Anodal Transcranial Direct Current Stimulation does not induce Analgesic Effects on

Experimentally Induced Pain

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Declaration

I declare that this thesis is my own account of my research and contains as its main

content work that has not previously been submitted for a degree at any tertiary educational

institution.

Name*: Brandon Scherrer*   Signature*:* _____

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Abstract

Chronic pain is a disabling condition in which the adaptive link between pain intensity and tissue damage is lacking. Transcranial direct current stimulation (tDCS) is a non-invasive brain stimulation technique that induces analgesic effects on experimentally induced pain when applied at the primary motor cortex (M1), and the dorsolateral prefrontal cortex (DLPFC). However, whether greater analgesic effects occur when tDCS is applied simultaneously at the M1 and the DLPFC is unknown, and is the primary aim of the current study. Nineteen healthy adult volunteers (12 male; $M_{age} = 29.21$, $SD = 10.78$, range 20 to 52) participated in a double blinded, crossover, sham controlled, randomised design. Dependent variables were self-reported pain ratings to punctuate pinprick stimuli, and the current level required of electrical stimulation to elicit moderate pain. These ratings were obtained pretest, posttest, and follow up of 20 min of anodal tDCS applied at the M1, DLPFC, M1 + DLPFC, or sham tDCS. Results indicate that pain to pinprick stimuli and the current level required to elicit moderate increased from pretest, posttest, and follow up. However, this was irrespective of the tDCS condition administered. Methodological inconsistencies pertaining to the administration of tDCS in the current study include lower current intensity and smaller electrode size as compared to past research. Thus, the tDCS stimulation parameters employed in the current study may have not been efficacious to inducing analgesic effects. Therefore, the current study highlights theoretical implications for future research to employ established tDCS parameters.

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Chronic pain presents a significant global health problem at both social and economic levels. The Global Burden of Disease (2012) has highlighted chronic pain as being one of the leading causes of worldwide disability, and in 2007, the total cost of chronic pain in Australia was estimated at $34.3 billion (MBF foundation, 2007). Estimates of chronic pain prevalence in the Australian adult population range from 15.4% (Miller, Sanderson, Bruno, Breslin, & Neil, 2017) to 17.1% and 20.0% for males and females respectively (Blyth et al., 2001). The pain can be so severe that it interferes with daily activities (Marinus et al., 2011), and this is exacerbated by the fact that 31% of Australian adults that report severe to very severe pain also report high to very high levels of psychological distress (Australian Bureau of Statistics, 2011). This may include depression, anxiety, and poor self-esteem, which negatively impacts social and work functioning (Bair, Wu, Damush, Sutherland & Kroenke, 2008; Demyttenaerer et al., 2007).

Pain is commonly defined as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage" (Merskey & Bogduk, 1994). The inability to perceive pain is highly maladaptive, as it invariably leads to early death (Manfredi et al., 1981). Paradoxically, pain can develop into a debilitating condition in which the link between tissue damage and pain intensity is lacking. Chronic pain is defined as pain without biological value that has persisted beyond normal tissue healing time (Merskey & Bogduk, 1994). This is often longer than the 3 to 6 month healing time expected for various pain conditions (Apkarian, Baliki, & Geha, 2009), and frequently there may not be any identifiable cause (Ready & Edwards, 1992).

As the mechanisms of chronic pain are underpinned by maladaptive neuroplasticity at structural (e.g., Apkarian et al., 2004; DaSilva et al., 2008), functional (e.g., Youssef et al.,

2014; Kim et al., 2015), and molecular levels (e.g., DosSantos et al., 2012; Martikainen et al., 2015), pharmacological interventions may never provide adequate pain relief (Moloney & Witney, 2013). Transcranial direct current stimulation (tDCS) can reliably induce and modulate neuroplasticity in the human cerebral cortex (Nitsche & Paulus, 2000; Nitsche & Paulus 2001; Nitsche et al., 2003). Thus, tDCS is suited to directly targeting the neurophysiological dysfunction that underpins chronic pain. tDCS has been shown to induce analgesic effects on experimentally induced pain when applied at the primary motor cortex (M1; Boggio, Zaghi, Lopes, and Fregni, 2008; Borckartdt et al., 2011; Reidler et al., 2012), and the dorsolateral prefrontal cortex (DLPFC; Boggio et al., 2008; Mylius et al., 2012). However, whether the analgesic effect of tDCS is greater when applied simultaneously at the M1 and the DLPFC is unknown. This is an avenue worthy of study, as a growing body of research indicates the co-activation of the M1 and the DLPFC during pain processing (Baudewig, Nitsche, Paulus, & Frahm, 2001; Lang, Nitsche, Paulus, Rothwell, & Lemon, 2004; Lang et al., 2005). Thus, the main aim of the current study is to investigate whether the analgesic effect of tDCS applied simultaneously at the M1 and the DLPFC on experimentally induced pain is greater than stimulation of either region in isolation. In doing so, the current study offers implications for chronic pain suffers that are non-pharmacological and effective.

Chronic Pain and Central Sensitisation

Central sensitisation has been implicated as an underlying mechanism for chronic pain (e.g., Girbés, Nijs, Torres-Cueco, & Cubas, 2013; Roussel et al., 2013) by lowering pain thresholds and increasing sensitivity to pain at the injured site and beyond (Kilo, Schmelz, Koltzenburg, & Handwerker, 1994; LaMotte, Shain, Simone, & Tsai, 1991). Central sensitisation is defined by an increase in nociceptive responses of neurons in the central nervous system that elicits pain hypersensitivity (Woolf, 2011). This results from conditioning of the nociceptive neurons in the dorsal horns of the spinal cord as a result of

peripheral tissue damage or inflammation (Ji, Kohno, Moore, & Woolf, 2003). Thus, the neurons in the central nervous system remain in a constant "wind-up" state such that individuals experience a persistent state of high reactivity, even after an injury has healed (McAllister, 2018).

Two key symptoms of central sensitisation manifest as hyperalgesia and allodynia at both the conditioned site (primary) and surrounding area (secondary) of tissue damage or inflammation (Klein, Stahn, Magerl, Treede, 2008; Koppert et al., 2001; LaMotte, Thalhammer, Torebjork, & Robinson, 1982). Hyperalgesia can be defined as both a decrease in pain threshold and increase in suprathreshold response to noxious stimuli, whereas allodynia can be defined as pain in response to non-noxious stimuli (Sandkühlertask, 2009).

Pain researchers have been able to reliably mimic the effects of hyperalgesia in healthy individuals via electrical stimulation (Klein, Magerl, Hopf, Sandkühler & Treede, 2004; Sluka, Judge, McColley, Reveiz & Taylor, 2000; Vo & Drummond, 2013). Electrical stimulation is delivered transcutaneously via a purpose-built electrode that preferentially activates superficial Aδ and C nociceptors (Inui, Tran, Hoshiyama, & Kakigi, 2002; Nilsson & Schouenborg, 1999). This elicits a moderate to severe pinprick sensation that gradually increases after each train of stimulation (Klein, Stahn, Magerl & Treede, 2008; Lang, Klein, Magerl, & Treede, 2007). Wall and Woolf (1984) demonstrated that a C fibre electrically stimulated once could remain sensitised for up to 3 min, whilst a C fibre stimulated at 1 Hz for 20 seconds can remain hypersensitive for up to 90 min. The consequence to the nociceptive system is an increase in the synaptic strength of stimulated nociceptors and long-term potentiation of nociceptive fibres terminating at the dorsal horn (Klein et al., 2008; Lang et al., 2007; Pfau et al., 2011). The result is sensitivity to mechanical punctuate stimuli at both the conditioned site and adjacent skin area, consistent with the presence of primary and secondary hyperalgesia (Klein et al., 2008; Pfau et al., 2011). Thus, central sensitisation to

sensory input from Aδ fibers likely explains the response observed in the secondary zone of hyperalgesia (Ziegler, Magerl, Meyer & Treede, 1999). Electrical stimulation can then be used as a method to induce hyperalgesia in healthy participants, thus mimicking the underlying mechanisms of chronic pain.

Pain in the Brain

Distinct parallel neural pathways within the cerebral cortex mediate the sensory (e.g., intensity, location) and affective (e.g., emotional, unpleasantness) components of pain (Purves et al., 2008; Yantis & Abrams, 2016). The sensory component is projected from the ventral posterior lateral nucleus of the thalamus to the primary (S1) and secondary (S2) somatosensory cortices, and the affective component is projected from the midline thalamic nuclei to the anterior cingulate cortex and insula. However, there is little support for this sensory and affective distinction, apart from anatomically distinct neural pathways (Apkarian, Hashmi, & Baliki, 2011). Furthermore, given that pain is always associated with unpleasantness, it would be unclear as to what significance such a division would have (Vasylenko, 2012). Thus, the sensory component of pain may be partly anatomically distinct from the neural pathways that are involved in the affective component of pain (Duquette, Roy, Lepore, Peretz, & Rainville, 2007). Indeed, the use of non-invasive brain stimulation techniques for the treatment of pain may achieve its analgesic effects via a cascading of modulatory effects of neural networks involved in pain-related processing, rather than the targeting of specific neural networks (Fregni & Pascal-Leone, 2007; Garcia-Larrea et al., 1997; Garcia-Larrea et al., 1999; Knotkova & Cruciani, 2010; Tsubokawa, Katayama, Yamamoto, Hirayama, & Koyama 1993). Additionally, neuroimaging studies suggest that the activity of the thalamus is decreased through corticothalamic pathways, which leads to decreased pain perception (Garcia-Larrea et al., 2006; Nuti et al., 2005; Peyron, Faillenot, Mertens, Laurent, & Garcia-Larrea, 2007). Taken together, these results suggest that the

neural pathways involved in the processing of the sensory and affective components of pain are intertwined.

Maladaptive Neuroplasticity in Chronic Pain

The ability of the nervous system to reorganise itself throughout individual life is defined as neuroplasticity (Ruge, Liou, & Hoad, 2012). In chronic pain, neuroplasticity becomes maladaptive as reorganisation of the nervous system both peripherally and centrally induce central sensitisation (Deer, Leong, & Ray, 2015). This is supported by a growing body of research suggesting the role of neuronal hyperexcitability (Fregni et al., 2005; Fregni, Freedman, & Pascual-Leone, 2007, Yi & Zhang, 2011) and maladaptive plasticity in the development and maintenance of chronic pain conditions (Attal et al., 2015, Grachev, Fredrickson, & Apkarian, 2000; Roussel et al., 2013). In pain-related neural networks, this maladaptive neuroplasticity occurs via a cascading effect. For example, Stern, Jeanmonod, and Sarnthein (2006) found that patients with chronic neuropathic pain have over activation in the insula, anterior cingulate cortex, prefrontal cortex, inferior parietal cortex, S1, and S2. Therapeutic lesion (central lateral thalamotomy) to the thalamus reduced over activation of these areas, as well as pain intensity, suggesting that the thalamus mediates the abnormal dysrhythmic activity in pain-related areas. Thus, chronic pain is precipitated and perpetuated by maladaptive neuroplasticity, which occurs via a cascading effect of pain-related neural networks.

Pharmacological Treatment for Chronic Pain

Chronic pain conditions are often refractory to pharmacological treatments (Finnerup et al., 2015), and pharmacological treatments may never provide adequate pain relief due to the complex neurophysiological dysfunction that underpins chronic pain (Moloney & Witney, 2013). One review found that antidepressants and anticonvulsants were effective for neuropathic pain in 24 of 29 trials (Watson, Chipman, & Monks, 2006). However, closer

inspection revealed that some trials reported small effect sizes, thus warranting a cautious interpretation of these results. Additionally, pharmacological treatments are undesirable as they can exacerbate symptoms (Kaneria, 2014)

As the neural pathways involved in the processing of the sensory and affective components of pain intertwine, and chronic pain is precipitated and perpetuated by a spreading of maladaptive neuroplasticity involving pain-related networks, interventions that are able to target the sensory and affective components of pain via a cascading effect of neuroplasticity are theoretically suited as an intervention for chronic pain conditions. As such, transcranial direct current stimulation (tDCS) is a suited intervention for chronic pain as tDCS is able to: (1) provide a broad pattern of stimulation to motor, somatosensory, and frontal cortices implicated in pain sensitivity (Fregni et al,. 2006; Luedtke et al., 2012; O'Connell, Marston, Spencer, DeSouza, & Wand, 2018), and (2) is able to induce a reliable cortical and subcortical neurophysiological response (Polania, Paulus, & Nitsche, 2012; Antal, Polania, Schmidt-Samoa, Dechent, & Paulus, 2011). Thus, tDCS can target the sensory and affective neural networks of pain via a cascading effect of neuroplasticity.

Transcranial Direct Current Stimulation (tDCS)

Transcranial Direct Current Stimulation (tDCS) is a modulatory non-invasive brain stimulation technique that uses a battery-operated device to painlessly deliver low direct currents (generally between 1 – 2 mA; Bikson, Datta, & Elwassif, 2009) via electrodes on the scalp. The consequence is the induction and modulation of neuroplasticity in the human cerebral cortex, and such "after-effects" are stable for up to ~1 hr if the tDCS stimulation duration lasts between 9 and 13 min (Nitsche & Paulus, 2000; Nitsche & Paulus 2001; Nitsche et al., 2003). In research settings, tDCS can be used as an active control condition to obtain baseline data to compare with experimental tDCS condition(s). This active control condition is commonly referred to as "sham tDCS" and is achieved through an initial ramp-

up and ramp-down period of stimulation in which the current level slowly increases and then decreases over a short period of time (usually 30 s; Ambrus et al., 2012).

How does tDCS promote neuroplasticity?

The type of stimulation delivered by tDCS determines its modulatory effects on the brain. tDCS allows for two types of stimulation: anodal and cathodal. Anodal stimulation depolarises the neuronal membrane and increases cortical excitability, whereas cathodal stimulation hyperpolarises the neuronal membrane and decreases cortical excitability. Although the precise mechanisms through which tDCS achieves pain relief are not completely understood, it has been proposed that anodal and cathodal tDCS induces long-term potentiation (LTP) and long-term depression (LTD) like plasticity respectively (Hallet, 2007; Monte-Silva et al., 2013; Nitsche et al., 2008). LTP and LTD describe long-lasting modifications of synaptic efficacy. LTP mediates the persistent strengthening of synaptic activity that facilitates the signal transmission between two neurons, and LTD weakens specific synapses to make better use of the synaptic strengthening caused by LTP. The consequence is improvement of neurological diseases underpinned by maladaptive neural networks (Rozisky, Antunes, Brietzke, Sousa, & Caumo, 2015).

tDCS and the Primary Motor Cortex (M1)

Theoretical research. The M1 is located on Brodmann area 4 (refer figure 1; Brodmann, 1909) and is involved in the planning and execution of movements (Sanes & Donoghue, 2000). Nitsche and Paulus (2001) compared several electrode arrangements and found that when an active electrode is placed over the M1 and reference electrode over the contralateral orbita, large excitability changes result.

It has been postulated that stimulation of the M1 may alleviate pain via modulation of abnormal thalamic activity, by activating descending pain inhibitory M1-thalamic neural pathways (Fregni et al., 2007). For example, M1 stimulation has been shown to reduce

hyperactivity of nociceptive ventral posterolateral thalamic neurons after transection of the

spinal cord in cats (Koyama et al., 1993). Corroborating this, epidural stimulation of the M1

reduced pain and thalamic hyperactivity in human patients with spinal cord injury

(Tsubokawa et al., 1991). Consequently, the M1 has been a common site of anodal tDCS
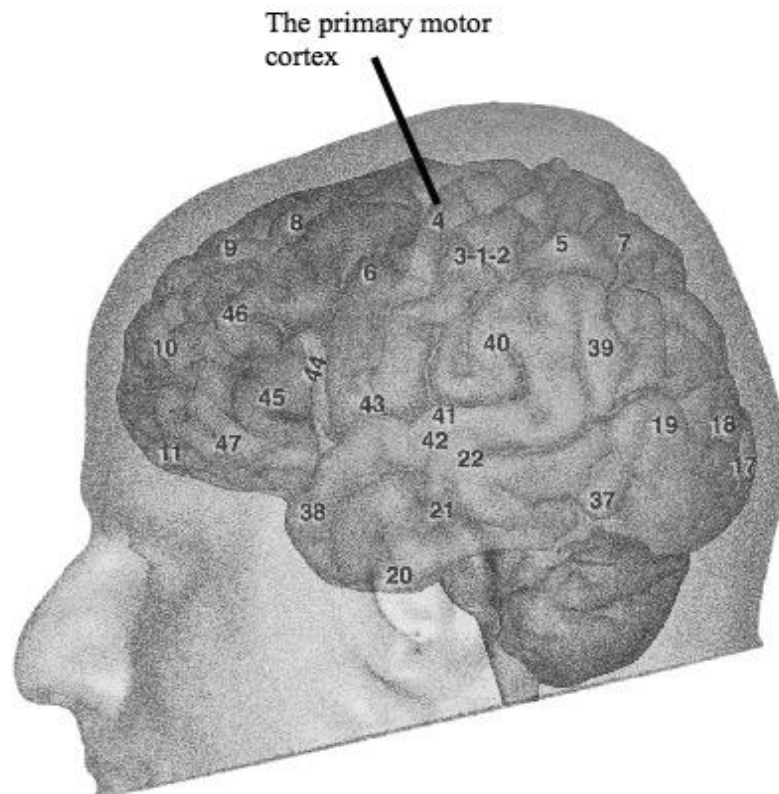
stimulation.

Figure 1. The M1 is located on Brodmann area 4 and is
involved in the planning and execution of movements (image
adapted from Catani & de Shotten, 2012).

