THE ROLE OF PLASTICITY IN COGNITION: A TMS-EEG STUDY

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Declaration

This thesis contains no material which has been accepted for the award of any other degree or diploma in any University, and, to the best of my knowledge, this thesis contains no materials previously published except where due reference is made. I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

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Abstract

Past studies have implicated a relationship between the Dorsolateral Prefrontal Cortex (DLPFC), and working memory and cognitive flexibility performance as measured via the N back and Trail Making tasks. It stands to reason that inducing plastic change to increase excitability of the DLPFC should result in improved performance on these tasks. This study used a 2 x 2 within groups single-blinded design with fourteen healthy participants (19 to 29 years old) attending two sessions, receiving iTBS in one, and sham in the other, investigating whether intermittent theta burst stimulation (iTBS) increased excitability of the DLPFC, and improved task performance. Cortical excitability was measured with TMS-evoked potentials (TEPs). Wilcoxon tests were used to determine the effect of iTBS on TEPs and psychometric performance, and the relationships between dependent variables were investigated using correlational analyses. Results show nonsignificant mild increases in 2-Back and Trail Making A tasks following iTBS relative to sham, and moderate correlations between changes in task performance and iTBS induced TEP changes. These findings go against previous research that support the iTBS to modulate TEP amplitudes, but are consistent with literature only finding mild effects of rTMS on improving working memory and cognitive flexibility.

1.1 Preamble

Plasticity is a fundamental part of human development; it is what allows the brain to grow and adapt as our environment changes, and bodies age (Jones, Nyberg, Sandblom, Neely, Ingvar, Petersson, & Bäckman, 2006). The magnitude of plastic change can be observed and measured via changes in excitability in underlying neuronal tissue (Bindman, Lippold, & Redfearn, 1962; Purpura, & McMurtry, 1965). Repetitive transcranial magnetic stimulation (rTMS) has been examined closely as a tool to induce plastic change, thus modulating cortical excitability. The use of rTMS has important implications clinically as part of rehabilitation after a head injury, and to improve function for people with organic brain injuries. Most commonly applied to the motor cortex, cognitive neuroscientists have since expanded upon this more traditional use of rTMS to induce plastic change in the prefrontal cortex (Chung, Rogasch, Hoy, & Fitzgerald, 2015). The applications of rTMS clinically are numerous; from depressive disorders to dementia and stroke patients, rTMS can be applied as a tangible tool to modulate and improve both cognitive function and memory. Already rTMS has seen success in improving mood for patients with drug resistant depression, and for improving motor function for stroke patients (Jorge et al., 2004; Wassermann et al., 1995; Takeuchi, Chuma, Matsuo, Watanabe, & Ikoma, 2005). While the effects of rTMS are mild at best, they still contribute to meaningful improvements in these different areas of function. Theoretically, there should be no difference in improving higher order cognitive processes. By applying rTMS to the Dorsolateral Prefrontal Cortex (DLPFC) we can study its effects on plasticity via Electroencephalography (EEG), and is fundamental effects to modulate behavioural performance via the N-back and Trail Making Tasks (TMT) A and B. rTMS can be an important tool for increasing the quality of life for people with cognitive deficits. Many mild forms of dementia and cognitive impairments that precede dementia

display significant losses of memory and cognitive function which can greatly decrease someone's quality of life. It is important in these clinical populations to attempt to positively and substantially improve higher order cognitive processes, such as working memory and cognitive flexibility; as these functions are fundamental in day to day functioning, and problem solving (Brunoni & Vanderhasselt, 2014; Hoy, Bailey, Michael, Fitzgibbon, Rogasch, Saeki, & Fitzgerald, 2016).

These constructs can be measured by variety of neuropsychological tests, however past literature studying the DLPFC and its association with both working memory and cognitive flexibility have found that the N-Back and TMT A and TMT B, specifically, reflect changes in these functions as well as physiological changes in the DLPFC (Moser et al., 2002; Hoy et al., 2016). This research examines cortical stimulation and the possibility of modulating behaviour; this means that there are practical implications of this research for improving recovery and performance for people with psychiatric illnesses, head injuries, and cognitive impairments.

1.2 Neuroplasticity

1.2.1 Defining neuroplasticity

Neuroplasticity is the ability for the brain to adapt, reorganise itself, and change throughout a lifetime (Classen, 2013; Pascual-Leone, Tarazona, Keenan, Tormos, Hamilton, & Catala, 1998; Rogasch, & Fitzgerald, 2013). Plastic change is a fundamental part of acquiring a new skill, rehabilitating from a head injury, and adapting to new environments and stimuli (Pascual-Leone et al., 1998). Neuroplasticity reflects changes in several physiological factors; synaptic weighting, the creation of new dendritic connections and synapses, and the modulation of the shape and number of dendritic spines (Classen, 2013;

Pascual-Leone et al., 1998). Neuroplasticity can occur through a variety of different processes, at a range of timescales, and between multiple cell types, and its effects can be adaptive or maladaptive depending upon the behavioural consequences of the plasticity (Classen, 2013). Adaptive plasticity is the response to external stimuli that results in new and increased repertoire of behaviour, whereas maladaptive plasticity reduces behavioural capacity (Classen, 2013). Plasticity that either increases or decreases synaptic efficacy as a response to external stimuli is specifically known as synaptic plasticity (Classen, 2013).

Synaptic plasticity can be considered either as potentiating or depressing synaptic strength, increasing or decreasing efficacy respectively (Classen, 2013). Potentiation or depression of synapses lasting thirty minutes or more is considered as long-term potentiation (LTP) and long-term depression (LTD); it is these changes in synaptic excitability that result in modulated plasticity (Classen, 2013). The literature defines LTP as long term increases in cortical excitability, while LTD are long term decreases in cortical excitability (Hoy et al., 2016). Most forms of LTP and LTD depend upon activation of N-methyl -D-aspartate (NMDA) glutamate receptors; these receptors are opened upon depolarisation as calcium (Ca^{2+}) enters the receptors (Artola, & Singer, 1993; Lisman, 1989). Fast and large increases in postsynaptic Ca^{2+} result in LTP, while in contrast, slow and smaller loads of Ca^{2+} cause LTD (Artola, & Singer, 1993; Lisman, 1989).

1.2.2 Inducing neuroplasticity

In both research and clinical practice, Transcranial Magnetic Stimulation (TMS) is fast gaining popularity in a wide variety of areas for its ability to both modulate and measure cortical excitability (Miniussi, Paulus, & Rossini, 2012). TMS involves the application of magnetic pulses to the scalp inducing an electric field in the underlying cortical tissue

(Miniussi et al., 2012). This electric field depolarises the neurons which in turn, elicits action potentials to fire (Miniussi, Paulus, & Rossini, 2012; Miniussi, & Ruzzoli, 2013).

Repetitive trains of pulses can induce plastic change, thus inhibiting or exciting the cortical tissue; this process is known as rTMS, and it can be administered at varying frequencies and intervals to modulate cortical tissue in different ways, either inducing LTP or LTD (Rogasch, & Fitzgerald, 2013; Huang, Edwards, Rounis, Bhatia, & Rothwell, 2005; Maeda, Keenan, Tormos, Topka, & Pascual-Leone, 2000; Nitsche, & Paulus, 2000; Stefan, Kunesch, Cohen, Benecke, & Classen, 2000). Generally, rTMS inhibits excitability with frequencies less than 3 Hz, or increases excitability with frequencies of 5 Hz or more (Fitzgerald, Fountain, & Daskalakis, 2006). The magnitude of a plastic response to rTMS protocol is influenced by many factors, such as a person's gender and specific genetic makeup, and the time of day (Ridding, & Ziemann, 2010). A popular form of rTMS is theta burst stimulation (TBS) (Miniussi et al., 2012). TBS administers TMS pulses in very short high frequency theta range bursts (4-7Hz); TBS can be applied intermittently for 2 s for 10 to 20 cycles, and this is known as intermittent TBS (iTBS) (Huang et al., 2005). iTBS is thought to more accurately mimic the natural firing patterns of pyramidal cells, and as such, induce cortical enhancements in the DLPFC more effectively than other forms of rTMS (Kandel, & Spencer, 1961; Eichenbaum, Kuperstein, Fagan, & Nagode, 1987; Oberman, Edwards, Eldaief, & Pascual-Leone, 2011). A large body of TBS studies have found that iTBS protocols consistently induced LTP modulation of the cortical tissue, while continuous TBS (cTBS) protocols consistently induced LTD modulation (Capocchi, Zampolini, & Larson, 1992; Hess, & Donoghue, 1995; Heynen, & Bear, 2001; Takita, Jay, Kaneko, & Suzuki, 1999; Heusler, Cebulla, Boehmer, & Dinse, 2000). While these studies were initially found from studies that measure neuroplasticity in the motor system, using Motor Evoked Potentials (MEPs), it was

hypothesised that iTBS modulation would be effective in other regions of the brain as well (Huang et al., 2005).

1.2.3 Measuring neuroplasticity

Recent studies have utilised EEG with TMS as a valuable method for monitoring excitability and cortical reactivity within the brain as evoked by TMS pulses (Chung et al., 2015). EEG is a non-invasive method of recording the electrical activity of neuronal tissue in microvolts, via electrodes that are placed upon the scalp. (Delorme, & Makeig, 2004). EEG recordings can be time locked around the presentation of stimuli to measure the extent to which the stimuli can affect neuronal electrical activity (Delorme, & Makeig, 2004). The administration of single pulses of TMS can be used for this purpose, as a test of excitability, of the neuronal electrical activity by evoking TMS evoked potentials (TEPs). (Rogasch, & Fitzgerald, 2013). TEPs are measured while administering the single pulses of TMS to the DLPFC via simultaneous EEG recordings (Chung et al., 2015). TEPs allow for measurement of cortical excitability, connectivity, and oscillatory activity, with increased TEP amplitudes reflecting increased neuronal excitability, while decreased TEP amplitudes reflect decreased neuronal excitability (Frantseva et al., 2012; Ilmoniemi & Kičić, 2010). TEPs are highly replicable over time, although they are very sensitive to experimental contexts, such as TMS intensity, the stimulation site, and the coil angle used (Veniero, Ponzo, & Koch, 2013; Bonato, Miniussi, & Rossini, 2006; Kähkönen, Komssi, Wilenius, & Ilmoniemi, 2005; Kähkönen, Wilenius, Komssi, & Ilmoniemi, 2004; Komssi et al., 2002; Komssi, Kähkönen, & Ilmoniemi, 2004; Casarotto et al., 2010; Lioumis, Kičić, Savolainen, Mäkelä, & Kähkönen, 2009). TEPs are composed of a variety of peaks and troughs labelled by the polarity and time lasting up to 300 milliseconds past the TMS pulse (Chung et al., 2015).

Different components of the TEP reflect a variety of different neuronal activity such as neuronal excitability or inhibition, as well as the release of different neurotransmitters (Chung et al., 2015). The change in the amplitudes of the several components which make up the TEP is the measure by which cortical excitability is measured as a marker of plastic change (Chung et al., 2015). The different components of TEPs, what they reflect, and how they are affected by induced plastic change, are largely not yet understood in the literature (Premoli et al., 2014). The literature suggests that early components, less than 50 ms, reflect activity of GABA-A, while later components of the TEP, more than 100 ms, reflect GABA-B activity (Connors, Malenka, & Silva, 1988; Deisz, 1999). For example, the N100 component of a TEP has been linked to cortical inhibition, mediated by the release of GABA-B (Rogasch, Daskalakis, Fitzgerald, 2013; Premoli et al., 2014; Farzan et al., 2013). Likewise, the N40 component of a TEP has been linked to the release of GABA-A (Rogasch et al, 2013; Premoli et al., 2014). Furthermore, the P60 component of a TEP has been found to reflect inhibition of excitatory mechanisms, as well as significantly correlate with MEP changes in past studies exploring the effect of rTMS on TEPs is in the motor cortex (Rogasch et al, 2013). Research exploring how the TEPs change in response to rTMS, typically found that the P60 and N100 components of the TEP are often the most significantly changed markers, reflecting rTMS induced plastic change (Cash et al., 2017). As these two components are most significantly reflective of changes in TEP amplitudes as a result of rTMS, this makes them useful for this study to use as markers, to observe and analyse the effects of rTMS on the DLPFC, in addition to the N40 which reflect the changes in GABA-A activity as induced by rTMS (Cash et al., 2017; Rogasch et al, 2013).

