Towards the use of transcranial direct current stimulation to improve motor function

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Abstract

This study investigated the effect of anodal tDCS on motor control and corticomotor excitability in healthy controls, with the long-term goal of investigating the use of anodal tDCS to improve motor function in covertly aware vegetative state patients. Experiment I investigated the effects of anodal tDCS on a motor reaction time task, and found no effect of tDCS on performance, whether or not participants trained on the task or were at rest during the stimulation. Experiment II looked at the effects of anodal tDCS paired with passive movements on corticomotor excitability, and found no significant difference in corticomotor excitability, as measured by motor evoked potentials (MEPs), between the placebo and anodal conditions. Future investigation is needed to understand if and when anodal tDCS can be used to improve motor function in this patient group.

Keywords: Transcranial direct current stimulation, Motor control, Covertly aware vegetative state patients, Passive movements
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Chapter 1

1 Introduction

1.1 Disorders of consciousness

Consciousness is a complex psychological concept that primarily relies on two major components: arousal and awareness. Arousal (or wakefulness) is defined as the level of consciousness. Sleeping is a low state of arousal, whereas being awake is a high state of arousal. Arousal is supported by the brainstem, specifically the reticular activating system, which projects to the thalamus and then to cortical regions (Laureys, Boly, Moonen, & Maquet, 2009). Damage to the brainstem or extensive damage to the cortex can lead to lower levels of arousal. Awareness is defined as the content of consciousness. An individual shows signs of awareness if they are capable of processing and responding to their environment. The neural mechanisms supporting awareness are not well understood, but it is hypothesized that they depend on the integrity of the cortex and its subcortical connections (Laureys et al., 2009). Several theories suggest that awareness is supported by fronto-parietal networks (Cavinato et al., 2015; Fernández-Espejo et al., 2012; Laureys et al., 2009) and thalamocortical networks (Fernández-Espejo et al., 2012; Laureys et al., 2000; White & Alkire, 2003). Patients who are not awake and do not show any signs of awareness are defined as being unconscious (Posner, Saper, Schiff, & Plum, 2007).

More often than not arousal and awareness are not dissociable because awareness is usually not present without a reasonable degree of arousal (Posner et al., 2007). However, in rare cases it is possible for these two components to dissociate. For example, the vegetative state (VS) and minimally conscious state (MCS), which are both disorders of consciousness (DoC), lead to a dissociation of awareness and arousal. VS and MCS patients have intact wakefulness (arousal), but show a disruption in their awareness. Specifically, VS patients show no behavioural signs of awareness of themselves or the environment, whereas MCS patients show fluctuating but minimal levels of awareness (Laureys, 2005; Posner et al., 2007).
The most common cause of VS and MCS is a severe brain injury that leads to a comatose, or unconscious state. Patients in a coma do not show signs of awareness, nor do they have sleep-wake cycles (Laureys et al., 2009). After a short period of time (between hours and a few weeks), patients in a coma will either recover or permanently lose all brain function (brain death). However, a small number of patients progress into a VS or MCS, in which they regain wakefulness, but still show minimal to no awareness (Laureys, Owen, & Schiff, 2004). The brain injury etiology that typically leads to these disorders involves extensive damage to the thalamus or bilateral hemispheric damage, with little to no damage in the brainstem (Posner et al., 2007).

The progression and prognosis of these two disorders can vary from patient to patient. For example, VS and MCS can be a transitional state indicating recovery from a coma or a worsening of a neurological disease, but it can also be a permanent state for some patients. Depending on the source of injury, a patient is considered to be in a permanent vegetative state if they have been in that state for 3 months after a non-traumatic brain injury or 1 year after traumatic brain injury. These permanent VS patients rarely recover after that period of time (Posner et al., 2007). Patients who are permanently in this state are severely disabled, and need constant care (Wilson, Harpur, Watson, & Morrow, 2002).

The diagnosis of VS and MCS is complex, as determining whether a patient is lacking awareness is significantly more difficult than diagnosing lack of wakefulness. The clinical diagnosis of VS and MCS relies on the observation of the patients’ voluntary behaviours by the examiner (Laureys et al., 2004; Monti, Laureys, & Owen, 2010). The assessment tool most commonly used to diagnose VS and MCS patients is the Coma Recovery Scale-Revised (CRS-R) (Giacino, Kalmar, & Whyte, 2004). These patients often display substantial involuntary movement, thus, it is difficult to differentiate between voluntary and involuntary movements (Laureys et al., 2009). Moreover, patients may have a comorbid disability (e.g. blindness, motor deficits), which may make it more difficult to respond to the commands of the examiner. Even when these patients are able to engage in purposeful behaviour, they often fatigue quickly or the behaviours that they are able to produce are inconsistent (Laureys et al., 2004). Additionally, given the rarity
of this condition, examiners often lack experience with these diagnoses, and may be confused about terminology (Monti, Laureys, & Owen, 2010). For these reasons, relying on behavioural measures to diagnose these patients has led to a high rate of misdiagnoses. Wilson and colleagues (2002) found that when they re-assessed patients using behavioural measures, 33% of VS and MCS patients were discovered to have been misdiagnosed by their health care practitioner upon entry to studies. These patients showed signs of awareness upon entry to the study that their health care practitioners had missed. Other studies have found similar rates of misdiagnosis (Andrews, Murphy, Munday, & Littlewood, 1996; Childs, Mercer, & Childs, 1993; Schnakers et al., 2009), and it is estimated that up to one in three patients diagnosed as VS should be diagnosed as MCS (Laureys et al., 2009). Therefore, relying on behavioral measures to diagnose these patients has led to a high rate of misdiagnoses (Laureys et al., 2004).

### 1.2 Covertly aware patients

Even when the behavioural assessment is conducted properly, behavioural assessments cannot discriminate between a correct diagnosis of VS and a patient who is aware but lacks the motor capabilities to respond to the examiner. These two very different patient groups would appear indistinguishable on the basis of a behavioural assessment. By using neuroimaging, it was discovered that there is a subset of patients who are diagnosed as VS because they show no signs of behavioural awareness but do show signs of covert awareness (Fernández-Espejo & Owen, 2013; Owen et al., 2006). Owen et al. (2006) discovered a patient who was diagnosed as VS but was able to modulate her brain activity based on commands using functional magnetic resonance imaging (fMRI). More specifically, this patient was asked to imagine playing tennis and to walk around her house (two tasks that recruit distinct brain areas), and her brain activity during these two tasks was compared to healthy controls engaging in the same tasks. During the tennis task, the patient showed activity in the supplementary motor area; in contrast, when imagining walking around her house, the patient showed activity in parahippocampal gyrus, posterior parietal lobe, and the lateral premotor cortex. This brain activation was similar to the activation seen when healthy controls imagined these two activities. Since this breakthrough, multiple studies have shown that there is a subset of patients,
estimated at approximately 20%, who are diagnosed as VS but who can modulate their brain activity based on their environment (Fernández-Espejo & Owen, 2013; Monti, Vanhaudenhuyse, et al., 2010). The behavioural measures used to diagnose these patients use command following as an indication of awareness. A patient who is able to complete this motor imagery task is able to follow commands, and therefore shows signs of awareness.

Cruse et al. (2011) were able to use another neuroimaging technique, electroencephalography (EEG), to replicate the finding that some VS patients show signs of covert command following, despite being diagnosed as not aware. In this study, Cruse et al. (2011) asked patients who were diagnosed as VS to imagine moving their hand in some trials, and imagine moving their toe in other trials, which are processes that can be distinguished from each other using EEG. Three VS patients were able to consistently imagine these two behaviours based on commands given by the experimenter over multiple trials. Additionally, Monti et al. (2010) used a paradigm involving motor imagery to allow covertly aware patients to answer yes-no questions by modulating their brain activity, which has now been replicated in two other studies (Fernández-Espejo & Owen, 2013; Naci & Owen, 2013). These patients were able to reliably and correctly respond to questions that had answers that were known by the experimenter (e.g. “are you in the grocery store” versus “are you in the hospital”). Using these techniques, some patients have also been able to answer non-verifiable questions, for example what they preferred to watch on television or if they were in pain (Fernández-Espejo & Owen, 2013). The findings from these neuroimaging studies suggest that some VS patients are being misdiagnosed as unaware because they are unable to indicate their awareness with voluntary motor behaviors.

Tasks used to assess covert awareness with neuroimaging have typically involve some form of motor imagery. These motor imagery paradigms engage complex cognitive processes, and require the patient to encode verbal instructions, produce the mental imagery for a prolonged period of time, and reproduce this imagery multiple times in a session. These tasks entail many aspects of cognitive control, including sustained attention, selection of the appropriate response, comprehension of the task instructions,
and memory of what to do for each trial (Cruse et al., 2011). Given the complexity of these motor imagery tasks, and that these responses can be replicated many times in the same patient, this is definitive evidence that the patients who can complete the tasks are aware.

The discovery of covertly aware patients raises questions as to why these patients are able to imagine motor behaviour, but unable to produce these same movements voluntarily. One possible explanation for these motor deficits would be a disruption in the connections between the thalamus and motor areas, as these connections are important for initiating simple and more complex volitional movement (Magoun, 1949). Moreover, Fernández-Espejo, Rossit, & Owen (2015) conducted a study to investigate the neural underpinnings that lead to these patients having no voluntary control of their motor responses. First, they compared the effective connectivity of healthy participants’ motor networks during motor imagery and motor execution while in the fMRI, and found that motor execution, but not motor imagery, requires an excitatory coupling from the thalamus to the motor cortex. Second, they compared motor network structural connectivity of a covertly aware patient to a non-VS patient with a similar injury etiology. This non-VS patient had a similar clinical history as the VS patient, but had emerged from MCS and was able to follow commands with motor responses. They found that there was a selective disruption of the fibers connecting the motor cortex and thalamus in the covertly aware patient but not in the non-VS patient. This observation suggests that a disruption in connectivity between the thalamus and motor cortex may explain the lack of volitional motor responses in covertly aware patients. This finding indicates that this disruption may be a possible route for rehabilitation. Specifically, if it were possible to enhance the connections between the thalamus and motor cortex, it may be possible to improve these patients’ voluntary motor control, which would allow these patients to respond to their environment behaviourally.

