Anodal transcranial direct current stimulation: A potential treatment for chronic 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.

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Abstract

Purpose: Current treatments available for chronic pain either do not provide patients with adequate pain relief, are invasive, expensive or cause negative side effects. Transcranial direct current stimulation (tDCS) delivered to the primary motor cortex (M1) and dorsolateral prefrontal cortex (DLPFC) brain regions has been identified as a potential treatment. However, the literature regarding is effectiveness is mixed. This study aimed to clarify if tDCS at M1 and DLPFC reduces healthy participants’ pain. In addition, it aimed to identify whether simultaneous stimulation of M1 and DLPFC results in greater pain reduction than stimulation at one cortical site alone.

Method: A randomized, crossover, within-subjects, double-blinded sham controlled design was utilized. Twenty healthy participants (10 female; aged 18 to 59) underwent four conditions, 20 minutes of 1 mA anodal tDCS at M1 and DLPFC concurrently, M1, DLPFC and sham. A low-frequency electrical current administered to participants’ right volar forearm induced pain. Pain was assessed pre and post tDCS by pain ratings to pinprick and the electrical current level required during electrical stimulation to induce moderate level pain.

Results: Analysis revealed a significant difference between pre and post tDCS pain assessment, however, this difference was present irrespective of tDCS condition. Participant habituation to low-frequency electrical stimulation may explain these results.

Conclusions: TDCS within this study did not reduce healthy participants’ pain. This study identified methodological considerations and tDCS parameters that should be implemented in future replication studies to further explore tDCS as a potential chronic pain treatment.
Anodal transcranial direct current stimulation: A potential treatment for chronic pain

Acute pain is an unpleasant physiological response of the central nervous system (CNS) triggered by potential tissue damaging stimulus, termed noxious stimuli (Chang, McDonnell & Gershwin, 2019). It is an adaptive response, alerting the individual to prevent/ minimize injury (Chang et al., 2019; Latremoliere & Woolf, 2009; Moseley & Flor, 2012). Pain becomes chronic when it persists longer than three months or beyond normal tissue healing (Chou et al., 2015). Thus, chronic pain is maladaptive, as it no longer serves a protective function. Chronic pain elicits central sensitization, a hypersensitivity of the CNS, which results in lower pain thresholds and the activation of pain signals initiated by stimuli that would not typically cause a pain response (Fregni, Freedman & Pascual-Leone, 2007; Ji, Kohno, Moore & Woolf, 2003; Millan, 2002; Nickel et al., 2014; Woolf, 2011). Central sensitization manifests as a consequence of neuroplastic changes that occur within the CNS, including cortical structures as a response to chronic pain (Woolf, 2011). Chronic pain can cause the individual significant distress and disability by negatively impacting wellbeing, quality of life and ability to function (Ataoğlu et al., 2013; Chizh et al., 2007; Deloitte Access Economics, 2019; Latremoliere & Woolf, 2009; Lefaucheur et al., 2008). Approximately 20% of individuals with chronic pain are diagnosed with depression, which is significantly higher than the general population prevalence rate of six percent (Currie & Wang, 2004). Additionally, those with chronic pain are 30% more likely to be unemployed (Deloitte Access Economics, 2019). Chronic pain can develop from multiple aetiologies, with individuals experiencing differing symptoms (Henrich, Magerl, Klein, Greffrath & Treede, 2015). The exact cause or onset is unclear, as not all individuals experience the same level of symptomology or develop chronic pain following similar injuries or illnesses (Henrich et al., 2015; Kuner &
Flor, 2017). Additionally, each individual’s response to treatment is unique, making it difficult to treat (Henrich et al., 2015; Kuner & Flor, 2017). It is estimated 3.2 million Australians live with chronic pain, costing $73.2 billion dollars each year (Deloitte Access Economics, 2019). Various pain treatments are available, including antidepressant and opioid medication, however, no more than 30% - 50% of patients experience pain relief (Magrinelli, Zanette & Tamburin, 2013; Turk, Wilson & Cahana, 2011). Furthermore, 80% of patients experience adverse side effects from these treatments (Turk et al., 2011). Surgical implantation of an electrode into the scalp to electrically stimulate the primary motor cortex (M1) has significantly reduced chronic pain symptoms for some patients (Garcia-Larrea et al., 1999; Saitoh et al., 2001). The mechanism underlying this pain reduction is unclear, however it has been proposed M1 stimulation induces thalamic blood flow, which is a brain region implicated with chronic pain (Garcia-Larrea et al., 1999; Saitoh et al., 2001). However, this procedure is invasive, expensive, and not effective for all individuals (Garcia-Larrea et al., 1999). These findings have generated a body of research into the non-invasive brain stimulation techniques, repetitive transcranial magnetic stimulation (rTMS) and transcranial direct current stimulation (tDCS), which elicit neuromodulation within targeted cortical regions (Mylius, Borckardt & Lefaucheur, 2012). Past research has identified M1 and the dorsolateral prefrontal cortex (DLPFC) brain regions as potential stimulation targets to reduce chronic pain symptoms (Boggio, Zaghi, Lopes & Fregni, 2008; Lefaucheur et al., 2017). Research has suggested rTMS may reduce symptoms of chronic pain, however it is expensive and not easily accessible, as patients are required to attend hospital for treatment (Lefaucheur et al., 2008). TDCS is inexpensive, safe and easy to administer, however, further research is required to establish its efficacy for chronic pain (O’Connell,
Marston, Spencer, Desouza & Wand, 2018). The development of efficacious, non-pharmacological, non-invasive and inexpensive treatments are urgently needed to treat chronic pain.

Pain processing comprises both descending and ascending pathways, involving both inhibitory and facilitatory neurotransmission processes in both the cortex and spinal dorsal horn (Bingel & Tracey 2008; Millan, 2002; Nickel et al., 2014).

**Ascending pain pathway**

The ascending pathway of pain occurs when noxious stimuli is detected in the periphery, which is known as nociception (Latremoliere & Woolf, 2009). Nociception is an adaptive process as it assists in the prevention of further injury by initiating a withdrawal reflex from the noxious stimuli (Latremoliere & Woolf, 2009). Additionally, it generates a range of unpleasant sensory, cognitive and emotive sensations that results in the avoidance of further contact (Latremoliere & Woolf, 2009). Myelinated Aδ and unmyelinated C afferent fibers located in the epidermis, convey nociceptive messages via synaptic transmissions terminating on the superficial and deep lamina of the dorsal horn of the spinal cord (Ji et al., 2003). Facilitatory ascending synaptic transmissions are then projected from the dorsal horn to the brain stem, where messages are conveyed to higher cortical regions (Bingel & Tracey, 2008). Once the torrent of nociceptive neuronal transitions activated by noxious stimuli enter the spinal cord, they excite a widespread network of neurons, leading to central sensitization, a central excitatory state (Van Den Broeke, 2018). Primary and secondary hyperalgesia, a hypersensitivity to painful stimuli, are characteristics of central sensitization, with primary hyperalgesia manifesting at the injured site and secondary hyperalgesia induced in the adjacent area (LaMotte, Shain, Simone & Tsai,
Experimental low-frequency subcutaneous electrical stimulation elicits central sensitization by activating both Aδ and C fibers (Beissner et al., 2010; Liu & Sandkühler, 1997; Ziegler, et al., 1999). Sauerstein et al. (2018) initiated central sensitization in healthy participants by inducing both primary and secondary hyperalgesia via the administration of a painful low-frequency electrical current of 1 Hz, to the dorsal aspect of participants’ feet (Sauerstein et al., 2018). Following electrical stimulation, participants reported greater pain to pinprick stimuli within the primary (site of stimulation) and secondary (adjacent to the stimulated site) areas compared to pre stimulation ratings (Sauerstein et al., 2018). These findings indicate low-frequency electrical stimulation induces primary and secondary hyperalgesia in healthy participants (Sauerstein et al., 2018). Validated experimental pain models tested on healthy participants allow for the assessment of pain processes, without the confounding factors of the multiple aetiologies chronic pain arises from and the various treatments and medications used to relieve symptoms; possibly leading to the discovery of more treatment avenues for chronic pain (Chizh et al., 2007; Koppert et al., 2005).