1.3 Dorsolateral Prefrontal Cortex

1.3.1 Functions

The DLPFC is an important neural structure for both working memory and cognitive flexibility (Brunoni & Vanderhasselt, 2014). Functional near-infrared spectroscopy (fNIRS) investigations have revealed that the DLPFC is activated when people complete TMT A and TMT B (Hagen et al., 2014). A 2011 functional magnetic resonance imaging (fMRI) analysis of the TMT found strong clusters of activation throughout a variety of neural pathways, including the DLPFC, in healthy participants aged between 20 and 39 years (Allen, Owens, Fong, & Richards, 2011). EEG studies have found that tasks demanding flexible thinking elicits increases in theta oscillations within the frontal lobe (Yeung, Han, Sze, & Chan, 2016).

1.3.2 Working memory

Working memory is a psychological construct and system regarding the ability to consciously store, manipulate, and control information (Baddeley, Logie, Bressi, Sala, & Spinnler, 1986). It consists of three fundamental components; the attentional controller, the central executive system, and it's two subsidiary systems; the visuospatial sketchpad of visual information, and the phonological loop for auditory information (Baddeley et al., 1986). Working memory has a limited capacity, and can only store information for a brief period of time in the absence of reliable external cues (Lezak, 2004; Goldman-Rackie, 1993).

A recent study by Hoy et al. (2016) found improvements on the N back task following iTBS. Hoy et al. (2016) measured performance on both the 2-Back and 3-Back tasks by two measures; correct discrimination rate and accurate reaction time. The N-Back was administered post sham and real stimulation at 0, 20, and 40 minutes. iTBS significantly

increased gamma power during both the 2-Back and 3-Back tasks, and significantly improved correct discrimination on the 2 Back task, with this enhancement affect being maintained 40 minutes post stimulation (Hoy et al., 2016). The 2-Back task was also correlated with increased theta connectivity between the F3 to P3 and P4 electrodes following active stimulation compared with sham, suggesting that increased theta activity in the fronto-parietal neural network could be a causal factor in increased working memory (Hoy et al., 2016; Baddeley, 2003; Lisman, 2010). iTBS did not significantly improve either theta connectivity or the proportion of correct responses for the 3-Back (Hoy et al., 2016). This was hypothesised by the authors to be due to suspected 'ceiling effect'; this suggests that iTBS can only affect performance up to a certain memory loading (Hoy et al., 2016). The ceiling effect reflects the phenomena that TEP amplitudes cannot increase or decrease past a certain point, as participants are already operating with the strongest possible activation of the relevant neural mechanisms (Hoy et al., 2013).

A 2014 systematic review by Brunori & Vanderhesselt found that rTMS administration was significantly associated with both faster response times and higher percentages of correct responses on both the 2-Back and 3-Back tasks. This evidence stands in contrast with Hoy at al.'s findings that 3-Back performance is too cognitively demanding to be improved via rTMS. There were methodological differences between the different studies that may account for the contrasting outcomes. The systematic review included data from studies involving clinical patients; this may be a significant reason behind the discrepancy results as clinical populations have been found by several studies be more responsive to rTMS than healthy participants (Brunoni & Vanderhasselt, 2014). This is likely because medicated participants are in an inhibited neurophysiological state, making them more susceptible to the effects of rTMS (Brunoni & Vanderhasselt, 2014). Furthermore,

increased N-Back performance is associated with prefrontal activation as measured by functional neuroimaging (Brunoni & Vanderhasselt, 2014; Owen, McMillan, Laird, & Bullmore, 2005).

1.3.3 Cognitive flexibility

Cognitive flexibility is the ability to adapt appropriately to environmental stimuli; this means being able to adapt and adjust to difference situations (Oshiro, Nagaoka, & Shimizu, 2016). Cognitive flexibility underpins other cognitively demanding functions such as problem solving and meeting task demands where shifts in attention are necessary (Lezak, 2004). Neuroimaging studies have linked cognitive flexibility with the DLPFC, as it is involved in monitoring and actively manipulating information, thus mediating flexible thinking (Yeung et al., 2016). A 2002 study by Moser et al. investigated the ability for TMS to improve cognitive flexibility and mood in middle-aged and elderly people with refractory depression. They found that rTMS significantly improved performance on TMT B without significantly affecting mood or other cognitive factors (Moser et al., 2002). The researchers noted that other cognitive abilities not associated with the dorsolateral prefrontal cortex were largely unaffected by the stimulation (Moser et al., 2002). This suggests that stimulation of the dorsolateral prefrontal directly improved the cognitive functions that it is associated with (Moser et al., 2002).

In a 2010 systematic review, Guse, Falkai, & Wobrock found that the TMT was one of the few tasks to see significant improvements as a result of rTMS applied to the DLPFC. In reviewing studies where cognitive improvement was found, the authors noted that high-frequency rTMS between 80 and 110% Resting Motor Threshold (RMT) over 10 to 15 sessions was the most effective protocol in inducing significant improvements (Guse, et al.,

2010). However, none of these experiments used healthy participants, but rather, used clinically depressed or schizophrenic patients. While only two studies found significantly improved results in TMT, many still reported trends of increased TMT performance post rTMS stimulation to the DLFPC in these clinical populations (Moser et al., 2002; Jorge et al., 2004; Boggio et al., 2005; Hausmann, et al., 2004)

1.4 Aims and Hypotheses

1.4.1 Aims

This study aims to build upon the existing literature by measuring differences between baseline TEPs, and post iTBS and sham TEPs. Differences in TEP component amplitudes between baseline and post iTBS reflect the extent to which excitability has been modulated; this reflects the extent to which plastic change has successfully been induced. While past studies have not always found significant effects on task performance as a result of rTMS, the literature does consistently see improvement in N-back and TMT scores due to rTMS as a trend; because of these trends, we hypothesised that rTMS would have a positive effect in improvement both N-back and TMT performance. Past studies examining modulation of the DLPFC via rTMS have found that the N-back and TMT are effective measures in observing changes of both working memory and cognitive flexibility respectively. Furthermore, by recording TEPs, we will be able to compare changes in TEP amplitudes and behavioural performance. This will inform us of possible relationships that might exist between modulated TEP components, and either increases or decreases in task performance. As such, we predict that iTBS of the DLPFC will significantly increase TEP amplitudes, and increase performance on both the N-back and TMT.

1.4.2 Hypotheses

With this aim in mind, we have three hypotheses. We hypothesise that;

I. application of iTBS to the DLPFC will increase TEP amplitudes compared to sham

II. application of iTBS to the DLPFC will improve performance on the N-Back and TMT compared to sham

III. improved performance on the psychometric tasks will be positively correlated with increased amplitudes of TEPs.

Chapter 2: Methods

2.1 Participants

A total of 14 healthy participants were included in this study, ranging in age from 19 to 29 (*M* = 24.07, *SD* = 3.29) years to control for age related effects of neuroplasticity induction. One participant's TEP data were excluded due to missing data, leaving 13 participants with complete data sets. Exclusion criteria included a history of epilepsy, being on medications affecting the central nervous system, and suffering any neurological conditions. All experimental procedures were approved by The University of Adelaide Human Research Ethics Committee (See Appendix A).

Participants were recruited privately by the researchers. Individuals who expressed interest were given an information sheet, and a TMS safety screen to complete that included a checklist of all exclusion criteria (See Appendixes B and C) (Rossi et al., 2009). Participants were predominantly male (10 male and 4 female), and mostly born in Australia (9 born in Australia, 5 born overseas).

Participants signed both the safety screen and consent form prior to the experiment beginning. Participants were advised of their right to withdraw at any time with no negative consequences before the experiment began, and were asked if they wanted to be informed of their results upon completion of the experiment.

2.2 Materials

2.2.1 TMS

TMS was administered with both a single pulse TMS coil to record TEPs, as well as with an rTMS coil for the iTBS active and sham stimulation. iTBS was applied using a

Magstim Rapid^2 stimulator with a 70-mm diameter figure-of-8 air film coil. The sham coil produces the same auditory stimulus associated with real stimulation, but without applying any magnetic pulse to the scalp. iTBS was applied to the left DLPFC at an intensity of 70% RMT. The iTBS protocol applied a 2 s train repeated every 10 s. In every 2 s train, bursts of 3 pulses of stimulation were given at 50 Hz, with bursts repeated at 5 Hz (Huang et al., 2005).

2.2.2 Trail making A and B

The Trail Making Task (TMT) (See Appendix D) is a fundamental component of a range of neuropsychological batteries (Lezak, 2004; Kortte, Horner, & Windham, 2002). The test has two parts; part A which requires participants to connect 25 numbers consecutively as quickly and as accurately as possible, and part B in which the participant must alternatively connect numbers and letters in a consecutive order (Lezak, 2004; Kortte et al., 2002). The TMT reflects several cognitive functions such as complex visual scanning, attention, and cognitive flexibility (Kortte et al., 2002).

2.2.3 N – Back

The N-back is extensively used within psychological literature as a measure of working memory (Jaeggi, Buschkuehl, Perrig, & Meier, 2010). The N-back, unlike many other working memory tasks, can use different loadings to increase or decrease the difficulty of the task (Jaeggi et al., 2010). The N-back task requires participants to remember a series of stimuli, in this case letters, and type the previous letter when it is replaced by a new letter. The load (N) of the task can be manipulated, so that a participant has to remember one letter, two letters, or three letters, per the administration of the 1-back, the 2 back, and the 3 -back respectively.

2.3 Procedure

This study used a randomised repeated measures single blind design. Each participant attended 2 sessions at the NeuroPAD laboratories in the Robinson Research Institute. The experiment was conducted in a quiet room with the door closed. The order in which both the psychometric tasks and type of stimulation (sham or active) administered were randomised across participants. Prior to participation, participants a NeuroPAD standard form (See Appendix E). At the beginning of each session, participants were seated in a comfortable chair and an EEG cap was fitted to their head.

The protocol began with the intensity of both single-pulse TMS and rTMS being set based on each participants RMT (see Figure 1). RMT was the minimum intensity required to elicit MEPs with a peak-to-peak amplitude of 50 µV three times out of five (Carroll, Riek, & Carson, 2001). MEPs were measured via Electromyographic (EMG) activity which was recorded from the first right dorsal interosseous (FDI) muscle via single use patient electrodes after cleaning the hand, and applying ultrasound gel to the underside of the electrode. Participants were asked to push against the researcher's finger with their forefinger to precisely identify the FDI muscle, so the electrodes could be positioned in a belly-tendon montage held in place with hypafix medical tape; with the active electrode placed over the muscle belly, and the reference electrode over the adjacent muscle. A ground strap was soaked in water and placed around the right wrist. Signals were amplified x1000, then band filtered between 20-1000Hz using a Cambridge Electrical Design (CED, Cambridge, UK), and digitised at a rate of 5kHz using Cambridge Electrical Design 1401 (CED, Cambridge, UK). For stimulation of the left DLPFC, the centre of the coil was positioned between the F3 and F5 electrode, with the handle pointing posterolaterally at a 45° angle to

the sagittal plane (producing a posterior-anterior current flow in the underlying cortical tissue). Without the use of neuronavigational equipment, this is the best way to estimate the position of the DLPFC (Fitzgerald, Maller, Hoy, Thomson, & Daskalakis, 2009; Rusjan et al., 2010). One hundred baseline TEPs were recorded before administering either sham or active iTBS. One hundred post stimulation TEPs at 120% RMT at 0.2Hz were recorded immediately after iTBS, followed by administration of both the N-back and TMT.

Figure 1 iTBS and Sham Protocol

The order in which TMT A, B, and the N back was administered after the post stimulation TEP recordings were randomised. The N back was administered by a desktop computer running Windows 10 and took participants approximately 50 minutes to complete the task. In both sham and iTBS conditions, participants were given verbal and written instructions how to complete the task before they undertook three practice trials, and subsequently completed the 12 tested trials. Trials were loaded as either 1- back, 2- back, or 3- back. Of the 12 trials completed by participants, two were loaded as 1- back, five were loaded as 2- back, and 5 were loaded as 3- back. Output was produced by the N back program as an Excel spreadsheet.

The TMT A and B were administered via pen and paper, with time to complete measured in seconds. Time was recorded with a stopwatch in the lab by the experimenter. Before completing each part of the test, participants were given a sample of both the TMT A and TMT B, along with verbal instructions on how to complete the task. Participants were

advised that the test was timed, and that they had to complete the task as quickly and as accurately as they could. The time it took complete each section was recorded in seconds my by the experimenter, and these times were later recorded in an Excel spreadsheet.