1.3 Brain stimulation

One way to further understand and possibly rehabilitate the motor deficits in covertly aware patients would be to alter the dynamics of the motor system and study the resulting behavioral and neural effects. There are various forms of brain stimulation that influence
or modulate brain activity, such as deep brain stimulation (DBS) and transcranial magnetic stimulation (TMS). DBS involves the surgical implantation of a device that delivers electrical current to a specified brain area, and can be effective at ameliorating motor symptoms in several neurological disorders (Grill, 2005). One drawback of DBS is that it involves neurosurgery to implant the device, thus it is invasive and expensive (Gardner, 2013). In contrast, TMS is a non-invasive brain stimulation technique that involves using magnetic fields to stimulate a cortical area of interest. Specifically, TMS delivers electrical current through a magnetic coil placed on an individual’s head, and this current produces a magnetic field that lasts approximately a millisecond. The magnetic field creates an electrical field that can be large enough to lead to changes in neuronal activity and, with enough current, cause an action potential in the neurons being stimulated (Sandrini, Umilta, & Rusconi, 2011). Repetitive-TMS (r-TMS) can cause decreases or increases in cortical excitability that can lead to plasticity changes in the brain (Hallett, 2007), and has been used in many different clinical populations to improve functioning, such as in Parkinson’s disease (Lefaucheur et al., 2004) and stroke patients (Kim et al., 2006). However, TMS, especially r-TMS, is not without its risks, as r-TMS has a risk of seizures (Hallet, 2007). DoC patients have a very high rate of seizures (Posner, Saper, Schiff, & Plum, 2007; Tresch, Sims, Duthie, Goldstein, & Lane, 1991) and epileptic activity is related to worsening of clinical symptoms (Chen & Wasterlain, 2006; Posner, Saper, Schiff, & Plum, 2007), consequently r-TMS would be too risky to be implement with these patients. While some studies have investigated the effects of DBS and TMS in vegetative state patients (Ragazzoni et al., 2013; Schiff et al., 2007), there are risks and difficulties associated with implementing these procedures with this patient group.

Another non-invasive brain stimulation technique that is gaining popularity in research and clinical worlds is transcranial direct current stimulation (tDCS). tDCS modulates the excitability of target brain areas by using electrodes placed on the scalp. These electrodes deliver a weak electrical current (usually between 0.5 to 2 mA), which can increase or decrease the likelihood of an action potential in the neurons being stimulated (Filmer, Dux, & Mattingley, 2014). tDCS has two types of electrodes: anodal and cathodal. Anodal tDCS, where the anodal electrode is placed on the area of interest,
is considered to be excitatory. Cathodal tDCS, where the cathode electrode is placed on the area of interest, is considered inhibitory. There is also a sham tDCS that mimics the sensations of active tDCS without stimulating the brain. The purpose of the sham tDCS is to control for factors unrelated to the stimulation that may lead to changes in behavior, for example learning and placebo effects (Filmer et al., 2014). The advantages of tDCS allow for it to be a better suited method to use in DoC patients. Specifically, tDCS can be easily transported, is non-invasive, and does not carry a risk of inducing seizures (Poreisz, Boros, Antal, & Paulus, 2007).

1.3.1 Mechanisms of action of tDCS

Anodal tDCS can modulate corticomotor excitability (Nitsche & Paulus, 2000), improve motor performance (Waters-Metenier, Husain, Wiestler, & Diedrichsen, 2014), and improve cognitive functions, including attention, motor learning, and working memory (Fregni et al., 2005; Jacobson, Koslowsky, & Lavidor, 2012; Kang & Paik, 2011). How tDCS is able to influence performance on numerous cognitive and motor tasks is not well understood, although there are some theories on its underlying mechanisms. Nitsche & Paulus (2011) and Nitsche et al. (2008) suggests that tDCS differs from other forms of brain stimulation, for example TMS, because the static fields of tDCS are not large enough to cause depolarization quickly enough to lead to an action potential. Instead, tDCS changes the resting membrane potential of the neurons near the stimulation site, which leads to an increase or decrease in the likelihood of an action potential firing. In support of this theory, low current excitatory stimulation of animal hippocampal slices decreased the threshold needed for these neurons to fire (Bikson et al., 2004). According to this theory, the changes seen during tDCS are thought to be dependent on changes in the permeability of neuronal ion channels, specifically sodium and calcium channels (Nitsche et al., 2003). In animals, anodal direct current stimulation leads to increases in calcium levels in cells (Islam, Aftabuddin, Moriwaki, Hattori, & Hori, 1995). Additionally, when a sodium channel-blocking drug is administered to healthy participants before anodal tDCS, no changes in corticomotor excitability are observed during the stimulation (Liebetanz, Nitsche, Tergau, & Paulus, 2002). Nitsche’s et al. (2008) theory that tDCS modulates the resting membrane potential of neurons suggests
that tDCS will be most effective when it is paired with an action or task that involves the brain area being stimulated, so that an action potential is initiated by that task.

In addition, N-methyl-D-aspartate (NMDA) receptors, which respond to the neurotransmitter glutamate, have been implicated in the learning effects seen after tDCS. Specifically, it is believed that the effect of tDCS on NMDA receptors leads to changes in neuronal plasticity through long-term potentiation and long-term depression (Luft, Pereda, Banissy, & Bhattacharya, 2014; Nitsche et al., 2008). In support of this idea, when drugs that block NMDA receptors are administered before stimulation, tDCS is less effective at modulating MEP amplitude (Nitsche et al., 2003), and a reduction of the long-term effects of tDCS is observed (Liebetanz et al., 2002). Additionally, after anodal stimulation of the motor cortex, increases in myoinositol, a chemical involved in the long-term potentiation second messenger system, are observed in the area being stimulated (Rango et al., 2008). Various other studies have implicated changes in NMDA receptors and/or glutamate as a key mechanism of action in the learning effects observed with tDCS (Clark, Coffman, Trumbo, & Gasparovic, 2011; Hunter et al., 2015).

Other neurotransmitters have also been implicated in the effects of tDCS. For example, Kim, Stephenson, Morris, & Jackson (2014) used fMRI to investigate neurotransmitter changes during a motor task paired with anodal tDCS over the motor cortex. They found a significant reduction in gamma-Aminobutyric acid (GABA) concentrations in the motor cortex during anodal stimulation, and that the concentration of GABA predicted inter-individual differences in motor learning memory on the motor task performed during stimulation. tDCS has also been shown to influence levels of brain-derived neurotropic factors (Fritsch et al., 2010), acetylcholine (Kuo, Grosch, Fregni, Paulus, & Nitsche, 2007), and dopamine (Tanaka et al., 2013).

Another relatively new theory on the mechanisms underlying tDCS suggests that the stimulation may influence glial cells in the area being stimulated. Ruohonen & Karhu (2012) used mathematical modeling to estimate transmembrane potentials in neuron and glial cells during tDCS. They showed that the changes in transmembrane potentials caused by tDCS display many similarities to the changes seen in glial cells, specifically astrocytes, during neuronal activation. This theory suggests that the current typically used
with tDCS, ~2mV, would be enough current to cause glial cells to undergo depolarization, and this would lead to changes in the regulation of neurotransmitter uptake and release. To my knowledge, there is no experimental evidence to support – or detract from – this theory.

1.3.2 tDCS and motor function

The effects of tDCS on motor function have been investigated extensively (Pavlova, Kuo, Nitsche, & Borg, 2014; Saucedo Marquez, Zhang, Swinnen, Meesen, & Wenderoth, 2013; Simonetta-Moreau, 2014; Vines, Cerruti, & Schlaug, 2008). These studies have shown that tDCS can modulate the excitability of the motor cortex (López-Alonso, Fernández-del-Olmo, Costantini, Gonzalez-Henriquez, & Cheeran, 2015; M.A. Nitsche & Paulus, 2000), improve motor learning (Waters-Metenier et al., 2014), and influence brain activity in the motor network (Polanña, Paulus, & Nitsche, 2012), when stimulating the motor cortex. Moreover, tDCS has been shown to improve motor function in stroke patients and in patients with other neurological disorders (Bastani & Jaberzadeh, 2012; Boggio et al., 2007).

**tDCS and corticomotor excitability.** The most consistent and robust finding from the tDCS literature is that tDCS modulates cortical excitability of the motor cortex, as measured by TMS induced motor evoked potentials (Lang, Nitsche, Paulus, Rothwell, & Lemon, 2004; Nitsche & Paulus, 2000). As mentioned previously, TMS is another type of brain stimulation method that delivers magnetic pulses to the brain area being stimulated and, unlike tDCS, these pulses can cause an action potential. When these pulses are delivered to the motor cortex, a motor evoked potential (MEP) can be produced. A MEP is muscle activity that has been caused by a TMS pulse, and MEP amplitude (how large the muscle activity is) is considered to be a measure of excitability in the motor cortex (Hallet, 2000). When anodal tDCS is applied over the motor cortex at rest, an increase in MEP amplitude is observed. When cathodal tDCS is applied over the motor cortex at rest, a decrease in MEP amplitude is observed (Nitsche & Paulus, 2000; Pellicciari, Brignani, & Miniussi, 2013). This change in cortical excitability lasts up to 90 to 120 minutes after the application of 13 minutes of tDCS (Nitsche & Paulus, 2001).
When anodal tDCS is applied daily, cumulative effects of tDCS are observed on MEP amplitude (Alonzo, Brassil, Taylor, Martin, & Loo, 2012). Furthermore, when tDCS over the motor cortex is paired with active movement, MEP amplitude is modulated to a greater degree than when tDCS is applied at rest (Kim & Ko, 2013). For example, Kim & Ko (2013) compared the effects of anodal tDCS and sham tDCS during rest and a grip exercise. They found that pairing anodal tDCS with the grip exercise led to the greatest increase in MEP amplitude after stimulation, whereas anodal tDCS at rest and the grip exercise with sham tDCS led to a similar smaller increase in MEP amplitude. Sham tDCS at rest led to no increase in MEP amplitude. These findings provide evidence that anodal tDCS increases corticomotor excitability when applied to the motor cortex, whether the subject is at rest or engaging in a motor behaviour.

There are some inconsistencies in the literature on the amount and direction of change in MEP amplitude after movements are paired with anodal tDCS over the motor cortex. Miyaguchi and colleagues (2013) investigated the effects of anodal tDCS over the motor cortex on MEP amplitude when the stimulation was paired with a finger abductor-adduction task or applied at rest. They found that anodal tDCS paired with rest led to an increase in MEP amplitude, but the active movements paired with tDCS and without tDCS led to a decrease in MEP amplitude. One possible explanation for these inconsistent findings may be related to effects of different types of movements on MEP amplitude. For example, Bortoletto, Pellicciari, Rodella, & Miniussi (2015) compared the effects of fast and slow thumb movements on MEP amplitude when paired with anodal tDCS over the motor cortex. They found that anodal tDCS paired with slow movements increased MEP amplitude to a greater degree than sham tDCS and anodal tDCS paired with fast movements. They concluded that combining anodal tDCS with another event that increases corticomotor excitability to a large degree (i.e. fast, exhaustive movements) reversed the facilitatory effect of anodal tDCS on MEP amplitude.

More generally, there are some inconsistent findings on the assumption that anodal tDCS enhances cortical excitability and cathodal tDCS diminishes it (Batsikadze, Moliadze, Paulus, Kuo, & Nitsche, 2013; Rosenkranz, Nitsche, Tergau, & Paulus, 2000). Jacobson et al. (2012) suggests that this notion cannot be assumed in all conditions. Even
within studies that have found results consistent with this assumption, large inter-
individual variability on changes in excitability after tDCS have been observed (Nitsche & Paulus, 2000; Strube, Bunse, Malchow, & Hasan, 2015; Wiethoff, Hamada, & Rothwell, 2014), although there is support that the intra-individual variability in response to tDCS is small (Alonzo et al., 2012). Wiethoff and colleagues (2013) observed only half of their participants’ had increased MEP amplitude after anodal tDCS. Despite these findings, there have been numerous studies under many different experimental conditions that support the assumption that anodal tDCS increases excitability and cathodal tDCS decreases it when stimulating the motor cortex (Alonzo et al., 2012; Nitsche & Paulus, 2000).