**Descending pain pathway**

The descending pain pathway begins with neurotransmission originating from specific brain regions converging at the brain stem, which then projects inhibitory or facilitatory mechanisms to the spinal cord, modulating incoming nociceptive messages from the periphery (Bannister & Dickenson, 2017; Yarnitsky, 2015). Initially, the brain processes nociceptive messages received from Aδ and C fibers via the synaptic transition of neurotransmitter glutamate, which can interact with both N-methyl-D-aspartate (NMDA) and non-NMDA excitatory amino acid receptors (Argoff, 2011; Vanderah, 2007). Incoming messages from the periphery are then modulated
and either facilitated or inhibited via the release of endorphins through opioid
receptors activated at the periaqueductal gray (PAG) region of the midbrain
(Vanderah, 2007). The key neurotransmitters involved within this process are
serotonin and norepinephrine, dopamine and \( \gamma \)-aminobutyric acid (GABA)
(Vanderah, 2007; Woolf & Salter, 2000; Yam et al., 2018). The “pain matrix” is the
grouping of brain regions deemed responsible for the descending pathway of pain in
experimental models (Bingel & Tracey, 2008; Moisset & Bouhassira, 2007). These
brain regions include the primary and secondary somatosensory cortices, the insular
cortex, the anterior cingulate cortex, the thalamus and the prefrontal cortex (Moisset
& Bouhassira, 2007). Broad and sustained cortical neuronal firing responses to acute
injury can lead to neuroplastic changes within the cortex, which assists with the
development and maintenance of chronic pain (Hashmi et al., 2013; Moseley & Flor,
2012; Ossipov, Morimura & Porreca, 2014).

Transcranial Direct Current Stimulation

TDCS is a non-invasive brain stimulation technique that is safe, inexpensive,
has minimal side effects and is accessible to a large portion of the population
(Kessler, Turkeltaub, Benson & Hamilton, 2012; Lefaucheur et al., 2017; Rossi,
Hallett, Rossini & Pascual-Leone, 2009). TDCS elicits neuromodulation by sending a
weak constant electrical current to targeted brain regions via anodal electrodes
(emitting a positive current) and cathodal electrodes (projecting a negative current),
affixed to the scalp (Antal et al., 2017; Cruccu et al., 2016; Fregni et al., 2005; Fregni
et al., 2007; Moisset & Lefaucheur, 2019). The strength of the electrical current
typically applied is between 1 mA to 2 mA, which is not strong enough to elicit action
potentials (Lefaucheur et al., 2017). Rather, neuronal membrane resting voltages are
augmented and brought either closer to (depolarized) or further away from
(hyperpolarized) firing (Lefaucheur et al., 2017; Thair, Holloway, Newport & Smith, 2017). Nitsche & Paulus (2000) examined cerebral excitability by recording motor evoked potentials following 1 mA anodal (a-tDCS) and cathodal (c-tDCS) tDCS delivered to M1. Results indicated a-tDCS was associated with a significant increase in motor-cortex excitability, conversely, c-tDCS elicited a decrease in excitability (Nitsche & Paulus, 2000). These findings confirm that a-tDCS depolarizes (excites) neurons, whilst c-tDCS hyperpolarizes (inhibits) neurons (Naegel et al., 2018; Thair et al., 2017). Thirteen minutes of tDCS induces neuromodulation that lasts up to 90 minutes post stimulation (Ihle, Rodriguez-Raecke, Luedtke & May, 2014; Nitsche & Paulus, 2001).

Neuroimaging studies have implied that a-tDCS administered to M1 and DLPFC may decrease pain by influencing the descending inhibitory pain process (Ong, Stohler & Herr 2019; Meeker et al., 2019; Moisset & Bouhassira, 2007). Polania, Nitsche & Paulus (2011) conducted a seed functional connectivity analysis following 10 minutes of 1 mA a-tDCS, c-tDCS and sham (a control condition, where active tDCS is delivered for 30 seconds before being ramped down for the remainder of the session) to M1. Analysis revealed greater functional connectivity between the thalamus and M1 following a-tDCS, this connectivity was not present within the c-tDCS and sham conditions (Polania et al., 2011). These results suggest a-tDCS of M1 modulates cortico-striato-thalamo-cortical circuits, inhibiting synaptic thalamic sensory pathways, which are involved in the descending inhibitory pain pathway (Millan, 2002; Moisset & Bouhassira, 2007; Polania et al., 2011). However, as this study did not record brain responses to painful stimuli, direct observations regarding the effect of tDCS on neuronal activity responding to pain cannot be made. This was addressed by Sankarasubramanian et al. (2017), who compared changes in
connectivity via functional magnetic resonance imaging (fMRI) to painful heat stimuli in a sham controlled study, following 20 minutes of 1 mA a-tDCS at M1 and in addition, to DLPFC. A-tDCS at both M1 and DLPFC induced functional connectivity changes between the ventroposterolateral and sensory nucleus of the thalamus, however functional connectivity strength was greater following M1 compared to DLPFC stimulation (Sankarasubramanian et al., 2017). This suggests that M1 stimulation may modulate thalamic brain activity during pain perception. However, functional connectivity strength was greater following DLPFC compared to M1 stimulation between the medial dorsal, the affective nucleus, and brain regions associated with affective information processing (Sankarasubramanian et al., 2017). These findings insinuate that M1 tDCS mainly modulates functional connectivity within sensory networks, whereas DLPFC stimulation modulates both sensory and affective networks (Sankarasubramanian et al., 2017). These neuroimaging studies imply that a-tDCS at M1 and DLPFC may reduce pain by differentially modulating neuroplastic changes within the descending inhibitory pain pathway, although the exact cortical mechanisms involved still remain unclear (Mylius et al., 2012; Polania et al., 2011; Sankarasubramanian et al., 2017; Wiech, Ploner & Tracey, 2008). However, these studies did not directly test participants’ level of pain pre and post tDCS, therefore definitive inferences cannot be drawn regarding the effectiveness of tDCS for pain reduction.

**Transcranial Direct Current Stimulation Primary Motor Cortex**

The application of a-tDCS at M1 has been studied in clinical populations to establish whether it reduces chronic pain, however results have been mixed (David, Moraes, Costa & Franco, 2018; O’Connell et al., 2018; Wrigley et al., 2013). Khedr et al. (2017) examined whether a-tDCS at M1 would decrease pain symptoms in
individuals diagnosed with fibromyalgia (which causes widespread chronic pain). Forty participants either received 10 sessions of 2 mA a-tDCS for 20 minutes or sham administered over 10 consecutive days (Khedr et al., 2017). Participants rated their level of pain on an 11-point visual analog scale (VAS), where a rating of 0 indicated no pain and 10, the worst imaginable pain (Khedr et al., 2017). Following the tenth session, participants in the a-tDCS condition reported significantly less pain than those in the sham group (Khedr et al., 2017). A study conducted by Ngernyam et al. (2015) established similar findings in participants with neuropathic chronic pain. Twenty participants received a single session of both sham and a-tDCS of 2 mA for 20 minutes at M1 (Ngernyam et al., 2015). Sessions were conducted at least one week apart and order of conditions was randomized to prevent carry over and order effects (Ngernyam et al., 2015). Participants in the a-tDCS condition reported significantly lower scores on a numerical rating scale for pain compared to sham, suggesting a-tDCS was successful at reducing chronic pain within this study (Ngernyam et al., 2015). However, findings produced by Thibaut, Carvalho, Morse, Zafonte & Fregni (2017) were mixed. Thirty-three participants who developed chronic pain following spinal cord injury either received sham or a-tDCS of 2 mA for 20 minutes at M1 (Thibaut et al., 2017). The study was conducted over two phases, during phase one, participants underwent five sessions over five consecutive days, and during phase two attended 10 sessions over 10 days (Thibaut et al., 2017). Participants’ reported VAS pain ratings were significantly lower following five sessions of a-tDCS compared to sham (Thibaut et al., 2017). There was no significant difference in VAS pain ratings following 10 sessions (Thibaut et al., 2017). Results suggest chronic pain was reduced following five consecutive sessions of a-tDCS, but not 10 (Thibaut et al., 2017). These results contradict the findings of Khedr et al. (2017), where a significant
reduction in pain was observed following 10 sessions of a-tDCS. Furthermore, Lewis, Rice, Kluger & McNair (2018) found participants’ chronic pain was not reduced following five consecutive sessions of 1 mA a-tDCS for 20 minutes at M1, contradicting results from the Thibaut et al. (2017) study. It is difficult to interpret the current results, as studies have not implemented methodological considerations which control for the confounding factors of clinical populations; such as the multiple aetologies of chronic pain, differing symptoms and patient treatment plans (Henrich et al., 2015). Experimental studies have examined a-tDCS on healthy populations, however results remain mixed.