2.4 Analysis

Analysis of the EEG data was performed using EEGLAB (Delorme, & Makeig, 2004) and custom scripts through MATLAB, and the TMS-EEG signal analyser (TESA), an open-source extension for EEGLAB (R2017a, The Mathworks, USA; Rogasch et al., 2017). The data had interfering channels and artefacts removed using custom Matlab scripts. The data also underwent an independent component analysis to remove additional artefacts, such as eye, auditory, and muscle artefacts (Rogasch et al., 2014). The data was filtered, with the output used for analysis being centred around the region of interest (ROI); which in this case, were the F3, FC5, FC1, AF3, F5, F1, and FC3 electrodes placed upon the DLPFC. The output provided amplitudes for the N40, P60, and N100 components of the TEP at the ROI (see Figure 2). The procedure to locate the TEP components was consistent with previous research (Rogasch, Daskalakis, & Fitzgerald, 2015). The N40 slope was calculated at the negative peak closest to 40 msec (25-55 msec), the P60 was calculated at the positive peak closest to 60 msec (45-75 msec), and the N100 was calculated at the negative peak closest to 100 msec (85-145 msec).

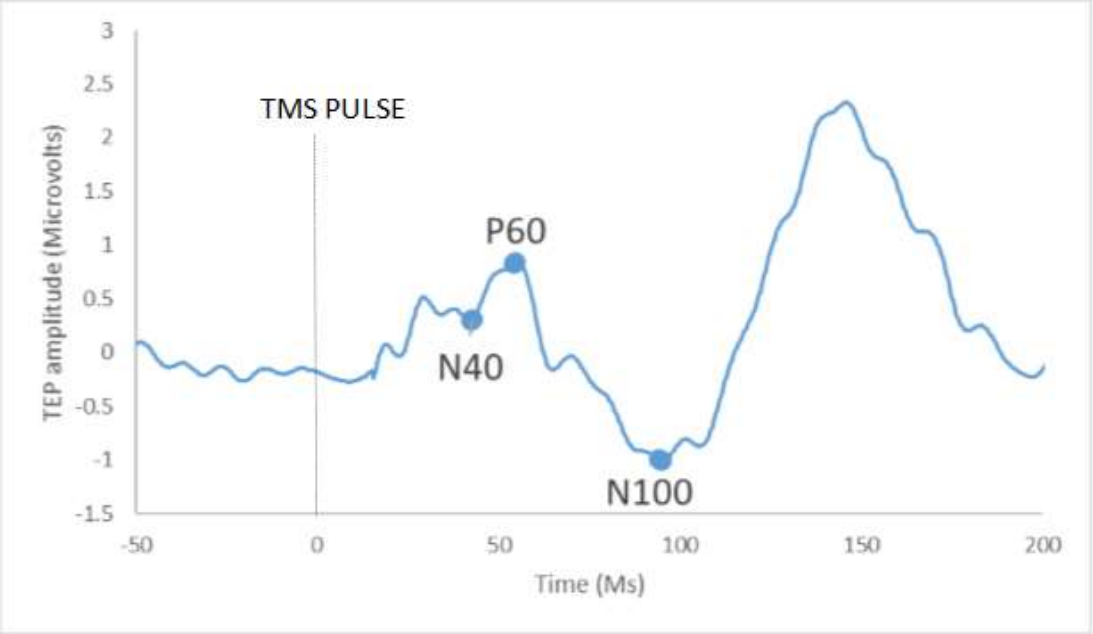

Figure 2 A TEP highlighting the TMS pulse, N40, P60, and N100 components

SPSS was used to conduct the statistical analysis, with an alpha level of .05 employed to judge for statistical significance for all tests. Conditions were compared via Wilcoxon signed-rank tests. Participants' response to iTBS was observed using both TEPs and behavioural tests. To calculate participants' response to iTBS, change in N40 amplitude (ΔN40), ΔP60, and ΔN100, following sham and iTBS were calculated. Pearson correlations were also calculated to determine if the TEP response to iTBS was associated with behavioural differences observed on the N-Back and TMT A and B.

Statistical analysis was performed to investigate if iTBS had an effect on the amplitudes of the N40 (distinguishable in 11 participants), P60 (distinguishable in 10 participants), and N100 (distinguishable in 13 participants) TEP components. Amplitudes were measured pre-stimulation and post stimulation, for both sham and real iTBS. To calculate the change in TEP amplitude per each condition, the difference between pre-stimulation and post stimulation amplitudes were calculated for each of the three TEP

components. This process created ΔN40, ΔP60, and ΔN100, for both iTBS and sham conditions. The differences between iTBS ΔN40, ΔP60, and ΔN100, and sham ΔN40, ΔP60, and ΔN100 were calculated to observe if a significant difference existed between sham TEP amplitudes and iTBS TEP amplitudes. Normality tests showed that the TEP data was often skewed, so normality could not be assumed.

Statistical analysis was performed to investigate if iTBS had an effect on performance for both 2-Back and 3-back performance, with performance defined as accurate recall. Performance was compared between sham and real conditions for both 2-Back and 3-Back tests. Time to complete the both TMT A and TMT B measured was compared between sham and real conditions for both TMT A and TMT B. Normality tests showed that the psychometric test data was often skewed, so normality could not be assumed for both N-back and TMT data.

2.5 Examination of testing order

The order in which participants were assigned to either receive sham or real iTBS was randomised via a random number generator, in which participants who would receive sham iTBS stimulation first were coded 0, and participants who would receive real iTBS stimulation first were coded 1. There appeared to be a bias towards more participants receiving sham in their initial session, and so a chi square of independence was performed to investigate the distribution. The chi square revealed that, despite concerns, the allocation to receiving either sham iTBS or real iTBS did not significantly differ from what would be expected by chance, indicating that the allocation was equally distributed x^2(df= 1, N=14) = 1.14, p=.29.

Likewise, the order in which participants were tested on N-back or TMT after stimulation, both real and sham, was randomised via a random number generator, with the TMT followed by the N-back coded as 0, and the N-Back followed by TMT protocol coded as 1.

Chapter 3: Results

3.1 The effect of iTBS on TEP amplitudes

There was no significant difference between sham baseline N40 amplitudes and experimental baseline N40 amplitudes Z = -3.14, *p* = .75. There was no significant difference between sham baseline P60 amplitudes and experimental baseline P60 amplitudes Z = .15, *p* = .88. There was a significant difference between sham baseline N100 amplitudes and experimental baseline N100 amplitudes Z = -2.06, *p* = .04 with a large effect size *r*= -.40 (see *Table 1*).

Table 1

Descriptive statistics for differences between baseline TEPs

N40 Baseline	M	SD
Sham	-0.023	0.94
iTBS	-0.04	1.8
P60 Baseline		
Sham	0.42	0.99
iTBS	1.13	1.81
N100 Baseline		
Sham	-1.11	1.22
iTBS	-1.85	1.61

Changes in amplitude between sham and iTBS conditions for each TEP component were compared to test whether application of iTBS to the DLPFC increased TEP amplitudes compared to sham. There was no significant difference between sham ΔN40 amplitudes (*M*= -.02, *SD*= 1.34) and experimental ΔN40 amplitudes (*M*= -.09, *SD*= .92) Z = .15, *p* = .88. There was however, a mild trend, with the experimental condition reporting more negative N40 amplitudes than the sham condition *r*=-.19 (see Figure 3).

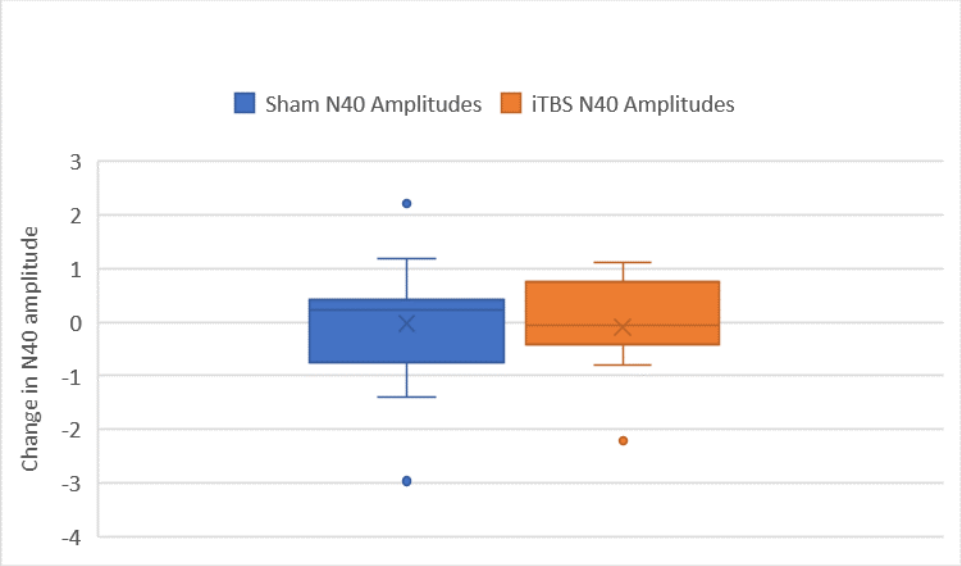

Figure 3 Differences in N40 change between Sham and iTBS. Data is expressed as mean ± Standard error of mean

There was no significant difference between sham ΔP60 amplitudes (*M*= .07, *SD*= 1.35) and experimental ΔP60 amplitudes (*M*= -.002, *SD*= .89) Z = .15, *p* = .88 (see Figure 4).

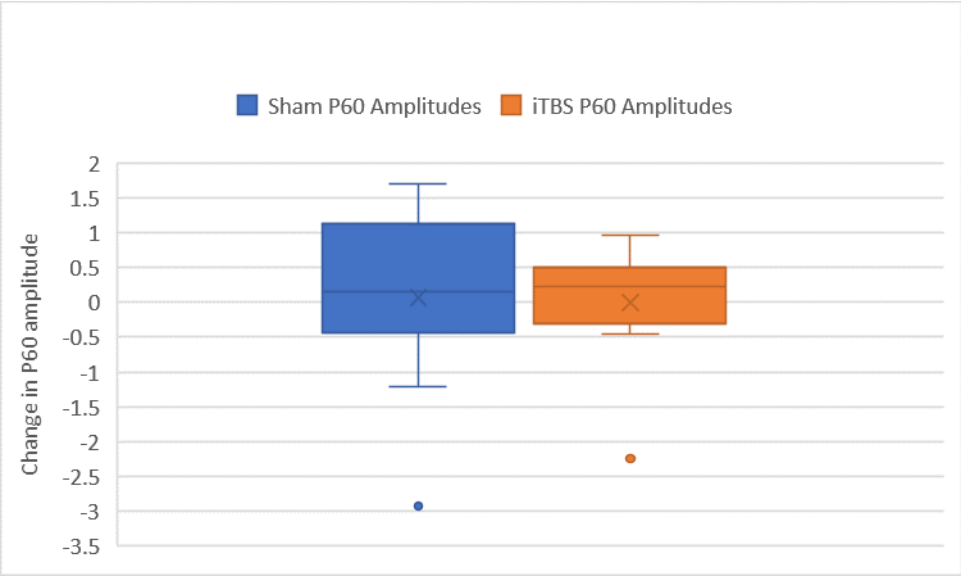

Figure 4 Differences in P60 change between Sham and iTBS. Data is expressed as mean ± Standard error of mean

There was no significant difference between sham ΔN100 amplitudes (*M*= .04, *SD*= .82) and experimental ΔN100 amplitudes (*M*= -.08, *SD*= .34) Z = -.45, *p* = .65 (see Figure 5).

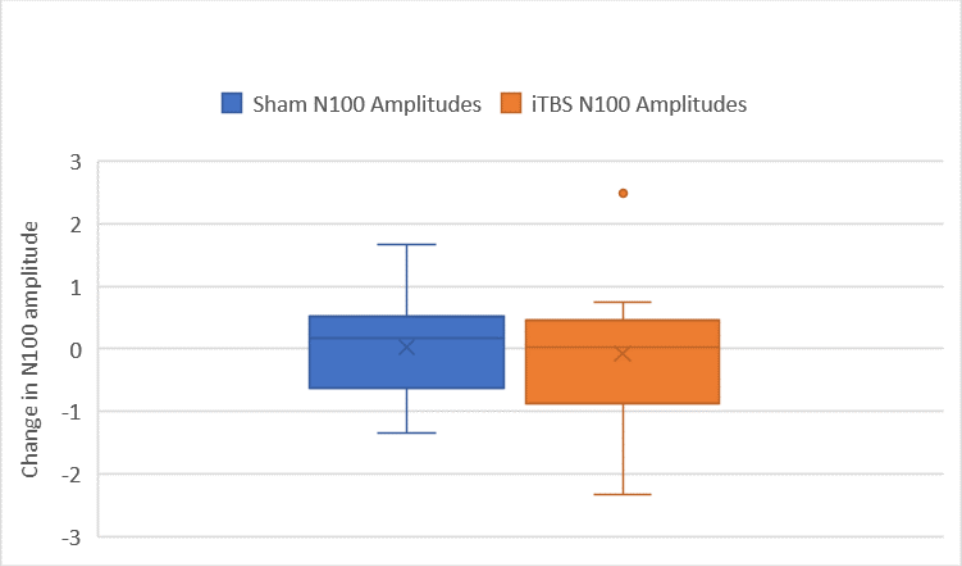

Figure 5 Differences in N100 change between Sham and iTBS. Data is expressed as mean ± Standard error of mean

3.2 The effect of iTBS on N-back performance

Changes in performance between sham and iTBS conditions for the 2-Back and 3-Back tasks were compared to test whether application of iTBS to the DLPFC improved performance on the N-Back compared to sham. There was no significant difference in 2-back accuracy between sham (*M*= .81, *SD*= .14) and experimental (*M*= .85, *SD*= .13) conditions Z = -1.35, *p* = .18 There was however, a mild trend, with the experimental condition showing greater 2-back accuracy than the sham condition r=-.26. There was no significant difference in 3-back accuracy between sham (*M*= .72, *SD*= .18) and experimental (*M*= .75, *SD*= .18) conditions Z = -1.2, *p* = .22. There was however, a mild trend, with the experimental condition reporting greater 3-back accuracy than the sham condition *r*= -.23 (see Figure 6).