Empirical research. Analgesic effects induced by tDCS applied at the M1 for

experimentally induced pain have been mixed. Borckartdt et al. (2012) reported significant

decreases in cold detection and cold pain thresholds, as well as increases in warm sensory

thresholds following 20 min of anodal tDCS (2mA) on M1. In contrast, Antal et al. (2008)

reported no difference in pain perception following 15 min of anodal tDCS (1mA) on laser-

induced pain. In a double blinded, randomised, sham controlled, cross over design, Boggio et

al. (2008) evaluated the effect of anodal tDCS on experimentally induced pain in 20 healthy

participants. Using electrical stimulation as the pain inducing stimulus, current supply was started 0 mA and was increased in steps of 0.1 mA until the intensity of current at which participants reported the perception of the electrical stimulus and the perception of pain was reached. tDCS (2 mA) was then applied for 5 min at the M1, DLPFC, primary visual cortex (V1), or a sham tDCS condition. tDCS of the M1 was found to increase the perception of electrical stimulation (threshold increase of 6.5%) and pain thresholds (threshold increase of 8.3%). In contrast, anodal stimulation of the DLPFC was found to increase pain threshold only (threshold increase of 10%). No significant effects for V1 or sham stimulation were reported. Boggio et al. (2008) suggested for further research exploring the effects of tDCS on pain, specifically, the simultaneous stimulation of the M1 and the DLPFC. Thus, the current study follows recommendations of Boggio et al. (2008)

In another study, Reidler et al. (2012) examined the effect of 20 min of anodal tDCS (2 mA) compared with sham tDCS on the M1. tDCS was found to increase the pain thresholds to von Frey's monofilament and pressure pain in 15 participants. However, this study administered active tDCS and sham tDCS within the same testing session, such that carry over effects of tDCS may have occurred. Thus, the current study seeks to remedy this limitation and clarify contradictions that may arise, by separating tDCS testing sessions by a minimum of seven days (Nitsche et al., 2008).

tDCS and the Dorsolateral Prefrontal Cortex (DLPFC)

Theoretical research. Another common site for tDCS stimulation is the DLPFC. The DLPFC is located on Brodmann areas 9 and 45 (refer Figure 2; Brodmann, 1909). In pain research, the DLPFC is commonly activated regardless of where pain is felt (Apkarian, Bushnell, Treede, & Zubieta, 2005). Specifically, the DLPFC has been suggested as a critical area where the neural circuit is involved in processing the affective component of pain (Duquette et al., 2007).
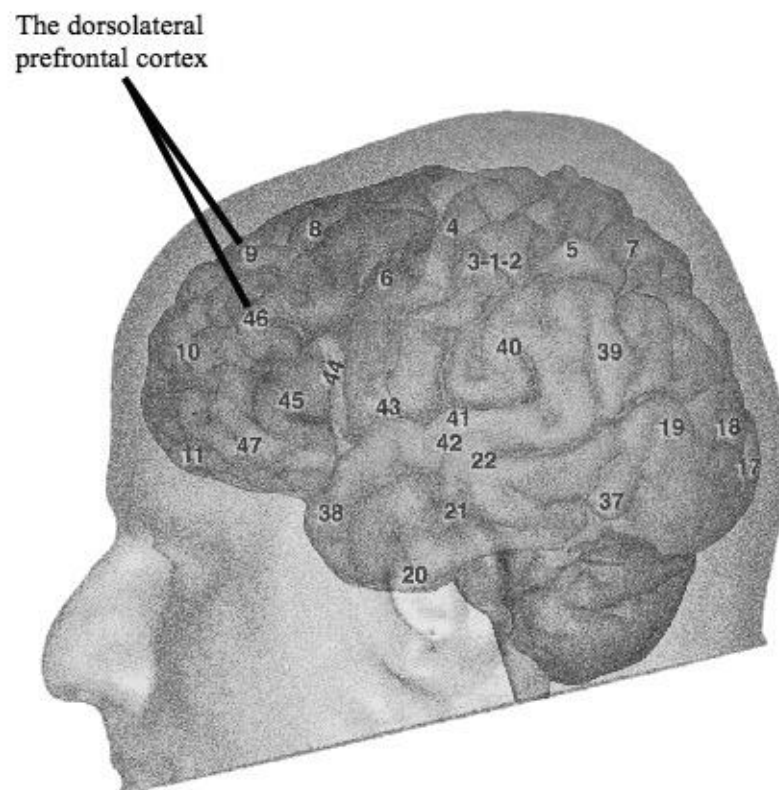
Figure 2. The DLPFC is located on Brodmann areas 9 and 46 and is involved in processing the affective component of pain (image adapted from Catani & de Shotten, 2012).

In light of this, Boggio, Zaghi, and Fregni (2009) found that anodal tDCS of the DLPFC decreases ratings of unpleasantness and discomfort/pain when viewing images of other humans in pain. Additionally, these ratings did not decrease during tDCS at the M1, V1, or sham tDCS. Further evidence for the role of the DLPFC in the affective component of pain comes from Boggio et al. (2008) aforementioned, finding that anodal tDCS at the M1 and the DLPFC in isolation can modulate pain thresholds, thus suggesting that the mechanisms of tDCS in modulating pain involves pathways that are independent of abnormal pain-related neural activity. Taken together, these results suggest a link between sensory pain modulation and the emotional processing of pain, and this corroborates that the sensory and affective neural pathways of pain are intertwined. Thus, the current study expects that anodal tDCS

applied to both the M1 and the DLPFC simultaneously will yield a greater reduction in pain than when both cortical regions are stimulated in isolation.

Empirical research. Research investigating the analgesic effect of tDCS applied at the DLPFC on experimentally induced pain is lacking. Mylius et al. (2012) reported an increased tolerance to heat pain following 20 min of anodal tDCS (2mA) at the DLPFC and Boggio et al. (2008) as aforementioned reported an increase in electrical pain threshold. However, research on the effects of tDCS at the DLPFC is warranted, especially as the refractoriness of chronic pain to current medical treatment may be due to neglecting its cognitive component (Tenenbaum et al., 2001).

The M1 and DLPFC Theoretical Link

A growing body of research indicates the co-activation of the M1 and the DLPFC during pain processing (Lang et al., 2004; Lang et al., 2005; Baudewig et al., 2001), suggesting evidence for the functional connectivity between both cortical areas. Additionally, research also suggests that the DLPFC coincides with increased M1 activity and modulation of the medial pain system (Ploghaus, Becerra, Borras, & Borsook, 2003; Wager et al., 2004), and both have been identified as being relatively superficial brain areas that contribute to the neural substrate of pain (Vaseghi, Zoghi, & Jaberzadeh, 2014). Thus, tDCS is ideally suited to delivering simultaneous stimulation of the M1 and the DLPFC, and this exact line of research has been recommended as an avenue for investigation (Boggio et al., 2008).

Aims and Hypotheses

The primary aim of the current study is to examine the analgesic effect of anodal tDCS applied at both the M1 and DLPFC simultaneously on experimentally induced hyperalgesia. Additionally, the current study seeks to clarify existing research on the analgesic effect of tDCS applied at the M1 and the DLPFC on experimentally induced pain in isolation. It is expected that anodal stimulation of both cortical areas simultaneously will

yield the greatest reductions in ratings of pain to pinprick stimuli, as well as require a higher

current level of electrical stimulation to elicit moderate pain. Following Boggio et al. (2008),

the current study adopts a double blinded, randomised, sham controlled, cross over design,

and uses electrical stimulation as a pain inducing stimulus, to increase comparability of

results. At the same time, the current study overcomes the limitation of Reidler et al. (2012)

that administered different tDCS conditions within the same testing session, allowing for the

influence of carry over effects, by separating tDCS testing sessions by a minimum of one

week (Nitsche et al., 2008).

Hypothesis one: primary and secondary hyperalgesia. To validate whether tDCS

induces analgesic effects on experimentally induced hyperalgesia, it is first necessary to

assess whether changes in central signalling processing occur following electrical

stimulation. In line with past research (Klein et al., 2008; Pfau et al., 2011), it was

hypothesised that electrical stimulation would result in an increasing sensitivity to

mechanical punctuate stimuli at both the conditioned site and adjacent skin area, consistent

with the presence of primary and secondary hyperalgesia.

Hypothesis two: the effect of tDCS on pain ratings elicited by pinprick stimuli. It

was further hypothesised that anodal tDCS applied to both the M1 and the DLPFC

simultaneously will yield the greatest decrease in self-reported ratings of pain to pinprick

stimuli at both the primary and secondary site, followed by anodal tDCS applied to the M1

and the DLPFC in isolation, as compared to sham tDCS. This is due to the co-activation of

the M1 and the DLPFC during pain processing (Lang, Nitsche, Paulus, Rothwell, & Lemon,

2004; Lang et al., 2005; Baudewig, Nitsche, Paulus, & Frahm, 2001).

Hypothesis three: the effect of tDCS on the modulation of pain thresholds. Lastly,

it was hypothesised that anodal tDCS applied at the M1 and the DLPFC simultaneously will

yield the greatest increase in pain threshold to electrical stimulation, followed by anodal

tDCS applied to the M1 and DLPFC in isolation, as compared to sham tDCS. This is due to the co-activation of the M1 and the DLPFC during pain processing (Lang, Nitsche, Paulus, Rothwell, & Lemon, 2004; Lang et al., 2005; Baudewig, Nitsche, Paulus, & Frahm, 2001).

As the clinical implications of tDCS are yet to be fully realised (Cruccu et al., 2016), perhaps due to the varying responses towards this particular treatment approach (Moreno-Duarte et al., 2014), positive outcomes from this investigation include supporting the existing literature on the analgesic effect of tDCS applied at the M1 and the DLPFC. Additionally, the current study will bridge the existing research gap regarding whether tDCS applied at the M1 and the DLPFC simultaneously will result in larger pain reductions than when either region is stimulated in isolation. In doing so, the current study may offer practical implications for chronic pain suffers.

Method

Study Design

The current study employed a double blinded, randomised, sham controlled, crossover design, to evaluate the effect of a single session of tDCS on experimentally induced hyperalgesia in healthy volunteers. The independent variables are the site of tDCS stimulation (M1, DLPFC, M1 + DLPFC, or sham tDCS) and time (pretest, posttest, and follow up), and the dependent variables are pain ratings in response to mechanical punctuate stimuli at the primary and secondary site, as well as the current level of electrical stimulation required to elicit moderate pain. Thus, the current study follows a two factor within-subjects design (tDCS condition: 4 [M1, DLPFC, M1 + DLPFC, Sham]) × (time: 3 [time 1, time 2, time 3]).

The within-subjects design was employed as it minimises the introduction of confounding factors that may the influence the inter-individual variability response to tDCS (for a review, see Li, Uehara, & Hanakawa et al., 2015). Such factors include age (Fujiyama

et al., 2014), hair thickness (Horvath, Carter, & Forte, 2014), and skull thickness (Datta, Truong, Minhas, Parra, & Bikson, 2012). However, the within-subjects design introduces the possibility of data being confounded by learning, practice, or order effects due to repeated sessions (Berryhill, Peterson, Jones, & Stephens, 2014). Thus, participants were randomised as to what tDCS condition they were to experience a priori via Latin square, in order to counterbalance the stimulation condition order.

To maintain double blinding, a secondary experimenter uninvolved in collecting pain-related data was responsible for administering tDCS. To maintain blinding to the participant, tDCS electrode placements were identical regardless of the tDCS condition administered. In the sham condition, 30 s of stimulation was applied (anodal stimulation of M1 + DLPFC at 1 mA), which provides the initial tingling and itching sensation experienced in the experimental tDCS conditions. As such, most individuals are unable to distinguish between experimental and sham tDCS (Ambrus et al., 2012). Furthermore, 30 s of tDCS is not sufficiently long enough to promote excitability changes (Nitsche & Paulus, 2000) or alter brain function (Nitsche et al., 2008), and thus serves as an active control condition.

Participants

A priori power analysis (conducted using G*power; Faul, Erdfelder, Lang, & Buchner, 2007) was conducted using a small-moderate effect size, as reported by similar tDCS studies as well as meta-analysis (Boggio et al., 2008; Luedtke et al., 2012; O'Connell et al., 2018; Vaseghi et al., 2014). The projected participant count was 76 to achieve 80% power with 4 levels of tDCS condition and 3 levels of time. However, Boggio et al. (2008) identified a significant reduction of experimentally induced pain in 20 healthy participants comparing 4 levels of tDCS condition and 2 levels of time. Thus, due to time constraints, the current study aimed to recruit a minimum of 20 participants.

Potential participants were recruited online through the psychology research participant pool at Murdoch University (Appendix A), via flyers placed around the university campus (Appendix B), and via word of mouth of experimenters. Potential participants were explained the risks and benefits of the study, and were screened for eligibility via a self-reported general health and tDCS safety screening form (Appendix C). Participants were regarded as healthy and suitable to participate if they fulfilled the following criteria: (1) aged between 18 – 65, (2) no clinically significant or unstable medical, neuropsychiatric, or chronic pain disorder, (3) no history of substance abuse or dependence, (4) no use of central nervous system-effective medication, (5) no history of brain surgery, tumour, or intracranial metal implantation, (6) not pregnant or breastfeeding, and (7) right-handed. Initially, 23 participants were recruited, however 4 were excluded due to: (1) Taking analgesic medication, (2) participated in the first testing session but did not return for the following sessions, (3) pain ratings were inconsistent in the initial testing session and were excluded for the remaining sessions and (4) did not return for the final testing session. The final participant count consisted of 19 right-handed; healthy, medication-free, adult volunteers (12 male; M_{age} = 29.21, SD = 10.78, range 20 to 52). Completion of a single session took ~2 hours and reimbursement for participation was provided in the form of course credit for psychology students at Murdoch University, or being in the draw for a $100 gift card.

Materials

Electrical stimulation. Electrical stimulation was generated by a constant current stimulator (DS7A; Digitimer, Welwyn Garden City, UK) and was delivered transcutaneously through a custom-built electrode. The electrode consisted of 25 copper pins mounted on a 2 cm x 3 cm perspex block. The pin tips projected from the perspex block at 0.5 mm, were separated by approximately 3.5 mm, and had a diameter of 0.2 mm. These electrode properties are ideally suited to activating superficial nociceptive Aδ and C fibers (Inui et al.,

2002; Kaube, Katsarava, Käufer, Diener, & Ellrich, 2000; Nilsson & Schouenborg, 1999). A 3.0 cm x 3.5 cm ground plate completed the electrical circuit.

Pinprick stimuli. A sharp tip with a calibrated spring mechanism exerting a force of 40g (Neuro-pen, Owen Mumforord, USA) was angled 90° to the surface of the skin and was applied with sufficient pressure to push the tip completely against the surface of the skin for 2 s. Doing so triggers a sharp pain sensation transmitted by myelinated Aδ nociceptors (Ziegler et al., 1999).

Transcranial direct current stimulation intervention (tDCS). Direct current was generated by a battery-driven 9-volt constant current stimulator (Chattanooga IontoTM Dual Channel Iontophoresis System; see Figure 3). Two electrodes delivered anodal stimulation and one electrode delivered cathodal stimulation (reference electrode) through thick (0.3 cm) rectangular surface sponge electrodes measuring 5.5 cm x 4.5 cm and 6.5 cm x 6.0 cm respectively. The choice for a larger sized reference electrode was to increase the focality of tDCS and reduce side effects (e.g., itching, burning sensation etc.) by decreasing the current density of the reference electrode (Nitsche et al., 2007). This effectively turns the cathode into an inactive electrode (Miranda, Faria, & Hallet, 2009). To facilitate the electrical conductivity of the current to the scalp, the electrode sponges were pre-saturated with ~5 ml of saline solution on each side and a small amount of conductive gel (10 mm circle diameter) was applied on the side of the electrode sponge that was to make contact with the scalp. The current density of the anode and cathode electrodes was 0.040 mA/cm^2 and 0.025 mA/cm^2 respectively, which is above the minimum threshold of 0.017 mA/cm^2 to actively modulate cortical activity (Nitsche & Paulus, 2000).

Figure 3. The tDCS was delivered using the Chattanooga Ionto^TM Dual Channel Iontophoresis System. The cathode electrode (left) was inserted through 6.5 cm x 6.0 cm surface sponge electrodes and the anode electrodes (middle and right) were inserted through 5.5 cm x 4.5 cm surface sponge electrodes.

Transcranial direct current stimulation parameters. One of four tDCS conditions was calibrated corresponding to one of the four testing sessions: anodal tDCS (20 min, 1 mA) at the left M1 (channel 1) or left DLPFC (channel 2), anodal tDCS at the left M1 and the left DLPC simultaneously (20 min, 1 mA), or sham tDCS (30 s, 1 mA at the left M1 and left DLPFC simultaneously). All tDCS stimulations began with a ramp-up of the current from 0 to 1 mA and ended with a ramp-down from 1 to 0 mA; this ramp-up and ramp-down lasted 30 s.

The tDCS intervention duration was based on findings that tDCS induces cortical excitability after-effects that are stable up to ~1 hr if tDCS duration is between 9 and 13 min (Nitsche & Paulus 2000; Nitsch & Paulus, 2001; Nitsche et al., 2003). Additionally, 20 min is a common tDCS duration in pain studies (e.g., Ahn et al., 2017; Magdalena, Volz, Farmer, & Siegmund, 2016; Yoon et al., 2013).

tDCS electrode placement was determined according to the European Chapter of the International Federation of Clinical Neurophysiology, which recommends that tDCS stimulation is administered with the anode over the M1 contralateral to the pain side and cathode over the supraorbital region contralateral to the M1 placement for efficacy among populations with chronic pain (Fregni & Pascual-Leone, 2007). As such, pain researchers have often administered anodal tDCS stimulation to the M1 hemisphere contralateral to the pain-affected area of the body and with the cathode electrode placed over the supraorbital region ipsilateral to the affected area (e.g., Ahn et al., 2017; Magdalena et al., 2016; Yoon et al., 2013). As such, the left M1 and the left DLPFC was used for all anodal simulations, with the reference electrode placed over the contralateral (right) supraorbital region.

As 1 - 2 mA is considered sufficient to achieve desired excitability, perceptual, and behavioural changes, and are regarded as safe if stimulation duration does not exceed 15 - 20 min as shown by behavioural measures, EEG, serum neuronspecific annuals concentration, and diffusion-weighted and contrast-enhanced magnetic response imaging measures (Nitsche & Paulus, 2001; Nitsche et al., 2003; Nitsche et al., 2004; Iyer et al., 2005), a current intensity of 1 mA was used for all stimulations.

Measures

Verbal numerical rating scale. Participants rated perceived pain on a 22-point Verbal Numerical Rating Scale (VNRS) from 0 (no pain/sharpness) to 10 (worst pain/sharpness imaginable) with a rating of 5 indicating moderate pain (Appendix D). Participants could provide ratings in either whole (e.g., 1) or half numbers (e.g., 0.5). The VNRS is a valid substitute for the Visual Analogue Scale (Bijur, Latimer, & Gallagher, 2003) used typically in pain research (e.g., Bolognini et al., 2015; Fregni et al., 2006; Magdalena et al., 2016). Additionally, self-report is the most reliable measure of pain (D'Amico &

Barbarito) and correlates well with functional magnetic resonance imaging used to measure

pain (Brown, Chatterjee, Younger, & Mackey, 2011)

      The edinburgh handedness inventory (Olfield, 1971). This 10-item inventory

indexes hand preference (Appendix E) as a laterality quotient with scores ranging from -100

(extreme left-handedness) to +100 (extreme right-handedness). Participants indicate their

hand preference for various activities (e.g., writing, using scissors, throwing, etc.) in the

following range: *strong* (++), *less strong* (+), to *indifferent* (+/+). This inventory exhibits

excellent internal consistency, Cronbach's α = .93 (Williams, 1991)

      Participants with a laterality quotient below +70 were not eligible to participate.

Participants were required to be right-handed in line with similar tDCS studies (e.g., Mylius

et al., 2016; Vaseghi, Zoghi, & Jaberzadeh, 2015), as well as to minimise confounds that may

arise from anatomical differences in the cerebral cortex between right and left handers.