Ihle et al. (2014) administered 1 mA a-tDCS, c-tDCS and sham at M1 for 15 minutes to 16 healthy participants. Participants’ pain ratings to heat and pinprick stimuli were obtained via a numerical rating scale at baseline and post tDCS (Ihle et al., 2014). There was no significant difference between participants’ pain ratings pre and post tDCS across all conditions (Ihle et al., 2014). However, there was a non-significant trend towards lower pain ratings within the a-tDCS condition (Ihle et al., 2014). These results imply tDCS did not reduce participants’ pain within this study (Ihle et al., 2014). Conversely, a study conducted by Meeker et al. (2019) contradicted results obtained by Ihle et al. (2014), where participants reported pain reduction to thermal stimuli following a-tDCS of 1 mA for 20 minutes at M1. Similar results were obtained by Zandieh et al. (2013), where a-tDCS to M1 at 2 mA for 15 minutes significantly increased cold pain thresholds compared to c-tDCS or sham in healthy participants. The findings of these studies suggest a-tDCS to M1 might reduce pain in healthy volunteers; however further studies are required to clarify the mixed results of prior research (Beissner et al., 2010; Granovsky, Raz & Defrin, 2017).
Transcranial Direct Current Stimulation of the Dorsolateral Prefrontal Cortex

Although the majority of research exploring the efficacy of tDCS for pain reduction has targeted M1, some neuroimaging and experimental studies suggest that DLPFC stimulation may reduce pain by activating descending inhibitory pain mechanisms (Boggio et al., 2008; Mylius et al., 2012; Sankarasubramanian et al., 2017; Wiech et al., 2008). To explore whether tDCS at DLPFC reduces pain Boggio et al. (2008) conducted a sham-controlled study administering 2 mA of a-tDCS for five minutes to DLPFC. In addition, Boggio et al. (2008) examined whether DLPFC a-tDCS would result in greater pain reduction than M1 a-tDCS. Pain was induced by electrically stimulating the right index finger of 20 healthy participants (Boggio et al., 2008). Results indicated that a-tDCS at DLPFC increased participants’ pain thresholds but did not decrease pain perception (Boggio et al., 2008). Additionally, a-tDCS at M1 decreased participants’ pain perception and increased pain thresholds, however, pain threshold increases within this condition were lower than following DLPFC stimulation (Boggio et al., 2008). Findings indicate that a-tDCS of both DLPFC and M1 reduced participants’ pain within this study (Boggio et al., 2008).

Due to the differing effect on pain perception and thresholds across conditions, it can be speculated that stimulation at M1 and DLPFC may concurrently but differentially influence the descending inhibitory pain process. Mylius et al. (2012) further investigated whether tDCS at DLPFC reduced healthy participants pain by assessing tolerance to heat. Twenty-four participants underwent three conditions, 20 minutes of 2 mA a-tDCS, c-tDCs and sham at DLPFC (Mylius et al., 2012). Pain tolerances to the heat stimuli were assessed pre and post tDCS (Mylius et al., 2012). Participants’ pain thresholds increased during DLPFC a-tDCS, no increase was observed in any other condition. The results of this study suggest DLPFC a-tDCS, but not c-tDCS,
reduces pain in healthy participants. There are no other known studies examining tDCS stimulation at DLPFC on healthy participants. Further studies are required to clarify current findings.

The current literature suggests a-tDCS of M1 and DLPFC could potentially reduce pain, however further research is required to clarify the results of published studies. This research needs to be conducted on healthy participants prior to continuing testing on clinical populations. This will establish whether tDCS can firstly reduce experimental pain, and if so, clarify the most effective tDCS parameters for pain reduction without the intervening confounding factors associated with chronic pain (Henrich et al., 2015). Once this has been clarified, further testing on clinical populations can recommence to establish its efficacy as a potential treatment for chronic pain. Furthermore, no known study has investigated whether concurrent a-tDCS of M1 and DLPFC reduces pain. This is worth exploring, as prior research has suggested a-tDCS of M1 influences pain perception and sensory networks, whereas DLPFC stimulation influences pain thresholds and affective networks, indicating these brain regions may differentially modulate activity in the descending inhibitory pain process (Boggio et al., 2008; Sankarasubramanian et al., 2017). By concurrently stimulating both brain regions, this may simultaneously influence several descending inhibitory pain processes, which may result in greater reductions of pain compared to stimulation at one site alone.

Firstly, the aim of this study was to clarify whether a-tDCS stimulation of M1 and DLPFC reduced pain in healthy individuals. Secondly, this study aimed to identify whether concurrent a-tDCS of M1 and DLPFC resulted in a greater reduction of pain than stimulation of each brain region independently. The current study administered a-tDCS to healthy adults across four conditions, M1 and DLPFC
concurrently, M1 alone, DLPFC alone and sham. Experimental pain was induced by low-frequency electrical stimulation administered to participants right volar forearm, this method has been validated to induce both primary and secondary hyperalgesia (core symptoms of chronic pain) by several studies (Sauerstein et al., 2018; Seifert, Kiefer, Decol, Schmelz & Maihofner, 2009). By clarifying the conflicting literature regarding whether a-tDCS at M1 and DLPFC reduces pain in healthy adults, and exploring concurrent stimulation of these brain regions as a potential treatment parameter, may lead to better treatments avenues for chronic pain.

Firstly, it was hypothesized a-tDCS at M1 and DLPFC concurrently would result in lower pain ratings to pinprick in the primary and secondary areas and a higher current level during electrical stimulation to elicit moderate pain, than M1 alone, DLPFC alone and sham. Secondly, it was hypothesized a-tDCS at M1 would result in lower pain ratings to pinprick in the primary and secondary areas and a higher current level during electrical stimulation to elicit moderate pain than sham. Thirdly, it was hypothesized a-tDCS at DLPFC would result in lower pain ratings to pinprick in the primary and secondary areas and a higher current level during electrical stimulation to elicit moderate pain than sham.

Method

Design

The following study employed a crossover, double-blinded, randomized, sham controlled design to evaluate the effect of tDCS on primary and secondary hyperalgesia induced by low-frequency electrical stimulation in healthy adults. The independent variables were the four tDCS conditions (a-tDCS at left M1 and DLPFC concurrently, a-tDCS at left M1, a-tDCS at left DLPFC and sham) and time (pre
tDCS (Time 1) and post tDCS (Time 2)). The first dependent variable was the electrical current level eliciting moderate (level 5) pain, applied to the right volar forearm. The second dependent variable was pain ratings to pinprick within the primary area, and the third, pain ratings to pinprick in the secondary area.

The order of tDCS conditions were counterbalanced and randomized between and within participants in accordance with the Latin square method to eliminate any potential order effects (Zeelenberg & Pecher, 2015). Conditions were scheduled at least one week apart to minimize carry over effects (Ihle et al., 2014; Samani, Agboada, Jamil, Kuo & Nitsche, 2019). Double-blinding was achieved as participants and the experimenter obtaining pain related data were not aware of which tDCS condition was administered during testing sessions. A within subjects design was utilized to minimize potential confounding factors arising from participant individual differences and to achieve greater statistical power with a small sample size (Charness, Gneezy & Kuhn, 2012).