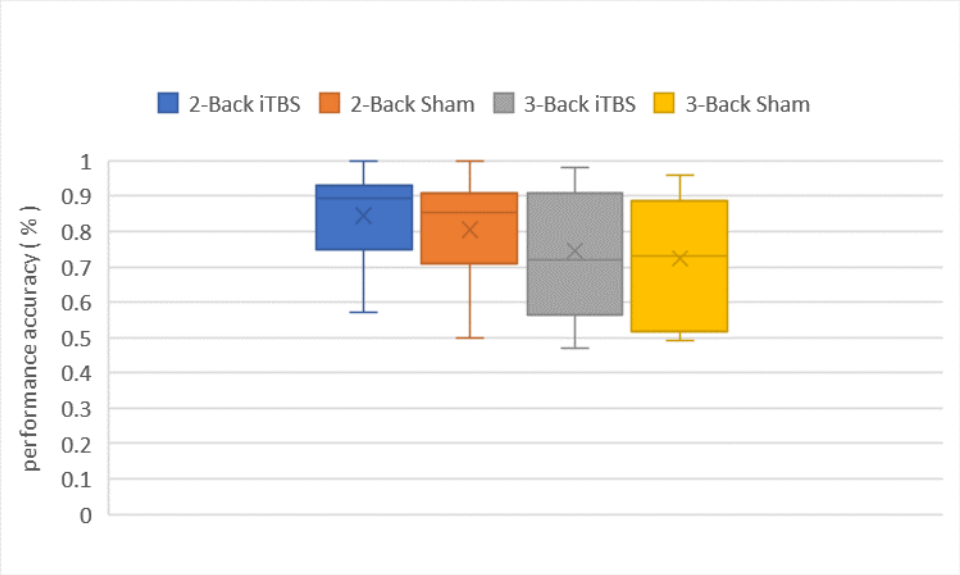

Figure 6 Differences in 2-Back and 3-Back performance between Sham and iTBS. Data is expressed as mean ± Standard error of mean

3.3 The effect of iTBS on TMT performance

Changes in performance between sham and iTBS conditions for the TMT A and TMT B were compared to test whether application of iTBS to the DLPFC improved performance on the TMT compared to sham. There was no significant difference between sham (*M*= 25.15, *SD*= 9.57) and experimental (*M*= 22.4, *SD*= 7.86) conditions on TMT A completion time Z = -1.73, *p* = .08 There was however, a mild trend, with the experimental condition reporting faster TMT A completion time after real iTBS than sham condition *r*= -.33. There was no significant difference between sham (*M*= 56.24, *SD*= 17.29) and experimental (*M*= 64.41, *SD*= 27.55) conditions on TMT B completion time Z = -.47, *p* = .64 (see Figure 7).

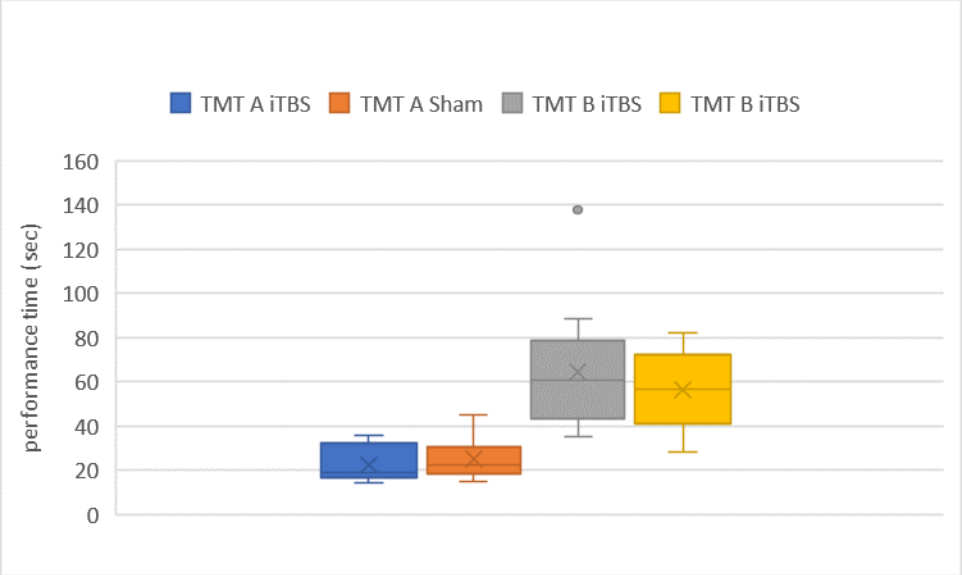

Figure 7 Differences in TMT A and TMT B performance between Sham and iTBS. Data is expressed as mean ± Standard error of mean

3.4 Correlations between task performance and changes in TEP amplitudes

Correlational analyses were performed to examine whether improved performance on the psychometric tasks was positively correlated with increased amplitudes of TEPs. Total TEP amplitude change were calculated for each TEP component by subtracting the sham amplitude from the real amplitude for all three measured TEP components. The results show that there was a moderate positive correlation between changes between real and sham TEP N40 amplitude and changes between real and sham performance on the 3-back task $r(9) = .32$, $p = .34$. The results show that two strong, albeit non-significant, correlations were also found. There was a strong positive correlation between changes between real and sham TEP P60 amplitude and changes between real and sham performance on the 3-back task $r(8) = .51$, $p = .13$. The results show that there was a significantly strong correlation between changes between real and sham TEP N100 amplitude and changes between real and sham performance on the 3-back task, with more negative N100 amplitudes being

associated with more accurate 3-back performance after real iTBS relative to sham, with r(11) =.61, *p*=.03 (see Figure 8). No other associations were observed. (see Table 2).

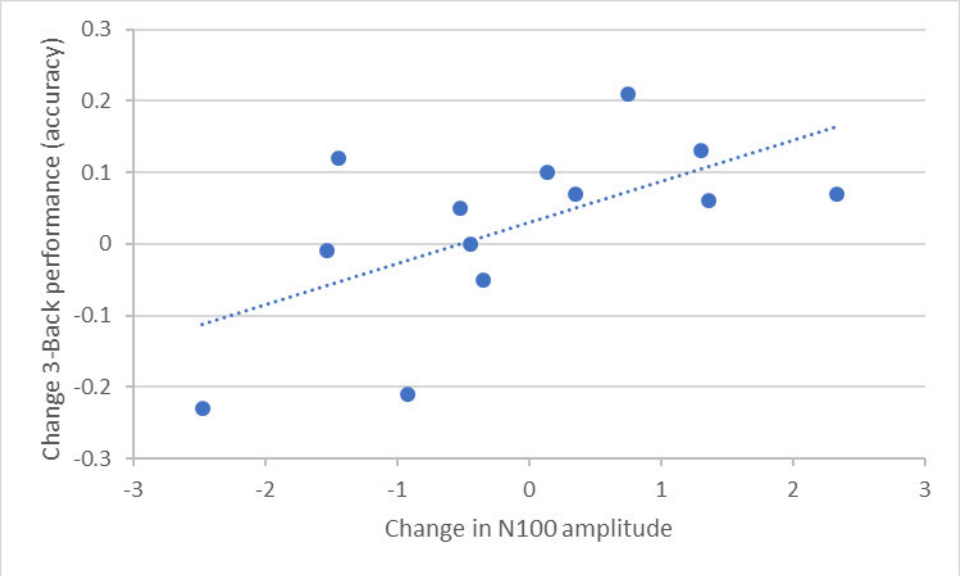

Figure 8 The relationship between changes in N100 amplitude and 3-Back performance

Table 2

Correlational Output between TEP amplitudes, and scores on 2-Back, 3-Back, and TMT B

		Changes in 2-BACK	Changes in 3-BACK	Changes in TMT B
N40	r	0.004	0.319	-0.021
	p value	0.991	0.34	0.95
	N	11	11	11
P60	r	-0.029	0.51	0.178
	p value	0.936	0.132	0.623
	N	10	10	10
N100	r	0.301	0.61	-0.152
	p value	0.318	0.027	0.62
	N	13	13	13

Chapter 4: Discussion

4.1 Summary of findings

The fundamental aim of this study was to investigate the relationship between changes in TEPs, and N-back, and TMT performance. We hypothesised that we would see increases in performance on the N-back and TMT as a result of iTBS, and that this increase in performance would be correlated with increases in the three TEP components; the N40, P60, and N100. However, in testing our three hypotheses, we saw no significant difference in either N40, P60, and N100 amplitudes, or psychometric performance, thus not supporting our initial three hypotheses. We expected to replicate past results by inducing improved N-back and TMT performance via iTBS, as is consistent with the past literature studying the effects of rTMS on working memory and cognitive flexibility (Moser et al., 2002; Hoy et al., 2016; Brunoni & Vanderhasselt, 2014). Fundamentally, the results obtained did not support any of the three original hypotheses.

Hypothesis one was not supported; there was no significant difference in changes in TEP amplitudes between sham and iTBS conditions. We did however, find mild trends between sham and iTBS conditions in amplitudes for the N40 and P60 TEP components suggesting that iTBS might successfully modulate cortical excitability to an extent. This stands in contrast to the existing body of literature which consistently finds iTBS to be effective in modulating TEP amplitudes (Kandel, & Spencer, 1961; Eichenbaum, Kuperstein, Fagan, & Nagode, 1987; Oberman, Edwards, Eldaief, & Pascual-Leone, 2011).

Hypothesis two was also not supported; there was no significant improvements in N-back and TMT performance between sham and iTBS conditions. We expected to build upon past results from Moser et al. (2002) and Esslinger et al. (2014) by inducing statistically

significant difference on TMT A and B via the use of iTBS. We predicted that iTBS would produce statistically significant improved performance, as rTMS has usually been found to improve TMT and N-Back scores (Jorge et al., 2004; Boggio et al., 2005; Hausmann, et al., 2004; Brunoni & Vanderhasselt, 2014). Given that iTBS is consistently found to be successful in inducing LTP plastic change, we expected that its application would significantly improve function of the DLPFC, and thus improve TMT and N-Back performance (Kandel, & Spencer, 1961; Eichenbaum, et al., 1987; Oberman, et al., 2011). However, our findings did not support this, as no significant differences in TMT or N-Back performance were found as a result of iTBS. Mild trends were found however, between sham and iTBS conditions for 2-back, 3-back, and TMT A performance, suggesting that iTBS might successfully improve behavioural performance excitability to an extent. This finding is supported by the literature, as many past studies often report mild nonsignificant improvements in performance, rather than statistically significant improvements in performance as a result of rTMS (Jorge et al., 2004; Boggio et al., 2005; Hausmann, et al., 2004; Brunoni & Vanderhasselt, 2014, Brunoni & Vanderhasselt, 2014; Gaudeau-Bosma et al., 2013; Guse et al., 2013).

Hypothesis three was also not supported; there were few statistically significant correlations between improvements in N-back and TMT performance, and modulation of TEPs between sham and iTBS conditions. Fundamentally, we aimed to see a linear relationship between changes in the TEP peak amplitudes, and modulated performance. To find such a relationship would tie together literature suggesting that iTBS effectively increases cortical excitability, as seen via modulated TEPs, as well as being able to successfully improve performance on both the two measures used (Kandel, & Spencer, 1961; Eichenbaum, Kuperstein, Fagan, & Nagode, 1987; Oberman, Edwards, Eldaief, &

Pascual-Leone, 2011; Moser et al., 2002; Esslinger et al. 2014; Li et al., 2017; Pascual-Leone et al., 1998). This finding is largely inconsistent with existing literature which indicates that both TEP amplitudes, working memory, and cognitive flexibility should be significantly modulated, as a result of iTBS plastic induced change to the DLPFC (Kandel, & Spencer, 1961; Eichenbaum, et al., 1987; Oberman, et al., 2011; Hoy et al. 2016; Moser et al. 2002). However, there were moderate correlations between changes on the three components of the TEP and performance on the 3-back task. In contrast to our predictions, the P60 TEP amplitude were observed to be lower in the experimental condition than the sham condition, though not significantly.