Procedure

      Ethics approval was obtained by the institutional ethics committee at Murdoch

University (2018/037; Appendix F). Testing sessions were conducted at the Mind and Body

lab at Murdoch University. Two experimenters were present at all times, one for collecting

pain-related data and one for administration of tDCS. At least one of the experimenters

obtained first aid qualifications prior to participant recruitment. Participants were tested

individually on four separate occasions, corresponding to the four tDCS conditions. Testing

sessions were separated by a minimum of seven days to a maximum of 14 days to minimise

possible carry-over effects resulting from tDCS (Nitsche et al., 2008).

      Upon arrival, participants were provided with an information letter outlining the

purpose and nature of the experiment, procedures, benefits, and risks (Appendix G). Potential

participants then provided written informed consent (Appendix H) before completing the The

Edinburgh Handedness Inventory (Olfield, 1971; Appendix E). Eligible participants were

provided with a self-reported general health and tDCS safety screening form (Appendix C) to ensure participants were healthy and met inclusion criteria. Participants were then advised that data would be treated anonymously and that withdrawal of the study without prejudice was possible any time during the experiment.

The procedure began with exfoliation of the right ventral forearm to facilitate electrical stimulation. The forearm was rinsed under running water and the experimenter used a pumice stone to lightly brush the area ten times. The forearm was again rinsed before being dried. Participants then sat in a comfortable chair. Figure 4 presents a timeline of the testing procedure.
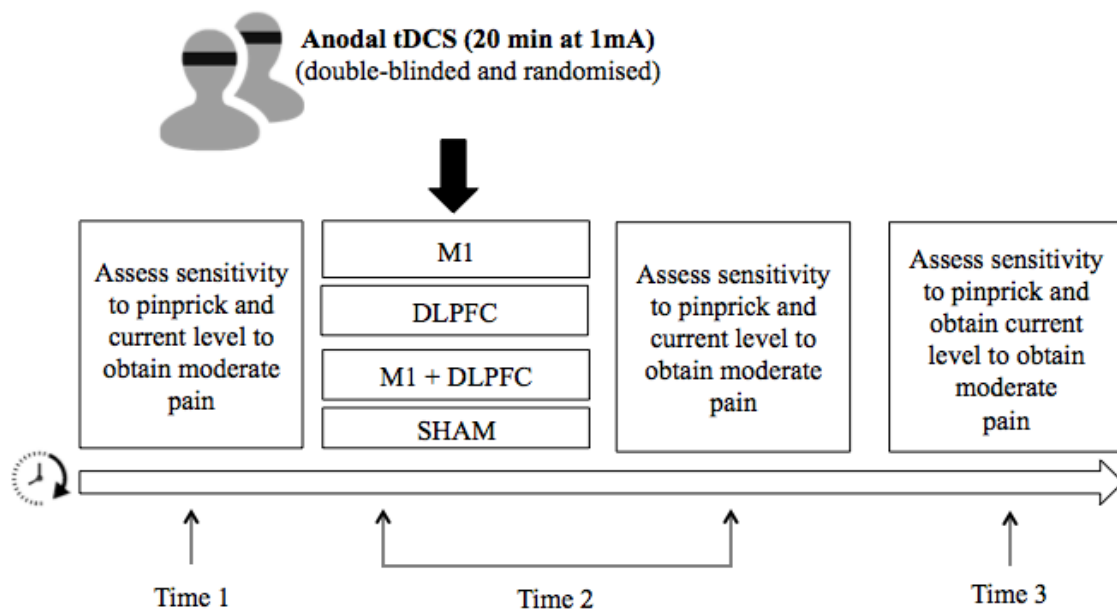
Figure 4. Schematic representations of the experimental procedure and timeline (figure adapted from (Vo, 2018).

Time one. Baseline assessment of pain to pinprick stimuli were obtained. To do this, the electrode of the electrical stimulator was positioned ventral of the right forearm ~3 cm below the middle of the right elbow. The border of the electrode was then traced with pen. The initial pinprick was administered inside the traced area (primary area) with an additional rating obtained ~2 cm distal from the border of the primary area in any direction (secondary

area). Participants verbally reported perceived pain on the VNRS. Prior to these baseline

pinprick tests, participants were trained until perceived pain ratings were stabilised on the

ventral wrist of the right hand as to provide more accurate ratings for testing. Two ratings

were obtained and averaged as the baseline test of pain to pinprick stimuli, as repeated testing

to a single area can enhance pain sensitivity (Vo, 2014).

Subsequently, the experimenter obtained the electrical current level required to elicit

moderate pain. The ground plate was mounted on the right ventral forearm positioned ~4 cm

right of the electrode (Figure 5). Positioning of the electrode and ground plate was kept in

place with an armband.
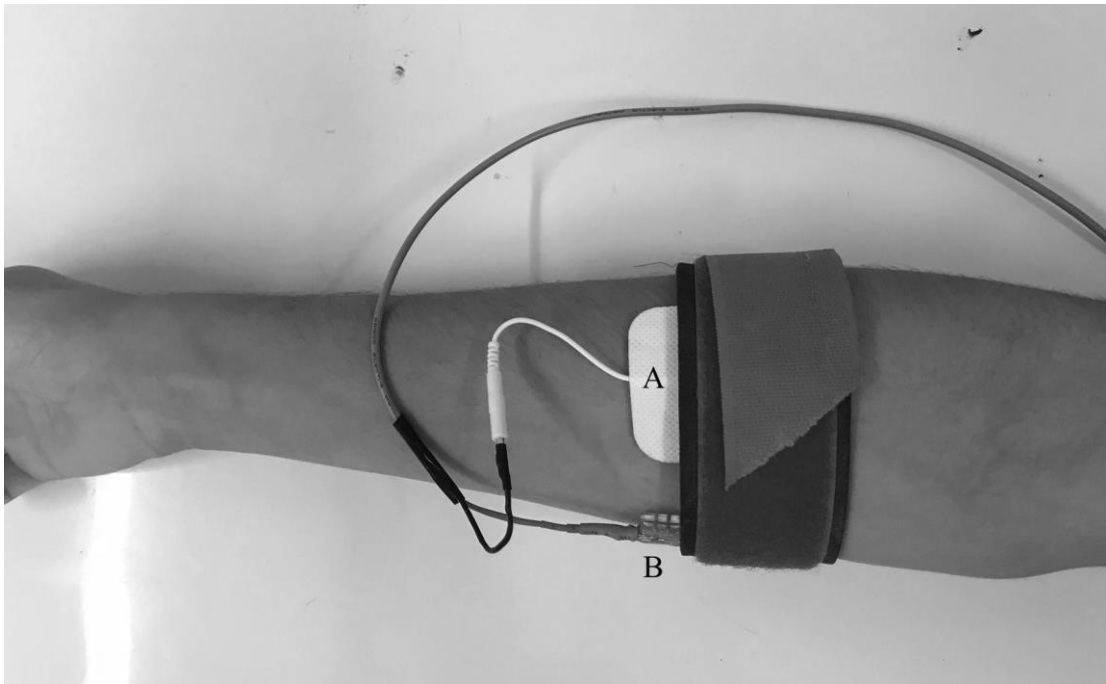
Figure 5. Configuration of electrode and ground plate placement for electrical
stimulation. The ground plate (A) was positioned 4 cm right of the electrode (B),
which was positioned 3 cm below the middle of the elbow.

The experimenter delivered repeated electrical pulses at 1 Hz and 0.5 ms pulse width,

beginning at a current level of 2.0 mA. As in the baseline tests of pinprick stimuli,

participants verbally reported ratings of pain on the VNRS. The experimenter then gradually

increased the current level by increments of 0.2 mA as the electrical pulses were delivered.

Participants continued to provide verbal ratings of pain until the current level rated as a pain

level of 5 (moderate pain) was obtained.

Time two. The tDCS device was then calibrated corresponding to one of four testing

conditions. The 10/20 electroencephalogram (EEG) system (Klem, Lüders, Jasper, & Elger,

1999) was used to determine electrode placements, as it is the most common way to do so in

tDCS studies (Thair, Holloway, Newport, Smith, 2017). In the 10/20 system, the left M1 and

the left DLPFC corresponds to C3 and F3 respectively, with the right supraorbital area

corresponding to Fp2 (see Figure 6).
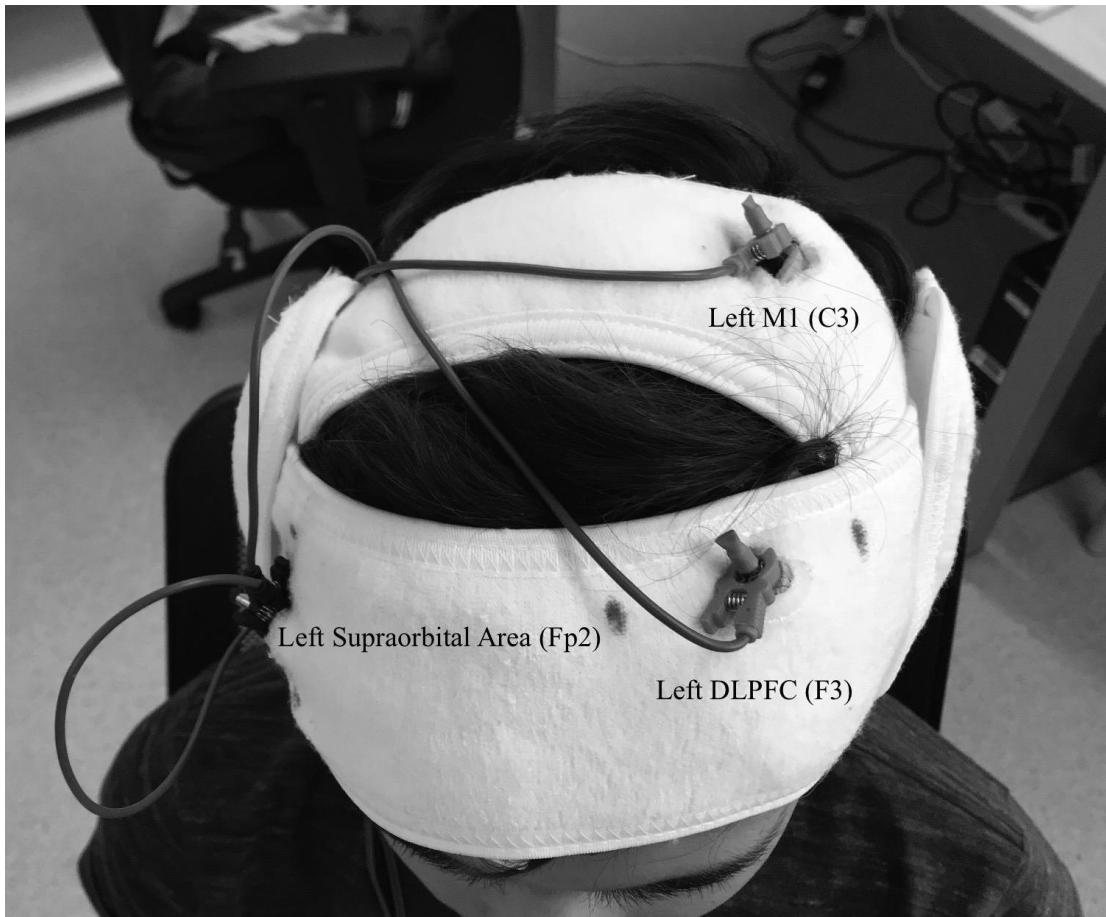
Figure 6. Electrodes secured with headbands. Channel 1 and channel 2 correspond to
the anode electrodes placed at the left M1 and the left DLPFC respectively, and the
cathode electrode was placed at the right supraorbital area.

The positioning of the electrodes was facilitated using the "Beam method" (Beam,

Borckardt, Reeves, & George, 2009; Appendix I). In this method, the F3 position is

calculated based on the measurements between the left and right preauricular point, naison -

inion, and head circumference. Measurements were entered into the Beam software and the

tDCS experimenter proceeded to make the necessary ink markings on the scalp to locate F3.

Neuroimaging studies have validated the use of the Beam method for locating F3 (Halper,

Yagi, Manevitz, Nishimoto & Onishi 2016; Mir-Moghtadaei et al., 2015). The beam method

also facilitated the electrode placement of C3. After electrodes were secured, participants

were asked to keep as relaxed and alert as possible to increase the reliability and validity of

the time course of cortical excitability (Nitsche et al., 2008). Participants then experienced

one of the four tDCS conditions during which all interactions between the experimenter and

the participant ceased, as active motor and/or cognitive activity during tDCS can negatively

interfere or completely abolish its effects (for a review, see Horvath et al., 2014)

Following this, tests of pinprick stimuli were repeated with procedures identical to

Time 1 with the exception of six and not two ratings obtained; four were obtained in the

secondary area and two obtained in the primary area. Four linear paths of 45° from the centre

of the primary area and ~2 cm distal from the border of the primary area (as traced in Time 1)

identified the secondary area. As electrical stimulation induces both primary and secondary

hyperalgesia (Klein et al., 2008; Pfau et al., 2011), pinprick tests were conducted at both the

primary and secondary area to assess primary and secondary hyperalgesia respectively. Pain

ratings obtained by tests of pinprick stimuli at the primary and secondary area was averaged

as the final reading.

Following tests of pinprick stimuli, the current level that elicited moderate pain was

reassessed at the right ventral forearm, beginning at the current level last rated as moderate

pain (rating of 5 on the VNRS) and using the same electrical stimulation parameters in Time

1. The experimenter then increased or decreased the current level by 0.2 mA to reobtain a

pain rating of 5.

Time three. After 10 min of rest, final tests of pinprick stimuli following identical procedures to Time 2 were carried out. The rest time allowed the arm to settle after stimulation as to not be oversensitive during final tests of pinprick stimuli, allowing for a more accurate rating. This was followed by reassessment of moderate pain via electrical stimulation, and was carried out following identical procedures to Time 2.

Results

All 19 participants were adequately right-handed as the laterality quotient range on the Edinburgh Handedness Inventory (Olfield, 1971) was between +71.40 to +100.00 ($M =$ 91.95, $SD = 11.63$). The tDCS sessions proceeded with no adverse effects. Moderate tingling and itching were reported and transient erythema to the area of stimulation was visible in some participants. This occurred across all tDCS conditions.

All statistical analyses were performed in the Statistic Package for Social Sciences version 24 (IBM SPSS Inc, Chicago, IL, USA). All hypotheses were evaluated using two factor within-subjects ANOVA (tDCS condition: 4 [M1, DLPFC, M1 + DLPFC, Sham]) × (time: 3 [Time 1, Time 2, Time 3]) on the following dependent variables: pain ratings elicited by pinprick stimuli at the primary and secondary area, and the current level required to elicit moderate pain. All relevant statistical output is presented in appendix J.

Due to human error in the recording of pain ratings elicited by pinprick stimuli at the primary site, two variables (0.88%) of data were missing. This is inconsequential, as the missing data compromises less than 5% of the data of all dependent variables (Schafer, 1999). Therefore, imputation was not necessary. Partial eta-squared (partial η^2) values are provided as measures of effect sizes with values of .01, .06, and .14 constituting small, medium, and large effect sizes respectively (Cohen, 1988). Where appropriate, significant main effects and interactions were followed-up with simple effects analyses applying Bonferroni correction, to correct for the inflation of type I error rate.

Prior to conducting each analysis, the assumptions of the ANOVA were evaluated. Due to small sample size ($N = 19$), the assumption of normality was evaluated with the Shapiro-Wilk test (Allen, Bennett & Heritage, 2014). On several variables the assumption was not satisfied ($p < .05$). However, examination of the skew and kurtosis of all study variables revealed all but three variables (current level required to elicit moderate pain during sham tDCS at Time 1, Time 2, and Time 3) had a skew and kurtosis less than 2.0 and 9.0 respectively, suggesting that the data was normal enough to conduct an ANOVA (Posten, 1984; Schmider, Ziegler, Dannay, Beyer, Bühner, 2010; see appendix J). Additionally, the repeated measures ANOVA is very robust to violations of the assumption of normality, with one study finding that the repeated measures ANOVA kept the type I error rate at .039 when skew was 3.0 and kurtosis was 21.0 (see Berkovits, Hancock, & Nevitt, 2000). Thus, the skew and kurtosis of the three study variables that were above 2.0 and 9.0 were not deemed a threat to the interpretation of the analyses.

To evaluate the assumption of sphericity, Maulchy's test was examined and the degrees of freedom were adjusted using the Greenhouse-Geisser Epsilon estimates of sphericity in the case of violations (where $p < .05$). Due to small sample size ($N = 19$) and mild deviations of variables not meeting the assumption of normality, the Greenhouse-Geisser correction is the most appropriate to keeping the type I error rate approximate to .05 (see Gignac, 2016). Statistical significance was determined at a two-tailed $p = 0.05$.

Electrical Stimulation and Primary Hyperalgesia and Pain Response to Pinprick Stimuli at the Primary Site across Time

In order to evaluate the hypothesis that electrical stimulation induces hyperalgesia at the primary site, and that tDCS induces analgesic effects on pain elicited by pinprick stimuli, the ANOVA was performed with the dependent variable being pain ratings elicited by pinprick stimuli at the primary area. Descriptive statistics are reported in Table 1.

Table 1

Means With Confidence Intervals (CIs) and Standard Deviations of Pain Ratings Elicited by Pinprick Stimuli at the Primary Area across tDCS

Conditions and Time

Time	M1		DLPFC		M1 + DLPFC		Sham	
	M (SD)	95% CI	*M (SD)*	95% CI	*M (SD)*	95% CI	*M (SD)*	95% CI
Time 1	.91 (.83)	[.48, 1.34]	1.38 (1.23)	[.74, 2.01]	1.17 (1.08)	[.61, 1.73]	1.35 (1.27)	[.69, 2.00]
Time 2	1.72 (1.14)	[1.13, 2.30]	1.66 (1.08)	[1.10, 2.22]	1.39 (.83)	[.96, 1.81]	1.92 (1.23)	[1.28, 2.55]
Time 3	1.64 (1.16)	[1.04, 2.24]	1.95 (1.37)	[1.24, 2.66]	1.47 (.94)	[.99, 1.96]	2.10 (1.08)	[1.54, 2.65]

Upon conducting the ANOVA, the assumption of sphericity was violated for the tDCS condition × time interaction ($p = .02$) but not for the tDCS condition or time main effects (ps < .05). Thus, the interaction effect was interpreted using the Greenhouse-Geisser correction. The ANOVA did not reveal a significant main effect of tDCS condition, $F(3, 48) = 2.32$, $p = .09$, partial $\eta^2 = .13$ but revealed a significant main effect of time, $F(2, 32) = 10.761$, $p < .001$, partial $\eta^2 = .402$. There was no tDCS condition × time interaction with respect to pain ratings elicited by pinprick stimuli at the primary site $F(3.02, 48.31) = .71$, $p = .55$, partial $\eta^2 = .04$.

To probe the effect of time, two pairwise comparisons were conducted with Bonferroni correction performed at $.05 / 2 = .025$. The analysis revealed that the pain ratings elicited by pinprick stimuli was significantly higher at both Time 2 ($p < .010$) and Time 3 ($p < .002$) than at Time 1, but did not differ between Time 3 and Time 2 ($p > .025$).