**tDCS and motor learning.** In addition to modulating corticomotor excitability, tDCS over the motor cortex also influences performance on motor tasks. One theory of anodal tDCS is that it increases long term potentiation, which leads to increased plasticity during a motor learning task (Nitsche et al., 2003). The beneficial effects of tDCS on task performance have been demonstrated in thumb movements (Bortoletto et al., 2015; Koyama, Tanaka, Tanabe, & Sadato, 2015), to more complex motor behaviours, for example sequence learning (Waters-Metenier et al., 2014), and robotic manipulandum (Hunter, Sacco, Nitsche, & Turner, 2009). For instance, Bortoletto et al. (2015) found participants receiving anodal tDCS over the motor cortex showed increased peak acceleration of thumb abduction movements when compared to a control condition. These results have been replicated in similar studies looking at increasing acceleration in a ballistic thumb movement task (Koyama et al., 2015), and improving hand dexterity with tDCS (Pavlova et al., 2014). Christova, Rafolt, & Gallasch (2015) had participants perform the grooved pegboard test, which assesses manual dexterity and coordination, and found that participants completed the task faster when stimulated with anodal tDCS compared with when they were given sham tDCS. In addition, anodal tDCS has also shown to improve swallowing (Zhao et al., 2015), whole body balance (Kaminski et al., 2013), and visuomotor tracking (Goodwill, Reynolds, Daly, & Kidgell, 2013).

A similar effect of tDCS on more complex motor learning tasks has also been demonstrated. With daily repeated stimulation, Waters-Meteneir et al. (2014) found that
anodal tDCS over motor areas improves finger sequence learning. They used stimulation in conjunction with daily training on a sequence learning task, and found that by the end of four days the anodal group was 40% faster in the task than the sham group. Similarly, Reis et al. (2009) had participants train on a sequential visual isometric pinch task for five days in conjunction with anodal or sham tDCS over the motor cortex, and found that the anodal tDCS group showed significantly more training improvements than the sham group. Numerous studies have shown that motor learning, and in particular sequence learning, can be significantly enhanced with anodal tDCS paired with training on the task (Gomes-Osman & Field-Fote, 2013; Saucedo Marquez et al., 2013; Stagg et al., 2011).

**Neural mechanisms underlying the behavioural effect of tDCS.** In addition to changing corticomotor excitability and influencing performance on motor tasks, simultaneous tDCS/fMRI studies have demonstrated that tDCS influences activity in brain networks. There is large variability in the findings from neuroimaging studies with tDCS. For example, Antal, Polania, Schmidt-Samoa, Dechent, & Paulus (2011) found that short periods of anodal tDCS and cathodal tDCS over the motor cortex during finger tapping did not have any effect on activity in the motor cortex or basal ganglia, although anodal tDCS during finger tapping led to reduced activity in supplementary motor cortex in the hemisphere being stimulated. In addition, Amadi, Ilie, Johansen-Berg, & Stagg (2014) investigated changes in connectivity 10 minutes after receiving anodal, cathodal, and sham tDCS separately over the motor cortex and found that although cathodal stimulation increased connectivity in the motor network, anodal tDCS did not produce significant changes in connectivity. In contrast, Baudewig, Nitsche, Paulus, & Frahm (2001) found reduced activation in the premotor cortex and the supplementary motor cortex after cathodal tDCS, and a trend to increased activation in these areas after anodal tDCS. In addition, Zheng, Alsop, & Schlaug (2011) observed an increase in regional cerebral blood flow during and after anodal tDCS. Sehm et al. (2012) observed increased functional connectivity in the motor network during anodal tDCS. Specifically, bihemispheric tDCS (where the anodal and cathodal electrode is positioned on the left and right hemispheres) modulated activation in prefrontal regions and the primary and secondary motor areas. Unihemispheric tDCS (where the active electrode is placed on the
area of interest, and the reference electrode is placed on the forehead) modulated prefrontal, parietal, and cerebellar areas. Furthermore, Polanía, Nitsche, & Paulus (2011) stimulated the motor cortex of participants with anodal tDCS and found there was an increase in functional coupling of the thalamus and the motor cortex during stimulation. While Polanía and colleagues’ (2011) results suggest that it would be theoretically possible to increase the connectivity between the motor cortex and the thalamus in covertly aware patients, the heterogeneity of the neuroimaging results warrants further investigation of the underlying neural mechanisms of tDCS.

**Clinical applications of tDCS.** In support of improving motor function in covertly aware patients, previous studies on neurological patients have shown that motor deficits can be improved with anodal tDCS (Boggio et al., 2007; Bolognini et al., 2011), and these improvements can last past the stimulation period (Angelakis et al., 2014). The effects of tDCS on stroke patients have been widely studied, and many of these studies show positive tDCS effects on motor function. For example, Boggio et al. (2007) investigated the effects of four weeks of tDCS on the motor cortex in chronic subcortical stroke patients with motor deficits. They had three stimulation conditions: sham tDCS, anodal tDCS of the affected hemisphere, and cathodal tDCS of the unaffected hemisphere. They found that participants in both active stimulation conditions showed improvements on motor tasks, and these improvements were greatest in the last two weeks of stimulation. Bolognini and colleagues (2011) investigated the effects of pairing bihemispheric tDCS with constraint movement therapy, a rehabilitation program used to improve motor function, in stroke patients. They found that active tDCS paired with this program led to functional improvements in participants’ motor abilities to a greater degree than when sham tDCS was paired with the program.

TDCS can improve motor functioning in other types of neurological patients with motor dysfunction, including patients with damaged spinal cords and Parkinson’s patients. For example, Murray et al. (2015) demonstrated that tDCS improves corticomotor excitability in patients with spinal cord injuries who had motor deficits in their wrist. Although they observed an increase in MEP amplitude after anodal tDCS but not sham, this increased amplitude did not lead to motor improvements in these patients.
This study involved only two sessions of active tDCS, which may explain why tDCS did not lead to functional motor improvements. Fregni et al. (2006) found that anodal tDCS over the motor cortex increased MEP amplitude and improved motor function, as measured by the Unified Parkinson's Disease Rating Scale, a simple reaction time task and Purdue Pegboard test, in Parkinson’s patients when compared with cathodal stimulation of the motor cortex as well as anodal stimulation of the dorsolateral prefrontal cortex. Kaski, Allum, Bronstein, & Dominguez (2014) paired anodal tDCS with dance therapy in one Parkinson’s patient, and found that when compared to sham, pairing anodal tDCS with tango dancing led to improvements in the patient’s dance ability and the patient’s gait and ability to walk for six minutes. These studies, among many others (Benninger et al., 2010; Hesse et al., 2007; Hummel, 2005), demonstrate that tDCS can improve motor function for many patient types, with different neurological profiles.

In addition, tDCS has also been investigated, with some success, in DoC patients. For example, Thibaut, Bruno, Ledoux, Demertzi, & Laureys (2014) used one session of anodal tDCS over the dorsolateral prefrontal cortex of 30 VS and MCS patients, and showed improvements on the Coma Recovery Scale. Specifically, they found a significant improvement during tDCS in scores on the Coma Recovery Scale in MCS patients, but no improvements were seen in VS patients during tDCS. They also found 2 out of 25 VS patients, and 13 out of 30 MCS patients, showed post-tDCS signs of consciousness. They concluded that anodal tDCS over the dorsolateral prefrontal cortex transiently improved signs of consciousness in some DoC patients.

Similarly, Angelakis et al. (2014) used anodal tDCS to stimulate the dorsolateral prefrontal cortex or the sensorimotor cortex for two weeks in MCS and VS patients, and investigated the effect of tDCS on performance on the Coma Recovery Scale. They found that all MCS patients showed an improvement in their scores, whether the dorsolateral prefrontal cortex or the sensorimotor cortex was stimulated. They did not find any improvement in scores after tDCS in the VS patients. In the patients that improved, these improvements included becoming able to swallow food, localizing noxious stimuli, and withdrawal of limbs to painful stimuli. Most recently, Naro et al. (2016) used oscillating
tDCS over the cerebellum of DoC patients, and saw transient clinical improvements on the CRS-R in MCS but not VS patients.

These three studies support the idea that tDCS can improve functioning in some DoC patients. One reason that only some patients in these studies improved could be that those patients who responded to tDCS had some intact awareness. Because none of these studies specifically examined covertly aware patients, or compared differences in patients who did and did not respond to tDCS, the reason why some patients and not others responded is not clear. Moreover, these studies did not look at motor functions specifically, and a targeted approach on motor function for covertly aware patients may lead to more clinical benefits for these patients.

1.4 Study Objectives.

As summarized above, previous research has shown that tDCS increases corticomotor excitability (Nitsche & Paulus, 2000) and improves motor learning in healthy controls (Waters-Metenier et al., 2014) and neurological patients (Angelakis et al., 2014), and provides evidence that stimulating the motor cortex may influence the functional connectivity between the motor cortex and the thalamus (Polanía, Paulus, & Nitsche, 2011). This suggests that anodal tDCS has the potential to increase the connectivity between these two areas in covertly aware patients. If this were possible, we do not know if this increase in connectivity may lead to improvements in motor execution in these patients. It is also presently unknown if tDCS can improve motor execution, without a sequence learning component.

The long-term goal of this study is to investigate the mechanisms underlying the effect of tDCS in covertly aware patients and study its potential to improve their motor functioning. Before investigating the effects of tDCS in covertly aware patients, it is important to investigate the efficacy of possible paradigms in healthy controls to find a paradigm with the greatest potential to improve motor function in patients. As a starting point, it is important to find a paradigm that improves motor functioning without sequence learning, and that can be implemented in covertly aware patients. Experiment I and II both investigated tDCS paradigms in healthy controls with this aim in mind.
Experiment I investigated the potential for tDCS to improve voluntary motor execution, as opposed to motor sequence learning, in healthy controls. Motor skill learning involves improving the spatial and temporal accuracy in motor tasks with practice, whereas motor control involves the planning and execution of movements. Motor skill learning is a more complex process that originates from the repeated practice of motor control, and leads to more efficient motor control for the task at hand (Willingham, 1998). Although there is strong support that anodal tDCS can improve motor skill learning in the form of sequence learning, whether anodal tDCS can improve voluntary motor control without sequence learning is not clear. The motor deficits covertly aware patients display are so severe that they have minimal to no control over their movements. Given these deficits, we would not expect that covertly aware patients would be able to learn and repeat a sequence or pattern of motor responses. The first step to improving these deficits would be to improve motor control, as opposed to sequence learning.

