No Difference in Motor Cortical Inhibition Between Young and Middle-Aged Adults: A TMS-EEG Study

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Declaration

I declare that this thesis is my own account of my research and contains as its main content work that has not previously been submitted for a degree at any tertiary educational institution.

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Abstract

It is well established that ageing is associated with a decline in manual dexterity. An important neural process for the control of manual dexterity is motor cortical inhibition, which is the process by which neural activity within the motor cortex is suppressed. Reductions in motor cortical inhibition may contribute to the age-related decline in manual dexterity. Paired-pulse transcranial magnetic stimulation (TMS) can be used to measure long-interval intracortical inhibition (LICI) in the motor cortex. Previous literature examining differences in LICI between young and older adults have produced conflicting results. In addition, none have included a middle-aged group of participants. The purpose of the current study was to determine whether there are differences in LICI between young and middle-aged adults. An emerging technique that combines TMS with electroencephalography (EEG) was used to measure LICI. In 12 young and 13 middle-aged participants, the TMS-evoked potential (TEP; recorded from EEG) reflected the motor cortical response to sham TMS, single-pulse TMS, and paired-pulse TMS. The TEPs generated by single- and paired-pulse TMS did not differ between young and middle-aged adults. Therefore, there is no evidence from the current study to suggest differences in motor cortical inhibition between young and middle-aged adults. However, these results are speculative as the TEPs generated by sham and single-pulse TMS were highly similar, suggesting that artefacts heavily influenced the TEPs. It is critical that future studies are able to minimise the artefacts during TMS-EEG recording, and reliably identify and remove artefacts from the EEG data.
No Difference in Motor Cortical Inhibition Between Young and Middle-Aged Adults: A TMS-EEG Study

Between 1996 and 2016 the proportion of the Australian population aged 65 years and over increased from 12.0% to 15.3%, and this group is expected to increase more rapidly over the next decade (Australian Bureau of Statistics, 2016). With advanced age comes a decline in motor control and functioning (Seidler et al., 2010). For example, research in motor control has demonstrated that older adults show longer reaction times (Bedard et al., 2002), reduced coordination (Fujiyama, Hinder, Schmidt, Garry, & Summers, 2012), and reduced manual dexterity (Calautti, Serrati, & Baron, 2001) compared to young adults. Coordination and manual dexterity are necessary for the successful execution of everyday tasks such as holding a pen and writing, or tying a shoelace. Therefore, it is not surprising that age-related deficits in motor control impact daily living, leading to reduced independence and a decrease in life satisfaction (Åberg, Sidenvall, Hepworth, O’Reilly, & Lithell, 2005). Evidence-based interventions that improve motor control in ageing populations are critical to minimise the financial burden placed on aged-care resources, and increase overall wellbeing in older adults. In order to develop these interventions, more research is needed to understand the neural mechanisms behind the age-related decline in motor control.

A number of brain areas contribute to the planning and execution of movement, including premotor areas, the supplementary motor area, and the cerebellum (Seidler et al., 2010). The primary motor cortex (M1), located in the frontal lobe of the brain along the precentral gyrus, is particularly important for the execution of motor plans that enable dexterous movement (Seidler et al., 2010). Neurons in M1 connect to fibres of the corticospinal tract, which descend through the brain stem and down the spine to activate skeletal muscles (Garret, 2003). Every muscle in the body has a representation in M1, and these representations are arranged somatotopically; for example hand
muscle representations are adjacent to the arm muscle representations, which are adjacent to the shoulder muscle representations, and so on (Garret, 2003). The muscles of the hand have a large representation in M1 (Garret, 2003), which is likely one factor that enables the dextrous control of the hands, necessary for tasks such as writing or tying a shoelace. Recent studies on the age-related decline in motor control suggest changes in the neural control of the muscles in M1 may contribute this decline (for a review see: Seidler et al., 2010).

One important neural process for motor control is cortical inhibition. Cortical inhibition is the process by which neural activity in the cortex of the brain is suppressed, mediated by a network of inhibitory neurons (Isaacson & Scanziani, 2011). Intracortical inhibition refers to the activity within the network of inhibitory neurons in M1. An example of intracortical inhibition in M1 is the suppression of excitability of muscles, a mechanism important for the planning and execution of dextrous movement (Sohn & Hallett, 2004).