**Participants**

Ethics approval was granted by the Murdoch University Human Ethics Research Committee (Appendix A). A convenience sampling approach was utilized to recruit 26 participants for this study. One participant did not complete all sessions and five participants were eliminated, as they did not reach moderate level pain at the maximum safe current level of 30 mA, during electrical stimulation. A final 20 right-handed healthy participants were included (10 males), aged between 18 and 59 ($M = 25.10, SD = 8.87$). We conducted a power analysis using G*Power 3.1, which indicated to achieve a medium effect size of 0.3 with power of 0.8 and a $p$-value of 0.05, we would require data from 24 participants (Faul, Erdfelder, Lang & Buchner, 2007). However, past studies achieved a significant difference with between 15 to 20
participants (Boggio et al., 2008; Meeker et al., 2019). Due to the time restraints of a one-year research project, it was agreed a final sample of 20 participants would be adequate. Individuals who were under the age of 18 or over 65, obtained a score of 60 or below on the Edinburg Handedness Inventory Short Form, suffered from a mental illness or medical condition, had epilepsy or experience seizures, had a cognitive impairment or intellectual disability, were pregnant or breastfeeding, taking any medication, wearing a pacemaker or suffering from chronic pain were ineligible to participate. Only right-handed participants were included within the study to avoid any confounding effects of handedness resulting in greater functional connectivity of the left hemisphere (Van Den Broeke, Hartgerink, Butler, Lambert & Mouraux, 2019; Polania et al., 2011).

Participants attended four separate two-hour experimental testing sessions held at the Murdoch University Body and Mind Laboratory, located in the Sports Sciences Building. Participants were required to reschedule their appointment if they had taken painkillers or drunk alcohol within 24 hours, or taken illicit drugs in the week prior to the scheduled session. As the experimental pain procedure would not exceed participants’ moderate level of pain, it would be unlikely they would feel physically or psychologically unwell following a testing session. However, should this occur, participants were provided with the contact details of the Murdoch Medical and/ or Counseling Centre to arrange an appointment at no cost to the participant. Psychology students who registered through the Murdoch University Research Participant Portal were given research credits for their course. Non-psychology students and external participants were incentivized by going into a draw to win a $100.00 voucher.
Apparatuses

Transcranial Direct Current Stimulation

TDCS was delivered to the scalp by a Chattanooga Ionto TM Dual Channel Iontophoresis System, powered by a 9 Volt battery or similar, with a maximum output of 8 mA (Guildford Surrey, UK; Appendix B). The positive electrical current was delivered by two active anodal rubber electrodes (5 cm x 5 cm). A cathodal rubber electrode (5 cm x 7 cm) completed the electrical circuit. All electrodes were encased in sponges. To assist with the conductance of the electrical current, 5 ml of saline was applied to the anodal sponges and 7 ml applied the cathodal sponge; additionally, gel was applied to the side of the sponges that made contact with the scalp (Appendix B).

Software

To locate participants’ M1 and DLPFC cortical regions the EZ-EEG program was utilized (Borckardt & Hanlon, 2015).

Low-frequency electrical stimulation

The low-frequency electrical stimulation pain model was generated by a constant current stimulator (DS7A; Digitimer, Welwyn Garden City, UK; Appendix B). The current was delivered by a custom built stimulating electrode with 25 copper pins, .2-mm-diameter tips, protruding .5 mm from the surface of a 2 cm x 2 cm perspex block, held in place by a firm velcro strap (Vo & Drummond, 2014; Vo & Drummond, 2016). A 3 cm x 3.5 cm ground plate was attached 2 cm to the left of the conditioning electrode to complete the electrical circuit (Vo & Drummond, 2016). The electrical current was set to the frequency of 1 Hz with a pulse width of 0.5 ms and a 10 ms delay, up to a maximum of 30 mA (these parameters active Aδ and C fibers; Sauerstein et al., 2018; Vo & Drummond, 2016). The strength of the electrical current was monitored via a Current Reader Box.
Pinprick sensitivity

To assess sensitivity to pain and sharpness a single use Neurotip was inserted into a calibrated spring mechanism exerting a force of 40 g (Appendix B). It was administered to participants right volar forearm at a 90° angle for 2 seconds (Neuropen®; Owen Mumford, Oxfordshire, UK) (Finch, Price & Drummond, 2019; Vo & Drummond, 2013).

Visual Analogue Scale

Participants rated pain and sharpness to electrical and pinprick stimuli using a Visual Analogue Scale (VAS), an 11-point scale ranging from 0 no pain/ sharpness to 10 worst pain/ sharpness imaginable, with a rating of 5 indicating a moderate level of pain/ sharpness (Appendix B; Vo & Drummond, 2014). The VAS is recognized as a valid and reliable measure of obtaining pain and sharpness ratings (Bijur, Silver & Gallagher, 2001).

Procedure

Participants attended four two-hour sessions, one session for each tDSCS condition. At the commencement of the first session participants were required to read an information letter, which detailed the nature and process of the study (Appendix C). They then completed a screening questionnaire consent form (Appendix D), Edinburg Handedness Inventory Short Form (Appendix E) and Transcranial Magnetic Stimulation (TMS) and Transcranial Direct Current Stimulation (tDSCS) Safety Screen (Appendix F), to ensure they met all inclusion criteria and it was safe to participate (Meeker et al., 2019; Veale, 2014).
Each session followed an identical procedure. Firstly, participants right volar forearm was exfoliated with Solvo soap, removing dead skin cells to reduce skin resistance to the electrical current (Sauerstein et al., 2018; Vo & Drummond, 2014). Secondly, the researcher obtaining pain related data, identified the primary and secondary areas on the right volar forearm for the low-frequency electrical stimulation procedure. The grounding electrode was affixed 2 cm to the left of the electrode.

Figure 1. Schematic representation of the experimental procedure and timeline
Figure 2. Visual representation of the primary and secondary areas for pinprick hyperalgesia induced by low-frequency electrical stimulation, with grounding electrode. Blue square marked on the right volar forearm is the primary area and 1 cm below the primary area is deemed the secondary area.

**Time 1**

Participants were trained to provide consistent pain and sharpness ratings in accordance with the VAS by responding to two Neuropen pinpricks administered to the right wrist. Two baseline pain and sharpness ratings were obtained, one from the primary area and one from the secondary area.

**Obtain moderate pain current level procedure**

Participants’ baseline current level to elicit moderate pain was then obtained. The stimulating electrode was placed over the primary area and secured with a velcro strap. Low-frequency electrical stimulation of the right volar forearm commenced, beginning at a 2 mA electrical current. The current was increased by 0.5 mA steps until the participant reported moderate pain (level 5 on the VAS).
Transcranial Direct Current Stimulation

The participant was required to sit still and quietly in a comfortable armchair. To maintain the double-blinded procedure, tDCS was carried out by a second researcher that did not obtain pain ratings to pinprick and electrical stimuli. Additionally, the anodes were placed over left M1 and left DLPFC across all four conditions, however, only the channel(s) relevant to the condition were activated. The cathode was placed over the contralateral (right) supraorbital area for all four conditions. The 10-20 international system for EEG electrode placement procedure was followed to located left M1 (location C3 according to the EEG system) and left DLPFC (location F3) for anode placement (DaSilva, Volz, Bikson & Fregni, 2011; Fregni et al., 2005). The tDCS operator administered an electrical current of 1 mA for 20 minutes to the target brain region(s) relevant to the condition (either at left M1 and DLPFC concurrently, left M1 or left DLPFC). During the sham condition a-tDCS of 1 mA was applied to both left M1 and DLPFC, the current was ramped up over 30 seconds and then ramped down over 10 seconds; no current was emitted for the
remainder of the 20 minutes. This sham procedure has been validated to mimic sensations associated with active tDCS to ensure participants remain blinded to conditions, without eliciting cortical excitability (Bachmann et al., 2010; Ihle et al., 2014). The tDCS administration of 1 mA for 20 minutes was chosen as prior studies had reported a significant increase in pain thresholds following these parameters, whilst minimizing side-effects to participants and maintaining the integrity of the blinding procedure (Kessler et al., 2012; Meeker et al., 2019).