In addition to not knowing if tDCS can improve motor control without sequence learning, it is not known if training on a motor task during stimulation is necessary to see significant improvements in motor control. Most studies investigating the effects of tDCS on motor function have combined practicing on a motor task with the stimulation (Reis et al., 2009; Waters-Metenier et al., 2014). As mentioned previously, VS patients who have minimal to no motor control would be unable to perform any motor task during the stimulation period. Experiment I investigated the effects of tDCS when participants are at rest versus when they are training on a motor control task. If training were necessary, one way that training would be possible in this patient group would be to passively move them during the stimulation period. Experiment II investigates the effects of anodal tDCS in healthy controls during passive movements on corticomotor excitability, specifically on changes in MEP amplitude.
Chapter 2

2 Experiment I – Investigating the use of transcranial direct current stimulation to improve motor performance with and without training

Many previous tDCS studies have shown performance improvements in motor skill learning, for example on a sequence reaction time task, with brain stimulation (Kang & Paik, 2011; Lefebvre et al., 2012; Reis et al., 2009). However, these studies involved training on the task at hand. Active motor training in vegetative state patients is not possible, as they have minimal to no voluntary control over their motor behaviors. Moreover, we do not know whether DoC patients can learn motor sequences, and motor skill learning involves having the individual engage in a task for long periods of time, which would likely not be possible for DoC patients due to fatigue. The current study investigated if tDCS can improve motor control without sequence learning, and explored if training during stimulation was necessary to see performance improvements.

Previous studies have shown that tDCS can improve accuracy and reaction time in explicit and implicit sequence learning tasks (Kang & Paik, 2011; Waters-Metenier et al., 2014). These tasks involve presenting numbers or symbols to participants, and having participants respond as quickly as possible by pressing a button that corresponds with that number or symbol. These numbers or symbols are presented in a predetermined order with a pattern or sequence, and participants can learn the sequence by practicing on the task (Schwarb & Schumacher, 2012). Since the current study was investigating if tDCS can improve motor control without sequence learning, a similar task was used in Experiment I except the numbers were presented in a random order instead of a predetermined sequence, so that participants would not be learning patterns of motor responses. Instead, participants would have no prior information of what button they will be expected to press until the numbers appeared on the screen. To my knowledge, no previous study has investigated the effect of tDCS on a random ordered sequence reaction time task.

The primary aim of this experiment was to determine if anodal tDCS can improve accuracy and reaction time of this motor reaction time task when compared to sham
tDCS. A secondary aim was to determine if active training is necessary to see improvements, or if tDCS can improve motor control while the participant is at rest. It was hypothesized that participants receiving anodal tDCS will improve on this task more than participants receiving sham tDCS. As well, it was predicted that participants receiving anodal tDCS in the training and no training group would improve on the task, but the improvement would be greater in the training group. This is based on previous research that shows anodal tDCS at rest shows increases in MEP amplitude greater than sham tDCS at rest (Nitsche & Paulus, 2000). However, motor training in combination with tDCS shows greater increases in MEP amplitude and motor performance improvement when compared to tDCS at rest (Kim & Ko, 2013).

2.1 Materials and Methods

2.1.1 Participant Demographics.

Forty-one healthy right-handed participants (25 female, 16 males) between the ages of 18 to 30 (M= 22, SD=2.4) participated in this study. Participants were recruited through posted flyers from the University of Western Ontario campus, and were screened prior to recruitment into study to ensure they did not meet any exclusion criteria. Exclusion criteria included: left-handed or mixed handedness, any neurological or psychiatric problems, implantation of metallic objects in the brain, use of psychoactive medication, active skin problems, unstable medical conditions, susceptible to migraines or other frequent headaches, any history of episodes of faintness, any metal implants or devices, current use of a hearing aid and/or pregnant or trying to become pregnant. Upon arriving for study, participants provided written informed consent. At the end of the study, participants received $10 for their participation. The Health Sciences Research Ethics Board of the University of Western Ontario provided ethical approval for the study. One participant was excluded because the experimenter was unable to reduce the impedance during stimulation due to the thickness of the participants’ hair.

2.1.2 Experimental Design

Participants were randomly assigned to one of four experimental conditions. Participants either received sham or anodal tDCS, and either practiced on a motor task (described
below) during stimulation or were at rest during stimulation. Participants completed the Edinburgh Handedness Questionnaire, and then were given instruction on how to perform the motor task, and performed a 1-minute practice session before beginning the study. Once participants felt comfortable with the task, the anodal and cathodal electrodes were positioned on the scalp. Before starting the stimulation, participants in all conditions performed the task for five minutes to measure baseline performance. Then the stimulation was turned on, and participants either trained on the task for 20 minutes, or watched a 20-minute video. This video was a 20-minute episode of the show “Friends”, which was chosen so participants would be in a relaxed state during the stimulation and to ensure all participants were doing the same thing during the rest period. They were also instructed to not move as much as possible during the video. Once the stimulation was complete, all participants performed the task again for five minutes to measure post-tDCS performance. Finally, participants completed a tDCS perceptual scale (modified from Waters-Metenier et al., 2014) to investigate if there were differences in perception between sham and anodal tDCS.

**Figure 1 Schematic of procedure.** All participants practiced on the task and performed the task before receiving tDCS to get a baseline measure of performance. Then participants received either anodal or sham tDCS, and either trained on the task or were at rest during stimulation. Then all participants performed the task after tDCS to get a post-tDCS measure of performance.
2.1.3 Transcranial direct current stimulation

The Chattanooga Ionto Dual Channel Electrophoresis System was used to deliver stimulation to participants. Carbon rubber electrodes (size: 3.81 cm x 5.72 cm) covered in saline soaked sponges were placed on participants’ heads. The anodal electrode was placed over the left motor cortex (C3) and the cathodal was placed over the right motor cortex (C4). The unit was set to a current for 2 mA for 20 minutes. In the sham tDCS condition, the unit was ramped up for 30 seconds, and then ramped down. In the anodal tDCS condition, participants received a total of 48 coulombs over the 20-minute period. Bihemispheric tDCS was used instead of the traditional unilateral montage because previous studies have demonstrated that this bihemispheric montage improves motor learning to a greater degree than unilateral montages (Kang & Paik, 2011). We speculated that since this montage shows greater performance improvements in motor learning tasks that it may also lead to greater performance gains on the motor control task used in this study.

2.1.4 Experimental Task

The task used for this experiment was modified based on the serial reaction time task. This task involves presenting numbers to participants, and asking participants to respond as quickly as possible by pressing the corresponding key on the keyboard. The numbers in the serial reaction time task are presented in predetermined order or sequence. In the current study, the numbers presented did not have a pattern or sequence, and were instead presented in a pseudorandom order. The task was presented using MATLAB R2013b on a Dell laptop (Windows 7). Participants were presented with random digits (1-4) on a computer screen, and asked to respond with their right hand by pressing the corresponding keys on the computer keyboard as quickly as possible. The ‘f’ key corresponded with ‘1’, the ‘g’ key corresponded with ‘2’, the ‘h’ key corresponded with ‘3’, the ‘j’ key corresponded with ‘4’, and these keys were labeled with their corresponding number. Participants were asked to use their index finger to indicate ‘1’, their middle finger to indicate ‘2’, their ring finger to indicate ‘3’, and their pinky finger to indicate ‘4’. Each number was pseudo randomized to appear the same number of times as the other numbers in each block. This was done to ensure there were no differences
within the blocks in which keys were pressed more or less. A block consisted of a total of 10 trials, where digits were presented for 20 seconds, and each trial had a 5 second break in between. For the participants who trained on the task during stimulation, they completed 4 blocks of 10 trials during the stimulation period. After each block they had a self-selected break of the participants’ choosing, which lasted on approximately 30 seconds.

2.1.5 Data Analysis

To investigate performance improvements, average reaction time (in seconds) and accuracy (correct responses / total responses) were calculated for the baseline and post-test for each participant. A mixed model Analysis of Variance (ANOVA) was conducted for both the training and non-training groups separately. Time point (baseline vs. post-test) was the within subject factor, and tDCS type (anodal vs. sham) was the between subject factor. To investigate if participants receiving sham tDCS differed in their responses on the tDCS perceptual scale from participants receiving anodal tDCS, a chi-square test of association was conducted.

2.2 Results

2.2.1 Performance on the motor control task before and after tDCS

Table 1 displays the means and standard deviations for accuracy and reaction time on the task before and after receiving tDCS for the group of participants who trained on the task. Table 2 displays means and standard deviations for accuracy and reaction time on the task before and after receiving tDCS for the group of participants who were at rest while receiving tDCS.

<table>
<thead>
<tr>
<th></th>
<th>Anodal tDCS</th>
<th></th>
<th>Sham tDCS</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Post Test</td>
<td>Baseline</td>
<td>Post Test</td>
</tr>
<tr>
<td>Accuracy</td>
<td>0.69(.08)</td>
<td>0.64(.17)</td>
<td>0.72(.09)</td>
<td>0.71(.07)</td>
</tr>
<tr>
<td>Reaction Time</td>
<td>0.53(.10)</td>
<td>0.47(.10)</td>
<td>0.60(.12)</td>
<td>0.55(.12)</td>
</tr>
</tbody>
</table>

Table 1 Performance values for training group. Means and standard deviations for baseline and post-test performance on the random sequence reaction time task for training group.
<table>
<thead>
<tr>
<th></th>
<th>Anodal tDCS</th>
<th></th>
<th>Sham tDCS</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Post Test</td>
<td>Baseline</td>
<td>Post Test</td>
</tr>
<tr>
<td>Accuracy</td>
<td>0.75(.06)</td>
<td>0.76(.06)</td>
<td>0.75(.04)</td>
<td>0.71(.07)</td>
</tr>
<tr>
<td>Reaction Time</td>
<td>0.55(.09)</td>
<td>0.54(.09)</td>
<td>0.51(.05)</td>
<td>0.50(.04)</td>
</tr>
</tbody>
</table>

**Table 2 Performance values for rest group.** Means and standard deviations for baseline and post-test performance on the random sequence reaction time task for rest group.

A mixed design ANOVA performed on the training group’s accuracy revealed no significant interaction for the difference between the sham and anodal group’s change in accuracy from baseline to post-test ($F_{(1,18)} = .583, p = .455$), which suggests tDCS did not have an effect on accuracy in this group. In addition, no significant main effect of time (i.e. baseline and post-test) was found for accuracy ($F_{(1,18)} = 1.456, p = .243$), nor was a significant main effect of tDCS type found in accuracy between the sham and anodal group ($F_{(1,18)} = 1.199, p = .288$). A follow up mixed design ANOVA was conducted after removing any participants who performed below chance (accuracy of less than .25), and they confirmed the previous reported results. There was no significant interaction for the difference between the sham and anodal group’s change in accuracy from baseline to post-test ($F_{(1,17)} = .006, p = .940$). Figure 2 displays the change in accuracy from baseline to post test for each participant.

