

Running Head: PHYSICAL ACTIVITY AND tsDCS MODULATION OF PAIN THRESHOLD

The Influence of Sex and Physical Activity Level on the Modulation of Pain Perception in
response to Transcutaneous Spinal Direct Current Stimulation

By

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Contributions

The work described in this thesis was carried out by the author (JG) under the supervision of Dr. Tremblay. The experiments and data analysis were carried out by the author with the assistance of the supervisor. Both the author and supervisor were involved in the drafting, writing, and editing of the final manuscript. The procedures in this study were approved by the Research Ethics Board at the Elisabeth-Bruyère Hospital (Appendix A).

List of abbreviations used in the text

Abbreviation	Explanation
ACC	Anterior cingulate cortex
BDNF	Brain-derived neurotrophic factor
CSEP	Canadian Sport and Exercise Physiology
CNS	Central nervous system
DNIC	Diffuse noxious inhibitory controls
DC	Direct current
DLPC	Dorsolateral prefrontal cortex
IASP	International Association for the Study of Pain
IPAQ	International Physical Activity Questionnaire
MTP	Myofascial trigger point
PPT	Pressure pain threshold
MI	Primary motor cortex
SI	Primary somatosensory cortex
SII	Secondary somatosensory cortex
tsDCS	Transcutaneous spinal direct current stimulation
tDCS	Transcranial direct current stimulation
TMS	Transcranial magnetic stimulation
VPL	Ventral posterior lateral nucleus
VPM	Ventral posterior medial nucleus

Summary

Transcutaneous spinal direct current stimulation (tsDCS) is a non-invasive technique that can be used to modulate spinal function. It consists of applying a low-level direct current (DC) across the skin to modulate spinal excitability using surface electrodes. Recent research indicates that this technique can relieve musculoskeletal pain. In this study, we investigate the effect of a 20-min anodal tsDCS (2.5 mA) protocol applied over the thoracic spine on pressure pain threshold (PPT) measured in the thigh, leg and foot in healthy young adults. One primary focus of this study was to determine whether physical activity level, as a potential modulator of pain perception, could influence individual responses to tsDCS. A secondary aim was to also address the role of sex as another potential modulator of pain response. Thirty-five healthy young adults (age 18-35) were recruited for this study. Participants were assigned to either a moderately active (n=21, 12 females), or highly active group (n=14, 8 females) based on a self-report questionnaire (International Physical Activity Questionnaire). The effects were determined by comparing PPT measures at the three sites (thigh, leg, foot) at three time points with respect to tsDCS application: T0: before, T1: immediately after, and T2: 30 min post-application. Results from a multivariate analysis of variance revealed a large main effect of time ($F=5.3$, $p<0.001$) on PPTs. Univariate tests ($F>16.2$, $p<0.001$) confirmed that PPTs were significantly elevated post-application. In addition, the analysis revealed a significant “Group X Time” interaction ($F=2.8$, $p=0.03$), which was explained by a larger elevation in PPTs (thigh site) in the highly active group when compared to the moderately active group. No main effect or interaction was found for sex. Altogether, these results confirmed the anti-nociceptive effects of tsDCS application on mechanical pain threshold and further point to the importance of physical activity as a personal factor susceptible to modulate response to tsDCS.

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Chapter I: Literature Review

1.1 Introduction to musculoskeletal pain

Chronic widespread musculoskeletal pain is a major health problem in society, with approximately 18.9% of the Canadian adult population having reported complaints of such pain (Schopflocher, Taenzer, and Jovey, 2011). For many of these individuals, musculoskeletal pain disorders may render them incapable of maintaining an independent lifestyle, as musculoskeletal pain affects their ability to move, work, participate in social activities, and even sleep (Brevik, Collett, Ventafridda, et al., 2006). Pain, according to the International Association for the Study of Pain (IASP), is defined as “an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage” (Merksey & Watson, 1979). Pain can be categorized as either acute or chronic depending on its onset and duration. While acute pain, which is generally manifested as a result of an external stimulus or trauma, can in most cases be easily diagnosed and treated, chronic pain is known to have psychological and social impacts in addition to the physical dimension, and therefore is much more complex and multidimensional than acute pain. Accordingly, the management of chronic pain states is more challenging and only partially successful, leaving many individuals with poorly managed symptoms and co-morbidities in relation to persistent pain syndromes in the long-term (Kwon, Altin, Duenas, & Alev, 2014).

1.1.1. Detection and perception of pain

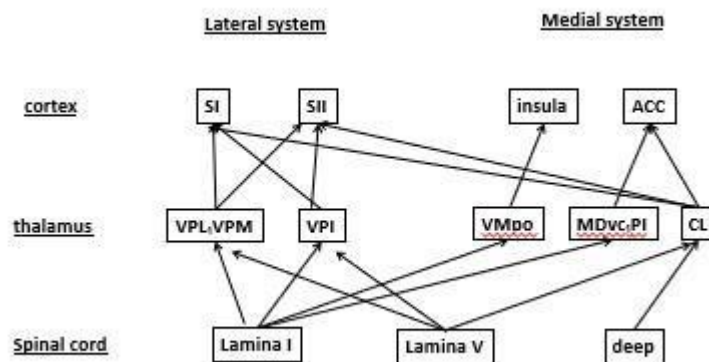
Detection and perception of nociceptive input functions similarly to that of any other somatic stimulus; stimulation is first detected by specialized peripheral sensors, sensitive to high intensity stimuli, i.e. the nociceptors. From there, the sensory information travels in the form of nerve impulses along the peripheral nerve to reach the spinal cord where the sensory endings make synapses with 2nd order sensory neurons, which will convey the impulses via the spinothalamic tract to the ventral posterior lateral nucleus (VPL) and, from there, to the somatosensory cortex, via 3rd order neurons. Only then, in the cortex, the stimuli can finally be interpreted and perceived

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as pain. The entire process occurs in a fraction of a second. However, it is not as simple as the body telling the brain that it is in pain. Pain, in reality, is more of an emotional construct that results from an activation of the nociceptors of the nervous system, much to the same degree that vision, smell, and hearing are all results of external stimuli but are more complicated than the simple detection of a stimulus (Marieb & Hoehn, 2013). The nociceptive stimulus must be relayed to the CNS where it is then processed and understood by the brain, creating the multimodal sensory experience referred to as pain (Treede, 2006).

There is still much to be discovered about the perception and processing of pain at the higher levels of the brain. It is well known that the nociceptive stimulus propagates along the peripheral nerves until it reaches the sensory thalamus (VPL, VPM nuclei), which is responsible for relaying sensory information to the cerebral cortex (Garland, 2012). From there, information is relayed to various different regions of the brain including somatosensory cortices (SI, SII, insula) and frontal executive areas (Anterior Cingulate, DLPC), which are responsible for interpretation of sensory input, and the limbic system (Ventral-medial Prefrontal cortex, Amygdala, Hypothalamus) which is responsible for mediating the emotional response linked with the stimuli, in addition to being involved in the processing of memory (Marieb & Hoehn, 2013). Although early theories on pain perception believed that pain was first processed in the thalamus, more current research has shown that much of pain processing occurs

early on in the spinal cord before information is relayed to the higher processing centers in the brain. For instance, sensory input received by the thalamus is first processed in laminae I-II and IV-V of the spinal



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cord, where second order neurons send projections through the antero-lateral system directed at either the thalamic nuclei (spino-thalamic tract) or at various areas of the brainstem (i.e., spinomesencephalic tract and spino-reticular tract). Upon reaching the cortex and other sub-cortical regions, the experience of pain gives rise to a large network of activation that is commonly referred to as the pain matrix; the numerous cortical and subcortical regions of the brain that have been shown to activate following nociceptive stimulation. The term 'pain matrix' commonly refers to the anterior cingulate cortex (ACC), insula, frontal cortices, primary and secondary somatosensory cortices (S1, S2), and the amygdala. The

pain matrix, represented in Figure 1, is **Figure 1**. Division of the pain matrix into medial and lateral systems (Adapted from typically subdivided into lateral and medial Treede et al, 1999).

systems to regroup regions of the brain that have similar roles in the processing and perception of pain (Brooks & Tracey, 2005). The insula and ACC have been shown to be consistently activated by nociceptive stimulation, and also play a role in the modulation of pain. The descending central pain modulatory system, which acts on the dorsal horn of the spine via the medulla encompasses structures such as the periaqueductal grey, rostral ventromedial medulla, and dorsolateral pons/tegmentum (Garland, 2012). The numerous regions of the CNS that are involved in the processing of nociceptive inputs shows that perception of pain is a complex process arising from multiple interactions at all stages from the periphery up to higher associative areas of the brain.