![Figure 4](image)

*Figure 4. Visual representation of the tDCS procedure. Yellow Sponge: cathodal electrode in the contralateral supraorbital area; blue sponge: anodal electrode at DLPFC; pink sponge: anodal electrode at M1.*

**Time 2**

**Low-Frequency Electrical Stimulation**

Following tDCS, the participant returned to the experimenter that collected pain data. Post tDCS, one pinprick pain and sharpness rating was obtained from both the primary and secondary areas. The procedure to obtain the moderate pain current level was again followed (as detailed above). However the electrical current
commenced at that level that elicited moderate pain (level 5 on the VAS) prior to tDCS (Time 1). The electrical current was increased by 0.5 mA steps until the participant reached moderate pain. Electrical stimulation continued at that current level for 20 minutes. The experimenter prompted the participant to provide pain and sharpness ratings every minute by taping their wrist. The participant then received a five-minute break to minimize the effect of a potential adaptive response the electrical stimulation (Sauerstein et al., 2018).

**Time 3**

Following the five-minute break, the experimenter that collected pain data obtained one pinprick pain and sharpness rating from both the primary and secondary areas. The procedure to obtain the moderate pain current level was again followed. However the current commenced at the level that elicited moderate pain (level 5 on the VAS) post t-DCS (Time 2). Finally the participant completed a Non-invasive Brain Stimulation (NiBS) Questionnaire to ensure they did not experience any adverse side effects during stimulation (Appendix G). In the last session participants completed an exit survey (Appendix H) and were asked to indicate in which session they thought they received sham.

**Emergency Procedure**

All experimenters were First Aid trained to ensure they were able to appropriately respond if a participant required basic medical attention. If urgent medical attention was required experimenters were to call an ambulance by dialing 000 and notify security.
Results

Inspection of the Edinburgh Handedness Inventory Short Form confirmed all participants met the criteria for right-handedness, with a reported minimum score of 75 and maximum of 100 ($M = 96.25$, $SD = 7.14$).

A chi-squared goodness-of-fit test (at $\alpha = .05$) was used to assess whether participants were able to correctly guess the sham condition. The chi-squared test was statistically significant $\chi^2(1, N = 20) = 9.80$, $p = .002$, indicating participants correctly guessed sham 15% of the time, which is less than chance. As an index of effect size, Cohen’s $w$ was 0.70, which can be considered a large effect. To further assess whether participants were blinded to the sham condition, the Non-invasive Brain Stimulation Questionnaire subscales, itching, tingling and burning (the most commonly reported sensations during tDCS) were examined (Kessler et al., 2012; Wrigley et al., 2013). A one-way repeated measures analysis of variance (ANOVA) was conducted to evaluate whether participants’ reported itching, tingling and burning sensations were statistically significantly different across M1 & DLPFC, M1, DLPFC and Sham conditions.
Table 1 presents the descriptive statistics across all conditions.

Table 1

Descriptive Statistics of Itching, Tingling and Burning Sensations Across All TDCS Conditions.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Itching M (SD)</th>
<th>Itching 95% CI</th>
<th>Tingling M (SD)</th>
<th>Tingling 95% CI</th>
<th>Burning M (SD)</th>
<th>Burning 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>tDCS M1 &amp; DLPFC</td>
<td>3.00 (1.12)</td>
<td>[2.47, 3.53]</td>
<td>2.45 (0.76)</td>
<td>[2.09, 2.81]</td>
<td>2.10 (0.79)</td>
<td>[1.73, 2.47]</td>
</tr>
<tr>
<td>M1</td>
<td>2.55 (1.00)</td>
<td>[2.08, 3.02]</td>
<td>2.15 (0.81)</td>
<td>[1.77, 2.53]</td>
<td>1.45 (0.76)</td>
<td>[1.09, 1.81]</td>
</tr>
<tr>
<td>DLPFC</td>
<td>2.70 (1.08)</td>
<td>[2.19, 3.21]</td>
<td>2.20 (0.89)</td>
<td>[1.78, 2.62]</td>
<td>1.70 (0.86)</td>
<td>[1.30, 2.10]</td>
</tr>
<tr>
<td>Sham</td>
<td>2.5 (1.24)</td>
<td>[1.92, 3.08]</td>
<td>2.55 (1.00)</td>
<td>[2.08, 3.02]</td>
<td>1.60 (0.99)</td>
<td>[1.13, 2.07]</td>
</tr>
</tbody>
</table>

Note. M = mean; SD = standard deviation; CI = confidence interval.

The ANOVA revealed there was a non-significant difference between tDCS condition and itching sensations $F(3, 57) = 1.94, p = .133, \eta^2 = .09$ and tingling sensations $F(3, 57) = 2.36, p = 0.081, \eta^2 = .11$. However, there was a significant difference between condition and burning sensations $F(3, 57) = 4.28, p = .009, \eta^2 = .18$. Bonferroni-adjusted pairwise comparisons revealed burning sensations during the M1 & DLPFC condition ($M = 2.10, SD = .79$) were significantly higher than the M1 condition ($M = 1.45, SD = .76$), $p = .022$, this difference was large, with $d = 0.84$.

However, there was no significant difference between burning sensations and all other conditions. The above results demonstrate that the tDCS effects in the active conditions were more pronounced than sham, indicating the tDCS procedure was administered correctly. However, participants were not able to distinguish between sham and active conditions, which suggests that participant blinding was successful.
Statistical Analysis of Hypotheses and Assumption Tests

One-way repeated measures ANOVAs (with $\alpha = 0.5$) were utilized to evaluate the hypotheses of this study. Assumptions of the analysis were tested. Shapiro-Wilk statistic ($p > .05$) was inspected to assess normality, which indicated, in accordance with this test, the majority of variables were not normally distributed. However, the Shapiro-Wilk statistic is sensitive and signals departures from normality when it is inconsequential (Allen, Bennett & Heritage, 2014; Tabachnick & Fidell, 2007). To further assess normality, visual measures were inspected. Additionally, skewness and kurtosis fell within three times the standard error of the mean for all variables, except two. Furthermore, a repeated measures ANOVA is considered robust to moderate deviations from normality (Blanca, Alarcón, Arnau, Bono & Bendayan, 2017; Guiard & Rasch, 2004). Due to the subjective nature of pain and robustness of the ANOVA, it was considered appropriate to continue with interpretation rather than performing transformations of the data (Bannister & Dickenson, 2017). $F_{\text{max}}$ figures for variables were 2.7 (tDCS conditions), 1.60 (primary area pain ratings) and 2.39 (secondary area pain ratings), demonstrating the assumption homogeneity of variance was not violated. Mauchly’s test ($p > .05$) indicated the assumption of sphericity was violated for some variables, in these instances Greenhouse-Geisser corrections were applied (Field, 2015).

The Effect of tDCS on Current Level during Electrical Stimulation at Time 1 and Time 2

The current level between Time 1 and Time 2 across tDCS conditions was analyzed to establish whether participants’ tolerance to the current level eliciting moderate pain resulted from tDCS. Table 2 presents the descriptive statistics for the intensity of the electrical current level across tDCS conditions.
Table 2

Descriptive Statistics for Strength of Electrical Current at Time 1 and Time 2 Across All TDCS Conditions

<table>
<thead>
<tr>
<th>Condition</th>
<th>Time 1</th>
<th>Time 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>tDCS</td>
<td>M (SD) 95% CI</td>
<td>M (SD) 95% CI</td>
</tr>
<tr>
<td>M1 &amp; DLPFC</td>
<td>8.40 (3.82)</td>
<td>[6.61, 10.19]</td>
</tr>
<tr>
<td>M1</td>
<td>7.95 (4.15)</td>
<td>[6.01, 9.89]</td>
</tr>
<tr>
<td>DLPFC</td>
<td>8.40 (4.21)</td>
<td>[6.43, 10.37]</td>
</tr>
<tr>
<td>Sham</td>
<td>9.13 (5.46)</td>
<td>[6.57, 11.68]</td>
</tr>
</tbody>
</table>

Note. M = mean; SD = standard deviation; CI = confidence interval.