These results suggest that electrical stimulation induces hyperalgesia at the primary area, consistent with the first hypothesis. However, tDCS does not induce analgesic effects on pain elicited by pinprick stimuli, and is inconsistent with the second hypothesis.

Electrical Stimulation and Secondary Hyperalgesia, and Pain Response to Pinprick Stimuli at the Secondary Site across Time

In order to evaluate the hypothesis that electrical stimulation induces hyperalgesia at the secondary site, and that tDCS induces analgesic effects on pain elicited by pinprick stimuli, the ANOVA was performed with the dependent variable being pain ratings elicited by pinprick stimuli. Descriptive statistics are reported in Table 2.

Table 2

Means With Confidence Intervals (CIs) and Standard Deviations of Pain Ratings Elicited by Pinprick Stimuli at the Secondary Area across

tDCS Conditions and Time

	M1		DLPFC		M1 + DLPFC		Sham	
Time	*M (SD)*	95% CI	*M (SD)*	95% CI	*M (SD)*	95% CI	*M (SD)*	95% CI
Time 1	1.28 (1.15)	[.73, 1.84]	1.26 (1.14)	[.71, 1.81]	1.47 (1.21)	[.88, 2.06]	1.36 (1.12)	[.82, 1.91]
Time 2	1.46 (.94)	[1.01, 1.92]	1.76 (1.26)	[1.15, 2.38]	1.51 (.86)	[1.10, 1.93]	1.77 (1.23)	[1.18, 2.37]
Time 3	1.73 (.93)	[1.27, 2.18]	1.78 (1.07)	[1.27, 2.30]	1.55 (1.10)	[1.02, 2.08]	1.81 (1.10)	[1.28, 2.34]

Upon conducting the ANOVA, the assumption of sphericity was violated for the tDCS condition main effect ($p = .03$) but not for time main effect or the tDCS condition $\times$ time interaction effect ($ps > .05$). Thus, the main effect finding of tDCS condition were interpreted using the Greenhouse-Geisser correction. The ANOVA did not reveal a significant main effect of tDCS condition $F(3, 54) = .37$, $p < .78$, partial $\eta^2 = .02$ but did reveal a significant main effect of time $F(1.66, 29.98) = 7.75$, $p < .003$, partial $\eta^2 = .31$. There was no tDCS condition $\times$ time interaction with respect to pain ratings elicited by pinprick stimuli at the secondary site $F(6, 108) = .73$, $p < .63$, partial $\eta^2 = .04$.

To probe the effect of time, one pairwise comparison was conducted. The analysis revealed that the pain ratings elicited by pinprick stimuli was significantly higher at Time 3 than at Time 1 ($p = .005$).

These results suggest that electrical stimulation induces hyperalgesia at the secondary area, consistent with the first hypothesis. However, tDCS does not induce analgesic effects on pain elicited by pinprick stimuli, and is inconsistent with the second hypothesis.

tDCS and Modulation of Electrical Pain Threshold

Finally, to evaluate the hypothesis that tDCS modulates pain thresholds, the ANOVA was performed with the dependent variable being the current level required to elicit moderate pain. Descriptive statistics are reported in Table 3.

Table 3

Means With Confidence Intervals (CIs) and Standard Deviations of Current Level Required to Elicit Moderate Pain via Electrical Stimulation across tDCS Conditions and Time

Time	M1		DLPFC		M1 + DLPFC		Sham	
	M (*SD*)	95% CI	*M* (*SD*)	95% CI	*M* (*SD*)	95% CI	*M* (*SD*)	95% CI
Time 1	6.20 (4.11)	[4.22, 8.18]	6.26 (4.21)	[4.22, 8.29]	5.49 (3.03)	[4.03, 6.95]	5.95 (5.22)	[3.43, 8.47]
Time 2	6.95 (4.79)	[4.64, 9.26]	6.92 (4.62)	[4.69, 9.15]	6.17 (3.20)	[4.62, 7.71]	6.48 (5.03)	[4.05, 8.91]
Time 3	8.71 (6.41)	[5.62, 11.80]	8.04 (5.20)	[5.53, 10.55]	7.60 (3.62)	[5.85, 9.35]	6.93 (5.11)	[4.47, 9.40]

Upon conducting the ANOVA, the assumption of sphericity was violated for tDCS condition and time main effects, as well the tDCS condition $\times$ time interaction effect ($ps <$ .05). Thus, findings were interpreted using the Greenhouse-Geisser correction. The ANOVA did not reveal a significant main effect of tDCS condition, $F(2.25, 40.42) = .44$, $p = .67$, partial $\eta^2 = .02$ but did reveal a significant main effect of time $F(1.16, 20.90) = 31.95$, $p <$ .001, partial $\eta^2 = .64$. There was no tDCS condition $\times$ time interaction with respect to the current level required to achieve moderate pain $F(3.57, 64.55) = 2.42$, $p = .063$, partial $\eta^2 =$ .11.

To probe the effect of time, three pairwise comparisons were conducted with Bonferroni correction performed at $.05 / 3 = .017$. The analysis revealed that the current level required to elicit moderate pain was significantly higher at Time 3 ($p < .001$) and Time 2 ($p =$ .002) than at Time 1, and significantly higher at Time 3 than at Time 2 ($p < .001$).

These results suggest that the current level required to elicit moderate pain increases over time, irrespective of the tDCS condition administered, and is inconsistent with the final hypothesis.

Discussion

The aim of the current study was to evaluate the effect of tDCS on experimentally induced hyperalgesia in healthy volunteers. The first hypothesis that electrical stimulation induces primary and secondary hyperalgesia was supported, as sensitivity to pinprick stimuli at the primary site and secondary site following electrical stimulation increased. There was an unexpected finding that primary and secondary hyperalgesia do not co-occur. The second hypothesis that tDCS induces analgesic effects on experimentally induced pain was not supported, as sensitivity to pinprick stimuli increased following electrical stimulation, irrespective of the tDCS condition administered. The final hypothesis that tDCS modulates

pain thresholds was not supported, as the current level of electrical stimulation required to elicit moderate increased, irrespective of tDCS condition administered.

Hypothesis one: Primary and Secondary Hyperalgesia

It was hypothesised that following electrical stimulation, subjective ratings of pain at the primary and secondary site would increase in response to pinprick stimuli, consistent with the presence of primary and secondary hyperalgesia (Klein et al., 2008; Pfau et al., 2011). This was necessary to ensure that electrical stimulation was able to evoke the expected changes in central signalling processes, in order to draw valid conclusions about the effects of tDCS on hyperalgesia. This hypothesis was supported, indicating that hyperalgesia was successfully induced (Klein et al., 2004; Sluka et al., 2000; Vo & Drummond, 2013) and that central sensitisation to sensory input from Aδ fibers likely explains the response observed in the secondary zone of hyperalgesia (Ziegler et al., 1999). Additionally, these findings validate that electrical stimulation preferentially activates superficial Aδ and C nociceptors (Inui et al., 2002; Nilsson & Schouenborg, 1999) as a C fibre electrically stimulated at 1 Hz can cause sensitisation (Wall & Woolf, 1984).

Interestingly, results indicate that the primary and secondary hyperalgesia do not co-occur as post-hoc tests revealed higher ratings of pain to pinprick stimuli at the secondary site only at Time 3 when compared to Time 1, whereas pain ratings at the primary site was higher at both Time 2 and Time 3 when compared to Time 1. Therefore, secondary hyperalgesia may only be induced following longer periods of electrical stimulation. Additionally, post-hoc tests did not reveal a significant increase in sensitivity to pinprick stimuli at the primary site from Time 2 to Time 3, despite displaying a higher numerical mean at Time 3. This suggests that the longer the duration of electrical stimulation, the more gradual the increase to sensitivity of pinprick stimuli is. This is opposed to a more exponential increase in sensitivity that occurs soon following the administration of electrical stimulation. This corroborates a

neural habituation mechanism whereby nociceptive signalling becomes less intense to repeated administration of a stimulus over time, thus resulting in a more gradual increase in sensitivity. Thus, the decrease in pain sensitivity to pinprick stimuli observed between Time 2 and Time 3 at the primary site is expected to occur in healthy participants (Le Bars, 2002). However, further research is needed to assess the sensitivity to pinprick stimuli following electrical stimulation over more than three times of measurements at both the primary and secondary site to validate this interpretation.

Hypothesis two: The Effect of tDCS on Pain Ratings Elicited by Pinprick Stimuli.

Results do not support the hypothesis that administration of tDCS induces analgesic effects on experimentally induced pain, as indicated by pain ratings elicited by pinprick stimuli increasing over time at both the primary and secondary site, irrespective of the tDCS condition administered. Thus, findings are inconsistent with Borckardt et al. (2012) that reported decreases in cold detection and cold pain thresholds, as well as increases in warm sensory thresholds following anodal tDCS on M1. Additionally, findings are inconsistent with Reidler et al. (2012) that reported a decrease in pain ratings to von Frey's monofilament following anodal tDCS at M1, as well as Mylius et al. (2012), that reported an increased tolerance to heat pain following anodal tDCS at the DLPFC. However, results are consistent Antal et al. (2008) that reported no difference in pain perception of laser-induced pain following anodal tDCS at M1. The contradictory results of the current study and past research are collectively addressed with the final hypothesis.

Hypothesis three: Effects of tDCS on Pain Modulation

Results do not support the hypothesis that tDCS modulates pain thresholds as the current level required to elicit moderate pain at pretest, posttest, and follow up of tDCS increased, and this did not depend on the tDCS condition administered. These findings are

inconsistent with Boggio et al. (2008) that reported an increase in pain thresholds of electrical stimulation following anodal tDCS of the M1 and the DLPFC.

The increasing pain ratings to pinprick stimuli and an increasing current level required to elicit moderate pain over time, irrespective of the tDCS condition administered, can be interpreted in light of participants' emotional factors. There is substantial evidence that anxiety and stress can increase pain and block analgesia (for a review, see Wiech & Tracey, 2009). In one study, participants were given verbal suggestions of hyperalgesia or analgesia (Bingel et al. 2011). Participants given suggestions of hyperalgesia were found to have significantly higher levels of anxiety and this hindered the effect of opiod remifentanil on heat pain. This was further confirmed via fMRI. In another study, Lyby, Aslaksen, and Flaten (2010) found that heat pain was related to higher perceived pain intensity during subsequent painful stimulation and to higher stress levels both during anticipation and administration of pain stimulation. Thus, considering the nature of the current study in which participants are informed that they will begin the testing session with electrical stimulation designed to induce pain, participants may present with anxiety and stress, which decreases as participants become more familiar with the sensations of the electrical stimulation at Time 2 and even more so at Time 3, thus requiring a higher current level to elicit moderate pain over time. Additionally, this anxiety may block the analgesic effects of tDCS, thus explaining why tDCS did not decrease pain ratings to pinprick stimuli over time. Future research can measure participants' anxiety from the beginning to the end of each testing session to validate this interpretation. This can achieved via galvanic skin response, as it provides a reliable and valid measure of anxiety over short time intervals (Najafpour, Asl-Aminabadi, Nuroloyuni, Jamali, & Shirzai, 2017).

Another possible contribution for the trend towards an increasing current level required to elicit moderate pain over time can be explained by the neural habituation

mechanism that exists in healthy populations, whereby nociceptive signalling becomes less intense to noxious stimuli over time (Le Bars, 2002). This temporal pain adaptation can occur during sequences of widely spaced noxious stimuli (Jepma, Jones, & Wager, 2014), thus explaining the stronger current level required to elicit moderate pain over time. Ernst, Lee, Dworkin, and Zaretsky (1986) found that repetitive electrical stimulation (1 Hz) in healthy participants results in a decrease in pain sensation to an almost unnoticeable level within a few seconds, before disappearing completely by the end of 10 min. However, it is unclear as to the time interval that mechanisms of habituation return to baseline following repetitive electrical stimulation. Future research can investigate the time interval between noxious stimuli that the neural habituation mechanism ceases, to validate the interpretation that the neural habituations mechanism contributes to the increasing current level required to elicit moderate pain over time.

Methodological Limitations between Current and Past Research

Inconsistencies of the results of the current study and past findings may be due to the tDCS stimulation parameters employed. Boggio et al (2008), Borckatdt et al. (2012), Mylius et al (2012b), and Reidler et al. (2012) had all found significant reductions of pain following tDCS, and these studies employed a stimulation parameter of 2 mA. In contrast, results of the current study corroborate those of Antal et al. (2008) that had found no significant reduction of pain following tDCS and applied a stimulation parameter of 1 mA. The tDCS current level of 1 mA was employed as 1 - 2 mA is considered sufficient to achieve desired excitability changes (Iyer et al. 2005; Nitsche & Paulus, 2001; Nitsche et al., 2003a; Nitsche et al., 2004). However, increasing the current level (e.g., 2 mA) may increase efficacy of stimulation due to a larger membrane polarisation shift (Nitsche & Paulus, 2000). Additionally, a greater applied current intensity may affect additional neuronal populations because of a greater efficacy of the electrical field in deeper cortical layers and different sensitivities of specific

neural populations to stimulation (Purpura & Mcmurty, 1965). Indeed, studies employing 1 mA have shown no analgesic effects or smaller analgesic effects (Antal et al., 2008; Csifcsak et al., 2009; Hansen et al., 2011; Jürgens, Schulte, Klein, & May, 2012). Additionally, Rush and Driscoll (1968) demonstrated that in monkeys, ~50% of transcranially applied currents enter the brain through the skull, and these results have been replicated in humans (Dymond, Coger, & Serafetinides, 1975). Thus, a stronger current level may translate to a higher percentage of currents entering the brain. However, a stronger current level may compromise participants to the blinding of the tDCS condition applied, due to the sensory effects of tDCS itself (O'Connell et al., 2018).

Differences between the size of the tDCS anode electrode of the current study and those used in Boggio et al (2008), Mylius et al (2012), and Reidler et al. (2012) Antal et al. (2008) also exist. The aforementioned studies employed an anodal electrode size of $35cm^2$, as opposed to the current study, in which the size of the anodal electrode was $24.75cm^2$. Thus, tDCS applied in the current study was more focal by not stimulating a broader cortical area. The efficacy of tDCS in past studies then may be due to a greater spreading effect of neuronal modulation, due to a greater cortical area targeted. Additionally, smaller electrodes may over-proportionally reduce current flow into the brain, such that an increase of current intensity may be warranted (Miranda et al., 2009). This exacerbates the already weak tDCS current level applied. However, the setback of a larger electrode size is that it may compromise participant blinding due to inducing greater cutaneous discomfort (Turi et al., 2014).

In summary, tDCS stimulation parameters including the current level applied and size of the electrodes influence the efficacy of tDCS to induce cortical excitability. However, more efficacious stimulation parameters may compromise participant blinding, due to the sensory effects of tDCS itself. Therefore, the results of past findings could be partially due to

demand characteristics. It is a credit of the current study then to adhere to a more rigorous double blinding procedure.

To employ more efficacious stimulation parameters without compromise to participant blinding, researchers may deliver tDCS at irrelevant cortical areas as the sham tDCS condition. Pain researchers can apply the anodal tDCS electrode at the V1 however employ identical stimulation parameters as the experimental condition(s). This is because the V1 has not been found to reduce pain (e.g., Boggio et al., 2008). This will induce the same cutaneous discomfort as in the experimental condition(s), but without inducing cortical excitability at the region of interest. In doing so, the control group effectively becomes a comparison group, as the sensory effects of tDCS in the experimental condition(s) are as similar as possible to the control condition. This may better achieve double blinding as compared to the traditional sham tDCS, as the sensory effects of tDCS will be the same.

Sampling Limitation

There is evidence for an analgesic effect of tDCS in the current study, however due to sample size, could not be realised. The interaction effect of tDCS condition and time with respects to the current level required to elicit moderate pain was trending towards significance ($p = .063$), with a moderate effect size reported (partial $\eta^2 = .11$), suggesting that the current study was underpowered to reach significance (Trout, Kaufmann & Kallmes, 2007). If tests of sphericity had not been violated, the interaction effect would have reached significance level ($p = .031$). Additionally, the sham tDCS condition displayed the lowest current level required to elicit moderate pain and the highest pain ratings to pinprick stimuli at Time 3. The limitation of small sample size is substantiated by a priori power analysis, which suggested a sample size of 76 to achieve 80% power, although the sample size of the current study is based on Boggio et al. (2008) that had detected an effect of tDCS on pain. Unfortunately, recruitment of participants was hampered time constraints that were further

exacerbated by each session requiring 2.5 hours to conduct. Additionally, participants that satisfied eligibility criteria and that were willing to participate were difficult to source. Therefore, future research should recruit a sample size consistent with a priori power analysis as smaller sample sizes have been found to demonstrate larger effects for tDCS (O'Connell et al., 2018). It is possible then that the results of Boggio et al. (2008) reflect a false positive.

Conclusion

The current study concludes that pain to pinprick stimuli and the current level required to elicit moderate pain to electrical stimulation increases over time, and tDCS does not affect this. However, the current study employed less efficacious tDCS stimulation parameters, in favour of participant blinding. Therefore, the positive results of past studies may in be in part due to demand characteristics. Additionally, results of the current study may be limited by the presentation of participants' anxiety. Theoretical implications then include a discussed alternative method for participant blinding and measurement of participants' anxiety during testing to control for its effect on pain perception. The latter is generalisable to research involving brain stimulation or not. Additionally, results of the current study are limited by small sample size, such that future research should include larger sample sizes to be better able to detect an analgesic effect induced by tDCS. Despite these methodological considerations, the current study provided a double blinded, randomised, sham controlled, crossover design, such that the credibility of the results should not be undermined. Therefore, tDCS remains a promising intervention for chronic pain sufferers, and future studies can now be better equipped to addressing the practical implications of tDCS for chronic pain sufferers.

References

Ahn, H., Woods, A., Kunik, M., Bhattacharjee, A., Chen, Z., Choi, E., & Fillingim, R. (2017). Efficacy of transcranial direct current stimulation over primary motor cortex (anode) and contralateral supraorbital area (cathode) on clinical pain severity and mobility performance in persons with knee osteoarthritis: An experimenter- and participant-blinded, randomized, sham-controlled pilot clinical study. *Brain Stimulation*, *10*(5), 902-909. doi: 10.1016/j.brs.2017.05.007

Allen, P., Bennett, K., & Heritage, B. *SPSS statistics version 22* (p. London, United Kingdom). Cengage Learning EMEA.