4.2 Interpretation and Evaluation of findings

4.2.1 Effect of protocol on TEP amplitudes

No significant difference in TEP amplitudes was found between sham and iTBS, and as such, hypothesis one was not supported. This finding is in contrast to previous literature which consistently finds that rTMS significantly modulates TEP amplitudes (Kandel, & Spencer, 1961; Eichenbaum, Kuperstein, Fagan, & Nagode, 1987; Oberman, Edwards, Eldaief, & Pascual-Leone, 2011). However, our results did detect mild nonsignificant changes in N40 and P60 amplitudes between sham and iTBS, suggesting that iTBS had an effect on modulating these components of the TEP, though the effect was mild (Rogasch, Daskalakis, & Fitzgerald, 2015).

4.2.2 Modulated N-back and TMT performance

No significant difference in N-back and TMT performance was found between sham and iTBS, and as such, hypothesis two was not supported. Our results found a mild

nonsignificant improvement in N-back and TMT A performance as a result of iTBS. It is interesting to note that for the much easier TMT A and 2-Back, we found a moderate effect between real and sham stimulation, while no such effect was found for the more cognitively demanding TMT B and 3-Back tasks. While no significant effect was found, the mild improvement in TMT and N-Back performance we observed between sham and iTBS conditions is consistent with the majority of existing literature (Jorge et al., 2004; Boggio et al., 2005; Hausmann, et al., 2004; Gaudeau-Bosma et al., 2013; Guse et al., 2013).

4.2.3 Correlations between TEP amplitudes and task performance

A significant correlation was found to exist between changes in 3-Back performance, and changes in N100 amplitude, with higher performance being associated with more negative N100 amplitudes. However, as only one correlation of a possible nine were found to be significant, hypothesis three was also not supported. We also found mild nonsignificant correlations between changes in N40 and P60 amplitudes and 3-back performance. The combined presence of these findings might suggest that rTMS had a mild effect on 3-back and 2-back performance. The non-significance of these results could imply that a relationship exists, but would require a significantly bigger sample size with increased statistical power to observe. Our study exists in line with the bulk of literature as it supports the existence of an effect on performance, albeit that effect is very weak (Brunoni & Vanderhasselt, 2014).

Another possible explanation is that there was no relationship between rTMS and changes in either TEP amplitudes and psychometric performance, and that the correlations found here were simply due to chance. This is a possibility however, since TEP amplitudes are dynamic fluctuating structures that are always changing in value to some extent or

another (Chung et al., 2015). This would be in complete contrast though to the established literature, which consistently finds that rTMS affects both TEPs and task performance, and as such is unlikely (Kandel, & Spencer, 1961; Eichenbaum, et al., 1987; Oberman, et al., 2011; Hoy et al. 2016; Moser et al. 2002).

4.2.4 Low spatial resolution of EEG readings

Given the low spatial resolution of EEG readings, it was sometimes difficult to separate neural activity from artefacts, even with the use of the TESA program created by Rogasch et al. (2017) which detects and removes some of these artefacts automatically, and provides a guide for the removal of the components that require operator judgement. For example, in the data there existed a large proportion of auditory and eye blink artefacts as the TMS coil was positioned directly over the DLPFC, which is very close to the ears, and muscles in the scalp and eyes. This meant we had to exclude large amounts of data to remove artefacts, and possibly means that as a result we did not see the full breadth of the neural response, and thus were unable to support our hypothesis that iTBS would significantly increase TEP amplitudes compared to sham. Furthermore, while we had participants listen to white noise whilst recording TEP responses, as the coil was so close to the ear, it is very likely that they could still hear the coil fire. To have the white noise loud enough to the point of completely blocking out the noise of the coil firing would likely have very uncomfortable for the participants.

This loss of data might have resulted in us not being able to observe the full effect of iTBS on the TEPs, explaining why we saw such little difference between sham and iTBS TEP amplitudes. One way to control for this issue in future studies would be to use other neurophysiological measures in addition to EEG, such as fMRI. A paired EEG/rFMRI protocol

could capture and record both EEG events, and localise areas of increased blood flow associated with these events simultaneously (Allen, Josephs, & Turner, 2000).

4.2.5 Uncontrolled physiological variables

A variety of physiological variables could not be adequately controlled for, and might have affected the results obtained. This physiological variability might have been a reason as to why we did not see improved performance on the N-Back and TMT after iTBS compared to sham as hypothesised. For example, if participants were well rested and alert at one session and not another, they would likely have performed better regardless of condition. Likewise, participants who are well rested are more susceptible to the effects of rTMS, and meaning that if participants were, by and large, not well rested during the iTBS sessions, then that could explain why the iTBS condition did not report a greater response as predicted to the intervention (Huber et al., 2012). In future research, extraneous variables such as sleep could also be considered as a factor statistically, by having participants fill out a sleep diary in the days preceding stimulation to collect relevant data regarding sleep quality, and quantity, in the week leading up to their testing.

The experiment took place in the afternoon to try to control for cortisol levels, as cortisol has, been shown to affect a subject's susceptibility to plasticity as elicited by rTMS (Sale, Ridding, & Nordstrom, 2008). However, this does not necessarily mean that cortisol levels were completely controlled for; to most effectively control for cortisol, cortisol levels would have to have been tested by saliva swabs at each TMS session. The results of these cortisol readings would then have had to be factored into the statistical analyses.

Another physiological variable that could have affected susceptibility to plasticity and performance was the presence of alcohol or caffeine as the presence of such

substances were not controlled between participants. The ingestion of these substances could have affected task performance, susceptibility to plasticity, or both (Kähkönen, Wilenius, Nikulin, Ollikainen, & Ilmoniemi, 2003; Kähkönen, & Wilenius, 2007; Murd, Aru, Hiio, Luiga, & Bachmann, 2010; Cerqueira, De Mendonça, Minez, Dias, & De Carvalho, 2006). In future studies, participants could also be asked to avoid alcohol and caffeine for a specific period leading up to testing to try and avoid any effect that these substances may have on plasticity and performance. While such measures would be highly demanding for participants, in theory, they would be important in ensuring validity of the results of experiments.

4.2.6 Lack of precise neuroimaging techniques

Without advanced neuroimaging techniques, it is not possible to precisely locate, and completely ensure stimulation of, the DLPFC (Rusjan et al., 2010). This means that it is not possible with the method used here to be completely sure that we were effectively inducing plasticity in the DLPFC as the rTMS coil might not have been placed in the correct position on the scalp. This might mean that iTBS might not have been directly stimulating the DLPFC as is necessary to induce the plastic change we expected to see. This contrasts with studies concerning the motor cortex, in which an immediate physiological response to TMS, the MEP, can be easily and directly observed and measured. This difficulty in ensuring stimulation of the DLPFC makes it more difficult to directly study in comparison to the motor cortex. If the DLPFC was not subjected to iTBS due to imprecise neuroimaging techniques, this might have been a reason as to why we did not see increased TEP amplitudes iTBS condition compared to sham as hypothesised.

4.3 Limitations

4.3.1 Culturally biased measures

Another possibility is that the effects of performance on both the N back and TMT could have been biased by participants born overseas with English as a second language. In this study, a large proportion (36%) of the 14 participants were born overseas with English not being their primary language. The N-back used letters of the English alphabet, whilst the TMT task used a combination of letters and numbers. For people with English as a second language, this could have made the tasks too difficult to be improved via rTMS, as there is a ceiling effect to the extent to which performance can be improved, if a task is too demanding, as the participants would have to operate and engage the relevant neural mechanisms to complete the task at a very high level (Hoy et al., 2013; Hoy et al., 2016). This might have resulted in participants with English as a second language not having their performance as effectively modulated as participants whose primary language is English as the task was too difficult to be effectively affected by rTMS.

Other studies that measure cognitive flexibility and working memory used participants with English as their first language, and this further differentiates past samples from the one used here. Overwhelmingly, in measuring cognitive flexibility in working memory the TMT and N-back tasks have been successfully used as measures (Moser et al., 2002; Hoy et al., 2016). However, both these tasks contained elements that assumed an English-speaking background (Dugbartey, Townes, & Mahurin, 2000; Chan et al., 2008). The TMT requires an innate memory of the alphabet, while the N back used letters as the symbols to remember, assuming a natural knowledge of the letters positioning on the western keyboard. This assumption cannot be made of participants with English as a second

language backgrounds, and as such, this compromises the validity of the result obtained. To compensate for this limitation, future studies could use adapted versions of the TMT and N back that use more universally recognised symbols. This could perhaps mean using an N back with numbers instead of letters, or by using culturally generic symbols such as shapes or colours. Likewise, a culturally neutral equivalent of the TMT exists using colours could be used instead of the TMT (Dugbartey et al., 2000). This will allow researchers to test participants from a broader variety of cultural backgrounds.

4.3.2 Small sample size and low power

This study suffered several key limitations which compromise the extent to which the results reflects its chosen sample and population. The most significant of these limitations is its small sample size and low statistical power. Many other studies in this field report similar issues, presumably this is a fundamental reason why most studies report trends as a result of rTMS rather than significant results (Jorge et al., 2004; Boggio et al., 2005; Hausmann, et al., 2004). The effects of iTBS effect may have been too mild to observe on tests with more demanding cognitive loads, such as the TMT B and 3-Back, in comparison to the easier TMT A and 2-Back which reported mild improvements as a result of iTBS. Furthermore, rTMS can be highly variable in its effects from person to person, and it is possible that the sample recruited were persons who specifically do not respond to rTMS (Ridding, & Rothwell, 2007). To control for this variability between participants, a larger sample would be needed, however it is difficult to recruit larger samples, due to the resource intensive nature of rTMS protocol. Furthermore, several participants could not participate or complete their participation due to reported discomfort during the rTMS procedure, or due to the strict nature of the medical screening.

4.3.3 Time between sessions was uncontrolled

Furthermore, while there was a minimum seven days between sham and real protocols, there is no upper limit for this timeframe; meaning that some participants had seven days between sessions, while others had closer to a month between sessions. While the order in which participants were subjected to either sham or iTBS was randomised and controlled, the degree to which participants had recently completed the tasks was not properly controlled for. This means that participants who completed the sessions within the minimum seven-day period could have worked out strategies in how to most effectively complete the tasks, specifically the N-back in preparation for the second session, as the nature of the tests was still fresh in the minds. This means that these participants, would have likely scored higher on their second testing session, regardless of which condition they were assigned to, in comparison to participants who had close to a month pass between sessions. To prevent this in future studies, time frames between testing sessions should use both upper and lower limits to protect against some participants having this familiarity, and thus control for this effect.

4.3.4 Significant difference in N100 baseline TEPs

We observed significant differences between sham and iTBS conditions for baseline N100 TEP components, with the iTBS condition reporting the more negative baseline. Increased inhibition as a result of significantly more negative N100 peaks might induce a ceiling effect, reducing the efficacy of iTBS to induce plastic change in the brain (Nikulin, Kičić, Kähkönen, & Ilmoniemi, 2003; Bender et al., 2005). The implication of this ceiling effect means that before iTBS, N100 amplitudes were already as strongly negative as they could be in the iTBS condition, which likely resulted in a weaker effect as engagement of the

N100 related neural components were already operating at their maximum potential. This means that the condition with the more negative N100 peak would be less susceptible to induced plastic change (Nikulin et al., 2003; Bender et al., 2005). This might mean that the difference in N100 amplitudes were greater between sham and iTBS conditions because of this confounding variable, rather than due to truly modulated change because of the iTBS intervention. This would possibly explain why the results saw such a strong relationship between changes in N100 amplitudes and 3-Back performance, in comparison to the N40 and P60 components.

4.3.5 Inadequate sham

Furthermore, while a sham coil was used it did not accurately replicate the tactile sensation of real stimulation. This might have meant that participants knew they were receiving sham stimulation, compromising the ability of participants to remain blind to the study's procedure and aims. The inadequate sham might have unconsciously biased these participants to score better on real sessions rather than sham sessions due an experimenter effect. This is a fundamental weakness in all rTMS research, as there is no sham technique that accurately replicates the tactile sensations of real stimulation without inducing some degree of cortical modulation (Lisanby, Gutman, Luber, Schroeder, & Sackeim, 2001; Loo et al., 2000).