**Figure 2 Accuracy in training group.** Difference in average accuracy from baseline to post-test for each participant in the training group.
A mixed design ANOVA performed on the trained group’s reaction time revealed no significant interaction for the difference between the sham and anodal group’s change in speed from baseline to post test ($F_{(1,18)} = .200, p =.660$), which suggests tDCS did not have an effect on reaction time in this group. However, a main effect of time ($F_{(1,18)} = 7.497, p =.014$) revealed a significant decreased in reaction time between the baseline (M = .562, SD = .10) and post-test (M = .511, SD = .12). No main effect of tDCS type was found ($F_{(1,18)} = 2.871, p =.107$) . A follow up mixed design ANOVA was conducted after removing any participants who performed below chance (accuracy of less than .25), and they confirmed the previous reported results. There was no significant interaction for the difference between the sham and anodal group’s change in reaction time from baseline to post-test ($F_{(1,17)} = .392, p=.540$). Figure 3 displays the change in reaction time from baseline to post-test for each participant.

**Figure 3 Reaction times in training group.** Difference in average reaction time from baseline to post-test for each participant in the training group.

A mixed design ANOVA performed on the rest group’s accuracy revealed no significant interaction between the sham and anodal group’s change in accuracy from baseline to post-test ($F_{(1,18)} = 2.043, p =.170$), which suggests tDCS did not have an effect on accuracy in this group. In addition, no significant main effect of time (i.e. baseline to post-test) was found for change in accuracy ($F_{(1,18)} = .740, p =.401$), nor was a significant main effect of tDCS type found in accuracy between the sham and anodal group ($F_{(1,18)} = .2043, p =.170$).
1.397, p = .253). Figure 4 displays the change in accuracy from baseline to post-test for each participant.

![Graph showing change in accuracy from baseline to post-test for each participant.]

**Figure 4 Accuracy in rest group.** Change in average accuracy from baseline to post-test for participants in the rest group.

A mixed design ANOVA performed on the rest group’s reaction time revealed no significant interaction for the differences between the sham and anodal group’s change in speed from baseline to post-test ($F_{(1,18)} = .677, p = .422$), which suggests tDCS did not have an effect on reaction time in this group. A main effect of time ($F_{(1,18)} = 31.046, p < .000$) revealed a significant decrease in reaction time between baseline ($M = .533, SD = .071$) and post tDCS ($M = .516, SD = .074$). No significant main effect of tDCS type was found between the anodal and sham group ($F_{(1,18)} = 1.850, p = .422$). Figure 5 displays the change in reaction time from baseline to post-test for each participant.
2.2.2 Performance on motor control task during tDCS

As no difference in performance was found from baseline to post-test in the anodal and sham tDCS groups, an exploratory post-hoc analysis of the training group’s performance improvement during the stimulation period was conducted to investigate if online tDCS led to improvement on this task. A mixed model ANOVA revealed no significant interaction for the difference in accuracy from the first training block to the last training block between the sham and anodal groups ($F_{(1,18)} = .000, p = .997$). A mixed model ANOVA revealed no significant interaction for the difference in reaction time from the first training block to the last training block between the sham and anodal groups ($F_{(1,18)} = 3.998, p = .061$).

2.2.3 Perception of tDCS

Table 3 displays participants’ responses to each question about the sensations from tDCS in the tDCS perception questionnaire, which was completed at the end of the experiment. No significant difference in responses on the questionnaire between the sham and anodal group was found. A chi square test of association was performed to determine if there was a difference between the sham and anodal tDCS condition in participants’ belief that they received real versus placebo tDCS, with no difference found in responses between the two conditions ($X^2 (1) = .114, p = .736$) (displayed in figure 6).
Table 3 Perception of tDCS sensations. Mean ratings on a scale from 1 to 10 for anodal and sham tDCS group from the tDCS perception questionnaire. No significant differences were found in responses between anodal and sham conditions.

<table>
<thead>
<tr>
<th>Question</th>
<th>Anodal tDCS</th>
<th>Sham tDCS</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>tDCS intensity level</td>
<td>4.55</td>
<td>4.00</td>
<td>.252</td>
</tr>
<tr>
<td>Distraction due to tDCS</td>
<td>2.15</td>
<td>2.25</td>
<td>.806</td>
</tr>
<tr>
<td>Discomfort level from tDCS</td>
<td>2.95</td>
<td>2.25</td>
<td>.222</td>
</tr>
<tr>
<td>Feeling of tingling from tDCS</td>
<td>4.00</td>
<td>4.20</td>
<td>.795</td>
</tr>
<tr>
<td>Pain from tDCS</td>
<td>2.05</td>
<td>2.10</td>
<td>.947</td>
</tr>
<tr>
<td>Feeling of burning from tDCS</td>
<td>3.18</td>
<td>2.55</td>
<td>.494</td>
</tr>
<tr>
<td>Feeling of itching from tDCS</td>
<td>2.65</td>
<td>2.70</td>
<td>.954</td>
</tr>
<tr>
<td>Feeling of dizziness from tDCS</td>
<td>0.55</td>
<td>0.50</td>
<td>.904</td>
</tr>
</tbody>
</table>

Figure 6 Belief as to whether participants received real tDCS. Number of participants who responded yes versus no as to whether or not they believed they received real tDCS for anodal and sham condition. No significant difference was found in responses between the two groups.

2.3 Discussion

This experiment did not find evidence that anodal tDCS improves reaction time or accuracy on a random sequence reaction time task when compared to sham tDCS. Participants in both tDCS groups did not improve in accuracy from baseline to post test. The results indicate that participants did tend to decrease their speed significantly on the task from baseline to the post-test, whether or not they had trained on the task. This decrease in speed happened whether participants received anodal or sham tDCS, signifying that this improvement in reaction time was unrelated to the tDCS.
In addition, no group differences between the anodal and sham tDCS conditions were found in terms of hours of sleep the night before and perception of tDCS sensations. There was also no significant difference in belief as to whether participants had received sham versus anodal tDCS, which suggests that the sham condition was effective at mimicking the sensations of real tDCS. This finding supports the use of the sham condition as an appropriate placebo condition.

There may be multiple explanations for why anodal tDCS did not improve performance on this task. Firstly, it is possible that multiple sessions of tDCS would be needed. Studies that stimulate participants over a number of days find more robust tDCS effects on performance compared to when tDCS is applied over one day (Alonzo et al., 2012; Reis et al., 2009). Additionally, studies have found that on some motor tasks, tDCS leads to greater improvement when tested on the task the next day compared to immediately after stimulation (Saucedo Marquez et al., 2013). Nevertheless, even if multiple days were required to see significant improvement on this task with tDCS, a pattern of improvement with tDCS, even if not significant, would likely still appear with one session of tDCS. For example, Reis and colleagues (2009) investigated the effects of anodal tDCS on motor skill learning over five days. Although the greatest improvement due to anodal tDCS was observed on the fifth day, there was still improvement within the first day. No pattern of improvement was observed when comparing performance in the anodal and sham tDCS condition for the current study, suggesting that even with multiple days, performance would likely not be improved by tDCS on this task.

Another possibility as to why we did not see a significant effect of tDCS is because of a lack of power to detect an effect, had it been there. We included 10 participants in each condition, which is typical for other tDCS studies that have about 10 to 15 participants in each condition (Alonzo et al., 2012; Baarbé et al., 2014; Gálvez, Alonzo, Martin, & Loo, 2012). In addition, if this were the case, a pattern would likely have emerged from the data despite it not being significant. No discernable pattern emerged from the data when comparing the change in performance in the sham and anodal tDCS (refer to figures 3, 4, 5, & 6). Based on these figures, very few participants showed large changes in their performance, and there was no difference in pattern for this improvement between the sham and anodal groups.
A further potential reason a lack of improvement due to tDCS was observed might be because the measures used to determine performance, accuracy and reaction time. Previous tDCS studies involving tasks that measure reaction time and accuracy often have participants learn a motor sequence. These studies have shown that anodal tDCS enhances the learning of the sequence (Kang & Paik, 2011; Kantak, Mummidisetty, & Stinear, 2012; Waters-Metenier et al., 2014), but it was not clear if tDCS would improve performance when there was no sequence to learn. We chose the task in Experiment I because it shared many similarities to the sequence tasks that are improved by tDCS (Waters-Metenier et al., 2014), but it did not measure sequence learning, and instead measured a rapid motor response to the presentation of a stimuli. The difference in the task used in this experiment was that while participants were making a motor response to a command, they had no information prior to the appearance of the command on the screen as to what finger would be making the response. It was hypothesized that improvements in speed or accuracy would still be shown even when the sequence learning component of the task was removed. As explained previously, DoC patients show very little ability for motor control or active training, which made it important to investigate the effects of tDCS with a simple motor task, instead of a sequence motor task. It is possible that because there was no sequence for participants to learn, reaction time and accuracy were not sensitive to changes due to anodal tDCS.

Due to the lack of information participants had on the expected motor response prior to the presentation of the number, it is possible that participants could not improve significantly on this task. In line with this, it is likely that participants were performing close to their best from the beginning of the experiment, and were not able to improve much further than their starting performance. This is supported by the fact that even when participants trained on the task for 20 minutes, they did not become more accurate on the task, whether they received real tDCS or not. Despite showing a significant improvement in reaction time from pre to post-test in the training group, when collapsed across all groups this difference amounts to a decrease in average speed of approximately 35 milliseconds. This significant, but small, decrease in speed was found in the training group and the rest group, although a greater decrease in reaction time was observed in the training group than in the rest group (51 milliseconds versus 18 milliseconds).
addition, this decrease in reaction time also coincided with a small non-significant decrease in average accuracy for three out of the four conditions. This could reflect participants changing their strategy from baseline to post-test, as opposed to a performance benefit in reaction time. This evidence suggests that the task used may not be a task participants are able to improve very much on, whether or not they received anodal or sham tDCS.

In addition, tDCS as a brain stimulation method has limitations that may have influenced the findings of this study. There is large variability in how individuals respond to tDCS, with some individuals showing no effects on corticomotor excitability after tDCS and even some individuals showing effects opposite to the predicted direction of corticomotor excitability (Chew, Ho, & Loo, 2015; Horvath, Carter, & Forte, 2014). It is not, however, likely that inter-subject variability entirely explains these results, as a visual inspection of the individual data shows that apart from one outlier whose reaction time and accuracy both decreased substantially from baseline to post-test, most participants’ scores did not change notably.

In order to understand why participants did not improve on this task, a follow up study comparing performance on a reaction time task that involves both random appearances of numbers and sequences of numbers that have a pattern with them would be needed. However, since it is not expected that covertly aware patients will be able to train on new motor behaviours (or sequences), improvements in accuracy and speed on this motor sequence task may not be applicable to patients. For instance, causing a patient to voluntarily move a finger is qualitatively different than having healthy participants become faster at producing sequences with their fingers. As such, improvements on a motor sequence task would be getting further away from the aim of the current project to improve motor function in covertly aware patients.