1.1.2. Pain as a multimodal sensory experience

The concept of pain as a product of the brain in response to a physical event dates back to the Syriac Empire in 200 B.C. In fact, many theories of pain presented themselves early on in research. One such theory, the specificity theory of pain, defined pain as a unique sensation, detected independently from other sensations such as heat, cold, or pressure. In contrast, the intensity theory

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of pain defined pain not as a unique sensation, but rather a reaction to a stimulus being stronger than normal (Moayedi & Davis, 2012). However, despite the amount of valuable research these theories have yielded, they also presented many shortcomings; mainly a lack of understanding of the complexity of pain and its integration into the nervous system. While research has indicated that pain is certainly associated with the nociceptive system, it is possible that low intensity nociceptive stimuli do not always lead to pain, thus introducing the idea of variability of pain; some individuals may perceive an incredibly strong stimulus as painless, while others who suffer from a neuropathic condition may experience unbearable pain from even the gentlest of touches (Treede, 2006). In this same regard, Goodwin (2001) discusses the possibility that the difference between pain and other senses lies in the emotional component of pain, explaining that nociception is closely linked with the sensory-discriminative component of pain, while the negative emotion is linked with the unpleasantness of the pain experience.

The strong emotional component of pain introduces an interesting element to the discussion of the perception of pain. While some may argue that the minimum intensity at which a stimulus is perceived as painful, commonly referred to as the pain threshold, is universal, such a notion is hard to reconcile with pain being a highly individualized experience. In fact, research has shown that the pain experience can be modulated by individual factors linked with prior experiences and expectations, allowing one to either increase or decrease the perceived intensity of a painful stimulus (Moayedi & Davis, 2012). This poses a challenge for the argument for the existence of a “universal pain threshold”. Nowadays, pain is recognized as a multidimensional experience influenced by a multitude of factors linked with genetics, emotions, and cognition, which all contribute to individualize the nature of the experience (Tracey, 2010). In fact, Singer and colleagues (2004) demonstrated that emotion alone is sufficient in activating brain regions that are

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associated with nociception and perception of pain, as stimulation of a participant's loved one showed activation of portions of the pain matrix even when the subjects themselves were not being exposed to the painful stimulus. Summarizing, pain is not only a sensation in response to a stimulus but also an emotion which can interact with cognitive factors linked with prior experiences and memory, making the pain experience unique to each individual so that any given level of stimulation can be perceived as painful depending on the person's health status, genetic makeup, personal history, and the context in which the stimulation is applied.

1.2 Factors influencing pain perception threshold

The concept of individual variability leads us to question as to which are the factors that determine an individual's subjective pain experience. As stated earlier, past experience is certainly a major factor, as the pain that individuals have already experienced forms their frame of reference with which they will compare all future painful stimuli (Mcgrath, 1994). For instance, young children who have not been exposed to a large quantity of nociceptive stimuli are likely to rate every nociceptive experience as the most painful experience of their lives. However, as these children mature, they will develop a greater frame of reference for the pain they are experiencing and will have a larger and more diverse history of pain experiences with which to compare new pain (Mcgrath, 1994). We can therefore confidently hypothesize that an adult population will generally be able to tolerate more pain than a child population. However, additional factors exist that have the potential to modulate pain perception. In the context of this study, we were primarily interested in examining the role of physical activity as a modulator of pain perception. The role of biological sex in terms of male and female was also examined.

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1.2.1. Pain perception and tolerance in an athletic population

It is often suggested that athletes tend to exhibit a higher tolerance to pain than their sedentary counterparts due to the extensive training they partake in and the mentality of pushing through pain that is commonly seen in competitive sport. While an increase in pain tolerance seems likely to coincide with an increase in pain perception, previous studies have shown that these values exist independently of each other (Baker & Kirsch, 1991; Defrin, Shramm, & Eli, 2009). Essentially, while two individuals may exhibit the same pain threshold, their tolerance to pain may differ greatly. Therefore, while it can be expected that athletes will generally be able to tolerate painful stimulation for a greater amount of time than sedentary individuals (Flood, Waddington, Keegan, et al., 2017; Flood, Waddington, Thompson, & Cathcart, 2016), it is unclear to what degree the actual threshold at which pain is perceived is affected by physical activity level. Additionally, while studies conducted on endurance athletes and game sports athletes (basketball, soccer, football, baseball, etc.) reported an increase in pain tolerance when compared to inactive controls (Guieu, Blin, Pouget, & Serratrice, 1992; Egan, 1987; Scott & Gijbers, 1981), little data is available on differences in pain tolerance in strength and power athletes. However, one study reported that athletes participating in contact sports such as football exhibited a higher pain tolerance than athletes participating in endurance sports (Ryan & Kovacic, 1966). Furthermore, a study assessing pain tolerance in swimmers reported that pain tolerance of these athletes varied according to the stage of the training season, with highest pain tolerances being seen at the peak of the competition season, and dropping after the summer break when swimmers entered a new training cycle (Scott & Gijbers, 1981).

A systematic review of multiple studies that focused on pain threshold and tolerance between sedentary and athletic populations unveiled similar discrepancies; while much research shows pain tolerance to be significantly higher in an athletic population, a similar trend was not seen for pain

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threshold (Tesarz, Schuster, Hartmann, Gerhardt, & Eich, 2012). It has been suggested that differences in pain tolerance among athletes may be attributed to psychological factors rather than physiological factors, and that athletes are able to tolerate a greater amount of pain due to their extensive training and exposure to higher than average levels of pain during competition. Although one study has shown an increase in pain tolerance following a 12-week aerobic training program (Anshel & Russell, 1994), further research is required to determine whether athletes are capable of tolerating more pain because of their experience participating in physical activity, or whether these individuals choose to participate in physical activity because their elevated pain tolerance lends itself towards success in a high level of competition (Tesarz et al., 2012).

While we can safely conclude that athletes will generally exhibit a higher pain tolerance when compared to sedentary individuals, it is unclear whether these effects extend to pain threshold. Additionally, there is no universal definition for the quantity and type of physical activity that classifies an individual as an athlete, and therefore many studies use different criteria in their selection of athletes and non-athletes. While the Canadian Sport and Exercise Physiology (CSEP) guidelines are commonly used to classify individuals in terms of physical activity level, the 150 minutes of moderate to vigorous physical activity per week (Tremblay, Warburton, Janssen, et al., 2011) may not be sufficient enough for researchers to be able to notice a difference in pain threshold between physically active and sedentary participants. Further insight is required into the degree to which physical activity acts as a modulator for musculoskeletal pain, in particular whether physical activity can modulate pain threshold in addition to pain tolerance. It is also important to take sex differences into account when considering pain sensitivity in athletes, a detail which seems to be consistently lacking in the majority of studies, which use either exclusively male or exclusively female subjects.

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Another recent development regarding the interaction between pain response and physical activity is the link between physical exercise and brain plasticity. In recent years, evidence has accumulated that regular exercise can have long-term effects on neuroplasticity at the cellular and molecular level. As reviewed by El-Sayes et al. (2018), aerobic exercise results in an increase in uptake of brain-derived neurotrophic factor (BDNF), a growth factor promoting neuroplasticity, into the central nervous system. Additionally, Singh et al. (2014) found that a single bout of aerobic exercise altered receptor activity in the brain, resulting in an increased excitability in the primary motor cortex. This increase in BDNF and motor cortical excitability indicate the potential for highly active individuals to exhibit a greater susceptibility to non-invasive spinal and brain stimulation, including tsDCS-related modulation of pain.