4.4 Broader implications

Fundamentally, this study suffered from a small sample size; this makes it difficult to generalise its results on the broader discussions that surround iTBS and its effectiveness in inducing plastic change, and enhancing working memory and cognitive flexibility performance. While there is considerable evidence supporting the use of rTMS to improve

motor function in stroke patients, and to improve mood for patients with drug resistant depression, the results comparing the effects of sham and iTBS on task performance do not support the use of iTBS as a therapeutic tool to improve working memory and cognitive flexibility. The evidence suggests that the effects of iTBS and rTMS are generally more potent in clinical populations as opposed to healthy nonclinical populations, as investigated here (Brunoni & Vanderhasselt, 2014). To validate iTBS as a possible means of treatment, this claim would have to be investigated further in future studies by testing and comparing the effects of rTMS on clinical and nonclinical conditions.

In exploring the extent to which different populations respond, or do not respond, to iTBS, both clinical and nonclinical, young and old, its use in a clinical tool becomes better evaluated and understood. The effects of rTMS are very variable, and its application has different effects on different people; sometimes improving performance, sometimes making no difference, and sometimes decreasing performance (Ridding, & Rothwell, 2007). In this study, we saw a mix of all three of these effects. Given that so many external variables contribute to susceptibility to plastic change, it will take many more years of study before iTBS would likely see use clinical use to improve cognition and memory in either psychiatric patients, or patients with organic brain damage.

4.5 Future directions

This study is a building block within the literature, as it is a platform that future studies can build upon to answer questions raised by its results. Fundamentally, the study needs to be replicated with a larger sample. There is strong evidence that rTMS does induce plastic change which can have significantly meaningful effects on behavioural performance.

However, the power of this effect is, at best, very mild and would likely require a considerably larger sample size to see such results.

Future research could examine rTMS more specifically as a tool to modulate plasticity to improve cognition and memory in these clinical populations. The literature suggests that clinical participants are more susceptible to the effects of rTMS than healthy participants (Brunoni & Vanderhasselt, 2014). Such research could be done in conjunction with other treatments, such as drug treatments, to explore the extent to which rTMS satisfactorily functions as a clinical tool, and under what therapeutic contexts and combinations it is most effective in improving cognitive function. Furthermore, unlike the vast majority of other studies, this study used a particularly young sample, with the oldest participant being 29 years old. The effectiveness of rTMS on different age groups is not well understood within the literature, and this gap could be explored in future research (Jorge & Robinson, 2011).

4.6 Concluding Comments

The current study aimed to investigate the extent to which iTBS modulates both excitability in the DLPFC, and behavioural performance on tasks that measure working memory and cognitive flexibility; constructs regulated by the DLPFC. Most importantly, the current study aimed to observe what relationship, if any, existed between changes in the physiological and psychological measures. The results provide an insight into the effects of iTBS on the DLPFC and how iTBS affects performance on both N-back in TMT. Contrary to much of the literature, we did not find a substantial effect on TEP amplitudes, and only mild changes in some of the behavioural tasks. While there are other studies that suggest that

iTBS can substantially affect cortical excitability, and both these measures, we cannot support with confidence such claims based on the results attained.

As such, these results provide evidence that iTBS, as a means to modulate TEPs in the DLPFC and improve behavioural performance, is likely not effective, or at best, is very mild, in its effects on the population represented here. By understanding when iTBS does not work, as well as when it does work, will help both researchers and clinicians alike, to better screen for and identify subjects who will more readily respond to its effects. This is a fundamental step in the evaluation of any clinically meaningful medical or psychological intervention.

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Appendices (A-E)

Appendix A : Ethics Approval

THE UNIVERSITY of ADELAIDE

RESEARCH SERVICES
OFFICE OF RESEARCH ETHICS, COMPLIANCE AND INTEGRITY

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1 May 2017

Dr B Hordacre
School of Medicine

Dear Dr Hordacre

PROJECT NO: H-2015-172
Does motor reserve affect functional impairment induced with non-invasive brain stimulation in healthy adults to model stroke?

Thank you for the amendment request and revised ethics application provided my Professor Ridding dated 31.3.17. On behalf of the Human Research Ethics Committee I have approved the request to include: stimulation to the dorsolateral prefrontal cortex; a motor function test; and two Honours students as investigators as detailed in the submitted application.

The ethics expiry date for this project is: 31 August 2018

Participants in the study are to be given a copy of the Information Sheet and the signed Consent Form to retain. It is also a condition of approval that you **immediately report** anything which might warrant review of ethical approval including:

- serious or unexpected adverse effects on participants,
- previously unforeseen events which might affect continued ethical acceptability of the project,
- proposed changes to the protocol; and
- the project is discontinued before the expected date of completion.

Yours sincerely

[redacted]

Appendix B : Transcranial Magnetic Stimulation (TMS) Safety Screen

Transcranial Magnetic Stimulation† (TMS) Adult Safety Screen

Name:
Date:
Age:

Please answer the following:

Do you have epilepsy or have you ever had a convulsion or a seizure?	❍Yes	❍No
Have you ever had a fainting spell or syncope? If *yes*, please describe in which occasions in the space provided below.	❍Yes	❍No
Have you ever had severe (i.e., followed by loss of consciousness) head trauma?	❍Yes	❍No
Do you have any hearing problems or ringing in your ears?	❍Yes	❍No
Are you pregnant or is there a chance you might be?	❍Yes	❍No
Do you have cochlear implants?	❍Yes	❍No
Do you have an implanted neurostimulator? (e.g., DBS, epidural/subdural, VNS)	❍Yes	❍No
Do you have a cardiac pacemaker or intracardiac lines or metal in your body	❍Yes	❍No
Do you have a medication infusion device?	❍Yes	❍No
Are you taking any medications? *(Please list)*	❍Yes	❍No
Have you had a surgical procedure to your spinal cord?	❍Yes	❍No
Do you have spinal or ventricular derivations?	❍Yes	❍No
Did you ever undergo TMS in the past?	❍Yes	❍No
Did you ever undergo MRI in the past?	❍Yes	❍No

Subject signature:	
Experimenter name:	**Signature:**

*If you answered **yes** to any of the above, please provide details (use reverse if necessary):*

_____
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Appendix C : Participant information sheet

Participant Information Sheet

Does Motor Reserve Affect Post-Stroke Impairment?

Ethics approval number: (H-2015-172)
Principle Investigator: Brenton Hordacre

This information sheet is intended to provide you with sufficient information to make an informed decision about participating in this study. If there is any aspect that is not clear to you, please discuss this with one of the investigators.

Background of the Study
The human brain is capable of undergoing reorganisations throughout life. This ability is the basis for modification in human performance and behaviour. Recovery from brain injuries such as stroke relies heavily upon this ability of the brain to reorganise, allowing stroke survivors to regain function and improve performance.

However, some patients show greater capacity to compensate and recover following a stroke. Variable patterns of recovery may be related to individual differences in brain networks. Those patients with greater brain network function may have more 'reserve' pathways to compensate and recover following brain injuries such as stroke.

We can utilise brain stimulation techniques that allow us to induce safe, short lasting, reorganisations that are similar to those seen during stroke. One such technique is Theta Burst Stimulation. This experiment will measure brain reserve before and after Theta Burst Stimulation to determine if brain reserve is an important factor contributing to recovery from injuries such as stroke. We will also determine how movement is related to brain reserve by assessing hand function before and after Theta Burst Stimulation.

Why am I being invited to participate?
We are currently seeking healthy adults aged 18-50 years with no history of neurological or musculoskeletal impairments or injuries.

What will the study involve?
Three experiments will be conducted in the NeuroPAD Laboratories in the Norwich Centre (77 King William Road, North Adelaide – opposite the Women's and Children's Hospital). During each session you will be seated on a comfortable chair. Each experimental process will take approximately 2 hours. You will be reimbursed for your time at $15/ hour.

Measures of Brain Reserve
Questionnaires and Measures of Brain Function
At the first session, participants will complete standardised assessments of brain function which are thought to reflect brain reserve. These assessments include determining number of educational years, the Lifetime Experience Questionnaire and the Cognitive Reserve Index Questionnaire. These questionnaires measure history of occupation, education, sports, hobbies and recreation. This session will also include use of the Cambridge Neurocognitive

Testing Automated Battery (CANTAB). The CANTAB is a computer based assessment of memory, attention and problem solving.

Recording Brain Activity
At the second, third, fourth and fifth sessions, electroencephalography (EEG) will be used to measure electrical brain activity. This will be recorded using a flexible EEG cap. In order to obtain a good signal, a conductive gel will be applied to the skin beneath each EEG sensor and a Q-tip will be used to clean the skin surface.

Brain Stimulation
Recording from hand muscles
During the study we will make electromyographic (EMG) recordings from a hand muscle. The EMG activity will be measured by sticking small disc electrodes to the skin with sticky tape.

Transcranial Magnetic Brain Stimulation (TMS)
TMS is a technique that employs a magnetic field to activate the brain. A coil is held over the scalp by the experimenter and a brief current pulse flows through the coil. This in turn generates a magnetic field that activates the brain beneath the coil. When positioned over the part of the brain which controls the hand muscles, the opposite hand will twitch. These responses are recorded with electrodes taped to the skin overlying the muscles. The technique of TMS is painless and non-invasive. It has been in use for more than 15 years and is used routinely to investigate the motor system.

Repetitive transcranial magnetic stimulation (Theta Burst Stimulation)
rTMS is a modification of conventional TMS employing trains of stimuli at a range of frequencies. International guidelines have been established for its safe use. The form of rTMS we will be using in this experiment is called Theta Burst Stimulation. This stimulation is painless and we have had no adverse effects to its use in previous studies.

As a matter of policy we exclude any persons for our study who have a history epilepsy or stroke, or who have metal implants in the skull, or cardiac pacemakers. If you have any of these, please inform the investigators prior to commencing the study. If you have any doubts about whether you should participate, please discuss them with one of the investigators. Additionally, you will be required to complete a safety questionnaire that will identify any possible contraindications for the use of either TMS or rTMS

Assessment of Hand Function
In two sessions, three hand movement assessments will be performed as a measure of change in function following brain stimulation. These assessments will include a customised grip-lift task, a standard assessment of fine hand movement (the Perdue Pegboard Test) and a motor learning task involving making thumb movements as fast as possible. These tasks should take no longer than 20 min to complete.

Assessment of cognitive function
In two of the sessions cognitive function will be assessed by using a short-term memory test (n-back test) and a test that examines attention and planning (trail making test).

What are the risks?
We wish to make it clear that although these techniques are used both diagnostically and in research laboratories around the world, all experiments involve a small but finite risk. Very

occasionally it has been reported by other groups that subjects may experience a mild and transient headache after TMS. In our experience this is very rare. It is our policy to exclude any subjects with cardiac pacemakers, metal implants in the skull or a history of stroke or epilepsy.

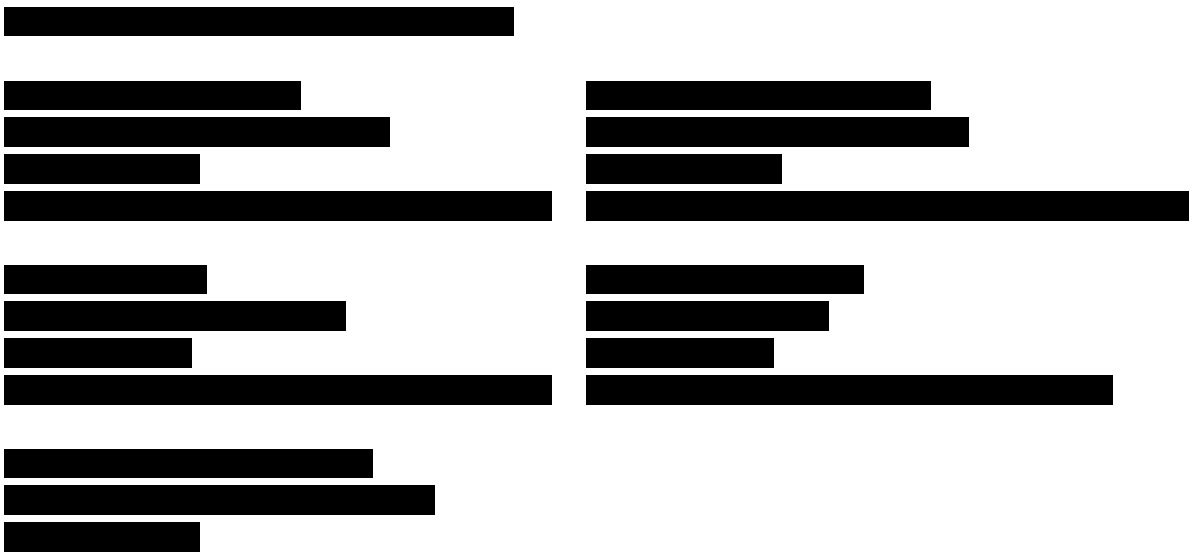
What are the benefits of the research project?
There are no direct benefits to participants in this study. Findings may result in improved rehabilitation approaches for stroke survivors in the future.

Confidentiality
All participants' details will remain confidential except as required by law. Information will be confidentially stored on password protected university computers, and only accessed by members of the research team. Although we plan to publish the results of this study, participants will only be identified by a participant number.

You are free to withdraw from this study at any time without having to explain your reasons for doing so.

Who do I contact if I have questions about the study?
You can contact any member of the research team via email or phone number (please see contact details at the end of the information sheet)

What if I have a complaint or any concerns?
The study has been approved by the Human Research Ethics Committee at the University of Adelaide (approval number H-2015-xxx). If you have questions or problems associated with the practical aspects of your participation in the project, or wish to raise a concern or complaint about the project, then you should consult the Principal Investigator. Contact the Human Research Ethics Committee's Secretariat on phone +61 8 8313 6028 or by email to hrec@adelaide.edu.au. if you wish to speak with an independent person regarding concerns or a complaint, the University's policy on research involving human participants, or your rights as a participant. Any complaint or concern will be treated in confidence and fully investigated. You will be informed of the outcome. (Also see attached Independent Complaints Procedure document)



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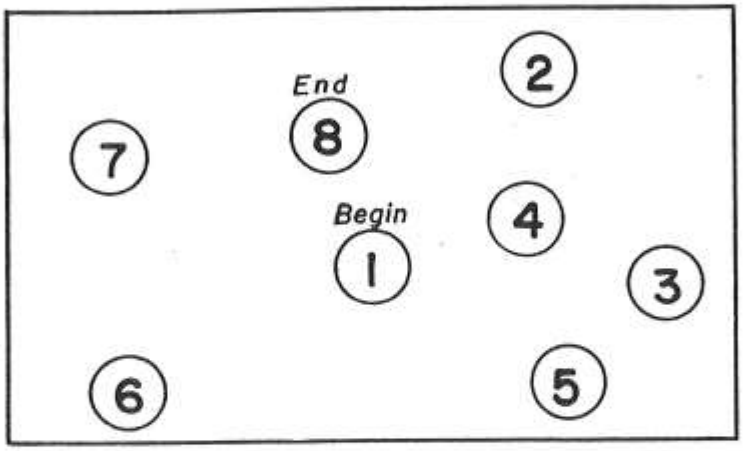
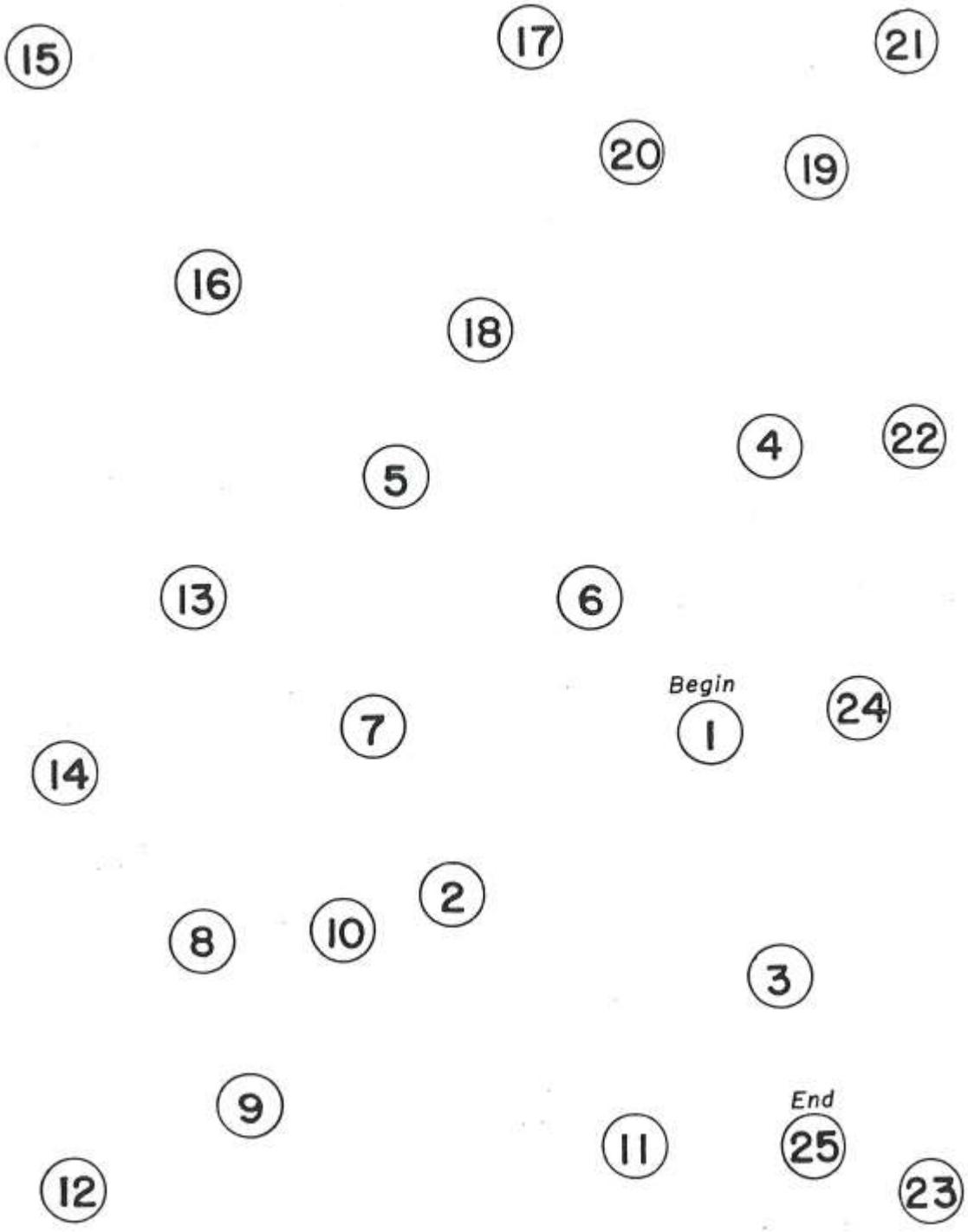
Appendix D : Trail Making Tasks A and B

TRAIL MAKING

Part A

SAMPLE

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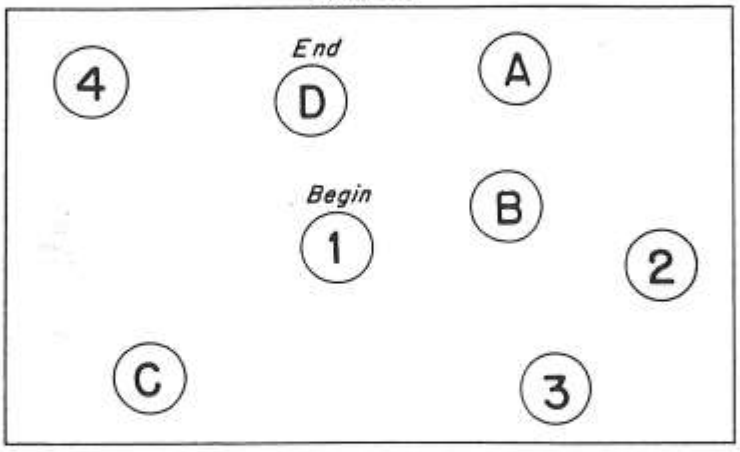
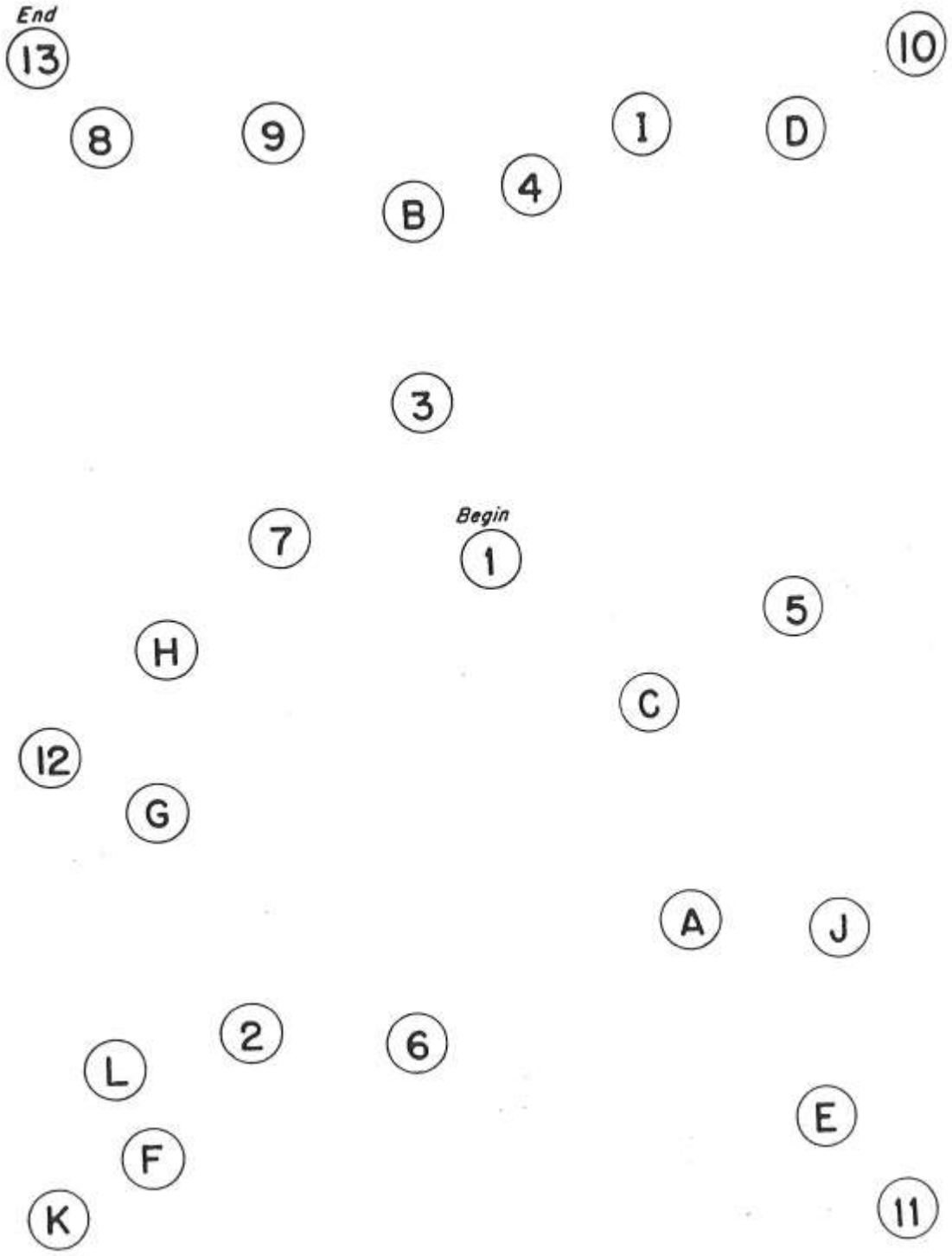
TRAIL MAKING

Part B

SAMPLE




End
13
10
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Appendix E: NeuroPAD Standard form

[redacted]

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Generic Participant Information

Instructions: If this is the first experiment you have undertaken at NeuroPAD, please complete all sections 1 and 2. If you have previously undertaken an experiment at NeuroPAD and completed this form, please complete section 2 only.