The implications of this experiment on patients are difficult to derive because no effect of tDCS was found. This does not indicate that tDCS will not improve motor function in covertly aware patients, but may instead reflect a limitation in the paradigm used to measure motor improvement. As previously mentioned, it is imperative to find a paradigm that is most likely to lead to improvements in covertly aware patients before investigating tDCS in these patients. To do this, multiple paradigms may need to be
investigated in healthy controls before finding the tDCS paradigm that has the greatest chance at leading to improvements in covertly aware patients.
Chapter 3

3 Experiment II – Investigating the use of transcranial direct current stimulation to improve corticomotor excitability during passive training

Experiment II measured the effects of tDCS paired with passive movements on corticomotor excitability. Specifically, Experiment II measured changes in MEP amplitude before and after tDCS. MEP amplitude is a more direct measure of changes in corticomotor excitability (Hallett, 2007). The most reliable effect of tDCS is a modulation of corticomotor excitability, as measured by TMS (Alonzo et al., 2012; Jacobson et al., 2012; Miyaguchi et al., 2013; Nitsche & Paulus, 2001; M.A. Nitsche & Paulus, 2000). When a TMS pulse is delivered to the motor cortex with enough intensity, a muscle activity in the body part represented in the brain area being stimulated is produced. The electrical activity produced in this muscle is called a motor evoked potential (MEP), and this activity can be measured using electromyography (EMG). The amplitude, or how large the electrical activity being produce is, of a MEP is used as a measure of corticomotor excitability (Hallett, 2007). Corticomotor excitability may be a better measure of improvement in motor function in these patients, as this is a measure used as a prognostic tool for motor rehabilitation in stroke patients (Rapisarda, Bastings, Maertens de Noordhout, Pennisi, & Delwaide, 1996). In addition, it may measure motor improvement more directly. For example, an increase in MEP amplitude is observed immediately before, and during a movement (Zaaroor, Pratt, & Starr, 2003). Interestingly, improvements in motor performance coincide with increases in MEP amplitude (Muellbacher, Ziemann, Boroojerdi, Cohen, & Hallett, 2001). These findings support the use of MEP amplitude to investigate the potential applications of tDCS for patients.

Although an aim of Experiment I was to determine if motor training was necessary to see improvement with anodal tDCS, we did not determine if training is important to elicit improvements due to tDCS because no improvement due to anodal tDCS was observed for either the training or the rest group. Previous research shows MEP amplitude is increased to a greater degree when tDCS is paired with non-exhaustive active movement than when tDCS is applied at rest (Bortoletto et al., 2015). As
previously discussed, covertly aware patients have minimal to no voluntary movement. To increase the likelihood of observing motor improvements if they are possible, it is important that the paradigm most likely to elicit improvements be used. Active training, which involves a participant producing voluntary movements, is not possible with this patient group. One possible way around this would be to pair tDCS with passive movements, which would involve an exogenous source moving the participant without any voluntary movements on the participants’ part. Most vegetative state patients receive some form of range of motion physiotherapy (Wheatley-Smith et al., 2012), where a family member or therapist would move different body parts of the patient to prevent rigidity and muscle atrophy. If passive movements increased covertly aware patents’ MEP amplitude more than when they are at rest, this type of motion therapy could be easily paired with tDCS to increase the effects of tDCS in these patients. Experiment II measured MEP amplitude, as opposed to behavioural performance, which allowed for the investigation of the effects of anodal tDCS with passive movements.

We theorize that passive movements paired with tDCS may have a similar effect on corticomotor excitability as active movements paired with tDCS. Previous research has shown that passive movements, where the participant is externally moved while they remain relaxed, lead to activation in similar motor areas as active movements (Alary et al., 1998; Lotze, Braun, Birbaumer, Anders, & Cohen, 2003), although this activation is weaker (Estévez et al., 2014; Wu et al., 2011). Passive movements, like active movements, also modulate MEP amplitude; passive muscle shortening leads to an increase in amplitude, and passive muscle lengthening leads to a reduction in amplitude (Lewis & Byblow, 2002). In addition, passive and active movements show similarities in MEP amplitude modulation, although active movements show a greater increase in amplitude during muscle shortening compared to passive movements (Chye, Nosaka, Murray, Edwards, & Thickbroom, 2010).

In support of using passive movements as a rehabilitation tool for motor deficits, Lindberg, Schmitz, Forssberg, Engardt, & Borg, (2004) found that passive motor training daily for four weeks helped stroke patients improve their upper limb movements. They theorized that these patients had incorporated the use of enhanced somatosensory input,
which led to motor improvements. They supported this theory with evidence from fMRI suggesting that these patients had cortical reorganization in the sensorimotor areas, and this reorganization was associated with improvements in motor functioning. This study indicates that passive movement training can lead to improvement in motor deficits, and if passive movement training is paired with anodal tDCS, even greater improvements may be possible.

The aim of Experiment II was to investigate if anodal tDCS paired with passive thumb movements would increase MEP amplitude as compared to sham tDCS. A similar design was employed for this experiment as for Experiment I; participants were randomly assigned to receive sham or anodal tDCS. MEPs were measured before and after tDCS to measure corticomotor excitability. It was hypothesized that anodal tDCS paired with passive movements will lead to a greater increase in MEP amplitude when compared to the sham condition.

3.1 Materials and Methods

3.1.1 Participant Demographics

Thirty-three healthy right-handed participants (21 female, 12 males) between the ages of 18 to 30 (M= 22.6, SD=3.21) participated in this study. Participants were recruited through posted flyers from the University of Western Ontario campus, and were screened prior to recruitment into study to insure they did not meet any exclusion criteria. Exclusion criteria included: left-handed or mixed handedness, any neurological or psychiatric problems, implantation of metallic objects in the brain, use of psychoactive medication, active skin problems, unstable medical conditions, susceptible to migraines or other frequent headaches, any history of episodes of faintness, any metal implants or devices, current use of a hearing aid and/or pregnant or trying to become pregnant. Upon arriving for study, participants provided written informed consent. At the end of the study, participants received $10 per hour for their participation. The Health Sciences Research Ethics Board of the University of Western Ontario provided ethical approval for the study. Nine participants were excluded; three participants stopped participation because they were not comfortable with tDCS, one participant stopped participation
because they felt faint after TMS, and five participants were excluded due to the equipment not working. The final sample included 24 healthy right-handed participants (15 females, 9 males) between the ages of 18 to 29 (M=23.1, SD=3.04).

3.1.2 Experimental Design

Participants were randomly assigned to receive sham or anodal tDCS. Participants first completed the Edinburgh Handedness Questionnaire. Surface EMG electrodes were placed on the right first dorsal interosseous (FDI) muscle in a belly-tendon montage, a reference electrode was placed on the ulna bone, and the Grass QP511 Quad AC amplifier system was used to pick up the signal. MEPs were recorded after stimulation by single-pulse TMS (Magstim 200 magnetic stimulator, Magstim, Whiteland, Dyfed, UK). First, the optimal spot for the FDI was marked on the participants scalp with a felt pen. Then, the resting motor threshold (RMT), defined as the minimum intensity needed to evoke an MEP of >50 µV in 5 out of 10 trials while the participant had their hand relaxed, was found for each participant. Once RMT was determined, the intensity was set at 120% of resting motor threshold for each participant, and 20 trials were recorded with this intensity. There was a five second delay between each TMS pulse for the 20 trials. Once 20 MEPs were recorded with this intensity, the anodal and cathodal electrodes were positioned on the scalp and the stimulation was turned on. Participants were instructed to not move as much as possible, and to not assist the experimenter with the movements of their thumb. During the 20-minute stimulation period, participants’ right thumb was passively moved in an abduction-adduction motion by the experimenter. This movement paired with anodal tDCS has previously show increases in MEP amplitude as compared to rest (Bortoletto et al., 2015). The experimenter wore headphones which made a beep each time they were to move the participant. Once the stimulation was complete, the TMS coil was positioned on the scalp in the same spot as before the tDCS, using the felt pen markings as guides. Twenty more MEPs were recorded with the 120% RMT intensity for each participant. Finally, participants completed a tDCS perceptual scale (modified from Waters-Metenier et al., 2014) to investigate if there were differences in perception between sham and anodal tDCS.
3.1.3 Transcranial direct current stimulation

The Chattanooga Ionto Dual Channel Electrophoresis System was used to delivered stimulation to participants. Carbon rubber electrodes (size: 3.81 cm x 5.71 cm) covered in saline soaked sponges were placed on participants’ heads. The anodal electrode was placed over the left motor cortex (C3) and the cathodal electrode was placed on the forehead contralaterally, above the orbit. The unit was set to a current for 2 mA for 20 minutes. In the sham tDCS condition, the unit was ramped up for 30 seconds, and then ramped down. In the anodal tDCS condition, participants received a total of 48 coulombs over the 20 minutes. Unihemispheric tDCS was used for this experiment because previous studies have suggested that, although bihemispheric tDCS leads to better outcomes for motor learning, it is less robust at modulating MEPs compared to unihemisphere tDCS (Mordillo-Mateos et al., 2012).

3.1.4 Experimental Task

During the stimulation period, participants were instructed that the experimenter would passively move their thumb and they were asked not to assist with the movement. To indicate when the experimenter should move the participant, beeps were presented using MATLAB R2013b on an Apple laptop (OS X Yosemite) while the experimenter wore headphones to ensure the participant was unable to hear the beeps and anticipate the movement. The beeps were presented at an average of every four seconds, but ranged between three and five seconds, to prevent participants’ from being able to predict when the movement would occur. During the 20 minutes, there were six blocks that included 160 seconds of movements and 40 seconds of rest which lead to each participant being moved a total of 240 times.

3.1.5 Data Analysis

To investigate changes in MEP amplitude, each participant’s average peak-to-peak MEP amplitude was calculated for pre and post tDCS using MATLAB R2013b. A post to pre-MEP ratio (post/pre mean amplitude in mV) was calculated for each participant, to standardize each participant’s MEP amplitude. A ratio of 1 indicated no change in MEP amplitude, a ratio that was greater than one indicated an increase in MEP amplitude, and
a ratio that was less than one indicated a decrease in MEP amplitude. MEP amplitude post to pre ratio has been used in previous studies (Cantarero, Tang, O’Malley, Salas, & Celnik, 2013). This ratio was used as a way to standardize the amplitude values, as there is large inter-subject variability in MEP amplitude (Wassermann, 2002). An independent t-test was used to investigate if there were differences in the MEP ratio for the sham and anodal tDCS groups. It was not possible to use a chi-square test to determine if participants receiving sham tDCS differed in their responses to whether they received real or placebo tDCS because less than five participants indicated that they believed they received placebo tDCS in both the anodal and sham group, so a Fischer’s exact test was conducted.

3.1.6 Follow up experiment

As a control experiment, ten participants (7 female, 3 male) with an average age of 23.7 (SD=4.1) were tested using the same procedure but instead of passively being moved during tDCS they were at rest. Previous studies have shown that this condition reliably leads to anodal tDCS increasing MEP amplitude (Bortoletto et al., 2015; Nitsche & Paulus, 2001).