1.2.2. Sex differences in pain perception

Multiple studies attempting to determine whether or not sex differences play a factor in pain perception have yielded inconclusive results, with some reporting a higher pain tolerance from a male population (Carter, McNeil, Vowles, Sorrell, Turk, Ries, & Hopko, 2002) and others reporting a variation in pain threshold between a male and female population depending on the modality used to experimentally induce pain. These differences between sexes have been shown to be inconsistent as certain modalities have elicited higher pain responses from females, while other modalities have shown there to be no significant difference between sexes (Riley, Robinson, Wise, Myers, & Fillingim, 1998).

A recent systematic review by Racine et al. (2012) surveyed 10 years of pain research comparing eight different types of noxious stimuli in an effort to determine whether or not a true difference exists in pain tolerance, i.e., how strong or how long one can tolerate painful stimuli beyond the pain threshold, between males and females. The researchers deemed their analysis to be inconclusive, explaining that 10 years of research have been unsuccessful in determining a

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consistent pattern of sex differences in pain tolerance. Females exhibited lower tolerance than males for certain pain modalities (pressure, heat pain, and cold pain), while there was no significant difference in pain threshold for modalities such as chemical pain and ischemic pain (Racine, Tousignant-Laflamme, Kloda, Dion, Dupuis & Choinière, 2012).

In the second part of their review, Racine et al. discussed numerous biopsychosocial confounding factors that can contribute to either increase or decrease sex effects in pain studies, including gender roles and experimenter sex. Gender roles are a set of stereotypical characteristics that describe the way members of a certain sex should behave. Typically, males are seen as tougher and more stoic, while females are seen as more sensitive and emotional. Researchers believe that, due to these social constructs, individuals will react to experimental pain according to their stereotypical gender roles, giving females a greater willingness to report pain than their male counterparts (Racine et al., 2012). In addition to the role of stereotypical gender roles in pain perception, Racine et al. discussed the potential of experimenter sex affecting pain tolerance. While this seemed to be an insignificant factor in many studies, some studies reported pain tolerance to differ with respect to the sex of the experimenter and the sex of the participant. This was most notable in male participants being tested by a female experimenter, the majority of whom reported a higher pain tolerance when being tested by a member of the opposite sex (Racine et al., 2012). While experimenter sex, for the most part, appears to be irrelevant in terms of the pain tolerance of participants, the few studies in which it made a difference show that it is still an important factor to take into account for studies involving experimental pain.

In addition to the results yielded from pain threshold and tolerance research, studies on chronic pain disorders have shown that an overwhelming majority of the individuals who seek treatment for chronic pain are female (Mogil, 2012). However, there are numerous factors that could potentially contribute to this, including a greater likelihood for females to report and seek treatment

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for pain. Additionally, although many studies have reported that females exhibit a higher pain threshold than males, it is unclear whether the observed sex differences in pain perception stem from hormonal and biological differences, or whether they are influenced by socio-cultural influence and sex expectations. A recent review that sought to determine which descending inhibitory mechanisms contribute to these differences in pain threshold concluded that males show greater diffuse noxious inhibitory controls (DNIC) than females in response to noxious stimuli, contributing to the likelihood for females to report greater pain threshold (Popescu, LeResche, Truelove, & Drangsholt, 2010). However, due to the inconclusive results provided by previous studies and the lack of focus on musculoskeletal pain in particular, further research is required to determine to what degree factors such as hormonal and biological differences, socio-cultural influence, and gender expectations influence the differences in pain threshold between males and females.

1.3 Pressure pain threshold

Pressure pain threshold (PPT) is a technique used to assess sensitivity to mechanical pain through the use of a pressure algometer, which is used to apply constant pressure to a stimulus area. The PPT is the point at which the amount of pressure being applied produces a local change in sensation in the stimulus area, from pressure to pain (Jones, Kilgour, & Comtois, 2007). PPT algometry has shown to be a reliable technique, and is used both clinically, in the assessment of fibromyalgia and other regional pain syndromes (Granges & Littlejohn, 1993), as well as in experimental trials involving sensitivity to mechanical pain.

While PPT measurements have been shown to be consistent between multiple examiners, PPT varies greatly depending on the region being examined (Keating, Lubke, Powell, Young, Souvlis,

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& Jull, 2001; Defrin, Ronat, Ravid, & Peretz, 2003), and can even vary along the length of a muscle such that two different PPT values can be obtained from the two different body areas on the same muscle (Binderup, Arendt-Nielsen, & Madeleine, 2010). Additionally, one study observed a decrease in PPT values taken in the same 8 locations over four consecutive days of testing, with PPT values on day 4 being significantly lower than baseline measures taken on day 1 (Jones et al., 2007).

Several studies have shown sex differences in PPTs elicited from males compared to those elicited from females, although these differences seem to be inconsistent. In one such study, lower PPTs were reported in females when the erector spinae muscles were assessed, but examination of the masseter muscle showed no difference in PPT between males and females (Binderup et al., 2010). Another study, however, tested PPT in three different body areas; the hand, pain-free back, and myofascial trigger points (MTPs) in the back, reporting no differences between male and female PPT in any of these three regions (Defrin et al., 2003). It is likely that the cause of sex differences with regards to PPT is multi-factorial, and is affected in part by the body area tested in addition to being affected by hormonal differences and socio-cultural expectations. It has been suggested that the menstrual cycle has the potential to affect PPT, although this has been challenged by a study showing no effect of the menstrual cycle on PPT in the trapezius muscle (Binderup et al., 2010). If the menstrual cycle does, in fact, affect PPT, it is possible that these effects only occur in certain muscles.

1.4 Neurostimulation in the management of pain

Historically, pharmacological interventions have been the primary method of pain relief for individuals with musculoskeletal pain disorders. However, in many cases, pharmacological treatment alone is not sufficient in improving quality of life among patients with chronic pain

disorders, and will sometimes even result in decreased quality of life due to adverse effects of medications (Hansson, Attal, Baron, & Cruccu, 2009). Also, medication can provide an unnecessary expense, particularly when there are less expensive alternatives that can be used to treat musculoskeletal pain. For this reason, there has been an increasing amount of interest surrounding neurostimulation techniques as an alternative to pharmacological interventions. Cortical stimulation as a method of pain relief was originally introduced as an invasive surgical procedure involving the implantation of an electrode in the motor cortex under general anaesthesia in an effort to treat individuals with thalamic pain (Tsubokawa, Katayama, Yamamoto, Hirayama, & Koyama, 1991). While the procedure was found to be effective, with most participants reporting improved pain management even in the absence of medications (Tsubokawa et al., 1991), recent research has turned to non-invasive brain stimulation techniques, such as transcranial magnetic stimulation (TMS) and transcranial direct current stimulation (tDCS) as a less costly and less invasive way of modulating pain perception.

1.4.1. Transcranial direct current stimulation

Trans-cranial direct current stimulation (tDCS) is a form of non-invasive neurostimulation commonly used to modulate pain in studies involving experimental pain. tDCS functions via a subthreshold modulation of the membrane potentials of cortical cells in the brain, resulting in an alteration in excitability in the cortex. Excitability is either increased or decreased depending on the direction of flow of the current and the target neurons of the stimulation (Woods, Antal, Bikson, Boggio, Brunoni, Celnik, ... & Nitsche, 2016). The tDCS technique involves the placement of two saline-soaked electrodes on the scalp, which deliver a weak electrical current (1-2 mA) from a stimulation device to the scalp. The current itself passes through the brain in a path determined by the placement of the two electrodes. The process is virtually painless; participants will feel nothing but a small tingling sensation at the beginning and end of the stimulation (Woods et al., 2016).

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Modulatory effects of tDCS can be achieved either via anodal or cathodal stimulation. Anodal stimulation tends to depolarize cortical cells and thus increases cortical excitability, whereas cathodal stimulation tends to hyperpolarize cortical cells and thus reduces cortical excitability (Dayan, Censor, Buch, Sandrini, & Cohen, 2013; Lefaucheur, 2013). However, many studies have found the effects of tDCS to be quite variable between people (Lefaucheur, 2013). Due largely in part to the complex nature of pain, many studies involving the effect of direct current stimulation on pain have yielded a variety of results. Direct current stimulation seems to carry the potential to either increase or decrease pain threshold depending on the form of stimulation used, the sample population, and the pain modality.