A one-way repeated measures ANOVA was conducted to evaluate whether tDCS condition had an effect on the intensity of the current level required for participants to report a moderate level of pain.
Figure 5 demonstrates the Sham condition required the highest current level for participants to report a moderate level of pain; this is followed by DLPFC, then M1 & DLPFC and finally the M1 condition.

The ANOVA results revealed that the current level to elicit moderate pain level varied statistically significantly between Time 1 and Time 2 $F(1.00, 19.00) = 23.39, p < .001, \eta^2 = .55$; however, the current level did not vary significantly across tDCS conditions, $F(1.95, 37.12) = 0.83, p = .441, \eta^2 = .042$; or the interaction between tDCS conditions and time $F(1.53, 29.10) = 0.88, p = .339, \eta^2 = .04$. Results revealed there was a significant main effect for time, indicating the current level to elicit moderate pain level increased significantly from Time 1 to Time 2 irrespective of tDCS condition. Participant habituation to the low-frequency electrical stimulation may provide an explanation for these findings (Milne, Kay & Irwin, 1991; Seifert et al., 2009).
The Effect of tDCS on Pinprick Pain Ratings at Primary and Secondary Areas Across Time 1 and Time 2

To assess whether tDCS decreased participants’ sensitivity to pinprick, participants’ pain ratings to pinprick within the primary and secondary areas at Time 1 and Time 2 were analyzed. Table 3 presents the descriptive statistics for participant pinprick pain ratings in the primary area across tDCS conditions.

Table 3

Descriptive Statistics of Pain Ratings to Pinprick in the Primary Area at Time 1 and Time 2 Across All TDCS Conditions

<table>
<thead>
<tr>
<th>tDCS Condition</th>
<th>Time 1</th>
<th>Time 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M (SD) 95% CI</td>
<td>M (SD) 95% CI</td>
</tr>
<tr>
<td>M1 &amp; DLPFC</td>
<td>1.40 (0.99) [0.93, 1.87]</td>
<td>1.63 (1.23) [1.05, 2.20]</td>
</tr>
<tr>
<td>M1</td>
<td>1.25 (1.06) [0.76, 1.75]</td>
<td>1.25 (1.19) [0.69, 1.81]</td>
</tr>
<tr>
<td>DLPFC</td>
<td>1.29 (1.18) [0.74, 1.84]</td>
<td>1.33 (0.94) [0.89, 1.76]</td>
</tr>
<tr>
<td>Sham</td>
<td>1.45 (1.16) [0.91, 1.99]</td>
<td>1.20 (1.08) [0.69, 1.71]</td>
</tr>
</tbody>
</table>

*Note.* M = mean; SD = standard deviation; CI = confidence interval.
Table 4 presents the descriptive statistics for participant pinprick pain ratings in the secondary area across tDCS conditions.

Table 4

*Descriptive Statistics of Pain Ratings to Pinprick in the Secondary Area at Time 1 and Time 2 Across All TDCS Conditions*

<table>
<thead>
<tr>
<th>Condition</th>
<th>Time 1</th>
<th>Time 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M (SD)</td>
<td>95% CI</td>
</tr>
<tr>
<td>tDCS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M1 &amp; DLPFC</td>
<td>1.48 (1.30)</td>
<td>[0.87, 2.08]</td>
</tr>
<tr>
<td>DLPFC</td>
<td>1.28 (1.18)</td>
<td>[0.73, 1.83]</td>
</tr>
<tr>
<td>M1</td>
<td>1.10 (0.97)</td>
<td>[0.65, 1.55]</td>
</tr>
<tr>
<td>Sham</td>
<td>1.23 (1.14)</td>
<td>[0.69, 1.76]</td>
</tr>
</tbody>
</table>

*Note.* M = mean; SD = standard deviation; CI = confidence interval.
A one-way repeated measures ANOVA was used to compare participants’ pain ratings to pinprick within the primary and secondary areas at Time 1 and Time 2 across all tDCS conditions.

**Primary area pinprick pain ratings**

![Primary area pinprick pain ratings](image)

*Figure 6.* Estimated marginal means for primary area pinprick pain ratings at Time 1 and Time 2 across M1 & DLPFC, M1, DLPFC and Sham conditions.

Figure 6 demonstrates Sham was the only condition that elicited a reduction in primary pain ratings post tDCS. There was no change within M1 and DLPFC conditions and an increase in the M1 & DLPFC condition. However, these differences were non-significant.

The ANOVA results show that pinprick pain ratings within the primary area did not vary statistically significantly between tDCS condition $F(3, 57) = 0.51, p = .677, \eta^2 = .03$, Time $F(1, 19) = 0.001, p = .979, \eta^2 = .00$ or the interaction between tDCS condition and time $F(3, 57) = 0.88, p = .456, \eta^2 = .04$. These results indicate the tDCS condition had no effect on participants’ primary area pain ratings.
Secondary area pinprick pain ratings

Figure 7. Estimated marginal means for secondary area pinprick pain ratings at Time 1 and Time 2 across M1 & DLPFC, M1, DLPFC and Sham conditions.

Figure 7 demonstrates M1 & DLPFC was the only condition that elicited a reduction in secondary area pain ratings post tDCS. An increase was observed across all other conditions. However, these differences were non-significant.

The ANOVA results indicate that pinprick pain ratings within the secondary area did not vary statistically significantly between tDCS condition $F(2.30, 43.77) = 2.32, p = .103, \eta^2 = .11$, Time $F(1, 19) = 0.09, p = .765, \eta^2 = .005$ or the interaction between tDCS condition and time $F(3, 57) = 0.99, p = .405, \eta^2 = .05$. These results indicate the tDCS condition had no effect on participants’ secondary area pain ratings.

Discussion

This study aimed to clarify the conflicting literature regarding whether a-tDCS at M1 and DLPFC reduces pain in healthy adults. Additionally, it aimed to identify whether concurrent a-tDCS of M1 and DLPFC results in a greater reduction of pain
than stimulation of each brain region alone. The findings of this study suggest 20
minutes of 1 mA a-tDCS at M1 and DLPFC concurrently, at M1 alone and DLPFC
alone, does not reduce experimental pain induced by-low frequency electrical
stimulation in healthy participants. These results are somewhat consistent with the
literature, due to the conflicting nature of current evidence (Boggio et al., 2008; Ihle
et al., 2014; Meeker et al., 2019; Mylius et al., 2012; Zandieh et al., 2013).

Hypothesis One

The hypothesis that a-tDCS at M1 and DLPFC concurrently would result in
lower pain ratings to pinprick in the primary and secondary area and the highest
electrical current during electrical stimulation to elicit moderate pain than M1 alone,
DLPFC alone and sham conditions was not supported. A very recent study, Henriques
et al. (2019), compared the administration of 20 minutes 2 mA a-tDCS to M1 and
DLPFC concurrently, with 20 minutes of 2 mA c-tDCS at M1 and a-tDCS to DLPFC
concurrently. Results indicated a-tDCS at both M1 and DLPFC did not reduce healthy
participants pain, whereas c-tDCS at M1 and a-tDCS at DLPFC reduced pain.
However, due to methodological differences, direct comparisons with our study
cannot be made. Firstly, Henriques et al. (2019) tested on 10 male participants. Our
participant sample included both males and females. Secondly, they administered a 2
mA current to each brain region, whereas our study applied 1 mA. Thirdly, pain was
not experimentally induced; rather participants rated pain perception on a subjective
rating scale in response to hip flexion and range of motion exercise tolerances. The
decrease of participant pain in the M1 c-tDCS and DLPFC a-tDCS montage may be
explained by findings suggesting c-tDCS at M1 increases hip range of motion
(Henriques et al., 2019; Lins, Lattari, Monteiro, Albuquerque Maranhão Neto &
Machado, 2019). If hip flexibility increases due to c-tDCS at M1, it would be
expected subsequent pain ratings in response to the same degree of hip flexion would decrease, therefore, this method of pain assessment may not be a true assessment of participant pain thresholds. However, without objectively testing this, definitive conclusions cannot be made. No other known studies have reported on healthy participants pain following tDCS administered to M1 and DLPFC concurrently. Due to the limited research within this area, future studies should replicate our study design but incorporate c-tDCS montages to establish whether alternative tDCS parameters may effectively reduce pain in healthy participants.