3.2 Results

3.2.1 MEP amplitude change

Table 4 displays the mean amplitude for pre and post tDCS MEP amplitude as well as the post/pre ratio for the sham and anodal tDCS condition. Figure 7 displays the raw MEP amplitude for each participant from baseline to post-tDCS. An independent t-test revealed no significant difference between the sham and anodal tDCS groups in MEP ratio (T_{22} = -.444, p = .662). Figure 8 displays the MEP ratio boxplot for the sham and anodal tDCS group, with the raw ratios plotted for each participant.
<table>
<thead>
<tr>
<th></th>
<th>Anodal tDCS</th>
<th></th>
<th>Sham tDCS</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Post Test</td>
<td>Ratio</td>
<td>Baseline</td>
</tr>
<tr>
<td>MEP amplitude</td>
<td>3.86(2.84)</td>
<td>4.09(3.32)</td>
<td>1.08(.61)</td>
<td>3.21(2.59)</td>
</tr>
</tbody>
</table>

Table 4 Group averaged MEP amplitude for the passive condition. MEP amplitude means, post/pre ratio, and standard deviations for anodal tDCS and sham tDCS group.

Figure 7 MEP amplitude for each passive group participant. MEP amplitude for pre and post tDCS for each participant.

Figure 8 MEP ratio boxplot for the passive group. MEP amplitude ratio displayed for each participant in the anodal and sham tDCS groups. No significant difference was found in MEP ratios between the anodal and sham tDCS group.
3.2.2 Perceptions of tDCS

Table 5 shows participants’ responses to each question about the sensations from tDCS in the tDCS perception questionnaire, which was completed at the end of the experiment. Figure 9 displays the number of participants who responded yes and no to whether they believed they had received real or placebo tDCS. The Fischer’s exact test revealed no significant difference in responses to this question for sham and anodal group (p=.217).

<table>
<thead>
<tr>
<th>Question</th>
<th>Anodal tDCS</th>
<th>Sham tDCS</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>tDCS intensity level</td>
<td>4.58</td>
<td>3.25</td>
<td>.087</td>
</tr>
<tr>
<td>Distraction due to tDCS</td>
<td>2.25</td>
<td>1.25</td>
<td>.127</td>
</tr>
<tr>
<td>Discomfort level from tDCS</td>
<td>2.58</td>
<td>1.92</td>
<td>.401</td>
</tr>
<tr>
<td>Feeling of tingling from tDCS</td>
<td>3.67</td>
<td>3.27</td>
<td>.698</td>
</tr>
<tr>
<td>Pain from tDCS</td>
<td>1.08</td>
<td>1.55</td>
<td>.580</td>
</tr>
<tr>
<td>Feeling of burning from tDCS</td>
<td>2.50</td>
<td>2.77</td>
<td>.812</td>
</tr>
<tr>
<td>Feeling of itching from tDCS</td>
<td>1.75</td>
<td>1.82</td>
<td>.950</td>
</tr>
<tr>
<td>Feeling of dizziness from tDCS</td>
<td>0.17</td>
<td>0.91</td>
<td>.192</td>
</tr>
</tbody>
</table>

Table 5 Perception of tDCS for sham and anodal groups. Mean ratings from 1 to 10 for anodal and sham tDCS group from tDCS perception questionnaire. No significant differences were found in responses between the two groups.

Figure 9 Belief as to whether participants received real tDCS. Number of participants who responded yes versus no to whether or not they believed they received real tDCS for anodal and sham condition. No significant difference was found in responses between the two groups.
3.2.3 MEP amplitude in rest group

To investigate further, 10 participants were run with the same paradigm but were at rest during tDCS. Table 7 displays the means, standard deviations and post/pre ratio for the MEP amplitude from this experiment. An independent t-test was conducted on the rest group, and revealed no significant difference between the sham and anodal group in MEP ratio \(T_{(8)} = -0.364, p = .725\). The raw MEP ratio for each participant is displayed in figure 11.

<table>
<thead>
<tr>
<th></th>
<th>Anodal tDCS</th>
<th>Sham tDCS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Post Test</td>
</tr>
<tr>
<td>MEP amplitude</td>
<td>4.01(3.29)</td>
<td>3.21(1.97)</td>
</tr>
</tbody>
</table>

Table 6 Group averaged MEP amplitude for the rest condition. MEP amplitude means, post/pre ratio, and standard deviations for anodal tDCS and sham tDCS group for the rest condition.

Figure 10 MEP amplitude for each rest group participant. MEP amplitude from baseline to post-tDCS for each participant.
3.3 Discussion

This experiment did not find a significant difference between MEP amplitude in the sham and anodal groups when participants were passively moved. Both the sham and anodal tDCS groups had a MEP ratio that was slightly larger than 1, but there were no significant differences between the two groups. This suggests that passive movements paired with anodal tDCS may not be an effective method at increasing corticomotor excitability.

Only one study that we are aware of has looked at the effect of passive movements when paired with tDCS. Miyaguchi et al. (2013), using a bihemispheric tDCS montage on nine participants, showed that when anodal tDCS was paired with fast passive finger movements, MEP amplitude stayed the same pre and post tDCS. This study also found that active exhaustive movements paired with tDCS and passive movements without any stimulation decreased MEP amplitude. This study used the same frequency at which movements were initiated for the passive and active movements, and as previously discussed, exhaustive movements when paired with tDCS decrease MEP amplitude whereas non-exhaustive movements increase MEP amplitude. One possible reason they did not find passive movements paired with anodal tDCS modulated MEP amplitude could be because of the interaction between fast passive movements and tDCS.
Previous research has shown that having a task during tDCS can sometimes interfere with the effects of tDCS, depending on the nature of the task (Horvath et al., 2014). For example, Quartarone et al. (2004) found that when participants were asked to imagine motor behaviours during tDCS, the excitatory effects of anodal tDCS on MEP amplitude disappeared. It may be that by pairing tDCS with the passive movements initiated at the same pace as exhaustive active movements, Miyaguchi et al. (2013) counteracted the typical excitatory response from anodal tDCS. Given fast active movements show a different pattern of MEP amplitude modulation than slow active movements, it was hypothesized that a similar pattern would be observed with fast versus slow passive movements.

The current experiment investigated the effect of slow passive movements on MEP amplitude when anodal tDCS is applied over the motor cortex. No significant difference was found between the sham and anodal conditions, and the average MEP post to pre ratio for the anodal group was very close to one (ratio = 1.08), suggesting very little change from pre to post. Despite the change in frequency at which passive movements were initiated, we found a similar pattern of results with passive movements and anodal tDCS as Miyaguchi et al. (2013), which supports the possibility that anodal tDCS paired with passive movements lead to no change in MEP amplitude.

To help elucidate these findings, a follow up experiment was conducted with participants at rest during the stimulation. An abundance of previous research has shown that anodal tDCS at rest increases MEP amplitude (Batsikadze et al., 2013; Nitsche et al., 2007), so if we were unable to replicate this finding, this may suggest a problem with the experimental design. This experiment showed no significant difference in MEP amplitude ratio between the sham and anodal group. For this condition, only ten participants were recruited, which was likely too few participants to find a significant effect had it been there. We stopped collecting data at ten participants because it was believed that since a pattern that was expected based on previous literature was not emerging from the data, there may be a problem with the study procedures. Instead of continuing to collect more data using the same procedures, it was decided to conduct a follow up study using a new study procedure. The anodal tDCS group was highly
variable in their change in MEP after tDCS, whereas the sham tDCS condition’s MEP post to pre ratio had much less variability and all participants were close to 1, showing very little change in MEP amplitude from pre to post tDCS. An opposite pattern was observed for the passive condition, where the sham group had a highly variable MEP ratio and the anodal condition had less variability. There may be multiple reasons why these patterns could have emerged.

First, there is large inter-subject variability in how individuals respond to tDCS (Horvath et al., 2014). Despite replicating the finding that passive movements paired with anodal tDCS led to little change in MEP amplitude, the rest conditions of the current study were not consistent with previous findings. The rest condition did not show significant differences in changes in MEP amplitude between the sham and anodal conditions. In the anodal tDCS condition, one participant out of five had a MEP ratio greater than one (e.g. increase in MEP amplitude), whereas the two participants had a MEP ratio less than one (e.g. decrease in MEP amplitude), and two other two participants had a ratio very close to one (e.g. no change in MEP amplitude). The large variability in how participants responded to anodal tDCS in the rest condition, along with a small sample size, has made in difficult to compare the current study to other tDCS studies.

Second, there may have been confounding factors in the experimental design that influenced the findings. We conducted the rest condition to determine if the findings from the passive movement experiment were reliable. Anodal tDCS increasing MEP amplitude in the literature is a robust finding that has been replicated multiple times (Batsikadze et al., 2013; Nitsche & Paulus, 2000). If we did not find this effect it would suggest there might be an issue with the experimental procedure being used. One limitation of this study was that a neuronavigation system was not in use to place the TMS coil. Although we made every effort to ensure the position and angle of the coil was kept constant between pre and post, a manual coil placement is not as accurate as neuronavigation and, thus, subtle differences in positioning may have affected the results. A neuronavigation system works by using a structural MRI scan of the participant’s brain to mark the exact location for the placement of the TMS coil, and allows the coil to be placed in the same spot on the participants head each time TMS is used (Herwig, Padberg, Unger, Spitzer, &
Instead, for the current experiment, an outline of the coil was marked on the participants' scalp, and the experimenter placed the coil based on these markings as precisely as possible. This was done because the neuronavigation system was not readily available for this experiment. In addition, various previous studies looking the effects of anodal tDCS on MEP amplitude did not use a neuronavigation system, and still found effects of tDCS on MEP amplitude (Gálvez et al., 2012; Nitsche & Paulus, 2001). One possible explanation for the large variability in MEP ratios observed in some of the conditions may be related to error due to the placement of the coil. Specifically, it is possible that there were small deviations in the placement of the coil from pre to post for some participants, and this may have affected MEP amplitude from pre to post, irrespective of the tDCS.

Third, we also do not know if there may be a cumulative effect of pairing anodal tDCS with passive motor training over a number of stimulation sessions that we may be missing by only investigating the effects of one session of tDCS. For example, Alonzo and colleagues (2012) observed that daily anodal tDCS leads to a cumulative effect on MEP amplitude, such that participants' changes MEP amplitude pre to post tDCS was larger on the fifth day of stimulation when compared to the first day of stimulation. Moreover, participants' MEP amplitude pre-tDCS was larger on the fifth day compared to the first day. This suggests there is some long-term and cumulative effect of anodal tDCS that we did not tap into by only measuring MEP amplitude immediately after the stimulation period and only having one stimulation session.