In general, past research has shown that tDCS can be successful in relieving both experimental and clinical pain models. One such study by Vaseghi et al. showed increases in both sensory threshold and pain threshold following cathodal tDCS of the primary motor cortex (M1), sensory cortex (S1), and dorsolateral prefrontal cortex (DLPFC) (Vaseghi, Zoghi, & Jaberzadeh, 2015). In line with these results, Boggio and colleagues found that pain threshold increased following anodal tDCS of M1 in healthy volunteers, although they observed no effect on either pain or sensory threshold after tDCS over DLPFC (Boggio, Zaghi, Lopes, & Fregni, 2008). Another study investigating tDCS of the cerebellum noted that anodal stimulation produced an analgesic effect in participants who were exposed to laser evoked potentials (LEPs) (Bocci et al., 2015). Additionally, yet another team reported an increase in mechanical pain threshold in a small number of subjects following tDCS (Mylius et al., 2012). In general, these studies considered the acute effects of tDCS on pain perception, and therefore it is uncertain what long-term effects the stimulation may have had. However, several studies that looked more closely at the long-term effects of clinical tDCS showed the potential for effects to last for up to two months following a series of 10 sessions of stimulation (Lefaucheur, 2013).

1.4.2. Transcutaneous spinal direct current stimulation

Transcutaneous spinal direct current stimulation (tsDCS) is a non-invasive technique that can be used to modulate spinal function and follows a protocol similar to that of tDCS. However, in the case of tsDCS, the direct current is directed to the back to reach the spine as opposed to the cortex via the scalp, with one electrode placed over the spine and one placed over the shoulder. Two recent studies have investigated the potential for tsDCS to modulate pain in experimental trials, yielding promising results. In an anodal tsDCS study conducted by Meyer-Frießem and colleagues., tsDCS applied over the thoracic spine was shown to decrease sensitivity to heavy mechanical stimuli for up to an hour following stimulation (Meyer-Frießem et al., 2015). Another study showed that anodal tsDCS, once again applied over the thoracic spine, was able to modulate laser evoked potentials, increasing pain tolerance in healthy participants (Truini, Vergari, Biasiotta, et al., 2012). Although the neural mechanism underlying the antinociceptive effects of tsDCS remains speculative, one likely possibility is through “anodal block”; anodal stimulation leading to a hyperpolarisation of axons in the ascending pathways relaying nociceptive inputs (Nardone, Höller, Taylor, Thomschewski, Orioli, et al., 2015). However, to date we have still limited observations on the therapeutic effects of tsDCS, particularly where musculoskeletal pain is concerned, and additional research is required to further define the analgesic potential of tsDCS in a clinical setting.

Objectives of the present work

In this study, we investigated how tsDCS application can modulate mechanical pain threshold as reflected in pressure pain threshold in the lower extremity. Additionally, we sought to determine whether sex and physical activity level influence tsDCS-induced modulation of pressure pain threshold in the lower extremity.

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Hypotheses

Given the evidence reviewed above with regards to physical activity level and pain threshold, it was anticipated that PPTs would be unaffected by physical activity level. With regards to tsDCS effects, it was hypothesized that PPTs would be elevated in all participants immediately after the application and up to 30 min post-application. In addition, given the role of physical activity in modulating neural plasticity, we also hypothesized that the extent of pain reduction post-tsDCS would be influenced by the level of physical activity, highly active individuals showing greater response than low or moderately active individuals. Finally, it was hypothesized that sex would have no effect on PPT measured pre- and post-tsDCS.

Chapter II: Methods

2.1. Participants

Based on the minimal clinically important differences (14.7 N/cm²) reported for PPT (Chesterton et al., 2007), we performed a power analysis. This analysis showed that a sample size of 12 in each group has a 90% power to detect a difference between means of 14.45 with a significance level (alpha) of 0.05 (two tailed). In addition, a recent meta-analysis (Luedtke et al., 2012) on the impact of tDCS application on experimental and clinical pain showed a favourable outcome on pain perception with a relatively large effect size of 2.39. Based on this report and our power analysis, we recruited 35 participants for this study.

Participants consisted of healthy young adults between the ages of 18 and 30 (n=35, 15 males; mean age = 22.5 ± 2.7 years), who were asked to come to the lab for one testing session lasting approximately 2 hours. For the purpose of this study, health was defined as the absence of chronic conditions such as multiple sclerosis or rheumatoid arthritis, and other musculoskeletal conditions such as back pain or leg pain. Prior to the experimental session, participants were screened for contraindications to spinal DC stimulation such as skin conditions (scars, lesions, psoriasis) that may have interfered with the application. Also, given that the PPT testing targeted the lower extremity, participants were also screened for any antecedents of recent fracture or other injuries to the lower extremity in the six months prior to enrolment. All participants received a \$20 honorarium to cover parking and transportation fees associated with their participation in the study. Handedness was determined using the Edinburg Hand Inventory Index (Appendix C).

Participants were recruited through the use of posters, and through community groups on the internet. In particular, our highly active participants were recruited primarily from local triathlon and running groups in the community. Potential participants who expressed interest in the study were given an information sheet and a consent form, which they were be asked to read carefully. Upon reading the information sheet and providing verbal consent, participants were

asked to sign a consent form prior to testing. The procedures in this study were approved by the Bruyère Research Ethics Board (Protocol #M16-17-025, Appendix A).

2.2. Group assignment and experimental design

Figure 1 illustrates the experimental design, which consisted in a repeated-measures prepost-test design. As shown in the Figure, after recruitment, participants were assigned to activity groups based on the International Physical Activity Questionnaire (IPAQ, Appendix B). This questionnaire was used to assess physical activity level of all participants. The IPAQ is a questionnaire composed of seven questions aiming to assess physical activity level by placing an individual in one of three categories (sedentary, moderately active, or highly active) based on the quantity of self-reported physical activity (moderate-intensity, vigorous-intensity, or walking) accumulated in one week. Although the IPAQ has been shown to over-estimate physical activity level (Lee, Macfarlane, Lam, & Stewart, 2011), it remains the recommended and most widely used method. As shown in Figure 2, according to the IPAQ, 14 participants (eight women) were classified as having a high physical activity profile, which consisted of vigorous-intensity activity on at least 3 days accumulation at least 1500 MET-minutes/week or 7 days of any combination of walking, moderate- or vigorous-intensity activities accumulating at least 3000 METminutes/week. These participants were therefore assigned to the highly active (HIGH) group. Most of these individuals (9/14) were endurance athletes involved in activities requiring high levels of endurance such as triathlon, marathon, or varsity cross country running. The remainder (5/14) were either varsity athletes involved in university sport (swimming) or athletes involved in high levels of competition (Crossfit, powerlifting). For the remaining participants (n=21), 16 were classified as moderately active, which corresponded to ≥ 3 days of vigorous-intensity activity or ≥ 5 days of moderate-intensity activity or walking (30 min/day), or ≥ 5 days of any combination of walking,

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moderate-, or vigorous-intensity activities accumulating at least 600 MET-minutes/week. Most of them were involved in sporting or training activity at a recreational level (e.g. recreational running or cycling, intramural sports). The last six participants reported minimal physical activity and thus, were classified in the low active category. However, given their small number, the latter individuals were included with the group of moderately active participants to form a LOW-MOD group (n=21, 12 women). The demographic characteristics of the participants in the two activity groups are shown in Table 1.