**Hypotheses Two and Three**

The hypotheses that a-tDCS at M1 alone, or DLPFC alone would result in lower pain ratings to pinprick in the primary and secondary areas and a higher current level during electrical stimulation to elicit moderate pain than sham, was not supported. As the results of prior studies are mixed, our results somewhat contradict the literature. Zandieh et al. (2013), who reported a reduction in participants’ pain following a-tDCS at M1, utilized a similar sample size, of 22 participants, and stimulation duration of 20 minutes. However, Zandieh et al. (2013) administered 2 mA of a-tDCS, whereas our study applied 1 mA. Additionally, pain was assessed by a cold pressor test by recording the duration participants were able to withstand their arm submerged into water at 3°C. Mylius et al. (2012) also observed a reduction of 24 participants pain following 20 minutes of 2 mA a-tDCS at DLPFC. Pain was assessed by recording participants’ tolerance thresholds to cold and heat stimuli. Again, the current intensity of a-tDCS and pain assessment procedures differs from our study. Ihle et al. (2014) reported no reduction to participants’ pain by applying the same current intensity of 1 mA a-tDCS at M1, as our study. Ihle et al. (2014) assessed pain post a-tDCS by recording responses to pinprick and pain tolerances to heat stimuli.
Our findings are consistent with this study, however a direct comparison cannot be made due to the shorter stimulation duration of 15 minutes. Meeker et al. (2019) also administered a-tDCS of 1 mA at M1 for 20 minutes to 15 participants, similar to our study. Participants pain to pinprick and heat stimuli decreased, contradicting the results of Ihle et al. (2014) and our study. Additionally, most prior studies administered a-tDCS via 35 cm² electrodes, however the electrodes within our study were 25 cm² (Zandieh et al., 2013; Boggio et al., 2008; Meeker et al., 2019). The methodological differences of a-tDCS current intensities, electrode size, experimental pain models and stimulation duration may explain the diverging findings of the literature.

To maintain the integrity of the sham procedure and minimize side effects to participants, tDCS at the current intensity of 1 mA was selected within this study (Bastani & Jaberzadeh, 2013; Gandiga, Hummel & Cohen, 2006). However, most prior studies that obtained a significant reduction in healthy participants’ pain administered 2 mA to M1 and DLPFC (Zandieh et al., 2013; Boggio et al., 2008; Meeker et al., 2019). The literature indicates the strength of the electrical current induces different levels of cortical excitability (Bastani & Jaberzadeh, 2013; Nitsche & Paulus, 2000). The results of prior tDCS studies applying 2 mA could suggest the 1 mA current utilized within our study may provide an explanation for the non-significant results obtained. However, a study conducted by Ho et al. (2016), who directly tested cortical excitability differences between 1 mA and 2 mA a-tDCS at M1, found no significant difference in excitability between current strengths. Despite no difference in cortical excitability, 2 mA might be more effective at reducing participants’ pain. It is important to consider, the studies that utilized 2 mA did not report the success of participant blinding to the sham condition. Current intensities of
2 mA may compromise participant blinding, which could influence results obtained (Horvath, Carter & Forte, 2014; O’Connell et al., 2018). To clarify whether 2 mA a-tDCS results in greater pain reduction than 1 mA, future research needs to directly compare currents intensities and success of participants blinding in a sham controlled study.

Anodal electrodes of 25 cm² were selected for this study as smaller electrodes stimulate a more focal area, minimizing stimulation of adjacent sites; and to blind participants to active tDCS (Gandiga et al., 2006; Thair et al., 2017). The literature indicates larger stimulating electrodes will produce greater tDCS sensations, compromising blinding (O’Connell et al., 2012; Palm et al., 2013). However, prior studies reporting a reduction in participants’ pain following a-tDCS at M1 and DLPFC have utilized 35 cm² electrodes (Boggio et al., 2008; Meeker et al., 2019; Mylius et al., 2012; Zandieh et al., 2013). The literature suggests the size of the stimulating electrode influences the level of induced cortical excitability (Nitsche & Paulus, 2000). Ho et al. (2016) measured and compared excitability induced by 16 cm² and 35 cm² electrodes during a-tDCS. Results indicated the 35cm² electrode induced significantly greater cortical excitability (Ho et al., 2016). It can be speculated that the smaller sized electrode utilized by our study may not have induced the same level of cortical excitability as prior studies utilizing 35cm² electrodes. However, direct comparisons between 25 cm² and 35cm² electrodes have not been made. Before definitive conclusions can be drawn, future studies need to assess whether 35 cm² electrodes elicit greater pain reduction than 25 cm² electrodes during a-tDCS at M1 and DLPFC. Additionally, future studies need to report the success of participant blinding to accurately assess whether any reported decrease in pain is greater than placebo.
The low-frequency electrical stimulation pain model was selected for this study as it evokes both primary and secondary hyperalgesia, which are characteristics of chronic pain (Beissner et al., 2010; Liu & Sandkühler, 1997; Ziegler, et al., 1999). No known study has employed low-frequency electrical stimulation to assess pain responses following tDCS. Most prior studies reporting a reduction to participants’ pain utilized cold and heat stimuli (Ihle et al., 2014; Meeker et al., 2019; Mylius et al., 2012; Zandieh et al., 2013). The different pain model utilized in our study may explain the contradicting findings. To clarify this, future studies should compare pain responses to thermal stimuli with low-frequency electrical stimulation following tDCS.

**Alternative Explanations**

Similar pain ratings to pinprick, and a significant increase in the current level required to elicit moderate pain was observed after tDCS irrespective of the brain region(s) stimulated. Suggesting that 20 minutes of a-tDCS at 1mA did not reduce pain. Habituation to the electrical stimuli may explain the significant increase in current level required to induce moderate level pain post tDCS (Bauch, Andreou, Rausch & Bunzeck, 2017). Habituation is a neural response, where exposure to repetitive nociceptive stimuli decreases pain over time (Bauch, et al., 2017; Milne et al., 1991). However, this process is only observed with low to moderate intensity pain stimuli (Ginzburg et al., 2015). Habituation is an adaptive process of the pain modulation system, which inhibits pain once the initial warning signal to the painful stimuli has been received (Bauch et al., 2017; Bingel, Schoell, Herken, Büchel & May, 2007; Ginzburg et al., 2015). A study conducted by Milne et al. (1991) indicated that two minutes of low intensity electrical stimulation at 1 Hz frequency and a 0.5 ms pulse width induced habituation to the stimuli. Additionally, Ginzburg et al. (2015)
observed that participants reported lower pain ratings following 60 seconds of electrical stimulation. Our participants were exposed to over 60 seconds of low-frequency electrical stimulation at 1 Hz and 0.5 ms pulse width to achieve moderate pain at baseline and after tDCS, which might have been sufficient to induce habituation, resulting in the significant increase in current level required to induce moderate pain after tDCS (Ginzburg et al., 2015; Milne et al., 1991).