There is evidence from literature using animal models that cortical inhibition is reduced with age. For example, studies have shown age-related reductions in the number of inhibitory neurons in the brain (Hua, Kao, Sun, Li, & Zhou, 2008), alterations in the molecular composition of inhibitory receptors (Rissman, Nocera, Fuller, Kordower, & Armstrong, 2006), and reduced activity of enzymes that are crucial for inhibitory neurotransmitter production (Ling, Hughes, & Caspary, 2005). Consistent with this evidence from animal studies, changes in the function of inhibitory networks have been reported in human studies using brain imaging and non-invasive stimulation techniques (Levin, Fujiyama, Boisgontier, Swinnen, & Summers, 2014). Importantly, the changes in cortical inhibition observed with age have been shown to be associated with an age-related decline in manual dexterity (Calautti et al., 2001). Despite the large and growing literature investigating how the ageing process modifies
activity of the inhibitory networks, the specific neural changes that occur within M1 across the lifespan are not fully understood.

Transcranial magnetic stimulation (TMS) is a non-invasive brain stimulation technique that can be used to gain information about the excitatory and inhibitory mechanisms of movement control (Barker, Jalinous, & Freeston, 1985). This method involves holding a coil, which is made of copper wire and an insulated outer casing, over the scalp, and delivering a brief, high current electrical pulse through the coil. The electrical pulse generates a magnetic field that passes through the scalp and skull and an induced current flow in the neurons beneath the coil (Hallett, 2000). If the pulse is sufficiently intense, the neurons will depolarise, resulting in action potentials (Barker et al., 1985). A single TMS pulse delivered over the area of the M1 that controls a peripheral muscle can be used to assess excitability: the action potentials result in a response in the muscle known as the motor evoked potential (MEP). The MEP is recorded via electrodes placed on the surface of the skin over the muscle (surface electromyography). The amplitude of the MEP reflects the excitation of neurons in the M1, and is mediated by activity in both the brain and the spinal cord (Barker et al., 1985).

Paired-pulse TMS can be used to assess the inhibitory networks of the M1. When two pulses are delivered through the same coil with an interstimulus interval (ISI) of 100ms, the amplitude of the resulting MEP in the target muscle is reduced compared to the MEP produced by a single pulse (Wassermann et al., 1996). The first pulse activates inhibitory networks in the M1, which are still active when the second pulse is delivered, supressing the MEP (Wassermann et al., 1996). This effect is known as long-interval intracortical inhibition (LICI; Valls-Sole, Pascual-Leone, Wassermann, & Hallett, 1992). Pharmacological studies provide strong evidence that LICI is mediated by inhibitory receptors known as γ-aminobutyric acid type B (GABA_B) receptors.
Evidence suggests that LICI plays a role in the control of voluntary movement. Hammond and Vallence (2007) used paired-pulse TMS to measure LICI in the first dorsal interosseous (FDI) muscle of the hand at rest and during different levels of sustained voluntary abduction force of the index finger. As the level of muscle contraction increased, the magnitude of LICI decreased, suggesting that LICI plays a role in the control of M1 output during sustained contraction (Hammond & Vallence, 2007). To measure task-dependent changes in intracortical inhibition, Kouchtir-Devanne, Capaday, Cassim, Derambure, and Devanne (2012) compared LICI in the FDI muscle during sustained abduction of the index finger to a precision grip of the thumb and index finger. The precision grip is a more complex task requiring muscle coordination, as opposed to a simple finger abduction (Kouchtir-Devanne et al., 2012). LICI was reduced during the precision grip compared to the finger abduction, indicating that the role of LICI varies between functionally meaningful tasks that require different patterns of muscle activation (Kouchtir-Devanne et al., 2012). These results support the functional importance of LICI in the control of manual dexterity, and support the suggestion that a change in these inhibitory networks may contribute to the age-related decline in manual dexterity (Opie, Sidhu, Rogasch, Ridding, & Semmler, 2018).

Paired-pulse TMS has been used to investigate differences in LICI between older adults over the age of 60 and young adults, however the results of these studies are inconsistent. Some studies show a decrease in LICI in older adults compared to young adults. For example, Opie and Semmler (2014) used paired-pulse TMS to compare LICI in the dominant M1 of young and older adults. MEPs were recorded from the relaxed dominant first dorsal interosseous (FDI) muscle in the hand. Their results show that LICI was reduced in the older adults compared to the young adults, suggesting a
decrease in slow intracortical inhibitory networks in the M1 with age (Opie & Semmler, 2014). In a follow up study using paired-pulse TMS, Opie, Ridding, and Semmler (2015) also found reduced LICI in older adults compared to young adults. More recently, a study conducted by Hermans, Levin, et al. (2018) using similar paired-pulse TMS methods found similar results, with reduced LICI in older adults compared to young adults, supporting an age-related decline in intracortical inhibition.