Ambrus, G., Al-Moyed, H., Chaieb, L., Sarp, L., Antal, A., & Paulus, W. (2012). The fade-in – Short stimulation – Fade out approach to sham tDCS – Reliable at 1 mA for naïve and experienced subjects, but not investigators. *Brain Stimulation*, *5*(4), 499-504. doi: 10.1016/j.brs.2011.12.001

Antal, A., Brepohl, N., Poreisz, C., Boros, K., Csifcsak, G., & Paulus, W. (2008). Transcranial direct current stimulation over somatosensory cortex decreases experimentally induced acute pain perception. *The Clinical Journal of Pain*, *24*(1), 56-63. doi: 10.1097/ajp.0b013e318157233b

Antal, A., Polania, R., Schmidt-Samoa, C., Dechent, P., & Paulus, W. (2011). Transcranial direct current stimulation over the primary motor cortex during fMRI. *Neuroimage*, *55*(2), 590-596. doi: 10.1016/j.neuroimage.2010.11.085

Apkarian, A., Baliki, M., & Geha, P. (2009). Towards a theory of chronic pain. *Progress in Neurobiology*, *87*(2), 81-97. doi: 10.1016/j.pneurobio.2008.09.018

Apkarian, A., Bushnell, M., Treede, R., & Zubieta, J. (2005). Human brain mechanisms of pain perception and regulation in health and disease. *European Journal of Pain*, *9*(4), 463-463. doi: 10.1016/j.ejpain.2004.11.001

Apkarian, A., Sosa, Y., Sonty, S., Levy, R., Harden, R., Parrish, T., & Gitelman, D. (2004). Chronic Back Pain Is Associated with Decreased Prefrontal and Thalamic Gray Matter Density. *Journal Of Neuroscience*, *24*(46), 10410-10415. doi: 10.1523/jneurosci.2541-04.2004

Apkarian, V., Hashmi, J., & Baliki, M. (2011). Pain and the brain: Specificity and plasticity of the brain in clinical chronic pain. *Pain*, *152*(Supplement), S49-S64. doi: 10.1016/j.pain.2010.11.010

Attal, N., Ayache, S., Ciampi de Andrade, D., Baudic, S., Jazat, F., & Mhalla, A. et al. (2015). Comparison of the analgesic effects of RTMS and TDCS in painful radiculopathy: a randomized double blind placebo controlled study. *Journal of the Neurological Sciences*, *357*, e357. doi: 10.1016/j.jns.2015.08.1278

Australian Bureau of Statistics. (2011). 4841.0 - Facts at your Fingertips: Health, 2011. Retrieved from http://www.abs.gov.au/ausstats/abs@.nsf/Lookup/4841.0Chapter12011

Bair, M., Wu, J., Damush, T., Sutherland, J., & Kroenke, K. (2008). Association of depression and anxiety alone and in combination with chronic musculoskeletal pain in primary care patients. *Psychosomatic Medicine*, *70*(8), 890-897. doi: 10.1097/psy.0b013e318185c510

Baudewig, J., Nitsche, M., Paulus, W., & Frahm, J. (2001). Regional modulation of BOLD MRI responses to human sensorimotor activation by transcranial direct current stimulation. *Magnetic Resonance in Medicine*, *45*(2), 196-201. doi: 10.1002/1522-2594(200102)45:2<196::aid-mrm1026>3.0.co;2-1

Beam, W., Borckardt, J., Reeves, S., & George, M. (2009). An efficient and accurate new method for locating the F3 position for prefrontal TMS applications. *Brain Stimulation*, *2*(1), 50-54. doi: 10.1016/j.brs.2008.09.006

Berkovits, I., Hancock, G., & Nevitt, J. (2000). Bootstrap Resampling Approaches for Repeated Measure Designs: Relative Robustness to Sphericity and Normality Violations. *Educational and Psychological Measurement*, *60*(6), 877-892. doi: 10.1177/00131640021970961

Berryhill, M., Peterson, D., Jones, K., & Stephens, J. (2014). Hits and misses: leveraging tDCS to advance cognitive research. *Frontiers in Psychology*, *5*. doi: 10.3389/fpsyg.2014.00800

Bijur, P., Latimer, C., & Gallagher, E. (2003). Validation of a Verbally Administered Numerical Rating Scale of Acute Pain for Use in the Emergency Department. *Academic Emergency Medicine*, *10*(4), 390-392. doi: 10.1197/aemj.10.4.390

Bikson, M., Datta, A., & Elwassif, M. (2009). Establishing safety limits for transcranial direct current stimulation. *Clinical Neurophysiology*, *120*(6), 1033-1034. doi: 10.1016/j.clinph.2009.03.018

Bingel, U., Wanigasekera, V., Wiech, K., Ni Mhuircheartaigh, R., Lee, M., Ploner, M., & Tracey, I. (2011). The Effect of Treatment Expectation on Drug Efficacy: Imaging the Analgesic Benefit of the Opioid Remifentanil. *Science Translational Medicine*, *3*(70), 1 - 9. doi: 10.1126/scitranslmed.3001244

Blyth, F., March, L., Brnabic, A., Jorm, L., Williamson, M., & Cousins, M. (2001). Chronic pain in Australia: a prevalence study. *Pain*, *89*(2), 127-134. doi: 10.1016/s0304-3959(00)00355-9

Boggio, P., Zaghi, S., & Fregni, F. (2009). Modulation of emotions associated with images of human pain using anodal transcranial direct current stimulation (tDCS). *Neuropsychologia*, *47*(1), 212-217. doi: 10.1016/j.neuropsychologia.2008.07.022

Boggio, P., Zaghi, S., Lopes, M., & Fregni, F. (2008). Modulatory effects of anodal transcranial direct current stimulation on perception and pain thresholds in healthy volunteers. *European Journal of Neurology*, *15*(10), 1124-1130. doi: 10.1111/j.1468-1331.2008.02270.x

Bolognini, N., Spandri, V., Ferraro, F., Salmaggi, A., Molinari, A., Fregni, F. and Maravita, A. (2015). Immediate and Sustained Effects of 5-Day Transcranial Direct Current Stimulation of the Motor Cortex in Phantom Limb Pain. *The Journal of Pain*, 16(7), 657-665.

Borckardt, J., Bikson, M., Frohman, H., Reeves, S., Datta, A., & Bansal, V. et al. (2011). A pilot study of the tolerability, safety and effects of high-definition transcranial direct current stimulation (HD-tDCS) on pain perception. *The Journal of Pain*, *12*(4), P52. doi: 10.1016/j.jpain.2011.02.212

Brodmann K. (1909). *Localisation in the cerebral cortex: The principles of comparative localisation in the cerebral cortex based on cytoarchitectonics* (L. J. Garey, Trans.). New York, NY: Springer.

Brown, J., Chatterjee, N., Younger, J., & Mackey, S. (2011). Towards a Physiology-Based Measure of Pain: Patterns of Human Brain Activity Distinguish Painful from Non-Painful Thermal Stimulation. *Plos ONE*, *6*(9), e24124. doi: 10.1371/journal.pone.0024124

Cohen, J. (1988). *Statistical power analysis for the behavioural sciences*. Hove: Lawrence

> Erlbaum Associates.

Cruccu, G., Garcia-Larrea, L., Hansson, P., Keindl, M., Lefaucheur, J., & Paulus, W. et al.

> (2016). EAN guidelines on central neurostimulation therapy in chronic pain
>
> conditions. *European Journal of Neurology*, *23*(10), 1489-1499. doi: 10.1111/ene.13103

Csifcsak, G., Antal, A., Hillers, F., Levold, M., Bachmann, C., & Happe, S. et al. (2009).

> Modulatory Effects of Transcranial Direct Current Stimulation on Laser-Evoked
>
> Potentials. *Pain Medicine*, *10*(1), 122-132. doi: 10.1111/j.1526-4637.2008.00508.x

D'Amico, D., & Barbarito, C. (2015). *Health & physical assessment in nursing* (3rd ed.).

> Pearson.

DaSilva, A., Becerra, L., Pendse, G., Chizh, B., Tully, S., & Borsook, D. (2008). Colocalized

> Structural and Functional Changes in the Cortex of Patients with Trigeminal
>
> Neuropathic Pain. *Plos ONE*, *3*(10), e3396. doi: 10.1371/journal.pone.0003396

Datta, A., Truong, D., Minhas, P., Parra, L., & Bikson, M. (2012). Inter-Individual Variation

> during Transcranial Direct Current Stimulation and Normalization of Dose Using MRI-
>
> Derived Computational Models. *Frontiers in Psychiatry*, *3*. doi:
>
> 10.3389/fpsyt.2012.00091

Deer, T., Leong, M., & Ray, A. (2015). *Treatment of Chronic Pain by Integrative Approaches*

> (1st ed., p. 16). New York, NY: Springer New York.

Demyttenaere, K., Bruffaerts, R., Lee, S., Posada-Villa, J., Kovess, V., & Angermeyer, M. et al.

> (2007). Mental disorders among persons with chronic back or neck pain: Results from
>
> the world mental health surveys. *Pain*, *129*(3), 332-342. doi: 10.1016/j.pain.2007.01.022

DosSantos, M., Martikainen, I., Nascimento, T., Love, T., Deboer, M., & Maslowski, E. et al.

    (2012). Reduced Basal Ganglia μ-Opioid Receptor Availability in Trigeminal

    Neuropathic Pain: A Pilot Study. *Molecular Pain*, *8*, 1744-8069-8-74. doi:

    10.1186/1744-8069-8-74

Duquette, M., Roy, M., Leporé, F., Peretz, I., & Rainville, P. (2007). Mécanismes cérébraux

    impliqués dans l'interaction entre la douleur et les émotions. *Revue*

    Neurologique, *163*(2), 169-179. doi: 10.1016/s0035-3787(07)90388-4

Dymond, A. M., Coger, R. W. & Serafetinides, E. A. (1975). Intracerebral current levels in man

    during electrosleep therapy. *Biological Psychiatry* 10, 101–104.

Ernst, M., Lee, M., Dworkin, B., & Zaretsky, H. (1986). Pain perception decrement produced

    through repeated stimulation. *Pain*, *26*(2), 221-231. doi: 10.1016/0304-3959(86)90077-1

Faul, F., Erdfelder, E., Lang, A., & Buchner, A. (2007). G*Power 3: A flexible statistical power

    analysis program for the social, behavioral, and biomedical sciences. *Behavior Research*

    Methods, *39*(2), 175-191. doi: 10.3758/bf03193146

Finnerup, N., Attal, N., Haroutounian, S., McNicol, E., Baron, R., & Dworkin, R. et al. (2015).

    Pharmacotherapy for neuropathic pain in adults: a systematic review and meta-

    analysis. *The Lancet Neurology*, *14*(2), 162-173. doi: 10.1016/s1474-4422(14)70251-0

Fregni, F., & Pascual-Leone, A. (2007). Technology Insight: noninvasive brain stimulation in

    neurology—perspectives on the therapeutic potential of rTMS and tDCS. *Nature*

    Clinical Practice Neurology, *3*(7), 383-393. doi: 10.1038/ncpneuro0530

Fregni, F., Boggio, P., Lima, M., Ferreira, M., Wagner, T., & Rigonatti, S. et al. (2006). A

    sham-controlled, phase II trial of transcranial direct current stimulation for the treatment

of central pain in traumatic spinal cord injury. *Pain*, *122*(1), 197-209. doi:

10.1016/j.pain.2006.02.023

Fregni, F., DaSilva, D., Potvin, K., Ramos-Estebanez, C., Cohen, D., Pascual-Leone, A., &

Freedman, S. (2005). Treatment of chronic visceral pain with brain stimulation. *Annals

of Neurology*, *58*(6), 971-972. doi: 10.1002/ana.20651

Fregni, F., Freedman, S., & Pascual-Leone, A. (2007). Recent advances in the treatment of

chronic pain with non-invasive brain stimulation techniques. *The Lancet

Neurology*, *6*(2), 188-191. doi: 10.1016/s1474-4422(07)70032-7

Fregni, F., Gimenes, R., Valle, A., Ferreira, M., Rocha, R., & Natalle, L. et al. (2006). A

randomized, sham-controlled, proof of principle study of transcranial direct current

stimulation for the treatment of pain in fibromyalgia. *Arthritis & Rheumatism*, *54*(12),

3988-3998. doi: 10.1002/art.22195

Fujiyama, H., Hyde, J., Hinder, M., Kim, S., McCormack, G., Vickers, J., & Summers, J.

(2014). Delayed plastic responses to anodal tDCS in older adults. *Frontiers in Aging

Neuroscience*, *6*. doi: 10.3389/fnagi.2014.00115

Garcia-Larrea, L., Maarrawi, J., Peyron, R., Costes, N., Mertens, P., Magnin, M., & Laurent, B.

(2006). On the relation between sensory deafferentation, pain and thalamic activity in

Wallenberg's syndrome: A PET-scan study before and after motor cortex

stimulation. *European Journal of Pain*, *10*(8), 677-677. doi:

10.1016/j.ejpain.2005.10.008

García-Larrea, L., Peyron, R., Mertens, P., Gregoire, C., Lavenne, F., & Le Bars, D. et al.

(1999). Electrical stimulation of motor cortex for pain control: a combined PET-scan and

electrophysiological study. *Pain*, *83*(2), 259-273. doi: 10.1016/s0304-3959(99)00114-1

Garcia-Larrea, L., Peyron, R., Mertens, P., Grégoire, M., Lavenne, F., & Bonnefoi, F. et al. (1997). Positron Emission Tomography during Motor Cortex Stimulation for Pain Control. *Stereotactic and Functional Neurosurgery*, *68*(1-4), 141-148. doi: 10.1159/000099915

Gignac, G. (2017). *Factorial Repeated Measures ANOVA* (1st ed., p. 21). Perth, Australia.

Girbés, E., Nijs, J., Torres-Cueco, R., & Cubas, C. (2013). Pain Treatment for Patients With Osteoarthritis and Central Sensitization. *Physical Therapy*, *93*(6), 842-851. doi: 10.2522/ptj.20120253

Grachev, I., Fredrickson, B., & Apkarian, V. (2000). Abnormal brain chemistry in chronic back pain: an in vivo proton magnetic resonance spectroscopy study. *Pain*, *89*(1), 7-18. doi: 10.1016/s0304-3959(00)00340-7

Hallett, M. (2007). Transcranial Magnetic Stimulation: A Primer. *Neuron*, *55*(2), 187-199. doi: 10.1016/j.neuron.2007.06.026

Halper, J., Yagi, C., Manevitz, A., Nishimoto, K., & Onishi, A. (2016). Comparison of beam technique and 5.5 centimeter rule for determing site of TMS stimulation for major depressive disorder. *Brain Stimulation*, *9*(5), e3. doi: 10.1016/j.brs.2016.06.012

Hansen, N., Obermann, M., Poitz, F., Holle, D., Diener, H., & Antal, A. et al. (2011). Modulation of human trigeminal and extracranial nociceptive processing by transcranial direct current stimulation of the motor cortex. *Cephalalgia*, *31*(6), 661-670. doi: 10.1177/0333102410390394

Horvath, J., Carter, O., & Forte, J. (2014). Transcranial direct current stimulation: five important issues we aren't discussing (but probably should be). *Frontiers in Systems Neuroscience*, *8*. doi: 10.3389/fnsys.2014.00002

Inui, K., Tran, T., Hoshiyama, M., & Kakigi, R. (2002). Preferential stimulation of Aδ fibers by intra-epidermal needle electrode in humans. *Pain*, *96*(3), 247-252. doi: 10.1016/s0304-3959(01)00453-5

Iyer, M., Mattu, U., Grafman, J., Lomarev, M., Sato, S., & Wassermann, E. (2005). Safety and cognitive effect of frontal DC brain polarization in healthy individuals. *Neurology*, *64*(5), 872-875. doi: 10.1212/01.wnl.0000152986.07469.e9

Jepma, M., Jones, M., & Wager, T. (2014). The Dynamics of Pain: Evidence for Simultaneous Site-Specific Habituation and Site-Nonspecific Sensitization in Thermal Pain. *The Journal of Pain*, *15*(7), 734-746. doi: 10.1016/j.jpain.2014.02.010

Ji, R., Kohno, T., Moore, K., & Woolf, C. (2003). Central sensitization and LTP: do pain and memory share similar mechanisms?. *Trends In Neurosciences*, *26*(12), 696-705. doi: 10.1016/j.tins.2003.09.017

Jürgens, T., Schulte, A., Klein, T., & May, A. (2012). Transcranial direct current stimulation does neither modulate results of a quantitative sensory testing protocol nor ratings of suprathreshold heat stimuli in healthy volunteers. *European Journal of Pain*, *16*(9), 1251-1263. doi: 10.1002/j.1532-2149.2012.00135.x

Kaneria, A. (2014). Opioid-induced hyperalgesia: when pain killers make pain worse. *Case Reports*, *2014*(jun04 1), bcr2014204551-bcr2014204551. doi: 10.1136/bcr-2014-204551

Kaube, H., Katsarava, Z., Käufer, T., Diener, H., & Ellrich, J. (2000). A new method to increase nociception specificity of the human blink reflex. *Clinical Neurophysiology*, *111*(3), 413-416. doi: 10.1016/s1388-2457(99)00295-3

Kilo, S., Schmelz, M., Koltzenburg, M., & Handwerker, H. (1994). Different patterns of

hyperalgesia induced by experimental inflammation in human skin. *Brain*, *117*(2), 385-

396. doi: 10.1093/brain/117.2.385

Kim, J., Loggia, M., Cahalan, C., Harris, R., Beissner, F., & Garcia, R. et al. (2015). The

Somatosensory Link in Fibromyalgia: Functional Connectivity of the Primary

Somatosensory Cortex Is Altered by Sustained Pain and Is Associated With

Clinical/Autonomic Dysfunction. *Arthritis & Rheumatology*, *67*(5), 1395-1405. doi:

10.1002/art.39043

Klein, T., Magerl, W., Hopf, H., Sandkühler, J., & Treede, R. (2004). Perceptual Correlates of

Nociceptive Long-Term Potentiation and Long-Term Depression in Humans. *Journal of

Neuroscience*, *24*(4), 964-971. doi: 10.1523/jneurosci.1222-03.2004

Klein, T., Stahn, S., Magerl, W., & Treede, R. (2008). The role of heterosynaptic facilitation in

long-term potentiation (LTP) of human pain sensation. *Pain*, *139*(3), 507-519. doi:

10.1016/j.pain.2008.06.001

Klem, G. H., Lüders, H. O., Jasper, H. H., & Elger, C. (1999). The ten-twenty electrode system

of the International Federation. The International Federation of Clinical

Neurophysiology. *Electroencephalography and clinical neurophysiology.

Supplement*, *52*, 3-6.

Knotkova, H., & Cruciani, R. (2010). Non-invasive Transcranial Direct Current Stimulation for

the Study and Treatment of Neuropathic Pain. *Methods In Molecular Biology*, 505-515.

doi: 10.1007/978-1-60327-323-7_37

Koppert, W., Dern, S., Sittl, R., Albrecht, S., Schüttler, J., & Schmelz, M. (2001). A New Model

    of Electrically Evoked Pain and Hyperalgesia in Human Skin. *Anesthesiology*, *95*(2),

    395-402. doi: 10.1097/00000542-200108000-00022

Koyama, S., Katayama, Y., Maejima, S., Hirayama, T., Fujii, M., & Tsubokawa, T. (1993).