If similar results as the current study were found in a follow up study using a neuronavigation system and a similar sample size for both the passive and rest conditions, this would clarify the effect of passive movements and tDCS on MEP amplitude. Specifically, it would suggest that we do not have evidence that passive movements paired with anodal tDCS modulate MEP amplitude, even when the passive movement is presented at a frequency that has been shown to increase MEP amplitude with active movements. In the follow up study, an equal number of participants would be in the rest and passive conditions, which would allow a further comparison between the rest and passive anodal tDCS conditions. If there were no significant difference between these
two conditions, it would suggest that passive movements may not be an effective method of enhancing the effects of tDCS over the motor cortex, and may not be a valuable paradigm to be investigated in covertly aware patients.

In addition to the implications this follow up study with a neuronavigation system would have for covertly aware patients, it may also have implications for passive movements in general. It would be interesting to compare the passive and rest conditions with sham tDCS. With a follow up study, the rest + sham condition could have a large enough sample size to compare across the two groups to determine if there is a difference on MEP modulation with passive movements versus rest. This would provide evidence as to whether passive movements over an extended period of time increase or decrease MEP amplitude when compared to being at rest.

Taken altogether, the current experiment suggests passive training paired with anodal tDCS may not be the right avenue to improve motor function in covertly aware patients, although further investigation is needed to determine if these findings are related to a limitation in the study design or caused by the experimental manipulations (i.e. passive movements and tDCS). If these findings do hold up in further experiments where the neuronavigation system is used and the effects of multiple days of tDCS are investigated, this may suggest that anodal tDCS may not improve functioning in covertly aware patients.
Chapter 4

4 General Discussion

Experiment I and Experiment II bring us closer to finding an appropriate paradigm for investigating the effects of anodal tDCS in covertly aware patients to improve motor functions. These covertly aware patients have minimal to no voluntary motor control, despite having relatively intact awareness of their environment (Fernández-Espejo & Owen, 2013; Owen et al., 2006). A selective structural disruption between the thalamus and motor cortex is implicated for these motor deficits (Fernández-Espejo, Rossit, & Owen, 2015). We theorized that anodal tDCS may enhance the functional connectivity between the thalamus and motor cortex in these patients, based on previous findings showing that anodal tDCS can enhance these connections in healthy controls (Polánia, Paulus, & Nitsche, 2012). Before investigating the effects of tDCS in covertly aware patients, it was important to find a paradigm that would be most likely to elicit improvements in motor control. This would ensure that when we implement a paradigm with these patients, it will be a paradigm that is most likely to lead to improvements of their motor control deficits.

This project investigated two possible avenues for improving motor control with tDCS, by comparing the efficacy of tDCS with participants at rest versus actively training on a motor task, and investigating the effects of passive movements on corticomotor excitability. Experiment I, which looked at the effects of anodal tDCS on a motor reaction time task with and without training, found no evidence of an effect of anodal tDCS on performance, whether or not participants trained on the task during the stimulation. Experiment II, which looked at the effects of anodal tDCS paired with passive movements on corticomotor excitability, found no evidence of a difference in corticomotor excitability, as measured by MEP amplitude, between the sham and anodal conditions. The results from these two experiments are difficult to interpret in terms of their implications for covertly aware patients.
Caution must be taken when interpreting null results. In both experiment I and II, no significant effects from tDCS were found, which may support the null hypothesis that there is no effect of tDCS on the dependent measures (task performance for experiment I and MEP amplitude for experiment II). The problem in interpreting null results is that there may be many other alternative explanations as to why no significant difference between the two tDCS groups was found. It is possible that there was an error in the way the data was collected, or with the equipment used in the experiments. Without multiple replications of these two experiments confirming the null results, it is not possible to know if these issues led to the null results. Furthermore, even if multiple replications confirm these null findings, interpreting why these results are null is difficult. Kluger & Tikochinsky, (2001) explain that null findings could be the result of three possibilities: 1) that the theory behind the experimental hypothesis was wrong, 2) that the experimental hypothesis was poorly operationalized (e.g. that the hypothesis does not capture the theory behind it), or 3) that the generalization from the theory to the experimental hypothesis was not specified properly (e.g. such that neither the hypothesis nor the null hypothesis is true). Because of this, they say it is only possible to accept the null hypothesis at the operationalized level, but that until all alternative interpretations have been rigorously investigated, scientists should refrain from the accepting the abstract or theoretical null hypothesis. Experiment I did not find evidence of an effect of anodal tDCS on performance of a motor reaction time task. Experiment II did not find evidence of an effect of anodal tDCS and passive movements on MEP amplitude.

Overall, further investigation is necessary to understand if and how anodal tDCS can improve motor control in covertly aware patients. Experiment I did not find evidence that one session of anodal tDCS can improve behavioural measures of performance on a random sequence reaction time task in healthy young adults. Experiment II suggests did not find evidence that one session of anodal tDCS paired with passive movements leads to an increase in MEP amplitude as compared to sham tDCS paired with passive movements. We do not have a conclusive answer as to what patients should be doing during the stimulation session (e.g. at rest versus passively moved). We also do not know if multiple sessions of tDCS are necessary to see improvements in motor control in these patients. Furthermore, we do not know if improving the functional connectivity of the
thalamus and motor cortex in these patients is possible with tDCS, and importantly if this increase in connectivity will lead to improvements in behavioural output. Further experiments assessing the potential of anodal tDCS to improve motor function in covertly aware patients would allow for a better understanding of these currently unanswered questions. Experiment I and II have helped in designing a more robust way to investigate this, by highlighting potential confounds with anodal tDCS and motor control.
References


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http://doi.org/10.3109/09638288.2012.720355


http://doi.org/10.1016/S0987-7053(03)00029-7

Appendix

Questionnaires

Date: ________________  Participant #: ______

tDCS Perceptual Scale

We would like to know how you experienced the task and tDCS. You may have received real tDCS or a placebo version that mimics the sensations evoked by real tDCS.

1) Please rate the difficulty level of the task from 0-10, where:

0= Extremely easy
1 =
2 = Very Easy
3 =
4 = Easy
5 =
6 = Challenging
7 =
8= Very challenging
9=
10= Extremely challenging

2) Please rate your attention level (how focused you were on the task) from 0-10, where:

0= Extremely unfocused
1 =
2 = Very unfocused
3 =
4 = Unfocused
5 =
6 = Focused
7 =
8= Very focused
9=
10= Extremely focused

3) Please rate the fatigue level of your hand from 0-10, where:

0= No fatigue (no pain, no cramping, no tiredness of muscles)
1 =
2 = Very little fatigue (no cramping, slight tiredness of muscles)
3 =
4 = Little fatigue (slight cramping, tiredness of muscles)
5 =
6 = Fatigue (cramping, tiredness of muscles, slight pain)
7 =
8= High fatigue (cramping, pain, hand use difficult)
9=
10= Very high fatigue (cramping, pain, hand use very difficult)
Date: ____________________  Participant #: _______

4) Please rate the overall intensity level of tDCS (how strong it felt to you) from 0-10, where:

0 = Extremely weak (could not feel/detect anything)
1 =
2 = Very weak
3 =
4 = Weak
5 =
6 = Intense
7 =
8 = Very intense
9 =
10 = Extremely intense (intolerable)

5) Please rate the distraction level due to tDCS from 0-10, where:

0 = Not distracted at all due to tDCS
1 =
2 = Minimally distracted due to tDCS
3 =
4 = Somewhat distracted due to tDCS
5 =
6 = Distracted due to tDCS
7 =
8 = Very distracted due to tDCS
9 =
10 = Extremely distracted due to tDCS

6) Please rate your level of discomfort due to tDCS from 0-10, where:

0 = No discomfort
1 =
2 = Very little discomfort
3 =
4 = Little discomfort
5 =
6 = Discomfort
7 =
8 = High discomfort
9 =
10 = Very high discomfort
Date: _______________  Participant #: ________

7) tDCS can be associated with side effects, such as itching and burning. The most common side effects are listed in the table below. For these side effects, please write the intensity to which you experienced that side effect in the table below from 0-10 and specify how long this side effect lasted in minutes. Please use a fraction if the effect lasted less than 1 minute (e.g. write '0.5' to specify 30 seconds):

0= Extremely weak (could not feel/detect side effect)
1 =
2 = Very weak
3 =
4 = Weak
5 =
6 = Intense
7 =
8= Very intense
9 =
10= Extremely intense (intolerable)

<table>
<thead>
<tr>
<th>Side effect</th>
<th>Rating</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tingling</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pain</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Burning</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Itching</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dizziness</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mental Fatigue</td>
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</tbody>
</table>

8) Did you receive REAL tDCS? If you believe that you received real tDCS, please select 'YES'. If you believe that you did not receive real tDCS (i.e. received the placebo version) please select 'NO'.

YES
NO

9) How certain are you that your response to the question above is correct? (Please rate from 0-10, as below)

0= I am completely uncertain.
1 =
2 = I am very uncertain.
3 =
4 = I am uncertain.
5 =
6 = I am certain.
7 =
8= I am very certain.
9 =
10= I am completely certain.
10) Regardless of whether you thought that you received real tDCS or not, what effect do you expect that real tDCS would have on performance in this task? (Please rate from 0-10, as below)

0 = tDCS will severely hurt performance
1 =
2 = tDCS will hurt performance
3 =
4 = tDCS will slightly hurt performance
5 = tDCS will have no effect on performance
6 = tDCS will slightly benefit performance
7 =
8 = tDCS will benefit performance
9 =
10 = tDCS will highly benefit performance

11) Have you ever had brain stimulation (including transcranial magnetic stimulation and/or transcranial direct current stimulation) before today? Circle one. If yes, please report how long ago it was and what kind of brain stimulation it was.

Yes  No

12) How many hours of sleep did you have last night? _________
**Edinburgh Handedness Inventory**¹

Participant #: __________

Please indicate with a check ( ) your preference in using your left or right hand in the following tasks.

Where the preference is so strong you would never use the other hand, unless absolutely forced to, put two checks (  )

If you are indifferent, put one check in each column ( | ).

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 parenthesis.

<table>
<thead>
<tr>
<th>Task/ Object</th>
<th>Left Hand</th>
<th>Right Hand</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Writing</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Drawing</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Throwing</td>
<td></td>
<td></td>
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<tr>
<td>4. Scissors</td>
<td></td>
<td></td>
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<tr>
<td>5. Toothbrush</td>
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<td></td>
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<tr>
<td>6. Knife (without fork)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7. Spoon</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8. Broom (upper hand)</td>
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<td></td>
</tr>
<tr>
<td>9. Striking a Match (match)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10. Opening a Box (lid)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

# Edinburgh Handedness Inventory

<table>
<thead>
<tr>
<th></th>
<th>LH=</th>
<th>RH=</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total checks:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cumulative Total</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Difference</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Result</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interpretation:</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(Lefthanded: R < -40)  
(Ambidextrous: -40 ≤ R ≤ +40)  
(Righthanded: R > +40)
Curriculum Vitae

Name: Kathleen Lyons

Post-secondary Education and Degrees:
Ryerson University
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2008-2013 B.A. (Psychology)

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Presentations:


