 1) Slightly cool/Comfortable, 2) Cool/Slightly uncomfortable, 3) Cold/Uncomfortable, 4) Very Cold/Very uncomfortable, 5) Extremely cold/Extremely uncomfortable.

Conditioned Protocols for SAI, SAF and SICI

The protocol to assess afferent-related modulation was similar to that reported in our previous studies (see Young-Bernier, Tanguay et al. 2014). Briefly, for SAI and SAF, afferent nerve conditioning was produced by electrically stimulating the median nerve at the wrist through bipolar electrodes (cathode proximal to the anode, 200-μsec pulse) connected to a Digitimer stimulator (DS7A, Digitimer Ltd, Hertfordshire, UK). The conditioning intensity was set at the motor threshold to induce a minimal visible twitch in thenar muscles (Tokimura, Di Lazzaro et al. 2000). For SAI, the conditioning interval between the peripheral and cortical stimulation was set to 20 ms, whereas for SAF, the conditioning interval was set to 50 or 60 ms after initial testing with participants indicating which interval seemed most effective. The latter intervals were selected based on the systematic investigation of Devanne, Degardin et al. (2009) on the effects of varying inter-stimulus intervals (ISIs) on afferent-induced modulation. For both afferent

conditioning protocols, the TMS test intensity was set to 120% rMT. For SICI, the conditioning protocol consisted of pairing subthreshold TMS pulses with suprathreshold pulses with a fixed ISI of 2 ms. The subthreshold conditioning was produced by delivering pulses equivalent to 70% rMT, as such subthreshold intensity is known to activate cortico-cortical circuits without eliciting descending activity (Lazzaro, Restuccia et al. 1998). For suprathreshold pulses, the test intensity was set at 130% of the rMT in line with recent evidence showing that SICI remains stable over a range of test intensity from 110-130% rMT (Miyaguchi, Kojima et al. 2017). In each protocol, 15-20 conditioned and unconditioned MEPs were recorded.

Data Analysis

For skin temperature, readings from the two thermocouple sensors were averaged in each participant to derive individual mean values for the neutral (baseline) and cooled condition, respectively. For MEPs, mean amplitude values were obtained for each participant by averaging the peak-to-peak amplitude recorded under each block for both conditioned and unconditioned trials. As in our previous reports, variations in corticomotor excitability in response to cooling stimulation were determined by computing percent change relative to baseline (MEP_{BL}-$MEP_{cool}/MEP_{BL}) \times 100$). From these relative percentages, individual responses were classified using the 10% cut-off value (Hinder, Goss et al. 2014) as either suppressed (<10% BL), enhanced (>10% BL) or not modulated (±10% BL). For SAI, SAF and SICI, estimated levels of inhibition

or facilitation were determined using MEP ratios obtained by expressing conditioned MEPs as percentage of unconditioned MEPs (i.e., MEPs recorded at baseline, neutral temperature). MEP ratios above 100% indicated facilitation, whereas MEP ratios below 100% signalled inhibition.

Statistical Analysis

Before analysis, all variables (TMS markers, MEP modulation) were checked for the normality of their distribution with the D'Agostino-Pearson-test. All variables were normally distributed ($p>0.08$). Then, we performed a linear regression analysis with each marker (i.e., SAI, SAF and SICI) to determine their respective ability to predict cooling-induced variations in corticomotor excitability, as reflected in MEP relative change. We also performed a series of correlations with the Pearson's r to examine associations between the different markers of intra-cortical excitability. For each set of correlations, we used Bonferroni corrections to adjust p-values to reduce the risk of type I error (see Results).GraphPad Prism version 8.00 for Windows (GraphPad Software, San Diego, CA, USA, www.graphpad.com) was used for the statistical analysis and preparation of figures. All data are reported as mean ± one standard deviation (MEAN ± SD).

Results

Corticomotor excitability, SAI, SAF and SICI at baseline

At baseline, the average rMTs (±SD) in our group of healthy participants was 39.4 ± 10.9% MSO (maximal stimulator output). The average test (unconditioned) MEP amplitude for the SAI/SAF and SICI protocols was respectively 1.11 ± 0.52 mV and 1.18 ± 0.53 mV. For SAI and SAF, the average intensity to condition the median nerve was 15.2 ± 5.6 mV, and the average intensity for TMS test pulses was 47.1 ± 12% MSO. For SICI, the average conditioning intensity was 27.4 ±7.6 % MSO and the test intensity 52.2 ± 14.2 %. Figure 2a shows the distribution of individual levels measured respectively for SICI, SAI and SAF when dichotomized according to the size of the test MEPs. It can be seen that the latter factor (i.e. test MEP size) had no major influence on observed levels, although SAI levels tended to be more variable in individuals exhibiting larger test MEPs (>1 mV). We applied independent t-tests for each marker to search for possible differences related to test MEP size, and these comparisons revealed no significant differences between groups ($t_{19}<1.7$, $p>0.09$). Inspection of Figure 2a also shows that participants exhibited greater inter-individual variations in SAI and SAF than in SICI. The SAF protocol was particularly variable with only about half of the participants (9/21) showing the expected facilitation, the other half exhibiting either no clear facilitation or even inhibition Figure 2b, c and

d illustrate the correlation found between the different pairs of markers at the individual level. While a trend was seen for association between SAI and SAF (Figure 2b), none of the pairs of correlation reached statistical significance (adjusted p-value (0.05/3) =0.017)

Variations in corticomotor excitability with cooling

Figure 3a shows individual variations in MEP amplitude measured in response to cooling as a function of the decrease in skin temperature. As expected, individual responses were quite variable with a relatively large subset of participants (11/21) exhibiting depressed MEPs, while another subset (7/21) exhibited the opposite pattern (i.e., enhanced MEPs). Another small subset (3/21) showed no clear modulation. Further inspection of Figure 3a shows that individual reductions in skin temperature at the cooling site had no significant (p=0.55) impact on cooling-induced MEP modulation. As shown in Figure 3b, the local cooling stimulation and the accompanying reduction in skin temperature was perceived as just "Cold" by most participants and elicited only mild discomfort.

SAI, SAF and SICI as predictors of cooling-induced variations in corticomotor excitability

Figure 4 illustrates the results of the regression analysis to examine the respective role of SAI, SAF and SICI as predictors of individual responses to cooling in terms of MEP modulation. As shown in Figure 4, both SAI and SAF levels were significant predictors (adjusted p-value (0.05/4)= 0.0125) of cooling-induced MEP modulation. For SAI, individual variations predicted

>30% of the variance (p=0.007) so that individuals with high SAI (i.e., strong inhibition) were also those that showed depressed MEPs in response to cooling, and correspondingly, those with low SAI (i.e., low inhibition) tended to show MEP facilitation. Similarly, SAF (p=0.011) explained 30% of the variance (p=0.011), individuals showing large SAF being those that exhibited the largest enhancement in response to cooling, whereas low or lack of SAF was associated with depression. In contrast, variations in SICI level poorly predicted cooling-induced modulation (p=0.25). The role of SAI and SAF, as predictors of cooling-induced modulation, can be further appreciated in Figure 5, where individual examples of MEP modulation in the form of either cooling-induced inhibition (5a) or facilitation (5b) are shown with corresponding levels of SAI and SAF measured in response to afferent conditioning.

Discussion

In the present study, we sought to determine whether individual differences in TMS markers of intra-cortical excitability could explain some of the variability observed in response to distal focal thermal stimulation. In accord with our predictions, variations in SAI and SAF levels proved to be good predictors of cooling-induced modulation, whereas variations in SICI were not. In the discussion, we will address the significance these findings for research examining the impact of sensory stimulation protocols on corticomotor excitability.

Variations in SAI, SAF and SICI at baseline and intercorrelation between markers

Consistent with previous reports, levels of SAI, SAF and SICI varied considerably between individuals. In the case of SAI, the relatively large inter-individual variability exhibited by our participants (range, 13-87%) was comparable to that seen in our earlier reports (Young-Bernier, Davidson et al. 2012) with young adults of the same age (range, 3-67% MEP inhibition) using the same conditioning protocol. In their report examining the impact of age on SAI, Yarnall, Ho et al. (2016) also observed a wide variability of SAI levels in young adults (<40 years, see their Figure 1), some subjects even showing facilitation. For variations in SAF levels, the variability between participants was large with only about half of the participants showing the expected facilitation, an observation consistent with our previous reports in young adults (Young-Bernier, Davidson et al. 2012, Davidson and Tremblay 2013). Such variability contrasts with the consistent effects reported by Devanne's group (Devanne, Degardin et al. 2009, Degardin, Devos et al. 2011) with >75% of their participants showing SAF in the FDI. The reason for the discrepancy between their observations and ours is not clear but may reflect differences between protocols. One source of variability may be at the peripheral level since SAF is thought to result form recruitment of muscle afferents not only in response to direct nerve stimulation but also from the induced muscle twitch (Devanne, Degardin et al. 2009). The latter source of afferents may be particularly susceptible to variations from trials to trials and between individuals (e.g. slight differences in the intensity of the twitch), thus accounting for some of the variability observed at the cortical level.

With regards to variations in SICI, both early (Boroojerdi, Kopylev et al. 2000) and more recent reports (e.g., Samusyte, Bostock et al. 2018) have insisted on the high inter-individual variability. Although SICI appeared to be less variable than either SAI or SAF in our group of participants, the between-subjects variability (Coefficient of variation SICI, 43%) was in the range reported in previous studies. As stressed by Wassermann, Greenberg et al. (2001), variations in SICI levels has been shown to correlate strongly with certain personality traits, such as those associated with anxiety disorders; pointing to the importance of individual factors in influencing TMS markers of intra-cortical excitability.

Another potential source for the variability observed in SICI, SAI and SAF is related to size of the test MEP for unconditioned trials. Indeed, previous reports (Garry and Thomson 2009, Udupa, Ni et al. 2009) have shown that TMS markers of intra-cortical motor inhibition (e.g. SAI, SICI) tended to decrease with higher test MEP amplitude (and higher intensity). In this study, we used a test intensity for each of our protocol (i.e., 120% rMT for SAI/SAF and 130% rMT for SICI) that was previously reported optimal for probing changes in intra-cortical motor excitability (e.g., see Garry and Thomson 2009, Miyaguchi, Kojima et al. 2017). Although our participants exhibited variations in test MEP size, this variability had no systematic influence on levels of SICI, SAI and SAF measured (see Figure 2a). From these considerations, we conclude that the size of the test MEP amplitude was not a major factor in influencing variations observed in SAI, SAI and SAF level in our group of participants.

When examining the association between the different markers, our analysis revealed no significant association between SICI and either SAI or SAF. The lack of association between SICI and SAI is consistent with pharmacological evidence showing that the two markers reflect different forms of GABAergic inhibition mediated by different circuits within the M1 (Ziemann, Reis et al. 2015). The same logic can be invoked to explain the absence of relationship between SICI and SAF, although the pharmacological basis of afferent-mediated facilitation remains largely unknown at the moment. Finally, the lack of a clear association between SAI and SAF suggests that the two markers, although they may share common properties, likely reflect activation of different circuits within the M1. As discussed above, one major difference between the two lies at the sensory level since SAI can be evoked with purely cutaneous stimulation (Bailey, Asmussen et al. 2016), whereas SAF requires mixed nerve stimulation pointing to a proprioceptive modulation in origin (Devanne, Degardin et al. 2009). In this respect, the lack of strong association between the two suggests that afferent inhibition may vary independently of afferent facilitation in a given individual, much like measures of intra-cortical inhibition and intra-cortical facilitation, as reported by Wassermann (2002).

Variations in MEP modulation in response to distal cooling.

Consistent with our previous investigations (Ansari, Remaud et al. 2018, Ansari, Remaud et al. 2018), MEP modulation in response to distal cooling was characterized by a mixed pattern of

response with participants showing either enhanced or suppressed MEPs. Also consistent with our earlier observations (Ansari, Remaud et al. 2018), variations in MEP amplitude were largely independent of individual changes in skin temperature, confirming that the degree of skin cooling is not a critical factor in determining the sign of modulation. The addition of subjective ratings in the present report provided an opportunity to assess how participants perceived the focal cooling stimulation. As reported earlier, the fact that sensation elicited no major discomfort and was perceived as "cold" by the majority of participants confirm that the cooling was effective in stimulating low-threshold cold receptors, while avoiding the noxious range. In sum, these observations provide further evidence demonstrating that that individual responses to innocuous cold stimulation are inherently variable, likely reflecting individual differences in the way thermal afferent information is processed centrally.

SAI, SAF and SICI as predictors of MEP modulation in response to cooling

A major finding of the present study is that variations in SAI and SAF levels proved to be good predictors of MEP modulation elicited in response to cooling, whereas variations in SICI level were not. For the two markers of sensorimotor integration, the relationship was such that those showing a combination of high SAI and low (or lack of) SAF tended to show depressed MEPs in response to cooling, whereas those with low SAI and high SAF tended to show facilitation. In this regard, our results converge with those of Mang, Bergquist et al. (2012) who

observed a parallel modulation in SAI and SAF levels (i.e., down and up-regulation, respectively) after inducing an increase in corticomotor excitability with prolonged nerve stimulation. While these authors did not directly test the predictive value of SAI and SAF at onset, their results still converge with ours in showing a correspondence between circuits mediating SAI/SAF and those mediating changes in corticomotor excitability in response to afferent stimulation. Such a link may seem evident when considering changes in corticomotor excitability induced by electrical nerve stimulation, but it seems less evident when considering changes induced by thermal stimulation, as reported here. While it may seem odd at first to consider a link between SAI and SAF, which reflect activation of large afferent fibres, and, cooling-induced effects, which reflect activation of small afferent fibres, it appears less odd when considering the polymodal nature of thermal afferents. For instance, recent investigations in rodents (Wang, Belanger et al. 2018) have shown that >50% of cooling-sensitive neurones in the dorsal root ganglia also respond to mechanical stimuli, indicating that cold afferent fibres can convey not only thermal but also mechanical information; allowing a certain degree of sensory convergence between tactile and thermal modalities at higher levels of the somatosensory system.

The present observations that individuals with high SAI and low SAF tended to show MEP inhibition in response to cooling suggest that in those cases the predominant mode of modulation in response to afferent stimulation is inhibition. Since the level of SAI has been shown to depend on both the volume of sensory afferent (Bailey, Asmussen et al. 2016), we can assume that in these

individuals, the peripheral nerve stimulation was particularly effective in eliciting somatosensory excitation, leading to a strong inhibition at the cortical level at both the SAI and SAF conditioning intervals. High SAI levels also suggest highly efficient cholinergic-activity within the M1 (Di Lazzaro, Oliviero et al. 2002), which likely contributes to enhance afferent mediated inhibition at the intra-cortical level. Thus, for individuals exhibiting high SAI, the depressed modulation with cold stimulation, may reflects a general predisposition to express inhibition in response to afferent stimulation, either tactile or thermal in origin. Conversely, for individuals in whom facilitation was detected both in response to afferent nerve conditioning and in response to cooling stimulation, the association with lower levels of SAI suggests an increased motor excitability at baseline. For reasons discussed earlier, it also possible that in these individuals the nerve stimulation was particularly effective in recruiting proprioceptive afferents and inducing a muscle twitch, thus explaining the facilitation detected. However, this explanation could hardly account for the facilitation observed with cooling in the same individuals. As suggested, the co-occurrence of lower levels of SAI in these individuals rather points to an increase in cortical excitability, possibly linked with reduced cholinergic-dependent inhibition. Such an increase in excitability is by no way indicative of abnormality, especially given that rMTs in this subgroup were typical of young adults, it just seems to predispose these individuals to express facilitation (or to show less inhibition) in response to afferent stimulation, including thermal stimulation. Here again, our observations align with those of Mang, Bergquist et al. (2012). In their report, MEPs elicited at the

afferent facilitation interval were comparable in amplitude to test MEPs, i.e. individuals did not exhibit clear facilitation. Only after motor cortical excitability was increased after 40-min of neuromuscular stimulation, did afferent-facilitation was detected with a corresponding decrease in SAI (Mang, Bergquist et al. 2012). In our subset of participants, lower SAI and higher SAF at baseline provided condition at the cortical level to allow facilitation to be expressed in response to cooling stimulation.

In the case of SICI, the lack of a clear association with cooling-induced modulation was somewhat expected given that this marker reflects interactions within the M1 between cortical interneurons rather that afferent-mediated modulation. While studies have shown that circuits mediating SAI interact with those mediating SICI and that both modulate late indirect-waves generated by TMS (Turco, El-Sayes et al. 2018), there is evidence that SICI is a poor predictor of afferent-mediated changes in corticomotor excitability (López-Alonso, Cheeran et al. 2014).

Study Limitations

The present study has certain limitations. For instance, our sample size, while typical of investigations in human physiology, could have been larger to include a larger spectrum of participants notably middle-aged adults and seniors since ageing can affect TMS markers of sensorimotor integration (Young-Bernier, Davidson et al. 2012). In terms of outcomes, we limited our investigation on three specific markers derived from TMS, but we could have extended the list

to include long-latency afferent inhibition, long-interval intra-cortical inhibition and intra-cortical facilitation (ICF). However, these measurements are time-consuming and imply more TMS pulses delivery, which is not always possible or suitable when planning a testing session with human participants. Also, some markers, such as ICF, have very poor reliability on repeated sessions (Dyke, Kim et al. 2018), which complicates interpretation of variations. Another possible limitation is the lack of strict control over the conditioning stimulation when assessing SAI and SAF. As we explained earlier, some of the variability in SAF and SAI could have reflected variations in the afferent volley generated by the mixed nerve stimulation. Greater control, for instance by using the "M" wave, could have provided more consistent stimulation and reduce variability, especially when assessing SAF. However, such considerations, while important for methodological studies, were not critical for this study since the goal was not to assess the source of SAI/SAF variability. Along the same line, one may ask why we did not investigate the impact of cooling stimulation on SAI, SAF and SICI? As stated, our goal in this study was to investigate potential factors contributing to the variability of responses to thermal stimulation at the individual level and not to determine how cooling could affect SAI, SAF or SICI. A problem that arises when examining the effect of an intervention aiming at modulating corticomotor excitability is how to account for changes in excitability post-stimulation. This requires manipulations and time to adjust the TMS test intensity to account for increase or decrease in excitability. However, when examining the effect of a thermal intervention like cooling, time is critical as tissues temperatures

change rapidly with time, thus limiting the ability to perform protocol adjustments and manipulations. The adoption of a threshold tracking approach to measure SICI (Samusyte, Bostock et al. 2018) would be a suitable strategy to address thermal effects in the future.

Conclusion

The present study provides novel evidence linking variations in SAI and SAF at baseline with those in corticospinal excitability elicited by focal thermal stimulation. In this regard, our results raised the possibility of using SAI and SAF, as markers of sensorimotor integration, to predict how individuals will respond to sensory afferent stimulation protocols aiming at inducing lasting changes in corticomotor excitability. In particular, our results point to high SAI as a strong predictor of individuals who will express inhibition in response to repeated afferent stimulation, whereas high SAF seems to predict those who will exhibit facilitation. Such observations have potential implications for the application of sensory stimulation protocols in neurorehabilitation.

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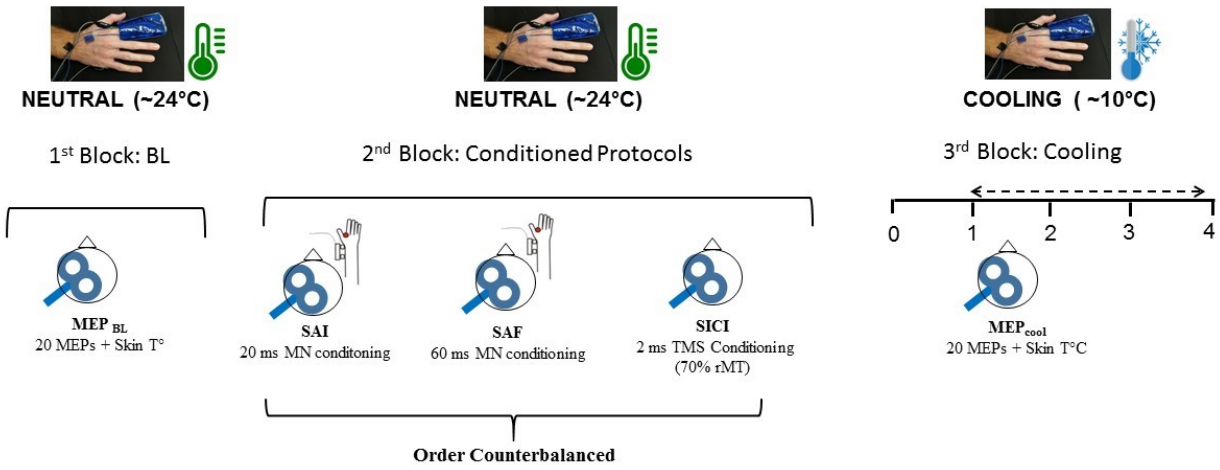
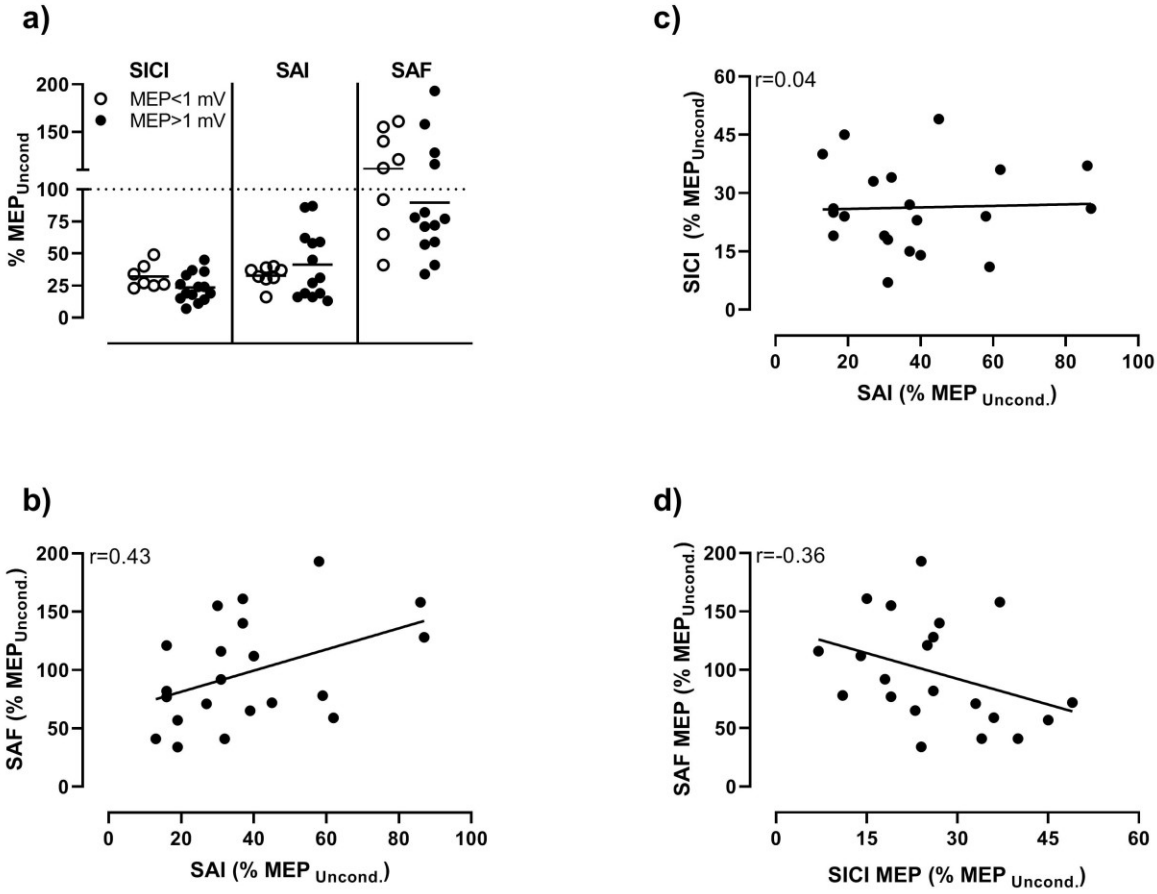
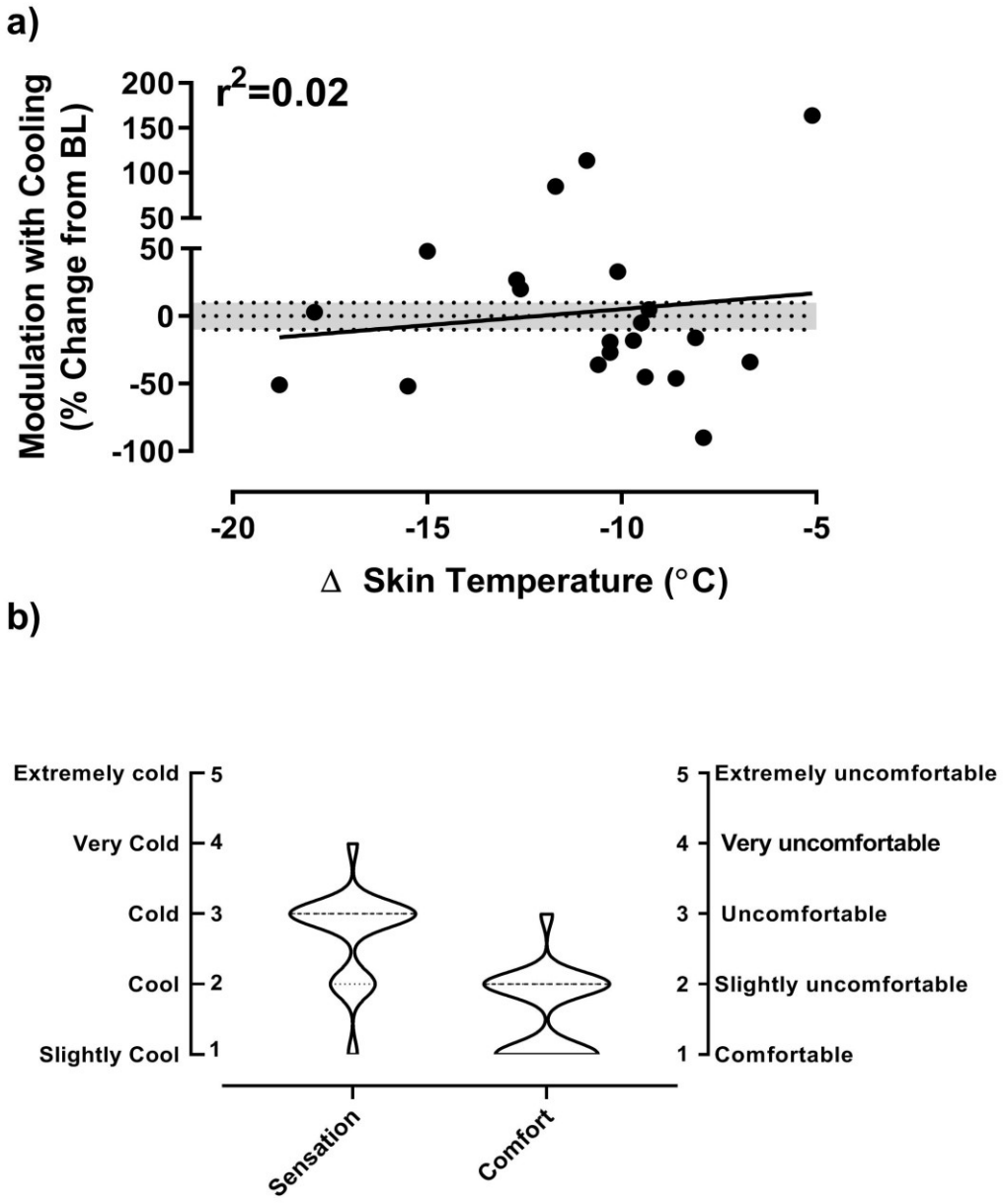


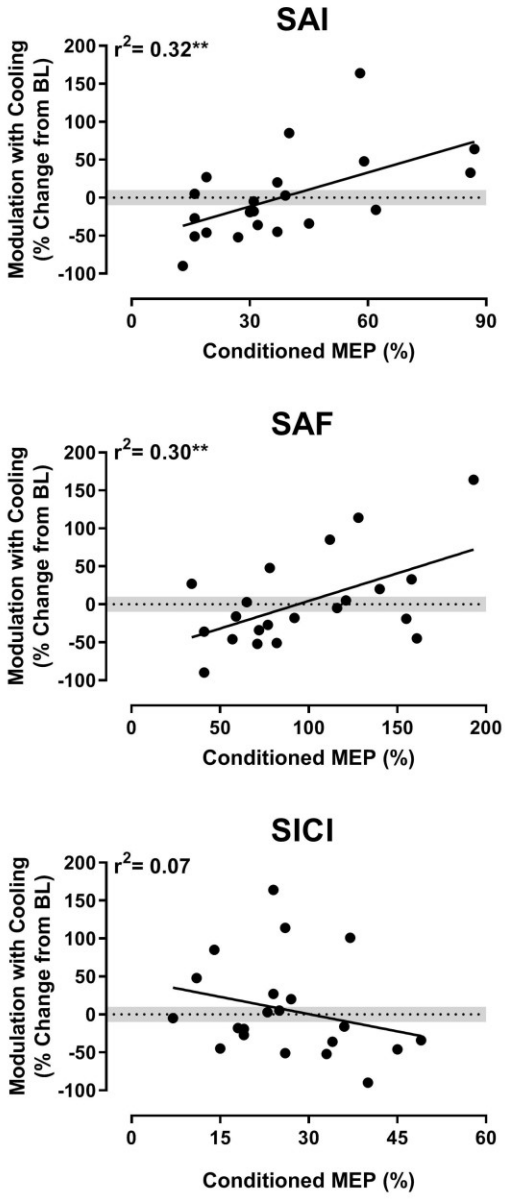
Paper 3: Figure 1. Schematic representation of the experimental protocol conducted in three steps. The first step consisted of establishing skin temperature (T°) and baseline (BL) values for corticomotor excitability as reflected in unconditioned MEPs at rest. The second step consisted of assessing short-latency afferent inhibition (SAI), short-latency afferent facilitation (SAF) and short-interval intra-cortical inhibition (SICI) with conditioned protocols. The intervals used for median nerve (MN) conditioning for SAI and SAF are indicated. For SICI, the interstimulus interval is indicated along with the TMS conditioning intensity (rMT: resting motor threshold). Note that all conditioned MEPs were recorded with the neutral gel pack sleeve in place to account for the tactile feedback associated with the finger wrapping. The final step consisted of assessing variations in skin temperature (T°) and MEP modulation in response to cooling.



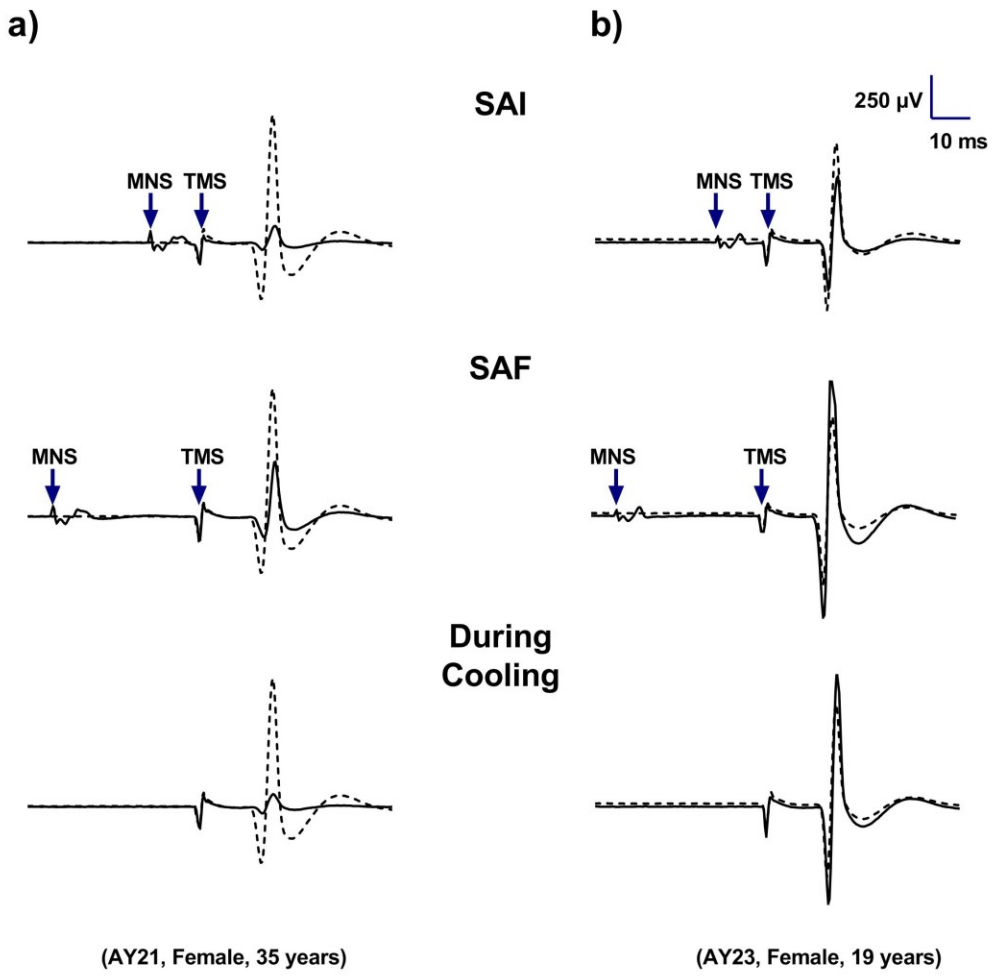
Paper 3: Figure 2. (a) Distribution of individual levels of SICI, SAI and SAF measured in all participants when dichotomized according to the size of the test (unconditioned) MEP (i.e. larger or smaller than 1 mV). (b, c and d) Results of the correlative analysis between the different markers (i.e., SAI, SAF and SICI) at baseline with corresponding Pearson's *r* values.



Paper 3: Figure 3. (a) Relationship between relative changes in MEP amplitude (% from baseline) in response to cooling and corresponding changes in skin temperature. Note that changes in temperature were not predictive of MEP modulation, as reflected in the very low coefficient of determination (r^2) (b) A violin plot showing the distribution frequency of subjective ratings for the perceive intensity and comfort associated with the cooling stimulation.



Paper 3: Figure 4. Results of the regression analysis showing the relationship between cooling-induced changes in MEP amplitude and individual variations in the different TMS markers at baseline. Note that variations in SAI and SAF levels, as reflected in r^2 values, were good predictors of corresponding cooling-induced variations in MEP amplitude, whereas variations in SICI were not. Asterisks denote significance with adjusted p-value at p=0.0125.



Paper 3: Figure 5. Individual examples showing the association between markers of sensorimotor integration and cooling-induced MEP Modulation. (a) This participant exhibited high SAI (strong inhibition) but no SAF (weak inhibition instead) and this was associated marked MEP inhibition during cooling. (b) This participant exhibited low SAI (weak inhibition) but clear SAF and correspondingly exhibited facilitation in response to cooling. In each trace, the dotted lines represent the baseline (pre-cooling) or unconditioned (SAI, SAF) MEP amplitude. All traces represent an average of 15-20 responses.

CHAPTER III. GENERAL DISCUSSION AND CONCLUSION

Our results demonstrate that focal thermal stimulation, in the form of either innocuous cooling or warming of a single digit, has a variable and short-lasting influence on corticospinal excitability, irrespective of the age (young and old), sex (men and women) and changes in skin temperature of participants. Our results also reveal that cooling is more likely to elicit modulation in corticospinal excitability than warming; a finding consistent with the greater sensitivity to cold stimulation in humans (Jones and Ho 2008) and the larger neurophysiological effects reported for local cooling stimuli (e.g. Chang, Arendt-Nielsen et al. 2005, Dewhurst, Riches et al. 2005). Consistent with previous reports regarding variability in response to sensory stimulation protocols; participants showed mixed patterns of modulation characterized by either inhibition or facilitation of corticospinal excitability only during actual cooling or warming stimulation, with the inhibition being the predominant pattern observed. Further, our observations indicate that tripling the cooling area in the distal hand still elicits variable responses in terms of facilitation and inhibition but is associated with a more sustained modulation in MEP amplitude in the post-cooling phase. These findings align with electrophysiological studies revealing substantial inter-individual variability in neurophysiologic responses to plasticity protocols (Maeda, Keenan et al. 2000, Charlton, Ridding et al. 2003, Brown, Williams et al. 2016). Such variability in motor output reflects high flexibility and dynamic nature of the nervous system (Berns, Song et al. 1999) and may depend on genetic factors (Cheeran, Talelli et al. 2008), daily fluctuations in the physiology of the circadian rhythms (Clow, Law et al. 2014), neural firing rates (Feldman 2012) or individual differences in the way sensory afferent inputs are processed centrally (Wiethoff, Hamada et al. 2014, Brown, Williams

et al. 2016). Of importance, the measures of resting corticospinal excitability (rMT and MEP) data obtained in our study in a separate testing session several weeks after the original one showed very good reproducibility over time in our group of participants; which further confirms that MEP modulation observed in our study reflected genuine heterogeneity in physiological responses to cooling stimulation rather than just random fluctuations in excitability. The exact mechanism underlying individuals' different reactions to thermal stimulation in terms of either inhibition or facilitation of MEP amplitude remains elusive, however it appears to be mainly central in origin. As discussed in the context of our paper (Ansari, Remaud et al. 2018), such a mechanism appears unlikely to have a peripheral origin given our observations regarding the lack of any association between the degree of skin cooling or warming and changes in M wave measurements and MEP characteristics. Indeed, thermal stimulation has the potential to activate multiple brain regions involved in motor planning such as the premotor, M1, the supplementary motor area (SMA) and posterior region of the ACC (Davis, Kwan et al. 1998, Kwan, Crawley et al. 2000, Tracey, Becerra et al. 2000, Brooks, Nurmikko et al. 2002, Chang, Arendt-Nielsen et al. 2005, Chen and Shaw 2006, Wu, Lin et al. 2010). The thermally-induced activation of these regions could facilitate sensorimotor reorganization, neuroplasticity and ultimately motor recovery in stroke survivors (Davis, Kwan et al. 1998, Gelnar, Krauss et al. 1999, Sheffler and Chae 2007, Chipchase, Schabrun et al. 2011, Chen, Tang et al. 2019). In addition, innocuous thermal stimulation leads to widespread activations in somatosensory regions including S1 and S2 (Craig, Reiman et al. 1996, Davis, Kwan et al. 1998), these activations could have contributed to suppressed motor excitability through functional and anatomical interactions between somatosensory cortices and the motor cortex pointing to an important role of input from S1 to M1 in altering the M1 excitability (Schabrun, Ridding et al. 2012). Such a mechanism is in line with observations that laser-induced thermal pain tends to suppress motor excitability when delivered at intervals reflecting sensorimotor

interactions at the cortical level (Valeriani, Restuccia et al. 1999, Suppa, Berardelli et al. 2012). Interestingly, a correlational analysis in our study revealed the potential role of markers of sensorimotor integration (SAI and SAF) in predicting cooling-induced modulation in corticospinal excitability ($r^2 \geq 0.30$) but not SICI. In particular, cooling-induced MEP inhibition was associated with high level of SAI, whereas facilitation was associated with high level of SAF. In other words, the predominant mode of modulation in individuals with high SAI and low SAF is inhibition in response to afferent stimulation, which seems to predispose them to exhibit inhibition in MEP amplitude with cooling stimulation. Given that the level of SAI is influenced by the level of somatosensory activation and the volume of sensory afferent (Bailey, Asmussen et al. 2016), we can assume that in these individuals, peripheral nerve stimulation was highly effective to provoke somatosensory excitation, resulting in strong inhibition at the cortical level. Moreover, high SAI levels, which is associated with highly efficient cholinergic activity within the M1 (Di Lazzaro, Oliviero et al. 2002), possibly contribute to enhance afferent mediated inhibition at the intra-cortical level. In contrast, for individuals exhibiting low SAI, the enhanced corticospinal excitability with cold stimulation, reflects an increase in cortical excitability likely associated with reduced cholinergic dependent inhibition. Given that rMTs in these individuals were typical of young adults, lower SAI and higher SAF at baseline do not indicate any abnormality, but rather allows facilitation to be expressed at the cortical level in response to thermal stimulation.

In brief, similar to studies reporting differential responses to painful stimulation (Coghill, McHaffie et al. 2003) or electrical nerve stimulation (Charlton, Ridding et al. 2003, Brown, Williams et al. 2016), individual responses to innocuous thermal stimulation were largely variable between participants. This indicates that cooling stimulation cannot be used as a one-size-fits-all treatment in clinical population, but depending on the desired therapeutic outcome, it may need to

be tailored to each individual patient according to the level of their markers of sensorimotor integration for a successful neuromodulatory treatment (Di Pino, Pellegrino et al. 2014). For instance, in stroke survivors presenting with spasticity linked with spinal hyperexcitability; finding a high level of SAI and low SAF may provide a neurophysiological basis for the use of focal cooling as a means to depress corticomotor excitability, thereby leading to reduced muscle hypertonia. Likewise, for patients where loss of dexterity and paresis are the primary impairments, finding a combination of low SAI and high SAF as detected with TMS, could provide a basis for cooling-induced enhancement in corticomotor excitability, thereby facilitating recruitment of paretic muscles.

Limitations

While specific limitations have been discussed in the context of each paper, there are other limitations that need to be addressed. First, given the functional importance of the hand for humans and its unique and large representation in the sensorimotor cortex (Léonard, Mercier et al. 2013), we limited our study to investigate the effect of thermal stimulation on the corticospinal excitability of the hand area only. However, we cannot assume that the changes observed during stimulation of the hand area will be the same as the changes occurring in the foot or the thigh. Future studies assessing the effect of thermal stimulation on the corticospinal excitability of the lower limb muscles are therefore warranted. We also must acknowledge that the effect of thermal stimulation may vary depending on the thermal agent used. This could be an interesting target for future studies to investigate the effect of alternative thermal agents such as topical menthol or capsaicin on corticospinal excitability. Another limitation is to not have investigated the possible effect of ovarian hormones in female participants, which could have influenced their cortical excitability at the time of testing and therefore interacted with the obtained results (Smith, Keel et al. 1999, Cahn, Herzog et al. 2003, Hattemer, Knake et al. 2007, Hermsen, Haag et al. 2016). Another important limitation is that we investigated the effect of single modality (i.e. either warm or cold stimulation) on corticospinal excitability in experiments I, II and III. The influence of both hot and noxious cold stimuli on corticospinal excitability of the paretic arm has been previously investigated in stroke patients by Tai et al (2014). These authors found significant increase in the MEP amplitude in the affected hemisphere in response to 30 min of alternating noxious cold and warm stimulation of the paretic arm in stroke patients (Tai, Lai et al. 2014).

This raises the question as to whether combining focal warm and cold stimuli could elicit stronger effect given evidence for the thermal gill illusion (TGI) where alternating innocuous cold

and warm stimulation leads to paradoxical burning heat sensation (Craig and Bushnell 1994). To address this issue, we designed a pilot experiment to explore the effects of distal focal simultaneous innocuous cold and warm stimulation on both perceived intensity of sensation and corticospinal excitability. Ten healthy young adults (29 ± 6 years, six males, eight right handed) were recruited for this pilot study. The experiment consisted of an assessment of sensation elicited by warm and cold stimulation alone and by combined stimulation (W+C) along with an assessment of MEP modulation. As in our other experiments, MEPs in the FDI and skin temperature (°T) were first recoded at baseline with the index and middle fingers of the preferred hand covered with two gel packs kept at room temperature (i.e. neutral temp. ~22 °C). Then, skin warming was performed by covering the index finger with a gel pack that had been pre-heated to 45 °C before application. The gel pack was applied for 2 min and then participants were asked to rate the intensity of sensation using a visual analogue scale (VAS). Immediately after ratings, the warm gel pack was removed to allow skin temperature to return to baseline with a waiting time of ~15 min. Then, skin cooling was performed using a gel pack that had been pre-cooled to~10 °C. After 2 min, the participants were asked again to rate the sensation with the VAS. Allowing another 15-20 min for the skin temperature to recover, the effect of combined stimulation was assessed by covering the index and middle fingers with a cold (10 °C) and a warm (45 °C) gel pack, respectively. After 2 min of stimulation, participants provided again ratings of sensation and MEPs were recorded from the FDI to assess the modulation in corticospinal excitability associated with the combined stimulation. Note that we did not assess MEP modulation with single cold or warm application since these observations have been collected before in our previous experiments (Exp I and III).

General Observations

The average rMTs corresponded to 40.3 ± 8.2% and the test stimulation intensity for TMS (130% rMT) corresponded on average to 52 ± 10.78% of the maximal stimulator output. Warming

and cooling stimulation induced the desired range of skin temperature (warming: 38.5-46.7 °C, cooling: 11.7-21.1 °C). Figure 7 shows the mean subjective rating of intensity computed respectively for the cooling, warming and combined (W+C) thermal stimulation. As shown in Figure 7, the combined stimulation on average elicited greater ratings than either the cooling or warming alone, which is consistent with psychophysical observations regarding thermal grill illusion. A non-parametric analysis with the Friedman test revealed a significant difference ($p=0.005$) between mean ratings and further post-hoc comparison with Dunn's test indicated a significant difference between warming stimulation and combined stimulation ($p=0.008$). Thus, participants perceived the combined warm and cold stimulation as more intense in general, especially when compared to the warming stimulation.

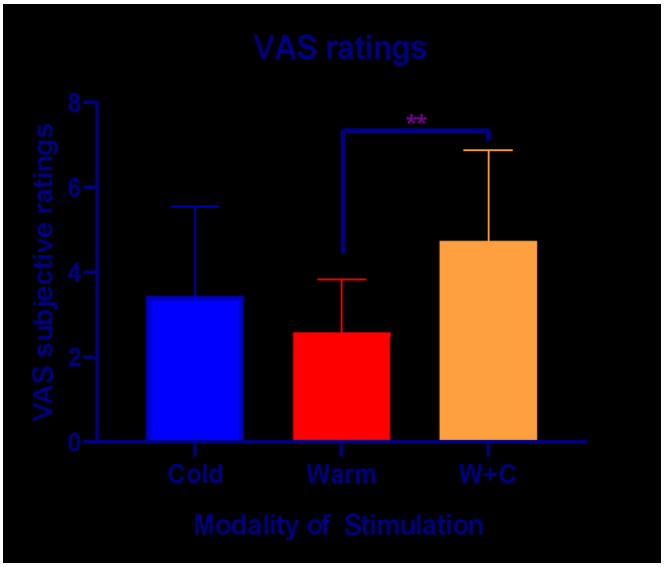


Figure 7. Bar chart demonstrating the subjective intensity of sensation of the cooling and warming stimulation alone and the combined stimulation using a VAS among participants.

As for MEP modulation and as evident in Figure 8, the combined focal stimulation was associated with much inter-individual variability. In fact, only 6/10 participants showed a clear modulation (i.e. Beyond ±10% variation in amplitude indicated by the shaded area) and of those,

half showed facilitated MEPs and half exhibited suppression. Thus, these observations, although still preliminary, are fully consistent with our previous observations derived from larger groups and further confirm that thermally-induced modulation in corticospinal excitability are inherently variable in nature. Thus, it seems that application of innocuous temperature stimuli in the distal extremity, either in the form of cold or warm, or even cold and warm combined, is susceptible to elicit variable responses in terms of motor excitability from one individual to the next.

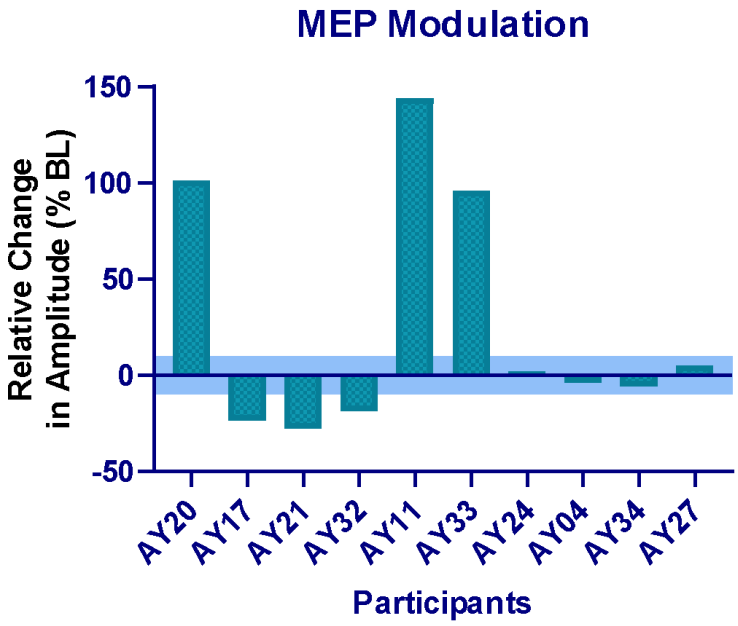


Figure 8. Bar chart demonstrating the variations in MEP amplitude (the relative change with respect to BL) in response to combined focal innocuous cold and warm stimulation among participants.

Conclusion

In conclusion, the present results highlight the need to consider individual differences in the way thermal sensory inputs are processed centrally and the fact that thermal stimulation can lead to either enhanced or suppressed excitability at the corticospinal level. Further, our results also show that responses in terms of MEP suppression or facilitation are fairly repeatable for a given individual, even with many weeks between sessions. Such fairly good within-subject repeatability seems critical for future therapeutic applications of cooling stimulation for rehabilitative purposes in neurological populations. Most notably, for the first time, the present work provides evidence linking variations in SAI and SAF levels measured at baseline with those in corticospinal excitability elicited by focal distal thermal stimulation. Indeed, individuals seem to exhibit differences in the excitability of circuits mediating sensorimotor integration, as reflected by variations in SAI level, which in turn may predispose them to express either suppression or enhancement in response to repeated afferent stimulation. This novel finding appears critical to foster any further development of thermal stimulation as an individualized patient-tailored neurofacilitation method in stroke rehabilitation. Overall, the findings of this research advance our understanding of the neurophysiological basis of thermally induced effects at the neural level and uniquely contribute to the development of more effective neurofacilitation method for patients with neurological conditions.

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February 28, 2017

Ms. Yekta Ansari
PhD Student
School of Rehabilitation Sciences
Faculty of Health Sciences
University of Ottawa

Re: Effects of Thermal Stimulation on Corticospinal Excitability
(Bruyère REB Protocol # M16-17-001)

Final Approval

Dear Ms. Ansari,

The Bruyère Continuing Care Research Ethics Board (REB) is pleased to give you ethical approval for the period February 28, 2017 to February 28, 2018.

Please note that this approval is for recruitment of English-speaking participants. The French recruitment materials and consent form will be reviewed and a separate letter of approval provided.

The following documents have been approved:

- COREB Form, received February 14, 2017
- Appendix B: English Information Sheet and Consent Form, dated February 14, 2017
- Appendix C: Questionnaire État de Santé/Health Status Questionnaire, received February 14, 2017
- Appendix D : Schematic of the Experimental Procedures, received January 9, 2017
- Questionnaire Pour La Stimulation Magnétique Transcrânienne/Questionnaire for Transcranial Magnetic Stimulation, received February 14, 2017
- Appendix E: English Script for Word of Mouth for Participants' Recruitment, received February 14, 2017
- Appendix F: English Recruitment Poster, received February 14, 2017
- Appendix G: Email Exchange with Pauline-Charron Centre, received February 14, 2017
- Appendix H: English First Contact Telephone Script or Email, received February 14, 2017
- Appendix I: Project Budget, received February 14, 2017
- Appendix J: Mini-Cog Test, received February 14, 2017

The Bruyère Continuing Care REB complies with the membership requirements and operates in compliance with the Tri-Council Policy Statement: Ethics Conduct for Research Involving Humans; the International Conference on Harmonization - Good Clinical Practice: Consolidated Guideline; the provisions of the Personal Health Information Protection Act 2004; and the Food and Drug Act of Health Canada and its applicable Regulations.

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,

Gordon DuVal, SJD
Chair, Bruyère Research Institute Research Ethics Board
University of Ottawa Faculty of Law
gduval@uottawa.ca
613-627-5399

CC: Dr. Francois Tremblay

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April 20, 2017

Ms. Yekta Ansari
PhD Student
School of Rehabilitation Sciences
Faculty of Health Sciences
University of Ottawa

Re: Effects of Thermal Stimulation on Corticospinal Excitability
(Bruyère REB Protocol # M16-17-001)

Final Approval – French Translations

Dear Ms. Ansari,

Thank you for providing copies of the French translations of study documents for the above noted study.

The following documents have been approved:

- Appendix B: Lettre d'information et formulaire de consentement, dated April 6, 2017
- Appendix C: Questionnaire état de santé, received April 6, 2017
- Questionnaire pour la stimulation magnétique transcrânienne (SMT), received April 6, 2017
- Appendix E: Script pour la bouche à oreille pour le recruitement des participants, received April 6, 2017
- Appendix F: French recruitment poster, received February 14, 2017
- Appendix H: Script pour le premier contact au téléphone ou par courriel, received April 6, 2017

With this approval you may now recruit French-speaking participants to participate the above noted study.

The Bruyère Continuing Care REB complies with the membership requirements and operates in compliance with the Tri-Council Policy Statement: Ethics Conduct for Research Involving Humans; the International Conference on Harmonization - Good Clinical Practice: Consolidated Guideline; the provisions of the Personal Health Information Protection Act 2004; and the Food and Drug Act of Health Canada and its applicable Regulations.

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,

Gordon DuVal, SJD
Chair, Bruyère Research Institute Research Ethics Board
University of Ottawa Faculty of Law
gduval@uottawa.ca
613-627-5399

CC: Dr. Francois Tremblay

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April 9, 2018

Ms. Yekta Ansari
Bruyère Research Institute

Re: **"**Effect of Localized Cooling on Cortical Inhibition and Sensori-Motor Integration**"** (Bruyère REB Protocol #M16-18-015)

Final Approval

Dear Ms. Ansari,

The Bruyère Continuing Care Research Ethics Board (REB) is pleased to give you ethical approval for the above noted study for the period of April 9, 2018 to April 9, 2019.

The following documents have been approved:

- BREB Application, received April 6, 2018;
- Participant Informed Consent Form English, version date: April 5, 2018;
- Participant Informed Consent Form French, version date: April 5, 2018;
- Health Status Questionnaire Bilingual, received April 6, 2018;
- Questionnaire for Transcranial Magnetic Stimulation English, received April 6, 2018;
- Questionnaire for Transcranial Magnetic Stimulation French, received April 6, 2018;
- Recruitment Script – word of mouth English, received April 6, 2018;
- Recruitment Script – word of mouth French, received April 6, 2018;
- Recruitment Script – telephone English, received April 6, 2018;
- Recruitment Script – telephone French, received April 6, 2018;
- Recruitment Flyer English, received April 6, 2018;
- Recruitment Flyer French, received April 6, 2018;
- Budget, received April 6, 2018.

The Bruyère Continuing Care REB complies with the membership requirements and operates in compliance with the Tri-Council Policy Statement II: Ethics Conduct for Research Involving Humans; the International Conference on Harmonization - Good Clinical Practice: Consolidated Guideline; the provisions of the Personal Health

Information Protection Act 2004; and the Food and Drug Act of Health Canada and its applicable Regulations.

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 6 weeks prior to the approval end date as noted above.

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

Sincerely,

Gordon DuVal, SJD
Chair, Bruyère Research Ethics Board
Bruyère Continuing Care
gduval@bruyere.org