    Thalamic neuronal hyperactivity following transection of the spinothalamic tract in the

    cat: involvement ofN-methyl-d-aspartate receptor. *Brain Research*, *612*(1-2), 345-350.

    doi: 10.1016/0006-8993(93)91684-k

LaMotte, R., Shain, C., Simone, D., & Tsai, E. (1991). Neurogenic hyperalgesia:

    psychophysical studies of underlying mechanisms. *Journal of Neurophysiology*, *66*(1),

    190-211. doi: 10.1152/jn.1991.66.1.190

LaMotte, R., Thalhammer, J., Torebjork, H., & Robinson, C. (1982). Peripheral neural

    mechanisms of cutaneous hyperalgesia following mild injury by heat. *The Journal of*

    Neuroscience, *2*(6), 765-781. doi: 10.1523/jneurosci.02-06-00765.1982

Lang, N., Nitsche, M., Paulus, W., Rothwell, J., & Lemon, R. (2004). Effects of transcranial

    direct current stimulation over the human motor cortex on corticospinal and transcallosal

    excitability. *Experimental Brain Research*, *156*(4), 439-443. doi: 10.1007/s00221-003-

    1800-2

Lang, N., Siebner, H., Ward, N., Lee, L., Nitsche, M., & Paulus, W. et al. (2005). How does

    transcranial DC stimulation of the primary motor cortex alter regional neuronal activity

    in the human brain?. *European Journal of Neuroscience*, *22*(2), 495-504. doi:

    10.1111/j.1460-9568.2005.04233.x

Lang, S., Klein, T., Magerl, W., & Treede, R. (2007). Modality-specific sensory changes in

humans after the induction of long-term potentiation (LTP) in cutaneous nociceptive

pathways. *Pain*, *128*(3), 254-263. doi: 10.1016/j.pain.2006.09.026

Le Bars, D. (2002). The whole body receptive field of dorsal horn multireceptive

neurones. *Brain Research Reviews*, *40*(1-3), 29-44. doi: 10.1016/s0165-0173(02)00186-

8

Li, L., Uehara, K., & Hanakawa, T. (2015). The contribution of interindividual factors to

variability of response in transcranial direct current stimulation studies. *Frontiers in

Cellular Neuroscience*, *9*. doi: 10.3389/fncel.2015.00181

Luedtke, K., Rushton, A., Wright, C., Geiss, B., Juergens, T., & May, A. (2012). Transcranial

Direct Current Stimulation for the Reduction of Clinical and Experimentally Induced

Pain: a systematic review and meta-analysis. *The Clinical Journal of Pain*, *28*(5), 452-

461. doi: 10.1097/ajp.0b013e31823853e3

Lyby, P., Aslaksen, P., & Flaten, M. (2010). Is fear of pain related to placebo

analgesia?. *Journal of Psychosomatic Research*, *68*(4), 369-377. doi:

10.1016/j.jpsychores.2009.10.009

Magdalena, S., Volz, M., Farmer, A. and Siegmund, B. (2016). Reduction of chronic abdominal

pain in patients with inflammatory bowel disease through transcranial direct current

stimulation. *Pain*, 157(2), pp.429-437.

http://dx.doi.org/10.1097/j.pain.0000000000000386

Manfredi, M., Bini, G., Cruccu, G., Accornero, N., Berardelli, A., & Medolago, L. (1981).

Congenital Absence of Pain. *Archives of Neurology*, *38*(8), 507-511. doi:

10.1001/archneur.1981.00510080069010

Marinus, J., Moseley, G., Birklein, F., Baron, R., Maihöfner, C., Kingery, W., & van Hilten, J. (2011). Clinical features and pathophysiology of complex regional pain syndrome. *The Lancet Neurology*, *10*(7), 637-648. doi: 10.1016/s1474-4422(11)70106-5

Martikainen, I., Nuechterlein, E., Pecina, M., Love, T., Cummiford, C., & Green, C. et al. (2015). Chronic Back Pain Is Associated with Alterations in Dopamine Neurotransmission in the Ventral Striatum. *Journal of Neuroscience*, *35*(27), 9957-9965. doi: 10.1523/jneurosci.4605-14.2015

MBF Foundation. (2018). The high price of pain: the economic impact of persistent pain in Australia. Retrieved from http://fpm.anzca.edu.au/documents/thehighpriceofpainfinal-185.pdf

McAllister, M. (2018). What is Central Sensitization?. Retrieved from http://www.instituteforchronicpain.org/understanding-chronic-pain/what-is-chronic-pain/central-sensitization

Merskey, H., & Bogduk, N. (1994). *Classification of Chronic Pain*. Seattle: IASP Press.

Miller, A., Sanderson, K., Bruno, R., Breslin, M., & Neil, A. (2017). The prevalence of pain and analgesia use in the Australian population: Findings from the 2011 to 2012 Australian National Health Survey. *Pharmacoepidemiology and Drug Safety*, *26*(11), 1403-1410. doi: 10.1002/pds.4301

Mir-Moghtadaei, A., Caballero, R., Fried, P., Fox, M., Lee, K., & Giacobbe, P. et al. (2015). Concordance Between BeamF3 and MRI-neuronavigated Target Sites for Repetitive Transcranial Magnetic Stimulation of the Left Dorsolateral Prefrontal Cortex. *Brain Stimulation*, *8*(5), 965-973. doi: 10.1016/j.brs.2015.05.008

Miranda, P., Faria, P., & Hallett, M. (2009). What does the ratio of injected current to electrode area tell us about current density in the brain during tDCS?. *Clinical Neurophysiology*, *120*(6), 1183-1187. doi: 10.1016/j.clinph.2009.03.023

Moloney, T., & Witney, A. (2013). Transcranial direct current stimulation (tDCS) priming of 1Hz repetitive transcranial magnetic stimulation (rTMS) modulates experimental pain thresholds. *Neuroscience Letters*, *534*, 289-294. doi: 10.1016/j.neulet.2012.11.049

Monte-Silva, K., Kuo, M., Hessenthaler, S., Fresnoza, S., Liebetanz, D., Paulus, W., & Nitsche, M. (2013). Induction of Late LTP-Like Plasticity in the Human Motor Cortex by Repeated Non-Invasive Brain Stimulation. *Brain Stimulation*, *6*(3), 424-432. doi: 10.1016/j.brs.2012.04.011

Moreno-Duarte, I., Morse, L., Alam, M., Bikson, M., Zafonte, R., & Fregni, F. (2014). Targeted therapies using electrical and magnetic neural stimulation for the treatment of chronic pain in spinal cord injury. *Neuroimage*, *85*, 1003-1013. doi: 10.1016/j.neuroimage.2013.05.097

Mylius, V., Jung, M., Menzler, K., Haag, A., Khader, P., & Oertel, W. et al. (2012). Effects of transcranial direct current stimulation on pain perception and working memory. *European Journal of Pain*, *16*(7), 974-982. doi: 10.1002/j.1532-2149.2011.00105.x

Najafpour, E., Asl-Aminabadi, N., Nuroloyuni, S., Jamali, Z., & Shirazi, S. (2017). Can galvanic skin conductance be used as an objective indicator of children's anxiety in the dental setting?. *Journal of Clinical and Experimental Dentistry*, 0-0. doi: 10.4317/jced.53419

Nilsson, H., & Schouenborg, J. (1999). Differential inhibitory effect on human nociceptive skin senses induced by local stimulation of thin cutaneous fibers. *Pain*, *80*(1), 103-112. doi: 10.1016/s0304-3959(98)00205-x

Nitsche, M., & Paulus, W. (2000). Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. *The Journal of Physiology*, *527*(3), 633-639. doi: 10.1111/j.1469-7793.2000.t01-1-00633.x

Nitsche, M., & Paulus, W. (2001). Sustained excitability elevations induced by transcranial DC motor cortex stimulation in humans. *Neurology*, *57*(10), 1899-1901. doi: 10.1212/wnl.57.10.1899

Nitsche, M., Cohen, L., Wassermann, E., Priori, A., Lang, N., & Antal, A. et al. (2008). Transcranial direct current stimulation: State of the art 2008. *Brain Stimulation*, *1*(3), 206-223. doi: 10.1016/j.brs.2008.06.004

Nitsche, M., Doemkes, S., Karaköse, T., Antal, A., Liebetanz, D., & Lang, N. et al. (2007). Shaping the Effects of Transcranial Direct Current Stimulation of the Human Motor Cortex. *Journal of Neurophysiology*, *97*(4), 3109-3117. doi: 10.1152/jn.01312.2006

Nitsche, M., Niehaus, L., Hoffmann, K., Hengst, S., Liebetanz, D., Paulus, W., & Meyer, B. (2004). MRI study of human brain exposed to weak direct current stimulation of the frontal cortex. *Clinical Neurophysiology*, *115*(10), 2419-2423. doi: 10.1016/j.clinph.2004.05.001

Nitsche, M., Nitsche, M., Klein, C., Tergau, F., Rothwell, J., & Paulus, W. (2003). Level of action of cathodal DC polarisation induced inhibition of the human motor cortex. *Clinical Neurophysiology*, *114*(4), 600-604. doi: 10.1016/s1388-2457(02)00412-1

Nuti, C., Peyron, R., Garcia-Larrea, L., Brunon, J., Laurent, B., Sindou, M., & Mertens, P. (2005). Motor cortex stimulation for refractory neuropathic pain: Four year outcome and predictors of efficacy. *Pain*, *118*(1), 43-52. doi: 10.1016/j.pain.2005.07.020

O'Connell, N., Marston, L., Spencer, S., DeSouza, L., & Wand, B. (2018). Non-invasive brain stimulation techniques for chronic pain. *Cochrane Database of Systematic Reviews*. doi: 10.1002/14651858.cd008208.pub4

Oldfield, R. (1971). The assessment and analysis of handedness: The Edinburgh inventory. *Neuropsychologia*, *9*(1), 97-113. doi: 10.1016/0028-3932(71)90067-4

Peyron, R., Faillenot, I., Mertens, P., Laurent, B., & Garcia-Larrea, L. (2007). Motor cortex stimulation in neuropathic pain. Correlations between analgesic effect and hemodynamic changes in the brain. A PET study. *Neuroimage*, *34*(1), 310-321. doi: 10.1016/j.neuroimage.2006.08.037

Pfau, D., Klein, T., Putzer, D., Pogatzki-Zahn, E., Treede, R., & Magerl, W. (2011). Analysis of hyperalgesia time courses in humans after painful electrical high-frequency stimulation identifies a possible transition from early to late LTP-like pain plasticity. *Pain*, *152*(7), 1532-1539. doi: 10.1016/j.pain.2011.02.037

Ploghaus, A., Becerra, L., Borras, C., & Borsook, D. (2003). Neural circuitry underlying pain modulation: expectation, hypnosis, placebo. *Trends in Cognitive Sciences*, *7*(5), 197-200. doi: 10.1016/s1364-6613(03)00061-5

Polania, R., Paulus, W., & Nitsche, M. (2012). Modulating cortico-striatal and thalamo-cortical functional connectivity with transcranial direct current stimulation. *Clinical Neurophysiology*, *122*, S146. doi: 10.1016/s1388-2457(11)60522-1

Posten, H. (1984). Robustness of the Two-Sample T-Test. *Robustness Of Statistical Methods And Nonparametric Statistics*, 92-99. doi: 10.1007/978-94-009-6528-7_23

Puri, R., Hinder, M., Fujiyama, H., Gomez, R., Carson, R., & Summers, J. (2015). Duration-dependent effects of the BDNF Val66Met polymorphism on anodal tDCS induced motor cortex plasticity in older adults: a group and individual perspective. *Frontiers in Aging Neuroscience*, *7*. doi: 10.3389/fnagi.2015.00107

Purpura, D., & McMurtry, J. (1965). Intracellular Activities and Evoked Potential Changes during Polarization of Motor Cortex. *Journal of Neurophysiology*, *28*(1), 166-185. doi: 10.1152/jn.1965.28.1.166

Purves, D., Augustine, G., Fitzpatrick, D., Hall, W., LaMantia, A., & White, L. (2008). *Neuroscience* (4th ed., pp. 231-251). Sunderland, Massachusetts.: Sinauer Associates, Inc.

Ready, L., Edwards, E. (1992). *Management of acute pain: A Practical Guide*. Seattle (Wash.): IASP Publications.

Reidler, J., Mendonca, M., Santana, M., Wang, X., Lenkinski, R., & Motta, A. et al. (2012). Effects of Motor Cortex Modulation and Descending Inhibitory Systems on Pain Thresholds in Healthy Subjects. *The Journal of Pain*, *13*(5), 450-458. doi: 10.1016/j.jpain.2012.01.005

Roussel, N., Nijs, J., Meeus, M., Mylius, V., Fayt, C., & Oostendorp, R. (2013). Central sensitization and altered central pain processing in chronic low back Pain: fact or myth?. *The Clinical Journal of Pain*, *29*(7), 625-638. doi: 10.1097/ajp.0b013e31826f9a71

Rozisky, J., Antunes, L., Brietzke, A., Sousa, A., & Caumo, W. (2015). Transcranial Direct
    Current Stimulation and Neuroplasticity. *Nova Science Publishers, Inc.*. Retrieved from
    https://www.researchgate.net/publication/305439421_TRANSCRANIAL_DIRECT_CU
    RRENT_STIMULATION_AND_NEUROPLASTICITY?enrichId=rgreq-
    faa106a9a2715003c3e22e387e3cc397-
    XXX&enrichSource=Y292ZXJQYWdlOzMwNTQzOTQyMTtBUzozODU3NDg0NDE
    yODg3MDRAMTQ2ODk4MTAwMzI3NQ%3D%3D&el=1_x_3&_esc=publicationCov
    erPdf

Ruge, D., Liou, L., & Hoad, D. (2012). Improving the Potential of Neuroplasticity. *Journal of
    Neuroscience*, *32*(17), 5705-5706. doi: 10.1523/jneurosci.0430-12.2012

Rush, S., & Driscoll, D. (1968). Current Distribution in the Brain From Surface
    Electrodes. *Anesthesia & Analgesia*, *47*(6), 717 - 7123. doi: 10.1213/00000539-
    196811000-00016

Sandkühler, J. (2009). Models and Mechanisms of Hyperalgesia and Allodynia. *Physiological
    Reviews*, *89*(2), 707-758. doi: 10.1152/physrev.00025.2008

Sanes, J., & Donoghue, J. (2000). Plasticity and Primary Motor Cortex. *Annual Review of
    Neuroscience*, *23*(1), 393-415. doi: 10.1146/annurev.neuro.23.1.393

Schafer, J. (1999). Multiple imputation: a primer. *Statistical Methods In Medical
    Research*, *8*(1), 3-15. doi: 10.1177/096228029900800102

Schmider, E., Ziegler, M., Danay, E., Beyer, L., & Bühner, M. (2010). Is It Really Robust?
    Reinvestigating the robustness of ANOVA against violations of the normal distribution
    assumption. *Methodology*, *6*(4), 147-151. doi: 10.1027/1614-2241/a000016

Sluka, K., Judge, M., McColley, M., Reveiz, P., & Taylor, B. (2000). Low frequency TENS is

    less effective than high frequency TENS at reducing inflammation-induced hyperalgesia

    in morphine-tolerant rats. *European Journal of Pain*, *4*(2), 185-193. doi:

    10.1053/eujp.2000.0172

Stern, J., Jeanmonod, D., & Sarnthein, J. (2006). Persistent EEG overactivation in the cortical

    pain matrix of neurogenic pain patients. *Neuroimage*, *31*(2), 721-731. doi:

    10.1016/j.neuroimage.2005.12.042

Tenenbaum, H., Mock, D., Gordon, A., Goklberg, M., Grossi, M., Locker, D., & Davis, K.

    (2001). Sensory and Affective Components of Orofacial Pain: Is it all in your

    Brain?. *Critical Reviews in Oral Biology & Medicine*, *12*(6), 455-468. doi:

    10.1177/10454411010120060101

Thair, H., Holloway, A., Newport, R., & Smith, A. (2017). Transcranial Direct Current

    Stimulation (tDCS): A Beginner's Guide for Design and Implementation. *Frontiers in

    Neuroscience*, *11*. doi: 10.3389/fnins.2017.00641

Trout, A. T., Kaufmann, T. J., & Kallmes, D. F. (2007). No significant difference. Says who?

    AJNR. American Journal of Neuroradiology, 28(2), 195-197. Retrieved from

    http://www.ajnr.org/content/28/2/195.full.pdf+html

Tsubokawa, T., Katayama, Y., Yamamoto, T., Hirayama, T., & Koyama, S. (1991). Motor

    cortex stimulation for control of thalamic pain. *Pain*, *41*, S491. doi: 10.1016/0304-

    3959(90)93093-d

Tsubokawa, T., Katayama, Y., Yamamoto, T., Hirayama, T., & Koyama, S. (1993). Chronic

    motor cortex stimulation in patients with thalamic pain. *Journal of Neurosurgery*, *78*(3),

    393-401. doi: 10.3171/jns.1993.78.3.0393

Turi, Z., Ambrus, G., Ho, K., Sengupta, T., Paulus, W., & Antal, A. (2014). When Size Matters: Large Electrodes Induce Greater Stimulation-related Cutaneous Discomfort Than Smaller Electrodes at Equivalent Current Density. *Brain Stimulation*, *7*(3), 460-467. doi: 10.1016/j.brs.2014.01.059

Vaseghi, B., Zoghi, M., & Jaberzadeh, S. (2014). Does anodal transcranial direct current stimulation modulate sensory perception and pain? A meta-analysis study. *Clinical Neurophysiology*, *125*(9), 1847-1858. doi: 10.1016/j.clinph.2014.01.020

Vaseghi, B., Zoghi, M., & Jaberzadeh, S. (2015). How Does Anodal Transcranial Direct Current Stimulation of the Pain Neuromatrix Affect Brain Excitability and Pain Perception? A Randomised, Double-Blind, Sham-Control Study. *PLOS ONE*, *10*(3), e0118340. doi: 10.1371/journal.pone.0118340

Vasylenko, O. (2012). *Effects of Anodal Transcranial Direct Current Stimulation on Experimentally Induced Heat Pain in Healthy Volunteers* (Masters). University of Tromsø.

Vo, L. (2014). *UNDERSTANDING PAIN: HOW IS PAIN PROCESSED IN HEALTHY HUMANS?* (Ph.D). Murdoch University.