In contrast to these studies showing reduced LICI in older adults than young adults, McGinley, Hoffman, Russ, Thomas, and Clark (2010) found an increase in LICI in older adults compared to young adults, suggesting an age-related increase in intracortical inhibition in the M1. However, in the study by McGinley et al. (2010), MEPs were recorded from the non-dominant flexor carpi radialis muscle in the wrist. The role of the flexor carpi radialis muscle is to stabilise the wrist during hand movements (Salvà-Coll, Garcia-Elias, Llusá-Pérez, & Rodríguez-Baeza, 2011), as opposed to the role of the FDI muscle in fine motor control (Infantolino & Challis, 2010). The different roles of these muscles may contribute to the difference in results found by McGinley et al. (2010) and the studies that measure MEPs from the FDI muscle. Stimulating the M1 of the non-dominant hemisphere may have also contributed to the contrasting results, as there is evidence for an increase in hemispheric asymmetry in LICI in older adults compared to young adults (Vallence, Smalley, Drummond, & Hammond, 2017). However, these suggestions are unlikely to fully explain the inconsistencies in the LICI literature, as in a recent study, Opie et al. (2018) found no differences in LICI between young and older adults when comparing the amplitude of MEPs recorded from the dominant FDI muscle. It is unclear why these results are inconsistent; however, there are two plausible explanations. First, the contribution of subcortical and spinal activity in the MEP elicited by paired-pulse TMS induces variability in the LICI measure. Second, changes in cortical inhibition likely
occur across the lifespan, not just from 60 years of age. Each of these explanations will be discussed in more detail below.

As demonstrated by the current literature, the effects of age on intracortical inhibition in the M1 remain unclear. An important limitation of conventional TMS studies is the nature of the measure, which involves recording MEPs in peripheral muscles (Opie et al., 2018). Although there is evidence that LICI is primarily mediated by cortical networks (Nakamura, Kitagawa, Kawaguchi, & Tsuji, 1997), activity in the spinal cord is a contributing factor in the amplitude of the MEPs, and may be a confounding factor in the measurement of LICI (McNeil, Martin, Gandevia, & Taylor, 2011). In order to overcome this limitation, researchers have combined TMS with electroencephalography (EEG) to directly record the brain’s response to TMS, removing the confound of variations in spinal cord activity (Opie, Rogasch, Goldsworthy, Ridding, & Semmler, 2017).

EEG is a method used to record electrical activity in the brain via electrodes placed on the scalp (Freberg, 2009). EEG can record evoked potentials, which is the common electrical activity of neurons in response to the application of a specific stimulus (Freberg, 2009). The neural activity measured with EEG is very small (on the order of microvolts), however with many trials of a specific stimulus it is possible to record an event-related potential (Freberg, 2009). TMS-EEG involves delivering a TMS pulse to the scalp while concurrently recording the neural response using EEG (Rogasch & Fitzgerald, 2013). TMS results in a highly reproducible evoked potential in EEG recordings known as the TMS-evoked potential (TEP; Casarotto et al., 2010). The TEP is made up of a series of positive and negative peaks occurring up to 300ms after the TMS pulse is delivered to the M1 (Bonato, Miniussi, & Rossini, 2006), which are thought to reflect different processes within the cortex (Rogasch & Fitzgerald, 2013). Figure 1 shows five commonly reported components of the TEP. The components are
named based on their latency in milliseconds after the TMS pulse, and whether their peak amplitude is positive (P) or negative (N). A positive peak at 30ms (P30) is thought to represent a general marker of cortical excitability (Ferreri et al., 2017). Negative peaks at 45ms (N45) and 100ms (N100) are thought to reflect activity of the inhibitory networks in the M1 (Premoli, Castellanos, et al., 2014). The nature of the P60 and P180 components is currently unknown.

![TMS-evoked potential](image-url)

*Figure 1.* A schematic diagram of the TMS-evoked potential. The dotted vertical line represents the application of the TMS pulse. The solid line represents the resulting event-related potential, with three positive components and two negative components occurring between 30 – 180 ms following the TMS pulse. Image used and edited with permission from Honours supervisor Ann-Maree Vallence.

The N100 component of the TEP is thought to specifically reflect the activity of GABA<sub>B</sub> inhibitory receptors (Premoli, Castellanos, et al., 2014), which mediate LICI and contribute to the control of manual dexterity. LICI can be measured using paired-pulse TMS-EEG, whereby the first pulse (known as the conditioning stimulus) recruits the GABA<sub>B</sub> inhibitory networks, which are still active when the second pulse (known as the test stimulus) is delivered, suppressing the TEP (Opie et al., 2017). As a result, the amplitude of the N100 component of the TEP generated by the test stimulus is smaller than the amplitude of the N100 of the TEP generated by single-pulse TMS (Opie et al., 2017). This process reflects intracortical inhibition in M1. It has been suggested that TMS-EEG measures of LICI offer greater selectivity in testing GABA<sub>B</sub> related
inhibition than can be derived from measuring LICI with MEPs (Premoli, Rivolta, et al., 2014).