Section 1

Handedness: ☐ Right ☐ Left Sex: ☐ Male ☐ Female

Current height?_____ Current weight?_____

Date of Birth:_____

Highest level of education: ☐ High school year 10 or less ☐ High school year 11/12
☐ TAFE/Apprenticeship ☐ Undergraduate ☐ Postgraduate

Number of education years:_____

Currently employed ☐ Yes ☐ No If yes, how many hours per week?_____

Job description: ☐ Managers & Administrative ☐ Professional ☐ Para-Professional
☐ Tradesperson ☐ Clerks ☐ Salesperson
☐ Plant & Machine Operator ☐ Labourer & related worker
☐ Other/home duties ☐ Unknown

Household income: ☐ $0-$40,000 ☐ $40,001-$60,000 ☐ $60,001-$80,000
☐ $80,001-$100,000 ☐ $100,000+

What was your birth gestational age (weeks)?_____

What was your birth weight?_____

What was your home address at time of birth?_____

What is your current home address?_____

[redacted]

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Section 2

Mode of transport to Neuropad today? ☐ Car ☐ Public transport ☐ Walk ☐ cycle ☐ Other

Days since last alcoholic beverage?_____

How many coffees or caffeinated drinks today?_____

Time of last coffee or caffeinated drink?_____

Do you currently smoke tobacco? ☐ Yes ☐ No

If yes, what is pack/year history (number packs per day x years smoked)?_____

How often do you watch TV: _____ (hours per day)

_____ (hours per week)

How often do you use a computer: _____ (hours per day)

_____ (hours per week)

How often do you use social media on your phone: _____ (hours per day)

_____ (hours per week)

How often do you use social media on a computer: _____ (hours per day)

_____ (hours per week)

How often do you read print (eg newspaper, book): _____ (hours per day)

_____ (hours per week)

How often do you read online: _____ (hours per day)

_____ (hours per week)

Research Use Only Below This Line

Participant ID: _____ Study Name/ID:_____

Session Number (If Applicable):_____

Experimental Participant ID (If Different To Lab Participant ID):_____

Date of testing:_____ Time:_____

TMS safety screen: ☐ completed ☐ safe to participate

Physical Activity (IPAQ)	☐ completed	Saliva sample	☐ completed
Perceived Stress (PSS)	☐ completed	Sleep quality (PSQI)	☐ completed

Perceived Stress Scale

The questions in this scale ask you about your feelings and thoughts **during the last month**. In each case, you will be asked to indicate by circling *how often* you felt or thought a certain way.

Name _____ Date _____

Age _____ Gender (*Circle*): **M F** Other _____

0 = Never 1 = Almost Never 2 = Sometimes 3 = Fairly Often 4 = Very Often

1. In the last month, how often have you been upset because of something that happened unexpectedly?	0	1	2	3	4
2. In the last month, how often have you felt that you were unable to control the important things in your life?	0	1	2	3	4
3. In the last month, how often have you felt nervous and "stressed"?	0	1	2	3	4
4. In the last month, how often have you felt confident about your ability to handle your personal problems?	0	1	2	3	4
5. In the last month, how often have you felt that things were going your way?	0	1	2	3	4
6. In the last month, how often have you found that you could not cope with all the things that you had to do?	0	1	2	3	4
7. In the last month, how often have you been able to control irritations in your life?	0	1	2	3	4
8. In the last month, how often have you felt that you were on top of things?	0	1	2	3	4
9. In the last month, how often have you been angered because of things that were outside of your control?	0	1	2	3	4
10. In the last month, how often have you felt difficulties were piling up so high that you could not overcome them?	0	1	2	3	4

Please feel free to use the *Perceived Stress Scale* for your research.

Mind Garden, Inc.
info@mindgarden.com
www.mindgarden.com

References

The PSS Scale is reprinted with permission of the American Sociological Association, from Cohen, S., Kamarck, T., and Mermelstein, R. (1983). A global measure of perceived stress. *Journal of Health and Social Behavior, 24*, 386-396.

Cohen, S. and Williamson, G. Perceived Stress in a Probability Sample of the United States. Spacapan, S. and Oskamp, S. (Eds.) *The Social Psychology of Health*. Newbury Park, CA: Sage, 1988.

Subject's Initials_____ ID#_____ Date_____ Time_____ AM PM

PITTSBURGH SLEEP QUALITY INDEX

INSTRUCTIONS:
The following questions relate to your usual sleep habits during the past month only. Your answers should indicate the most accurate reply for the majority of days and nights in the past month. Please answer all questions.

1. During the past month, what time have you usually gone to bed at night?

   BED TIME _____

2. During the past month, how long (in minutes) has it usually taken you to fall asleep each night?

   NUMBER OF MINUTES _____

3. During the past month, what time have you usually gotten up in the morning?

   GETTING UP TIME _____

4. During the past month, how many hours of actual sleep did you get at night? (This may be different than the number of hours you spent in bed.)

   HOURS OF SLEEP PER NIGHT _____

For each of the remaining questions, check the one best response. Please answer all questions.

5. During the past month, how often have you had trouble sleeping because you . . .

a) Cannot get to sleep within 30 minutes

Not during the past month_____	Less than once a week_____	Once or twice a week_____	Three or more times a week_____

b) Wake up in the middle of the night or early morning

Not during the past month_____	Less than once a week_____	Once or twice a week_____	Three or more times a week_____

c) Have to get up to use the bathroom

Not during the past month_____	Less than once a week_____	Once or twice a week_____	Three or more times a week_____

d) Cannot breathe comfortably

Not during the past month_____ Less than once a week_____ Once or twice a week_____ Three or more times a week_____

e) Cough or snore loudly

Not during the past month_____ Less than once a week_____ Once or twice a week_____ Three or more times a week_____

f) Feel too cold

Not during the past month_____ Less than once a week_____ Once or twice a week_____ Three or more times a week_____

g) Feel too hot

Not during the past month_____ Less than once a week_____ Once or twice a week_____ Three or more times a week_____

h) Had bad dreams

Not during the past month_____ Less than once a week_____ Once or twice a week_____ Three or more times a week_____

i) Have pain

Not during the past month_____ Less than once a week_____ Once or twice a week_____ Three or more times a week_____

j) Other reason(s), please describe_____

_____

How often during the past month have you had trouble sleeping because of this?

Not during the past month_____ Less than once a week_____ Once or twice a week_____ Three or more times a week_____

6. During the past month, how would you rate your sleep quality overall?

Very good _____

Fairly good _____

Fairly bad _____

Very bad _____

7. During the past month, how often have you taken medicine to help you sleep (prescribed or "over the counter")?

Not during the past month_____	Less than once a week_____	Once or twice a week_____	Three or more times a week_____

8. During the past month, how often have you had trouble staying awake while driving, eating meals, or engaging in social activity?

Not during the past month_____	Less than once a week_____	Once or twice a week_____	Three or more times a week_____

9. During the past month, how much of a problem has it been for you to keep up enough enthusiasm to get things done?

No problem at all _____

Only a very slight problem _____

Somewhat of a problem _____

A very big problem _____

10. Do you have a bed partner or room mate?

No bed partner or room mate _____

Partner/room mate in other room _____

Partner in same room, but not same bed _____

Partner in same bed _____

If you have a room mate or bed partner, ask him/her how often in the past month you have had . . .

a) Loud snoring

Not during the past month_____	Less than once a week_____	Once or twice a week_____	Three or more times a week_____

b) Long pauses between breaths while asleep

Not during the past month_____	Less than once a week_____	Once or twice a week_____	Three or more times a week_____

c) Legs twitching or jerking while you sleep

Not during the past month_____	Less than once a week_____	Once or twice a week_____	Three or more times a week_____

d) Episodes of disorientation or confusion during sleep

Not during the past month_____	Less than once a week_____	Once or twice a week_____	Three or more times a week_____

e) Other restlessness while you sleep; please describe_____

_____

Not during the past month_____	Less than once a week_____	Once or twice a week_____	Three or more times a week_____

Buysse DJ, Reynolds CF, Monk TH, Berman SR, Kupfer DJ: Psychiatry Research, 28:193-213, 1989.

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

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

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

PART 1: JOB-RELATED PHYSICAL ACTIVITY

The first section is about your work. This includes paid jobs, farming, volunteer work, course work, and any other unpaid work that you did outside your home. Do not include unpaid work you might do around your home, like housework, yard work, general maintenance, and caring for your family. These are asked in Part 3.

1. Do you currently have a job or do any unpaid work outside your home?

   ☐ Yes

   ☐ No → *Skip to PART 2: TRANSPORTATION*

The next questions are about all the physical activity you did in the **last 7 days** as part of your paid or unpaid work. This does not include traveling to and from work.

2. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, heavy construction, or climbing up stairs **as part of your work**? Think about only those physical activities that you did for at least 10 minutes at a time.

   _____ **days per week**

   ☐ No vigorous job-related physical activity → *Skip to question 4*

3. How much time did you usually spend on one of those days doing **vigorous** physical activities as part of your work?

   _____ **hours per day**
   _____ **minutes per day**

4. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads **as part of your work**? Please do not include walking.

   _____ **days per week**

   ☐ No moderate job-related physical activity → *Skip to question 6*

5. How much time did you usually spend on one of those days doing **moderate** physical activities as part of your work?

_____ **hours per day**
_____ **minutes per day**

6. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time **as part of your work**? Please do not count any walking you did to travel to or from work.

_____ **days per week**

☐ No job-related walking → *Skip to PART 2: TRANSPORTATION*

7. How much time did you usually spend on one of those days **walking** as part of your work?

_____ **hours per day**
_____ **minutes per day**

PART 2: TRANSPORTATION PHYSICAL ACTIVITY

These questions are about how you traveled from place to place, including to places like work, stores, movies, and so on.

8. During the **last 7 days**, on how many days did you **travel in a motor vehicle** like a train, bus, car, or tram?

_____ **days per week**

☐ No traveling in a motor vehicle → *Skip to question 10*

9. How much time did you usually spend on one of those days **traveling** in a train, bus, car, tram, or other kind of motor vehicle?

_____ **hours per day**
_____ **minutes per day**

Now think only about the **bicycling** and **walking** you might have done to travel to and from work, to do errands, or to go from place to place.

10. During the **last 7 days**, on how many days did you **bicycle** for at least 10 minutes at a time to go **from place to place**?

_____ **days per week**

☐ No bicycling from place to place → *Skip to question 12*

11. How much time did you usually spend on one of those days to **bicycle** from place to place?

_____ **hours per day**
_____ **minutes per day**

12. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time to go **from place to place**?

_____ **days per week**

☐ No walking from place to place → ***Skip to PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY***

13. How much time did you usually spend on one of those days **walking** from place to place?

_____ **hours per day**
_____ **minutes per day**

PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY

This section is about some of the physical activities you might have done in the **last 7 days** in and around your home, like housework, gardening, yard work, general maintenance work, and caring for your family.

14. Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, chopping wood, shoveling snow, or digging **in the garden or yard**?

_____ **days per week**

☐ No vigorous activity in garden or yard → ***Skip to question 16***

15. How much time did you usually spend on one of those days doing **vigorous** physical activities in the garden or yard?

_____ **hours per day**
_____ **minutes per day**

16. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, sweeping, washing windows, and raking **in the garden or yard**?

_____ **days per week**

☐ No moderate activity in garden or yard → ***Skip to question 18***

17. How much time did you usually spend on one of those days doing **moderate** physical activities in the garden or yard?

_____ **hours per day**
_____ **minutes per day**

18. Once again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, washing windows, scrubbing floors and sweeping **inside your home**?

_____ **days per week**

☐ No moderate activity inside home → ***Skip to PART 4: RECREATION, SPORT AND LEISURE-TIME PHYSICAL ACTIVITY***

19. How much time did you usually spend on one of those days doing **moderate** physical activities inside your home?

_____ **hours per day**
_____ **minutes per day**

PART 4: RECREATION, SPORT, AND LEISURE-TIME PHYSICAL ACTIVITY

This section is about all the physical activities that you did in the **last 7 days** solely for recreation, sport, exercise or leisure. Please do not include any activities you have already mentioned.

20. Not counting any walking you have already mentioned, during the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time **in your leisure time**?

_____ **days per week**

☐ No walking in leisure time → ***Skip to question 22***

21. How much time did you usually spend on one of those days **walking** in your leisure time?

_____ **hours per day**
_____ **minutes per day**

22. Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like aerobics, running, fast bicycling, or fast swimming **in your leisure time**?

_____ **days per week**

☐ No vigorous activity in leisure time → ***Skip to question 24***

23. How much time did you usually spend on one of those days doing **vigorous** physical activities in your leisure time?

_____ **hours per day**
_____ **minutes per day**

24. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** physical activities like bicycling at a regular pace, swimming at a regular pace, and doubles tennis **in your leisure time**?

_____ **days per week**

☐ No moderate activity in leisure time → ***Skip to PART 5: TIME SPENT SITTING***

25. How much time did you usually spend on one of those days doing **moderate** physical activities in your leisure time?
_____ **hours per day**
_____ **minutes per day**

PART 5: TIME SPENT SITTING

The last questions are about the time you spend sitting while at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading or sitting or lying down to watch television. Do not include any time spent sitting in a motor vehicle that you have already told me about.

26. During the **last 7 days**, how much time did you usually spend **sitting** on a **weekday**?

_____ **hours per day**
_____ **minutes per day**

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

_____ **hours per day**
_____ **minutes per day**

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