Vo, L. (2018). *The effects of transcranial direct current stimulation (tDCS) on central sensitisation in complex regional pain syndrome.* Murdoch University

Vo, L., & Drummond, P. (2012). High frequency electrical stimulation concurrently induces central sensitization and ipsilateral inhibitory pain modulation. *European Journal of Pain*, *17*(3), 357-368. doi: 10.1002/j.1532-2149.2012.00208.x

Vos, T., Flaxman, A., Naghavi, M., Lozano, R., Michaud, C., & Ezzati, M. et al. (2012). Years lived with disability (YLDs) for 1160 sequelae of 289 diseases and injuries 1990–2010:

a systematic analysis for the Global Burden of Disease Study 2010. *The*

Lancet, *380*(9859), 2163-2196. doi: 10.1016/s0140-6736(12)61729-2

Wager, T., Rilling, J., Smith, E., Sokolik, A., Casey, K., & Davidson, R. et al. (2004). Placebo-

Induced Changes in fMRI in the Anticipation and Experience of

Pain. *Science*, *303*(5661), 1162-1167. doi: 10.1126/science.1093065

Wall, P., & Woolf, C. (1984). Muscle but not cutaneous C-afferent input produces prolonged

increases in the excitability of the flexion reflex in the rat. *The Journal of*

Physiology, *356*(1), 443-458. doi: 10.1113/jphysiol.1984.sp015475

Watson, C. P. N., Chipman, M. L., & Monks, R. C. (2006). Antidepressant analgesics: a

systematic review and comparative study. In S. B. McMahon, & M. Koltzenburg (Eds.),

Wall and Melzack's textbook of pain. (5th ed., pp. 481-497). London, UK: Churchill

Livingstone.

Wiech, K., & Tracey, I. (2009). The influence of negative emotions on pain: Behavioral effects

and neural mechanisms. *Neuroimage*, *47*(3), 987-994. doi:

10.1016/j.neuroimage.2009.05.059

Williams, S. (1991). Handedness inventories: Edinburgh versus Annett. *Neuropsychology*, *5*(1),

43-48. doi: 10.1037//0894-4105.5.1.43

Woolf, C. (2011). Central sensitization: Implications for the diagnosis and treatment of

pain. *Pain*, *152*(Supplement), S2-S15. doi: 10.1016/j.pain.2010.09.030

Yantis, S., & Abrams, R. (2016). *Sensation and Perception* (2nd ed., p. 452). New York: Worth

Publishers.

Yi, M., & Zhang, H. (2011). Nociceptive Memory in the Brain: Cortical Mechanisms of Chronic Pain. *Journal of Neuroscience*, *31*(38), 13343-13345. doi: 10.1523/jneurosci.3279-11.2011

Yoon, E., Kim, Y., Kim, H., Kim, S., Lee, Y. and Shin, H. (2013). Transcranial direct current stimulation to lessen neuropathic pain after spinal cord injury. *Neurorehabilitation and Neural Repair*, 28(3), pp.250-259

Youssef, A., Gustin, S., Nash, P., Reeves, J., Petersen, E., & Peck, C. et al. (2014). Differential brain activity in subjects with painful trigeminal neuropathy and painful temporomandibular disorder. *Pain*, *155*(3), 467-475. doi: 10.1016/j.pain.2013.11.008

Ziegler, E., Magerl, W., Meyer, R., & Treede, R. (1999). Secondary hyperalgesia to punctate mechanical stimuli. *Brain*, *122*(12), 2245-2257. doi: 10.1093/brain/122.12.2245

Appendix A

Murdoch University research participant portal information

Abstract

This study aims to investigate the effect of tDCS on acute pain in healthy individuals. Positive outcomes imply that tDCS may offer an effective, non-pharmacological and non-invasive treatment for chronic pain sufferers.

Description

This experiment will consist of 4 SEPARATE WEEKLY SESSIONS. Each session will take APPROXIMATELY 2.5 HOURS TO COMPLETE, (IT IS NOT 1 x 10 HOURS SESSION AS IT APPEARS ON THE PORTAL). At each session the participant will be asked to complete some screening questionnaires. Participant will then be asked to rate pain and sharpness to a pinprick administered to the right forearm. The participant will then be asked to rate pain and sharpness to single electrical pulses delivered through an electrode to the right forearm. The pulses will commence at a low current level and increase gradually until a moderate pain level (level 5) is reached. The participant will then receive 20 minutes of tDCS, (a non-invasive, safe, painless brain stimulation that uses direct electrical currents to stimulate specific parts of the brain). The participant will then be fitted with an electroencephalography (EEG) cap and experience pain mild to moderate induced by low electricity stimulation to the right forearm for 20 minutes. When the electrical stimulation is completed, the EEG cap will be removed. The participant will be asked to provide verbal feedback ratings for pain and sharpness at different intervals throughout the procedure. At the end of each session, participants will be asked to complete some brief questionnaires. Please note that the participant's head and face will be touched at different times by the researchers during the experiment. The EEG cap and the tCDS head bands will be fitted snugly to the head and saline solution will be used which may wet the hair and scalp. If you are interested and would like to find out more, please contact us at tcdsresearchers@gmail.com, or phone Cavan: 0428830879, Jane: 0487340511, or Brandon: 0478667448.

Eligibility Requirements

Must be aged between 18 and 65, right handed, are healthy, having no medical condition (e.g., chronic pain), mental illness (e.g., depression), cognitive impairment, intellectual disability, be on any medication, have a pacemaker or be pregnant.

Preparation

No alcohol for 24 hours before testing. No painkillers on the day of testing. No illicit drugs for one week prior to testing. Hair must be washed the day of testing with shampoo only (no conditioner) and no make up to be worn

Appendix B

Flyer placed around the university campus for participant recruitment

RESEARCH STUDY: The Effect of Non-invasive Brain Stimulation on Acute Pain

Ethics Approval Number: 2018/037
Did you know that 20% of Australians are affected by chronic pain?

Chronic pain severely impacts quality of life and unfortunately treatments are often invasive, ineffective and costly.

Can you help us in searching for better treatment intervention for chronic pain?

Our research involves investigating the effect of a non-pharmacological and non-invasive brain stimulation on acute pain and we need healthy right-handed volunteers between the age of 18 and 65 years old!

- The study will take place in a laboratory and includes four sessions, each lasting approximately 2.5 hours.
- The study investigates the effect of weak electrical current applied to scalp on acute pain induced by electrical pulses administered in the forearm.
- Our testing procedures are harmless and have been used widely.

You will be free to withdraw from the study at any time without any consequences.

You should not participate in the experiment if you are pregnant or breastfeeding, have a pacemaker installed, suffer from chronic pain or epilepsy, or have a history of other medical and/or psychiatric conditions.

So you think you can help?

Please contact Jane, Brandon or Cavan

Murdoch Psychology Students can sign up through the Subject Pool website:
https://murdochpes.sona-systems.com/

This study has been reviewed and approved by the Murdoch University Human Research Ethics Committee (Approval 30/4/2018).

Investigators: **Jane Wilson** **Brandon Sherrer** **Cavan Cardile**
Phone: **0487340511** **0478667448** **0428830879**
Email: tcdresearchers@gmail.com Supervisor:L.Vo@murdoch.edu.au

Appendix C

General health and transcranial direct current stimulation safety screening form

Health and Transcranial Magnetic Stimulation (TMS) and transcranial direct current stimulation (tDCS) Safety Screening
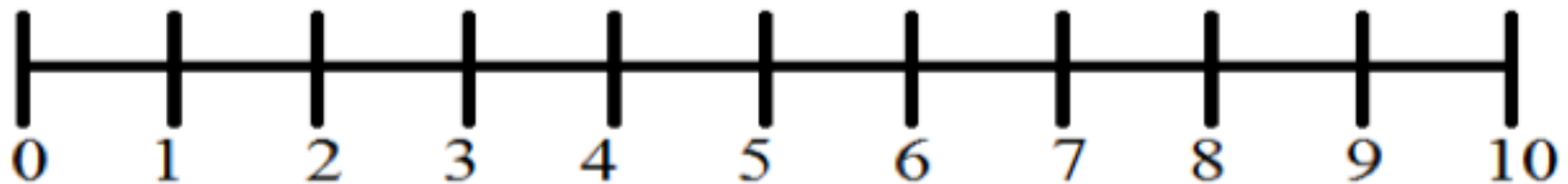
Name:
Date:
Age
Gender:

Please answer the following:

1. Do you suffer from any chronic pain conditions?	□ Yes	□ No
2. Do you suffer from any medical condition (e.g., heart condition, cancer, liver)?	□ Yes	□ No
3. Do you have epilepsy, or have you ever had a convulsion or a seizure?	□ Yes	□ No
4. Do you experience migraines/cluster headaches?	□ Yes	□ No
5. Have you ever had a fainting spell or syncope? If yes, please describe in which occasions in the space provided below.	□ Yes	□ No
6. Have you ever had a head trauma that was diagnosed as a concussion or was associated with loss of consciousness?	□ Yes	□ No
7. Have you ever undergone brain surgery?	□ Yes	□ No
8. Have you ever undergone neurological radiation treatment?	□ Yes	□ No
9. Do you have any hearing problems or ringing in your ears?	□ Yes	□ No
10. Do you have cochlear implants?	□ Yes	□ No
11. Are you pregnant or is there any chance that you might be, or breastfeeding?	□ Yes	□ No
12. Do you have metal in the brain, skull, or elsewhere in your body (e.g. splinters, fragments, clips, etc.)? If so, please specify type of metal?	□ Yes	□ No
13. Do you have an implanted neurostimulator (e.g., DBS, epidural/subdural, VNS?)	□ Yes	□ No
14. Do you have a cardiac pacemaker or intracardiac lines?	□ Yes	□ No
15. Do you have a medication infusion device?	□ Yes	□ No
16. Did you ever undergo TMS in the past? If so, were there any problems?	□ Yes	□ No
17. Did you ever undergo MRI in the past? If so, were there any problems?	□ Yes	□ No
18. Do you suffer from any psychiatric illness (e.g., depression, anxiety)?	□ Yes	□ No
19. Do you have any cognitive impairment (e.g., dementia, autism)?	□ Yes	□ No
20. Do you have any intellectual disability?	□ Yes	□ No
21. Have you taken any illicit drug with the past week (e.g., heroin, cocaine)?	□ Yes	□ No
22. Are you under the influence of alcohol, or have consumed alcohol today?	□ Yes	□ No
23. Do you take any substance, or any medication that may influence your pain perception (e.g., aspirin, paracetamol)	□ Yes	□ No

Appendix D

Verbal numerical rating scale used to assess pain sensitivity

PAIN / SHARPNESS RATING 0-10

0   1   2   3   4   5   6   7   8   9   10

No
pain /
sharpness

Moderate
pain / sharpness

Worst
pain /
sharpness
imaginable

Appendix E

The Edinburgh Handedness Inventory used to assess handedness

EDINBURGH HANDEDNESS INVENTORY

Name:………………………………………………….

Please indicate your preferences in the use of hands in the following activities by *putting* + 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* + +. If in any case you are really indifferent *put* + *in both columns*.
Some of the activities require both hands. In these cases the part of the task, or object, for which hand preference is wanted is indicated in brackets.
Please try to answer all the questions, and only leave a blank if you have no experience at all of the object or task.

		LEFT	RIGHT
1	Writing		
2	Drawing		
3	Throwing		
4	Scissors		
5	Toothbrush		
6	Knife (without fork)		
7	Spoon		
8	Broom (upper hand)		
9	Striking match (match)		
10	Opening box (lid)		

Appendix F

Ethics approval letter

Division of Research & Development
Research Ethics and Integrity Office

Monday, 30 April 2018

Chancellery Building
South Street
MURDOCH WA 6150
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Dr Lechi Vo
School of Psychology & Exercise Science
Murdoch University

Dear Lechi,

Project No.   2018/037
Project Title   The effects of transcranial direct current stimulation (tDCS) on acute pain in healthy individuals

Thank you for addressing the conditions placed on the above application to the Murdoch University Human Research Ethics Committee. On behalf of the Committee, I am pleased to advise the application now has:

OUTRIGHT APPROVAL

Approval is granted on the understanding that research will be conducted according the standards of the ***National Statement on Ethical Conduct in Human Research* (2007), the *Australian Code for the Responsible Conduct of Research* (2007)** and **Murdoch University policies** at all times. You must also abide by the **Human Research Ethics Committee's standard conditions of approval (see attached).** All reporting forms are available on the Research Ethics and Integrity web-site.

I wish you every success for your research.

Please quote your ethics project number in all correspondence.

Kind Regards,

Dr. Yvonne Haigh   Dr. Erich von Dietze
Chair   Manager
HREC Committee   Research Ethics and Integrity

cc: Dr Hakuei Fujiyama, Prof Peter Drummond, Dr Phillip Finch ; Brandon Scherrer, Jane Wilson, Cavan Cardile, Ashfaq Asif, Sarah Blakiston, Minouck Charlotte Duin. Benjamin James Hall, Kristin Knight, Ashleigh Louise Macri, Emma Leigh Taylor

Appendix G

Information letter outlining the purpose and nature of the experiment, procedures, benefits,

and risks

Participant Information Letter   www.murdoch.edu.au

Research Study: The effect of Transcranial Direct Current Stimulation on Central Sensitisation

Dear participants,

We invite you to participate in a research study that examines the effects of transcranial direct current stimulation (tDCS), a non-invasive and painless brain stimulation technique, on the sensation of pain. This study is part of our Honours Degree in psychology supervised by Dr Lechi Vo at Murdoch University.

Nature and Purpose of the Study

Twenty percent of the world population suffer from chronic pain. Unfortunately, the available treatments for chronic pain sufferers are often ineffective and may even exacerbate their symptoms. While the application of transcranial direct current stimulation (tDCS) to specific areas of brain has been shown to reduce acute pain in healthy individuals research in this area remains sparse so tDCS cannot be recommend as a treatment for chronic pain in current practice. Because tDCS potentially offers a more effective, affordable, and non-invasive alternative, which overcomes some of the limitations offered by alternative approaches (e.g., pharmacological) this study aims to further investigate the effect of tDCS on acute pain in healthy individuals.

If you are suffering from chronic pain, a mental illness (e.g., depression, anxiety), a medical condition (e.g., heart condition, cancer), experience epilepsy/seizures, cognitive impairment (e.g. dementia, autism), intellectual disability, are pregnant or breast feeding, wearing a pacemaker, or are taking any medications you should NOT participate in the current experiment. If you are unsure if you can participate in this study, please speak to one of the investigators now.

What the Study Will Involve

The experiment will consist of 3 separate weekly sessions. Each session will take about 2 hours to be completed. The investigator will show you and explain the relevant apparatus before the experiment commences. If you consent to participate in this study, you will:

· Complete a screening questionnaire to ensure that you are eligible to participate in the current study;
· Rate pain and sharpness on a scale of 0 to 10 from the application of a neuro-pen pressed against your forearm by the experimenter;
· Exfoliate your forearm with a pumice stone to prepare the sensitization sites;
· Be fitted with a 2cm x 3cm electrodes on one forearm and have area submitted to 0.5ms bursts of electrical stimulation, starting at a low amplitude and incremented at 0.1mA, until you rate a pain of 5 (a moderate pain level) on a scale of 0 to 10. This procedure (Figure 1) is harmless and has been used widely in laboratory settings.
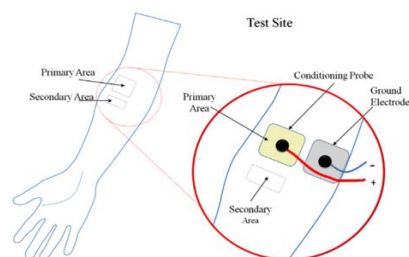
Figure 1-The sites on the forearm the electrodes will be placed.

· For the tCDS procedure, have four electrodes, which have been soaked in salt water (to help improve conductivity), placed on your head. Two will be placed on the upper left side of your head and two just above the right eyebrow. The electrodes will be held in place with a headband. For 20 minutes a mild electrical current at 2mA will be delivered through the electrodes. Only a small amount of the electricity reaches the brain, with the rest of the charge being dispersed harmlessly across the scalp. This procedure (Figure 2) has also widely been used in the laboratory;

Research Study: The effect of Transcranial Direct Current Stimulation on Central Sensitization

www.murdoch.edu.au

· Be fitted with a HydroCel Geodesic Sensor Net, consisting of electrodes being placed at 128 locations, and subjected to an Electroencephalography (EEG) to record the electrical activity of your brain during the remainder of the study after the tDCS;
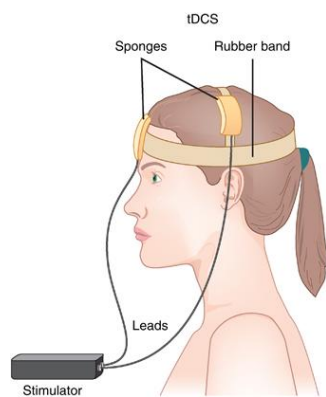

Figure 2-An example of what the tDCS will look like and it how it is applied.

· Once the tDCS is complete, be re-fitted with electrodes on the forearm and having the area submitted to 0.5ms bursts of electrical stimulation every second for 20 minutes. These electrical bursts will be at the amplitude that you previously identified as producing a moderate amount of pain (a rating of 5 on a scale of 0 to 10). You will be asked to provide a pain rating verbally to the experimenter on a scale of 0 to 10 every minute during the electrical stimulation. During the stimulation you may experience a "twitching" sensation in the muscles of the forearm and the area of stimulation may be sensitive to the touch for the next 24 hours;

· Be subjected to pinprick tests of hyperalgesia (an increased sensitivity to pain) at, and around the site of electrical stimulations, and being asked to provide a pain rating for each pinprick pen on a scale of 0-10.

Voluntary Participation and Withdrawal from the Study

Your participation in this study is entirely voluntary. You may withdraw at any time without discrimination or prejudice. All information is treated as confidential and no names or other details that might identify you will be used in any publication arising from the research. If you withdraw, all information you have provided will be destroyed.

Privacy

Your privacy is very important to us. Because some of the research team are staff members associated with this unit, whether you elect to participate or not will be kept entirely confidential. Any members of the research team who are associated with you in other roles will not know whether you have elected to participate and will view only anonymous data. It will thus not be possible to identify you; neither will you be identified in any publication arising out of this study.

Benefits of the Study

It is possible that there may be no direct benefit to you from participation in this study. While there is no guarantee that you will personally benefit, the knowledge gained from your participation may help others in the future. It will help improve the current knowledge about how pain is processed in the brain as well as the data being acquired helping to inform future studies exploring using tDCS as a treatment for chronic pain sufferers.

Possible Risks

As this a study about pain, there is some low risk and you will be experiencing ongoing moderate pain, but it will not have any long-term side effects. You should expect the areas that are electrically stimulated to be sensitive to the touch for up to 24 hours. However, if you find that you are becoming distressed or unwell, you are advised to seek support from a counsellor or medical practitioner. If these feelings persist after the completion of the session, arrangements will be made for you to access support from the Murdoch Counselling Centre at no expense to you. You can contact the Murdoch Counselling Centre on 9360 1227.