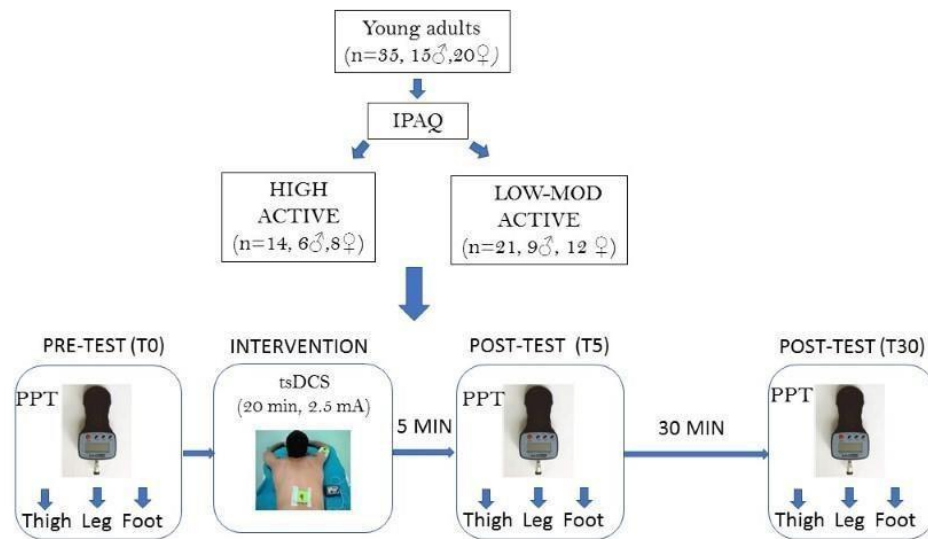


Figure 1: Group Assignment and Experimental Design

IPAQ: International Physical Activity Questionnaire, PPT: Pressure pain threshold, tsDCS: trans-spinal direct current stimulation

Table 1. Demographics of the participants in the two activity groups

<i>Activity Level</i>	Males (n)	Females (n)	Age (years)
			(Mean ± SD)

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High (n=14)	6	8	22.1 ± 3.7
Low-Mod (n=21)	9	12	22.8 ± 2.1
Total	15	20	22.5 ± 2.7

After being assigned to activity groups, participants underwent pre-testing to determine pain threshold. This was followed immediately by the tsDCS intervention for 20 min. After the intervention, pain thresholds were assessed again at two time points: 5- and 30-min postapplication (see Figure 1).

2.3. Pressure pain threshold (PPT) testing

PPT was determined at three time points: 1) prior to tsDCS (T0), 2) within 5 min after tsDCS (T5), and 3) 30 min post-tsDCS (T30). At each time point, the PPT was determined on the dominant leg (Right, n=31) at three different sites: 1) anterior thigh aspect (Thigh) over the *Rectus femoris*, 2) anterior upper leg (Leg) over the *Tibialis anterior*, and 3) dorsal aspect of the forefoot (Foot). At each location, the PPT was determined using a digital algometer (Wagner Ind., Force Ten™, CT, USA) by applying a gradual constant pressure until pain was elicited in the tested area. During testing, participants were instructed to report verbally as soon as they felt the sensation changing from mechanical pressure to painful stimulation. The amount of pressure in Newtons (N) at which pain was first perceived was then recorded. This procedure was repeated three times at each site proceeding sequentially from the thigh down to the foot. The PPT was determined for each site by averaging the three pressure recordings for each participant. All measurements were taken on the dominant side of the body, which was determined by the Edinburgh Handedness Questionnaire (Appendix C).

2.4. Spinal DC stimulation (tsDCS)

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As shown in Figure 2, the tsDCS application was performed with participants lying prone on a comfortable treatment table, using a constant direct current stimulator (SmartStim™ Model 1000, Neuraleve Inc, Ottawa, Ontario) connected to a pair of rectangular silicone-carbon electrodes (7 X 5 cm). The application consisted of anodal stimulation with the anode placed over the spinous process of the 10th thoracic vertebra and the cathode placed above the right shoulder (Figure 2). This montage was used according to previous studies showing anti-nociceptive effects (Meyer-Frießem et al., 2015; Truini et al., 2012). Prior to application, each electrode was enclosed in a saline-soaked (0.9% solution) sponge. Participants were asked to relax and limit movement during the stimulation, which was delivered for 20 min at 2.5 mA (0.0271 mA/cm² current density), in line with previous protocols reported to be effective in modulation pain perception (Meyer-Frießem et al., 2015; Truini et al., 2012).

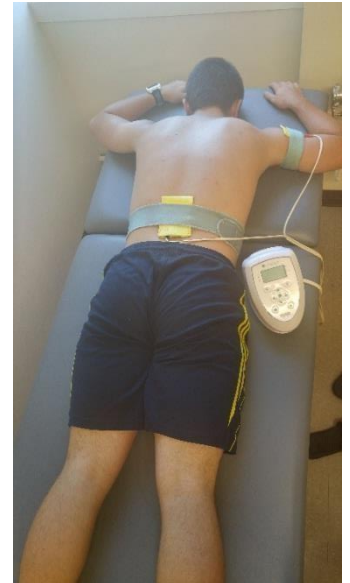


Figure 2. Illustration of the montage used for trans-spinal direct current stimulation. Note that the anode is placed over the thoracic spine and the cathode on the right shoulder. The application lasted for 20 min with a current of 2.5 mA corresponding to a current density of 0.071 mA/cm².

2.5. Data analysis

The statistical analysis was performed in two steps. First, to detect possible differences at baseline in relation to sex and physical activity, we applied a multivariate analysis of variance (MANOVA)

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on PPTs determined at the three sites (Thigh, Leg, Foot). Then, we applied a similar analysis to examine the effects of the tsDCS intervention. To this end, individual PPT measured at the three sites were entered into 3 X 2 X 2 MANOVA with Time as the within-subjects factor (T0; T5; T30) and Group (HIGH, LOW-MOD) and Sex (Male, Female) as the between-subjects factors. Upon detection of main effect or interaction, univariate tests were examined to determine the impact of factors on each dependant variable. The significance level was set at $p < 0.05$ for all tests. Analysis was performed with the SPSS software package 20.0. Graphs were produced using GraphPad Prism version 7.00 for Windows (GraphPad Software, La Jolla California, USA, www.graphpad.com).

Chapter III: Results

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3.1. General observations

Spinal stimulation was well tolerated by all participants, with minimal complaints of mild discomfort and itching following the 20-min tsDCS session. As mentioned, PPT determinations were performed on the right dominant leg for 31 participants. For four other participants, PPTs were determined on the left leg, with three being left handed. For one right-handed participant, the presence of blisters on the right thigh prevented PPT testing and thus, the left leg was tested. No lasting side effects of tsDCS or PPT testing were reported by any participants.

3.2. Comparison between groups at baseline (T0)

As mentioned in the Methods, we ran analysis on PPTs measured at T0 to assess baseline differences in pain thresholds in relation to physical activity level and sex. Figure 3 compares the mean PPTs measured at the three different sites for men and women according to their activity group. It can be seen that for female participants, PPTs at baseline were quite comparable between activity groups. For males, the HIGH group tended to show higher thresholds than the LOW-MOD group, but the small number of male participants in the HIGH group might have inflated the difference. Results of the MANOVA confirmed that PPTs at baseline (T0) were not significantly influenced either by Group or Sex ($F < 1.32$, $p > 0.28$). Thus, while there were some variations attributable to activity group (males only), overall participants presented with very similar PPTs at baseline at the three tested sites. All data within groups were normally distributed.

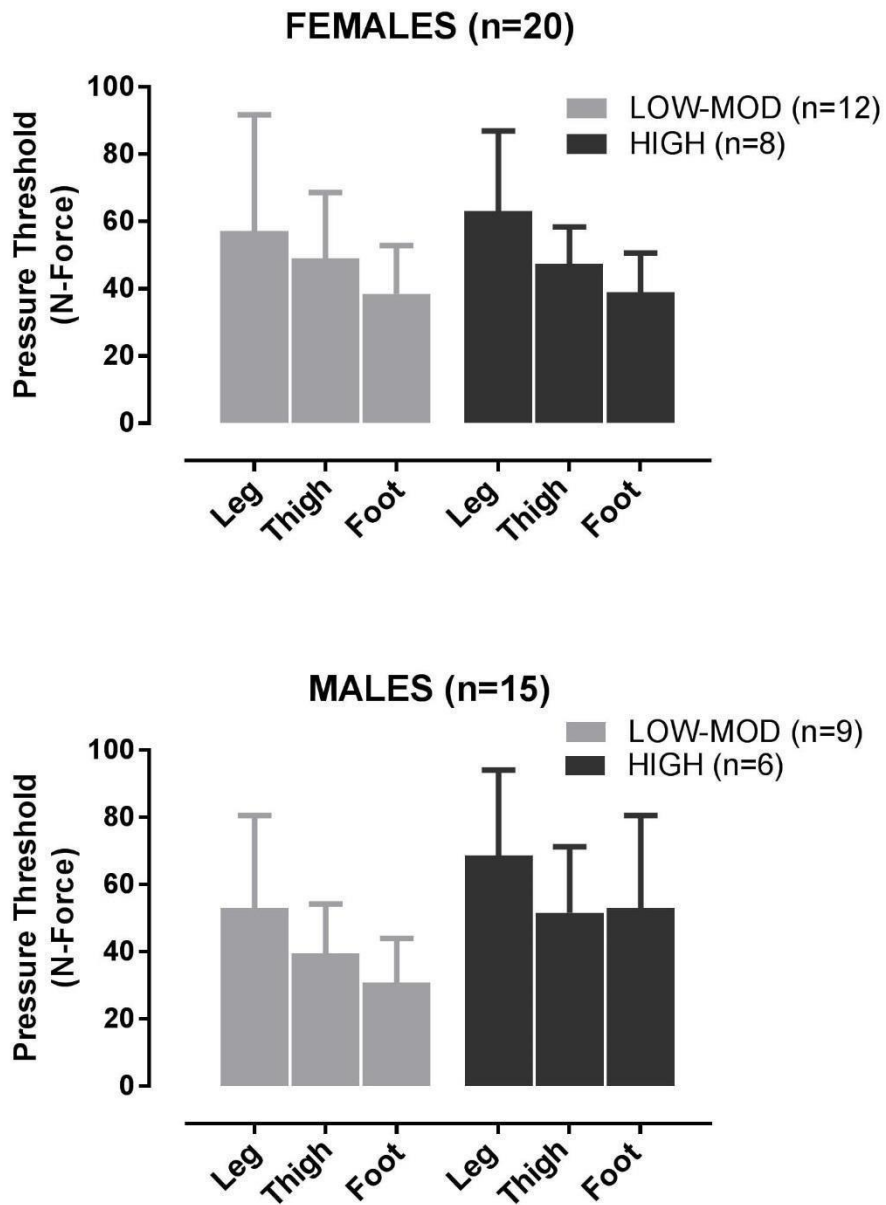


Figure 3. Mean (\pm SD) pressure pain threshold measured at the three sites at baseline (T0) in female and male participants of each activity group (LOW-MOD, Low-Moderately active; HIGH, highly active).

3.3. Effects of tsDCS

The mean PPTs computed at the different time points before and after tsDCS for the three sites are represented in Figure 4 for male and female participants of the two activity groups. Three

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important observations can be seen from inspection of Figure 4. First, irrespective of activity level, both men and women tended to show similar PPT across the three sites tested and at all time points. Second, with respect to the tsDCS intervention, while PPT tended to be elevated at T5 and T30 at all sites, the elevation was particularly noticeable at the leg and thigh. Third, with regards to activity level, participants in the HIGH group tended to show larger elevation than their counterparts in the LOW-MOD group, especially at the leg and thigh sites. The latter effects can be further appreciated by inspecting Figure 5, where variations in PPT at the three time points for the three sites are compared between the two groups when including male and female participants. The elevation of pain thresholds post-intervention was confirmed by the MANOVA, where a main effect of Time ($F = 5.3$; $p = 0.001$) was detected. In addition, a significant “Time X Group” interaction was detected ($F = 2.8$; $p = 0.03$), while no effect or interaction was found for Sex. The large main effect of Time on PPTs measured at all sites was confirmed by the univariate tests ($F > 16.2$, $p > 0.001$), owing to the raise in PPT noticed at T5 and T30. Post-hoc comparisons with the Bonferonni test further confirmed that PPT values were significantly elevated at T5 and T30 when compared to T0 ($p < 0.001$) at all sites. With regards to the “Time X Group” interaction, univariate tests indicated that the interaction reflected the larger elevation in PPT at the thigh site observed in the HIGH group post-tsDCS (i.e. at T5 and T30) when compared to the LOW-MOD group ($F = 4.399$; $p = 0.03$, Figure 5).

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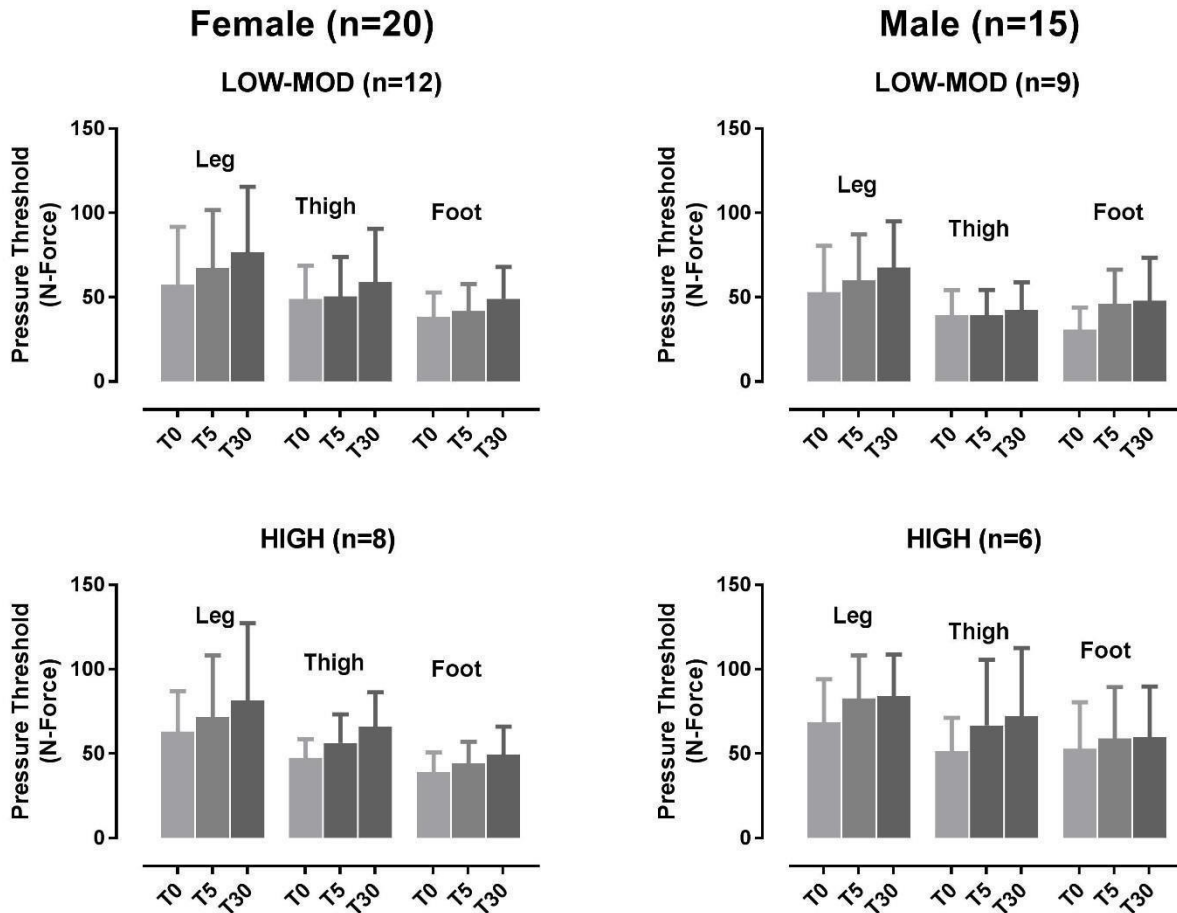


Figure 4. Mean (\pm SD) pressure pain threshold measured at the three sites at the different time points relative to the tsDCS (T0, pre-test, T5, 5 min post, T30, 30 min post) intervention in females and males of each activity group. Note the elevation of PPTs post-intervention at all sites, irrespective of sex and groups. There was a significant effect of Time overall ($F=5.3$, $p<0.001$).

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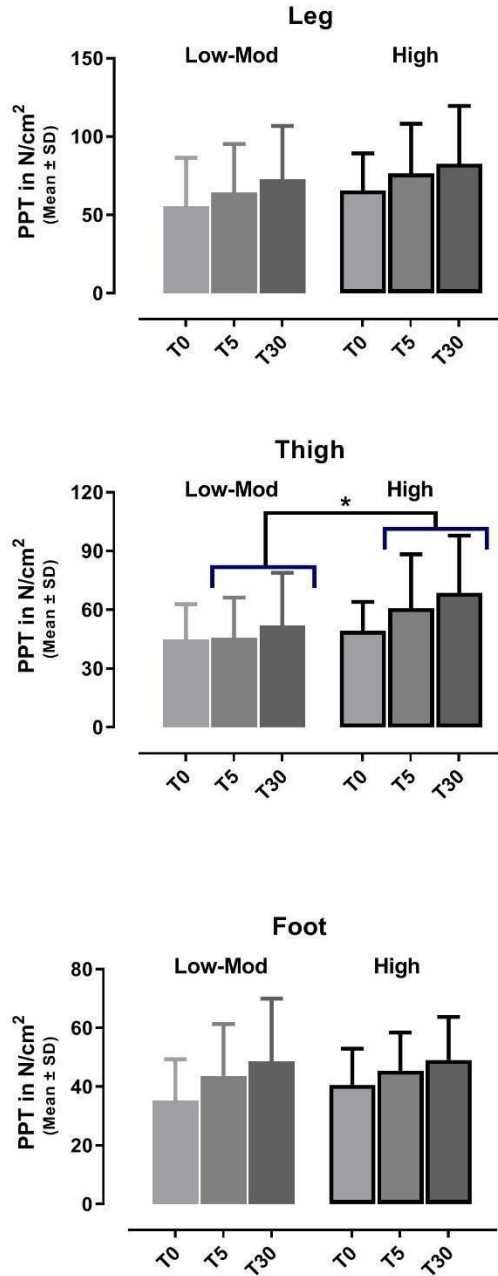


Figure 5. Mean pressure pain threshold measured in participants at each site according to activity level. Note the significant “Time X Group” interaction for the Thigh site owing to a larger increase in PPTs in the highly active group.

Chapter IV: Discussion

INFLUENCES OF tsDCS ON MECHANICAL PAIN THRESHOLD

4.1. Influence of physical activity level and sex on PPT at baseline

Consistent with previous research (Tesarz et al., 2012), we did not find differences in PPTs at baseline (T0) between the two physical activity groups (HIGH, LOW-MOD). Furthermore, no differences in baseline PPTs were noted between the two sex groups (Male, Female). These findings are once again consistent with previous studies, the majority of which reported no differences in pain threshold between males and females even when a difference in pain tolerance was observed (Riley et al., 1998; Racine et al., 2012).

Despite evidence that physically active individuals generally exhibit a higher pain tolerance than their sedentary counterparts (Flood et al., 2017; Flood et al., 2016; Anshel et al., 2014), it appears that these physiological adaptations do not extend to mechanical pain threshold. Rather, our findings were more consistent with those of Tesarz and colleagues, who suggested that pain threshold and pain tolerance reflects distinct processes, and that, although athletes are capable of training their bodies to tolerate greater amounts of pain, this learned effect does not extend to threshold when experiencing pain (Tesarz et al., 2012). The fact that our two groups (HIGH and LOW-MOD) exhibit similar PPT at T0 (Baseline) is consistent with the notion that greater tolerance to pain in athletes does not translate into higher pain threshold.

4.2. Effects of tsDCS

As expected, the influences of the tsDCS intervention on mechanical PPT in the lower limb were consistent with antinociceptive effects reported in previous studies (Boggio et al., 2008; Bocci et al., 2015; Mylius et al., 2012). In fact, in accord with our prediction, we found a large effect of Time, which was explained by the significant elevation of PPTs recorded immediately post-tsDCS in all three testing sites. Furthermore, the fact that these effects were still detectable at 30 min postintervention confirms the ability of anodal tsDCS to induce lasting modulation of nociceptive

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transmission. In fact, mean changes in PPT at the leg (18 N/cm²) and thigh (14 N/cm²) were at or above the minimum level reported for clinical important difference (i.e. 14 N/cm²), indicating that the observed changes in mechanical pain threshold were clinically meaningful. In many respects, our results are similar to those reported by Meyer-Frießem et al. (2015) who reported significant reduction in pain ratings and in response to heavy mechanical pinprick up to 60 min post tsDCS. In their study, reduction in pain ratings were not associated with reduction in mechanical pain threshold. However, their observations were based on measures of mechanical pain derived from pinprick stimulation with a very small probe (0.25 mm), which makes comparisons with our PPT measures obtained with a 100 mm probe difficult. Another report by Truini et al. (2015) showed that the application of the same tsDCS protocol as we used reduced nociceptive potentials evoked by laser stimulation on the foot and increased pain tolerance to immersion in cold water. Here again no effects were observed on actual pain thresholds evoked either by the laser or by the cold immersion. As suggested by Meyer-Frießem and colleagues (2015), the fact that pain thresholds seem to be less affected by tsDCS may indicate that the modality is more effective in response to stronger activation of nociceptive afferents. In the case of our study, as alluded before, the fact that our PPTs were obtained from a relatively large probe (compared to either mechanical pinprick or focal laser stimulation) may explain the effects we observed on pain thresholds since by virtue of spatial summation large populations of mechano-nociceptive afferents were likely activated by the pressure stimuli. In line with this, a recent report by Perrotta et al. (2016) showed that a 15 min anodal tsDCS application induced a long-lasting (up to 60 min) increase in the threshold for temporal summation of the nociceptive withdrawal reflex elicited at the ankle. Such results, along with the current observations, suggest that spatial and temporal summation of nociceptive inputs are likely critical factors influence tsDCS-induced pain modulation.

With regards to the neural mechanisms involved in changes in PPTs observed, in the absence of a sham stimulation condition, we cannot exclude the possibility that placebo effects might have contributed to tsDCS modulation. However, such effects were likely negligible given that all participants were naïve with regards to the technique, thereby reducing any anticipation. In addition, there is solid evidence from controlled studies, as reviewed earlier, regarding the physiological basis of tsDCS as a means to modulate spinal transmission non-invasively. Finally, the fact that we observed large and consistent effects across sites and across participants, irrespective of sex or group, strengthens our contention that the tsDCS modulation was real and physiologically meaningful. As proposed by Cogiamanian et al. (2016), hyperpolarisation of axons (i.e. “anodal block”) travelling along the posterior columns and the spinothalamic tract is likely the mechanism through which tsDCS can induce lasting reduction in pain perception.

4.3. Physical activity level

Consistent with our hypothesis, an interaction was found between physical activity level and tsDCS modulation of PPT. In response to the tsDCS application, participants in the HIGH group exhibited a lower degree of pain sensitivity in the thigh, as reflected in elevation in PPTs when compared to participants in the LOW-MOD group. The reason as to why this interaction was significant only for the thigh site is not clear but might be related to the greater pain sensitivity of this area (Mean PPT @ T0, 47 N/cm²) vs the other sites, notably the leg (Mean PPT @ T0, 69 N/cm²). However, the foot exhibited a similar sensitivity to that of the thigh (Mean PPT @ T0, 46 N/cm²) whereas no interaction was found. Yet, given that the area over the rectus femoris at the thigh is larger and softer than either the leg or foot, it is entirely possible that the stimulating probe produced a sharper stimulation during pressure testing leading to greater mechanical pain, hence the greater modulation at the thigh. In the absence of pain ratings, however, this possibility remains

speculative for now. Whatever the reasons, finding an interaction between tsDCS effects and activity level suggests that the latter factor may modulate responses to tsDCS interventions. In particular, our results suggest that highly active individuals, notably those engaged in endurance training, may exhibit enhanced response to neuromodulation induced by tsDCS over inactive or less active individuals. While the present results would be in line with the notion that athletes in general exhibit greater ability to modulate pain processing (e.g. more efficient pain inhibition) than non-athletes (Flood et al., 2017; Tesarz et al., 2012), the current observations pointing to physical activity level as a potential factor modulating pain relief in response to neuromodulation raises new perspectives.

As reviewed in the Introduction, there is now evidence that participation in regular aerobic physical activity is associated with enhanced neuroplasticity, notably through alterations in BDNF level (El-Sayes et al., 2018). Participation in regular exercises is also associated with changes in brain structure with increases in grey matter volume and white matter in several cortical areas. Given that our HIGH activity group was composed mainly of endurance athletes, it is likely that they exhibited some of the cellular and structural changes associated with practice of regular aerobic exercises. These changes, particularly more efficient BDNF CNS uptake, might have contributed to enhance their responsiveness to tsDCS, leading to a greater lasting modulation of nociceptive inputs after the intervention. In this respect, our results would be in line with reports showing that individual variations in BDNF level and in BDNF polymorphism influence brain plasticity in response to non-invasive brain stimulation protocols (e.g. Cheeran et al., 2008, Cirillo et al., 2012). Alternatively, it is also possible that tsDCS was more effective in modulating pain perception in the HIGH activity group simply because they exhibit greater local response at the spinal level leading to a more efficient “anodal block”, although such an explanation could hardly account for the fact that the interaction was found for only one site and was not affected by sex.

4.4. Clinical Relevance

The ability of the tsDCS intervention to produce a continued analgesic effect for up to thirty minutes following stimulation indicates the potential for tsDCS to be used as a therapeutic intervention in order to alleviate pain in a clinical setting. Additionally, while the majority of pain research has used electrical or thermal stimuli to induce pain, our study was centered around a mechanical model of pain, which is more similar to the type of pain that individuals suffering from chronic musculoskeletal pain disorders might experience. Furthermore, the sites we chose to use for PPT testing correspond to trigger points commonly used to diagnose fibromyalgia. The success of the tsDCS intervention at producing an analgesic effect in these specific areas points to its potential for alleviating pain among fibromyalgia patients. As we continue to explore the clinical potential of neurostimulation, it is important to consider the addition of a physical activity regimen in conjunction with neurostimulation, to ensure that individuals are receiving the greatest possible advantages from the stimulation.

4.5. Limitations

We originally hypothesized that the HIGH group would exhibit a greater response to the tsDCS intervention than the LOW-MOD group. Although this was only shown to be the case in one PPT testing location (Thigh), it is possible that our sample size of highly active participants was simply not large enough to yield significant results in the other two PPT testing locations. Furthermore, due to difficulty recruiting sedentary participants, it was necessary for us to combine the sedentary and moderately active individuals into a single physical activity group (LOW-MOD), despite their differences in activity level. In the future, a study with three different physical activity groups (HIGH, MOD, LOW) would indicate whether a similar difference in

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tsDCS-related modulation of PPTs to the one noted in our study exists between the HIGH and MOD groups alone in the absence of sedentary participants, which, in turn, could lend further insight into what level of physical activity is required in order to affect tsDCS modulation of pain.

While some might consider the absence of a sham condition a limitation of our study, we feel that sham stimulation has been tested on multiple occasions (Bocci et al., 2015; Boggio et al., 2008; Meyer-Frießem et al., 2015) where it has been shown to be ineffective with regards to the modulation of pain when compared with real stimulation. Since all of our participants were naïve with respect to the PPT testing protocol, it is unlikely that anticipation had any effect on the tsDCS-related modulation of PPT. However, future studies might consider the addition of a sham condition in order to clarify the effects of any anticipation that may have been present.

In addition, although we tested PPTs at multiple locations and time points throughout our study, we did not provide a way for participants to rate the severity of the pain experienced. Although we would not anticipate mechanical pain to be incredibly severe at an individual's pain threshold, the addition of pain ratings to our protocol would have added an additional layer of complexity to our results. Future studies might consider the addition of pain ratings to the protocol to provide insight into whether physical activity level or sex have any effects on the severity of the pain experienced at threshold.

Finally, although we took effort to ensure that our experimental pain model was as similar as possible to a clinical pain model through the use of mechanical stimuli in lieu of thermal or electrical stimuli, the fact that all of our participants were healthy adults with no history of chronic pain limits our understanding of how the tsDCS intervention would affect those with chronic pain disorders. Future studies should focus on the effects of tsDCS on pain modulation in a clinical

population, in addition to considering a physical activity intervention to aid in the tsDCS-related modulation of pain.

4.6 Conclusion

In conclusion, the present study provides further confirmatory evidence of the ability of anodal tsDCS to modulate experimental pain perception in human participants. In particular, our results point to the role of participation in physical activity as an important factor susceptible to modulate responses to tsDCS interventions at the individual level. As stressed before, the present results have also potential implications for tsDCS applications in clinical populations affected with chronic musculoskeletal pain. Future studies with chronic pain patients should consider adding physical activity as a means to boost analgesic effects linked with tsDCS interventions.

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Appendix B

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the **vigorous** activities that you did in the **last 7 days**. **Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. Think *only* about those physical activities that you did for at least 10 minutes at a time.

1. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, aerobics, or fast bicycling?

_____ days per week

No vigorous physical activities



Skip to question 3

2. How much time did you usually spend doing **vigorous** physical activities on one of those days?

_____ hours per day _____ minutes per day

Don't know/Not sure

Think about all the **moderate** activities that you did in the **last 7 days**. **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal.

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Think only about those physical activities that you did for at least 10 minutes at a time.

3. During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads, bicycling at a regular pace, or doubles tennis? Do not include walking.

_____ days per week

No moderate physical activities



Skip to question 5

4. How much time did you usually spend doing **moderate** physical activities on one of those days?

_____ hours per day _____ minutes per day

Don't know/Not sure

Think about the time you spent **walking** in the **last 7 days**. This includes at work and at home, walking to travel from place to place, and any other walking that you might do solely for recreation, sport, exercise, or leisure.

5. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time?

_____ days per week

No walking



Skip to question 7

6. How much time did you usually spend **walking** on one of those days?

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___ hours per day ___ minutes per day

Don't know/Not sure

The last question is about the time you spent **sitting** on weekdays during the **last 7 days**. Include time spent at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading, or sitting or lying down to watch television.

7. During the **last 7 days**, how much time did you spend **sitting** on a **week day**?

___ hours per day _____ minutes per day

Don't know/Not sure

This is the end of the questionnaire, thank you for participating.

Scoring protocol for IPAQ short and long

Continuous score a) IPAQ short:-

First the total minutes of walking, moderate and vigorous intensity activity per week was calculated by multiplying the minutes of an activity per day by the no of days per week the activity was reported.

- Walking MET-minutes/week = $3.3 * \text{walking minutes per day} * \text{no of days per week}$ in which walking was reported.
- Moderate MET-minutes/week = $4.0 * \text{moderate intensity minutes per day} * \text{no of days per week}$ in which moderate intensity activity was reported.
- Vigorous MET- minutes per week = $8.0 * \text{Vigorous intensity minutes per day} * \text{no of days per week}$ in which vigorous intensity activity was reported.
- Total physical activity MET-minutes/week = Walking + Moderate + Vigorous MET minutes/week scores.

The MET scores of 3.3, 4.0 and 8.0 for walking, moderate and vigorous intensity activity were assigned as per the IPAQ scoring protocol.

Categorical score

Three categories of physical activity were defined for the IPAQ short.

Category 1 - Low physical activity level:

Those individuals who did not meet criteria for Categories 2 or 3 were put in this category and considered to have a low physical activity level.

Category 2- Moderate physical activity level:

- At least 20 minutes of vigorous intensity activity per day for three or more days per week OR
- At least 30 minutes of moderate intensity activity per day for 5 or more days per week OR
- Five or more days of any combination of walking, moderate-intensity or vigorous intensity activities achieving a minimum Total physical activity of at least 600 METminutes/week.

Category 3- High Physical activity level

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- Vigorous-intensity activity on at least 3 days achieving a minimum Total physical activity of at least 1500 MET-minutes/week OR
- Five or more days of any combination of walking, moderate-intensity or vigorous intensity activities achieving a minimum Total physical activity of at least 3000 MET-minutes/week.

Appendix C

Handedness Questionnaire

Edinburgh Handedness Inventory

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

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

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

	Left	Right
1. Writing	<input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/>
2. Drawing	<input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/>
3. Throwing	<input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/>
4. Scissors	<input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/>
5. Toothbrush	<input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/>
6. Knife (without fork)	<input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/>
7. Spoon	<input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/>
8. Broom (upper hand)	<input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/>
9. Striking Match (match)	<input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/>

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10. Opening box (lid)	<input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/>
	<input type="checkbox"/>	<input type="checkbox"/>
<u>TOTAL(count X's in both columns)</u>		

Scoring:

Add up the checks in both left and right columns.

Whichever number is greater, would be considered your handedness.