Nickel et al. (2014) examined brain correlates of habituation to low-frequency electrical stimulation identical to the parameters of this study, by recording BOLD responses via fMRI. BOLD responses obtained during electrical stimulation indicated habituation modulated neuronal activity within brain regions associated with pain, including the DLPFC and thalamus (Nickel et al., 2014). As it is suggested a-tDCS at M1 may influence thalamic blood flow, which is potentially responsible for the decrease in pain observed following stimulation, habituation may have biased the data collected within this study (Garcia-Larrea et al., 1999; Saitoh et al., 2001). Neuro-modulation during habituation to the electrical stimuli may have confounded results obtained, as it potentially influenced participants’ pain perception. Therefore, any potential subtle effects of tDCS on pain within our study could not be observed. To eliminate this confounding variable, future studies should assess pain via measures that are reliable and consistent within and between experimental sessions, such as electronic pressure algometry applied to the C6 spinal segment (Frank, McLaughlin & Vaughan, 2013; Vatine, Shapira, Magora, Alder & Magora, 1993). Electronic pressure algometry objectively quantifies pain pressure thresholds in healthy participants via the application of a constant rate of 1 kg/s pressure; participants press a button when the pressure sensation changes to pain (Frank et al., 2013; Petersen-Felix & Arendt-Nielsen, 2002; Potter, McCarthy & Oldham, 2006). Prior studies
indicate this pain assessment method triggers nociceptive responses whilst providing high intra-rater and test-retest reliability, with consistent ratings obtained both five minutes and one week apart (Frank et al., 2013; Potter et al., 2006; Vatine et al., 1993). These findings indicate, that if any habituation to the pressure stimulus has occurred, these effects are non-significant, minimizing bias to obtained pain ratings.

In addition to habituation, female participants menstrual cycles could have influenced results (Iacovides, Avidon & Baker, 2015; Riley Iii, Robinson, Wise & Price, 1999). Tassorelli et al. (2002) examined nociceptive flexion reflexes, an objective measure of fluctuations within the pain control system, and VAS pain ratings, in response to high-frequency electrical stimulation administered to women during the different stages of their menstrual cycle. Results demonstrated women were more sensitive to painful electrical stimulation in their mid to late luteal phase (days six to eight from ovulation) than their follicular phase (eight to 10 days from the first day of menstrual bleeding; Tassorelli et al., 2002). This pain sensitivity during the luteal phase has been demonstrated in other studies (Fillingim et al., 1997; Pfleeger, Straneva, Fillingim, Maixner & Girdler, 1997; Riley Iii et al., 1999). Although the exact mechanism is unclear, it is speculated the fluctuation of sex hormones estrogen and progesterone during the menstrual cycle may influence pain perception, which might have altered the results of the present study by influencing pain thresholds (Basbaum & Fields, 1984; Facchinetti et al., 1988; Tassorelli et al., 2002). Additionally, it is argued, estrogen might impact serotogenic pathways (a neurotransmitter implicated with pain) by controlling excitability at the brain stem, which projects inhibitory or facilitatory pain mechanisms to the spinal cord (Bannister & Dickenson, 2017; Taylor, Mathew, Ho & Weinman, 1984; Yarnitsky, 2015). Therefore, the neuromodulation effect of tCDS may be implicated in the luteal phase.
due to its influence on descending pain mechanisms. As 50% of our sample was female and menstrual cycle was not controlled, this could have significantly influenced the results obtained. To minimize this potential confounding variable, future studies should test females during the same period of their menstrual cycle (Tassorelli et al., 2002; Riley Iii et al., 1999).

**Limitations**

The methodology of the current study needs to be considered in the context of prior research findings to interpret results obtained. The current study employed the VAS rating scale to rate participants’ level of pain. Although the VAS is considered a valid and reliable measure of pain utilized within pain studies, the measure is based on participant subjective ratings (Kane, Bershadsky, Rockwood, Saleh & Islam, 2005; Price, McGrath, Rafii & Buckingham, 1983; Rosen, Ramkumar, Nguyen & Hoeft, 2009). Incorporating an objective measure of pain alongside the VAS within the study may have captured changes in participants’ pain sensations not demonstrated by the VAS. To address this limitation, future studies should incorporate objective measures to capture decreases in pain that may not be evident through the use of the VAS alone. A suggested measure is the recording of the nociceptive flexion reflex, a physiological withdrawal response to painful stimuli, which has been established as a reliable, and objective measure of pain, that is easily measurable within clinical settings (Skljarevski & Ramadan, 2002; Tassorelli et al., 2002).

An additional limitation of the current study was the small sample size. Our methodological decision to test 20 participants was based on past tDCS studies obtaining significant results with similar sample sizes and the one-year timeframe established for this study (Zandieh et al., 2013; Boggio et al., 2008; Meeker et al., 2019). Future studies should increase statistical power by employing larger participant
samples, reducing the risk of falsely rejecting the null hypothesis, committing a type I error (Field, 2015). A further limitation was the four tDCS operators measuring participants’ scalps to determine the location of target brain regions. The present study used the 10/20 EEG system to locate M1 and DLPFC brain regions (DaSilva et al., 2011). All experimenters were trained to use this system by an experienced researcher. However, it cannot be completely ruled out that scalp measurements obtained by different experimenters were not consistent, which might have caused inconsistent stimulation of cortical areas across testing sessions. To mitigate potential inconsistencies of locating brain regions, future studies should ensure only one suitably trained researcher locates cortical targets.

**Future Directions**

Future research replicating our study is needed to determine whether a-tDCS of M1 and DLPFC reduces pain, and whether concurrent stimulation results in greater pain reduction. This is required due to current conflicting findings and limited published literature exploring concurrent stimulation of M1 and DLPFC for pain reduction. Given that a-tDCS at 1 mA did not reduce pain, a 2 mA current should be considered. However, studies need to report whether participant blinding is successful to determine whether any observed reduction in pain is not due to placebo (Gandiga et al., 2006; O’Connell et al., 2018). Future studies should assess whether larger electrode sizes of 35 cm² induce greater pain reduction than 25 cm² during tDCS. Studies should incorporate objective measures of pain, such as recordings of the nociceptive flexion reflex or objective pain rating tools such as electronic pressure algometry in their designs, to capture any potential subtle decreases in pain following tDCS that subjective pain measures such as the VAS will not reveal (Petersen-Felix & Arendt-Nielsen, 2002; Tassorelli et al., 2002). To minimize habituation effects of pain
stimuli during the experimental procedure, pain ratings should be obtained by
electronic pressure algometry applied to the C6 spinal segment (Frank et al., 2013;
Potter et al., 2006; Vatine et al., 1993). Lastly, testing on female participants should
occur during the same period of their menstrual cycle to minimize this potential
confounding factor biasing results (Tassorelli et al., 2002).

Conclusion

The current study investigated tDCS as a potential treatment for chronic pain
by administering 1 mA a-tDCS for 20 minutes to brain regions M1 and DLPFC
concurrently, M1 alone, DLPFC alone and sham to healthy participants. Participant
VAS pain ratings to primary and secondary area pinpricks and current level during
low-frequency electrical stimulation to elicit moderate pain were not significantly
different between tDCS conditions. These results indicate tDCS did not reduce
participants’ pain within this study. However, electric current level to elicit pain was
statistically significant between pre and post tDCS, suggesting participant habituation
to the low-frequency electrical stimulation may have biased results obtained (Bauch,
et al., 2017; Milne et al., 1991). Additionally, no objective measures assessed
potential subtle changes of pain resulting from tDCS. Prior literature suggests the
administration of 2 mA a-tDCS utilizing 35 cm² anodal electrodes might decrease
participants pain, however, these studies have not assessed whether participants were
blinded to sham (Boggio et al., 2008; Meeker et al., 2019; Mylius et al., 2012;
Zandieh et al., 2013). Future research should replicate this study to determine
whether tDCS reduces healthy participants pain by administering 2 mA of a-tDCS for
20 minutes with 35 cm² anodal electrodes. Studies should report whether participants
are successfully blinded to sham, to assess whether any observed decrease in
participants’ pain is greater than placebo. Additionally, to minimize habituation
effects and to obtain objective pain related data, electronic pressure algometry administered to the C6 spinal segment should be utilized to measure participants’ pain. Limitations of our study and the current literature need to be addressed in replication studies before definitive conclusions as to the efficacy of a-tDCS at M1 and DLPFC for the treatment of chronic pain can be made.
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