Appendix H

Consent form

Murdoch UNIVERSITY

Consent Form for Volunteers

www.murdoch.edu.au

Research Study: The effect of Transcranial Direct Current Stimulation on Acute Pain

1. I agree voluntarily to take part in this study.

2. I confirm that I meet the criteria for participation in this study:

☐ I am over 18 years of age
☐ I do not suffer from chronic pain
☐ I do not suffer from a medical condition (e.g., cancer, heart, or liver condition)
☐ I do not wear a pacemaker
☐ I do not suffer from any psychiatric illness (e.g., depression, anxiety)
☐ I do not suffer from epilepsy/experience seizures
☐ I do not suffer from a cognitive impairment (e.g., dementia, autism)
☐ I do not suffer from an intellectual disability
☐ I am not pregnant or breastfeeding
☐ I have not taken any illicit drug in the past 7 days (e.g., heroin, ice, cocaine, etc.)
☐ I am not under the influence of alcohol and I have not consumed alcohol today
☐ I am not taking any substance or medication that may influence or mask pain perception (e.g., aspirin, paracetamol, voltaren, etc.)
☐ I am feeling well and willing to participate today

3. I have read the Information Letter provided and been given a full explanation of the procedures involved in the study.

4. Any potential risks have been thoroughly explained and understood by me.

5. The researcher(s) have answered all questions I may have about my participation in the experiment.

6. I understand that I will be asked to:

    · Complete screening questionnaires;
    · To have my forearm area exfoliated with a pumice stone;
    · Rate pain and sharpness on a scale of 0 to 10 to a pinprick administered in my forearm. This procedure will be repeated across 3 different time intervals;
    · To receive electrical pulses at moderate pain level through a small electrode placed in my forearm. This procedure will be repeated across 3 different time intervals;
    · To receive a weak electrical current applied in my scalp for 20 minutes;
    · To receive electrical pulses at moderate level for 20 minutes, during which you will be asked to provide a pain and sharpness rating every minute;
    · To wear an EEG cap to enable the electrical activity in my brain to be recorded; and
    · To Complete participant exit questionnaire.

7. I understand that data will only be accessible by researchers, and allow my data to be used for comparison purpose with data collected in a future study that examines the effect of non-invasive brain stimulation in chronic pain patients.

8. I understand that my name and identity will not be traceable to my data.

9. I understand that I will not be identified in any publication arising out of this study.

10. I understand that all information provided by me is confidential and will not be released by the researcher to a third party unless required to do so by law.

11. I have been provided with contact details and will inform the research project if I am unable to attend or need to change the appointment time.

12. I understand I am free to withdraw from the study and stop participating at any time without needing to give reason.

13. I would like to be contacted at the following e-mail address for the purpose of the next study session: _____

Name of participant: _____ _____Age:_____
Signature of participant: _____ _____Date: ...../...../.....

I confirm that I have provided the Information Letter concerning this study to the above participant; I have explained the study and have answered all questions/queries raised by the participant.
Signature of researcher: _____ Date: .... /...../.......

CRICOS Provider Code: 00125J
ABN 61 616 369 313

Research Study: The effect of Transcranial Direct Current Stimulation on Central Sensitization

Reimbursement

Murdoch Psychology participants will be given 10 hours of Subject Pool credit for participation in this study.

If you have any questions about this project or you need to change the date of your next experiment session, please contact us at tcdsresearchers@gmail.com or on one of our contact numbers (Jane: 0487340511; Brandon: 0478667448; Cavan; 0428830879) .

A summary of our findings will be available on the Murdoch School of Psychology and Exercise Science website by the end of 2018.

If you are willing to consent to participation in this study, please complete the Consent Form. Thank you for your assistance with this research project.

Sincerely,

Brandon Scherrer, Cavan Cardile and Jane Wilson

This study has been approved by the Murdoch University Human Research Ethics Committee (Approval xxxx/xxx). If you have any reservation or complaint about the ethical conduct of this research, and wish to talk with an independent person, you may contact Murdoch University's Research Ethics Office (Tel. 08 9360 6677) or e-mail ethics@murdoch.edu.au). Any issues you raise will be treated in confidence and investigated fully, and you will be informed of the outcome.
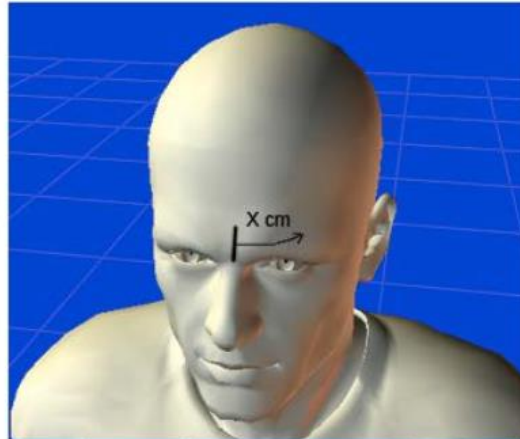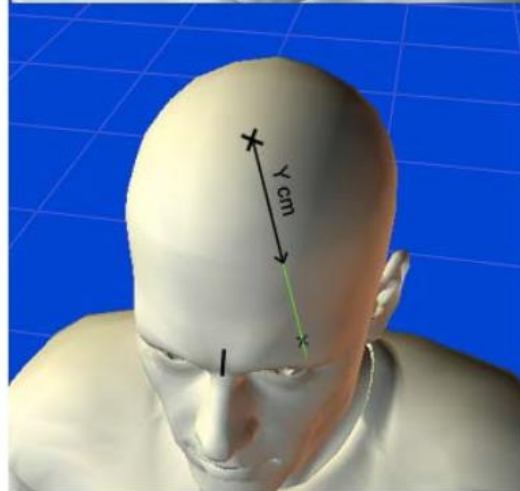
Appendix I

Beam F3 software

Appendix J

Skew and kurtosis of all study variables

Descriptive Statistics

	N	Skewness		Kurtosis	
	Statistic	Statistic	Std. Error	Statistic	Std. Error
M1_Time1_Pinprick_Pain_Primary_Mean	19	.22	.52	-1.35	1.01
M1_Time2_Pinprick_Pain_Primary_Mean	19	.84	.52	.20	1.01
M1_Time3_Pinprick_Pain_Primary_Mean	19	.79	.52	.91	1.01
M1_Time1_Pinprick_Pain_Secondary_Mean	19	.91	.52	.12	1.01
M1_Time2_Pinprick_Pain_Secondary_Mean	19	.43	.52	-.88	1.01
M1_Time3_Pinprick_Pain_Secondary_Mean	19	1.08	.52	1.15	1.01
M1_Time1_ModeratePain_CurrentLevel	19	1.83	.52	3.13	1.01
M1_Time2_ModeratePain_CurrentLevel	19	1.78	.52	2.75	1.01
M1_Time3_ModeratePain_CurrentLevel_Primary	19	2.08	.52	4.50	1.01
DLPFC_Time1_Pinprick_Pain_Primary_Mean	19	1.54	.52	4.02	1.01
DLPFC_Time2_Pinprick_Pain_Primary_Mean	19	.39	.52	-.81	1.01
DLPFC_Time3_Pinprick_Pain_Primary_Mean	18	.61	.54	-.43	1.04
DLPFC_Time1_Pinprick_Pain_Secondary_Mean	19	.93	.52	.70	1.01
DLPFC_Time2_Pinprick_Pain_Secondary_Mean	19	.67	.52	-.60	1.01
DLPFC_Time3_Pinprick_Pain_Secondary_Mean	19	.42	.52	-1.10	1.01
DLPFC_Time1_ModeratePain_CurrentLevel	19	1.75	.52	2.79	1.01
DLPFC_Time2_ModeratePain_CurrentLevel	19	1.89	.52	3.72	1.01
DLPFC_Time3_ModeratePain_CurrentLevel_Primary	19	2.07	.52	4.49	1.01
M1_DLPFC_Time1_Pinprick_Pain_Primary_Mean	19	.57	.52	-.63	1.01
M1_DLPFC_Time2_Pinprick_Pain_Primary_Mean	19	.49	.52	-.42	1.01
M1_DLPFC_Time3_Pinprick_Pain_Primary_Mean	18	1.13	.54	2.03	1.04

Descriptive Statistics

	N	Skewness		Kurtosis	
	Statistic	Statistic	Std. Error	Statistic	Std. Error
M1_DLPFC_Time1_Pinprick_Pain_Secondary_Mean	19	.99	.52	.19	1.01
M1_DLPFC_Time2_Pinprick_Pain_Secondary_Mean	19	.95	.52	.48	1.01
M1_DLPFC_Time3_Pinprick_Pain_Secondary_Mean	19	.17	.52	-1.59	1.01
M1_DLPFC_Time1_ModeratePain_CurrentLevel	19	1.19	.52	.67	1.01
M1_DLPFC_Time2_ModeratePain_CurrentLevel	19	.45	.52	-.58	1.01
M1_DLPFC_Time3_ModeratePain_CurrentLevel_Primary	19	.80	.52	-.50	1.01
SHAMTime1_Pinprick_Pain_Primary_Mean	19	1.66	.52	4.00	1.01
SHAMTime2_Pinprick_Pain_Primary_Mean	19	.87	.52	1.01	1.01
SHAMTime3_Pinprick_Pain_Primary_Mean	19	.81	.52	-.65	1.01
SHAMTime1_Pinprick_Pain_Secondary_Mean	19	.79	.52	.17	1.01
SHAMTime2_Pinprick_Pain_Secondary_Mean	19	.91	.52	.21	1.01
SHAMTime3_Pinprick_Pain_Secondary_Mean	19	.58	.52	-.63	1.01
SHAMTime1_ModeratePain_CurrentLevel	19	3.30	.52	12.68	1.01
SHAMTime2_ModeratePain_CurrentLevel	19	3.40	.52	13.30	1.01
SHAMTime3_ModeratePain_CurrentLevel_Primary	19	3.45	.52	13.47	1.01
Valid N (listwise)	17				

SPSS output for pain ratings elicited by pinprick stimuli at the primary site

Tests of Within-Subjects Effects

Measure: primarysubjectivepain

Source		F	Sig.	Partial Eta Squared
Treatment	Sphericity Assumed	2.318	.087	.127
	Greenhouse-Geisser	2.318	.103	.127
	Huynh-Feldt	2.318	.091	.127
	Lower-bound	2.318	.147	.127
Error(Treatment)	Sphericity Assumed			
	Greenhouse-Geisser			
	Huynh-Feldt			
	Lower-bound			
Time	Sphericity Assumed	10.761	.000	.402
	Greenhouse-Geisser	10.761	.000	.402
	Huynh-Feldt	10.761	.000	.402
	Lower-bound	10.761	.005	.402
Error(Time)	Sphericity Assumed			
	Greenhouse-Geisser			
	Huynh-Feldt			
	Lower-bound			
Treatment * Time	Sphericity Assumed	.713	.640	.043
	Greenhouse-Geisser	.713	.550	.043
	Huynh-Feldt	.713	.580	.043
	Lower-bound	.713	.411	.043
Error(Treatment*Time)	Sphericity Assumed			
	Greenhouse-Geisser			
	Huynh-Feldt			
	Lower-bound			

Pairwise comparisons for pain ratings elicited by pinprick stimuli at the primary site (time

main effect)

(I) Time	(J) Time	Mean Difference (I-J)	Std. Error	Sig.[b]
1	2	-.468*	.137	.010
	3	-.590*	.142	.002
2	1	.468*	.137	.010
	3	-.122	.123	1.000
3	1	.590*	.142	.002
	2	.122	.123	1.000

SPSS output for pain ratings elicited by pinprick stimuli at the secondary site

Tests of Within-Subjects Effects

Measure: painsecondary

Source		F	Sig.	Partial Eta Squared
Treatment	Sphericity Assumed	.365	.778	.020
	Greenhouse-Geisser	.365	.692	.020
	Huynh-Feldt	.365	.715	.020
	Lower-bound	.365	.553	.020
Error(Treatment)	Sphericity Assumed			
	Greenhouse-Geisser			
	Huynh-Feldt			
	Lower-bound			
Time	Sphericity Assumed	7.755	.002	.301
	Greenhouse-Geisser	7.755	.003	.301
	Huynh-Feldt	7.755	.002	.301
	Lower-bound	7.755	.012	.301
Error(Time)	Sphericity Assumed			
	Greenhouse-Geisser			
	Huynh-Feldt			
	Lower-bound			
Treatment * Time	Sphericity Assumed	.731	.626	.039
	Greenhouse-Geisser	.731	.566	.039
	Huynh-Feldt	.731	.599	.039
	Lower-bound	.731	.404	.039
Error(Treatment*Time)	Sphericity Assumed			
	Greenhouse-Geisser			
	Huynh-Feldt			
	Lower-bound			

Pairwise comparisons for pain ratings elicited by pinprick stimuli at the secondary site

(time main effect)

(I) Time	(J) Time	Mean Difference (I-J)	Std. Error	Sig.[b]
1	2	-.285	.116	.073
	3	-.373*	.101	.005
2	1	.285	.116	.073
	3	-.089	.076	.777
3	1	.373*	.101	.005
	2	.089	.076	.777

SPSS output for the current level required to elicited moderate pain over time

Tests of Within-Subjects Effects

Measure: currentlevel

Source		F	Sig.	Partial Eta Squared
Treatment	Sphericity Assumed	.435	.729	.024
	Greenhouse-Geisser	.435	.673	.024
	Huynh-Feldt	.435	.700	.024
	Lower-bound	.435	.518	.024
Error(Treatment)	Sphericity Assumed			
	Greenhouse-Geisser			
	Huynh-Feldt			
	Lower-bound			
Time	Sphericity Assumed	31.948	.000	.640
	Greenhouse-Geisser	31.948	.000	.640
	Huynh-Feldt	31.948	.000	.640
	Lower-bound	31.948	.000	.640
Error(Time)	Sphericity Assumed			
	Greenhouse-Geisser			
	Huynh-Feldt			
	Lower-bound			
Treatment * Time	Sphericity Assumed	2.424	.031	.119
	Greenhouse-Geisser	2.424	.063	.119
	Huynh-Feldt	2.424	.047	.119
	Lower-bound	2.424	.137	.119
Error(Treatment*Time)	Sphericity Assumed			
	Greenhouse-Geisser			
	Huynh-Feldt			
	Lower-bound			

Appendix K

Project Summary

Title: Anodal Transcranial Direct Current Stimulation does not induce Analgesic Effects on
Experimentally Induced Pain
Ethics Approval: 2018/037
Student Researcher: Brandon Scherrer
Supervisor: Dr Lechi Vo
Completed: October 2018

Chronic pain is a crippling condition that presents significant social and economic costs. In Australia, 15.4% of Australian adults are thought to suffer from chronic pain. The pain can often be so severe that sufferers are rendered disabled, as they are not able to engage in daily activates, such as work. Unfortunately, current treatments for chronic pain do not always provide adequate pain relief. This is worrying, as the prevalence of people with chronic pain will increase as the Australian population continues to age.

The main aim of the current study is to provide evidence for a new promising treatment for chronic pain. Transcranial Direct Current Stimulation (tDCS) is a device that uses a small battery to painlessly stimulate neural pathways. This is achieved via electrodes placed on the scalp. The result is neuroplasticity, which occurs when irregular brain pathways are pruned and new pathways are formed. Research has shown promise in the use of tDCS for reducing pain when applied at the primary motor cortex (M1) and the dorsolateral prefrontal cortex (DLPFC). However, no study has investigated the effect of tDCS in reducing pain when applied at both the M1 and the DLPFC simultaneously. This is surprising, as the M1 and the DLPFC both activate in response to pain. It the current study, it was predicted that tDCS applied at the M1 and the DLPFC simultaneously would reduce pain in to electrical stimulation and punctuate pinprick sensations, due to the activation of both areas during painful sensations. This is expected to be greater than when tDCS is applied the M1 or the DLPFC individually. To test this, application of tDCS at the M1, DLPFC, and M1 + DLPFC were compared to a fake tDCS condition in which the device was briefly turned on and then turned off. As such, the current study is exciting as it is the first of its kind, and may serve towards establishing tDCS as a treatment for chronic pain.

Method

There were 19 healthy participants in this study (12 male and 7 female). The average age was 29 years old. Participants completed all tDCS conditions on separate testing days and were all randomised as to what condition they were to experience first.

The first phase of the experiment involved obtaining pain ratings elicited by a pen-like instrument that had a sharp metal tip on the end. This is the pinprick sensation. The experimenter pushed the sharp tip of the pen on the skin for 2 seconds. Participants then rated pain on a 0 to 10 scale. The experimenter then obtained the current level required of electrical stimulation to elicit moderate pain. To do this, participants first received electrical stimulation at a very low current level to their forearm, and the experimenter gradually increased the current until participants reported a rating of 5 out of 10.

The next phase of the experiment involved administration of tDCS. The experimenter calibrated the tDCS device to either applying tDCS to the M1 only, DLPFC only, M1 + DLPFC simultaneously, or the fake tDCS. The tDCS stimulation went for 20 minutes, in

which participants sat quietly and relaxed. Following this, the pinprick instrument reobtained pain ratings, and the current level required to obtain moderate pain was reassessed.

The final phase of the experiment was conducted following 10 minutes of rest. Pain ratings were again obtained following the exact same procedures. The rest time allowed the forearm to settle, so it was not too sensitive at this last assessment. Therefore, more accurate ratings could be obtained.

Results

The ratings of pain to the pinprick instrument increased consistently from phase 1, phase 2, and phase 3. Additionally, the current level required to elicit moderate pain from electrical stimulation also increased consistently from phase 1, phase 2, and phase 3. This did not depend on tDCS as no effect of tDCS on the pain ratings was found when applied M1, DLPFC, M1 + DLPFC, as compared to the fake tDCS condition. These findings are surprising, as it is inconsistent with current research evidence finding that tDCS reduces pain.

Conclusion

The surprising results of the current study may be explained by the different settings of tDCS in the current study, compared with past research. In the current study, tDCS was applied with a lesser strength, meaning that less currents can enter the brain. Additionally, tDCS was delivered with electrodes that were smaller than those used in past research. Therefore, tDCS applied in the current study was more focused such that it did not stimulate a larger brain area, which may limit the neuroplasticity induced.

The findings that pain increased during phase 1, phase 2, and phase 3 in response to the punctuate pinprick instrument is explained by hyperalgesia, and is an unsurprising finding. As electrical stimulation is thought to sensitise an area, which results in increased sensitivity to mechanical punctuate stimuli, the increased pain ratings of the punctuate pinprick instrument was expected.

Finally, the unexpected finding of an increasing current level to elicit moderate pain irrespective of tDCS administration can be explained by participants' presenting with anxiety and stress, due to the nature of the current study. There is substantial evidence that anxiety and stress can increase pain and block analgesia. Thus, as participants become more familiar with the experiment throughout the testing session, anxiety and stress decreases, and thus they are able to tolerate a higher current level to elicit moderate pain.

Although the current study does not provide support in the use of tDCS to reduce pain, it may be due to the settings of tDCS itself, and due the nature of the current study, anxiety and stress, which decreased the ability of tDCS to induce neuroplasticity. Future researchers then should adhere to the settings of tDCS commonly employed in past studies, and investigate whether anxiety and stress is present in participants in pain research, and whether this declines throughout the testing session. This can validate whether stress and anxiety interfere with the ability of tDCS to reduce pain.