At the time the current experiment was designed, there was one existing study examining age-related changes in intracortical inhibition using TMS-EEG. Opie et al. (2018) applied single- and paired-pulse TMS to the dominant M1 of young and older adults, while recording the resulting TEPs using EEG, to assess age-related changes in LICI. When comparing the single-pulse TEPs, results showed the amplitude of the N45 component was greater in older adults compared to young adults (Opie et al., 2018). When comparing the paired-pulse TEPs, results showed a greater inhibition of the N100 and P180 components in older adults compared to young adults. These findings using TMS-EEG suggest that the ageing process is associated with an increase in intracortical inhibition, particularly in the GABA_{B} inhibitory network (Opie et al., 2018).

It is likely that changes in intracortical inhibition occur across the lifespan, not just from 60 years of age. Therefore, a limitation of the existing TMS literature on age-related changes in LICI is the lack of a middle-aged group to determine when the onset of these changes occurs. There is evidence of a nonlinear relationship between age and declines in manual dexterity, with the association becoming stronger with increasing age (Smith et al., 1999). It has been suggested that there is a critical period in the 6th decade of life when declines in manual dexterity abruptly worsen (Smith et al., 1999). In addition, studies using neuroimaging techniques suggest that the neural changes that underpin the age-related decline in motor control may occur earlier than the functional changes in manual dexterity performance (Seidler et al., 2010). However, there are currently no TMS studies investigating LICI across the lifespan (for example, by including middle-aged participants) so it remains unknown when age-related changes in LICI occur. Determining the point in the lifespan when age-related changes in LICI begin would have significant implications for the treatment of motor decline: to prevent
the age-related decline in motor control it is important to know the age at which to implement interventions.

Therefore, the aim of the present study was to compare LICI in young and middle-aged adults using TMS-EEG, to determine whether an age-related difference in LICI is evident in middle-aged adults. LICI was assessed by delivering single- and paired-pulse TMS to M1 while recording the resulting TEPs using EEG. The independent variable was the age group (young and middle-aged), and the primary dependent variable was the amplitude of the N100 component of the TEP. Given that the existing TMS-EEG study on age-related changes in LICI suggest the ageing process is associated with an increase in intracortical inhibition (Opie et al., 2018), it was expected that TMS-EEG indices of LICI would be increased in middle-aged adults compared to young adults. Specifically, it was hypothesised that there would be a greater inhibition of the N100 component following paired-pulse TMS in middle-aged adults compared to young adults, reflecting an age-related increase in LICI.

**Method**

**Subjects**

A power analysis was performed for sample size estimation, based on data from Opie et al. (2018), who compared TEPs in young (n = 17) and older (n = 17) adults. The effect size in Opie et al.’s study was considered to be large using Cohen's (1988) criteria. With an alpha = .05, power = .08, and expected effect size (f) = 0.4, the projected sample size needed for the current study was N = 32 to observe differences in TEPs between young and middle-aged adults. Fifty-four participants were recruited. After screening (outlined in detail below) 29 right-handed participants participated in the current study: 16 young adults (11 female; median = 23.44 years, range = 19 – 31 years), and 13 middle-aged adults (10 female; median = 46 years, range = 36 – 52 years). Participants were psychology students from Murdoch University, who received
participation credits as remuneration, and volunteers who were recruited from the wider community. The experiment was performed in accordance with the Declaration of Helsinki and was approved by the Murdoch University Research Ethics Committee (REF 2018-019). All participants gave written informed consent.

**Screening**

Prior to testing, all participants were screened for conditions that would contraindicate TMS. These included the current use of psychoactive medication, for example antidepressants, sedatives, antipsychotics etc., and a history of neurological or psychiatric condition (Rossi, Hallett, Rossini, & Pascual-Leone, 2009). In addition to those who participated in the experiment in full (N = 29), 9 people were screened for conditions that would contraindicate TMS and were excluded from participating (Rossi et al., 2009). The Montreal Cognitive Assessment (MoCA; Nasreddine et al., 2005) was used to screen for mild cognitive dysfunction. The total possible score is 30 points, and a score of 26 or more indicates no cognitive impairment (Nasreddine et al., 2005). The mean MoCA score was 28.14 (SD = 1.33). Two participants scored less than 26 on the MoCA and were therefore excluded from the experiment. Handedness was assessed with the Edinburgh Handedness inventory (Oldfield, 1971), which yields a laterality quotient (LQ) that ranges from -100 (indicating extreme left-handedness) to +100 (indicating extreme right-handedness). The mean LQ was 88.45 (SD = 15.18). Three participants were determined to be ambidextrous or left-handed after completing the Edinburgh Handedness inventory, and were therefore excluded from the experiment.

**Electromyography**

During the experiment, participants were seated in a comfortable chair with a pillow on their lap to rest their hands on, and were asked to keep their eyes open. EMG activity was recorded from the relaxed FDI muscle of the dominant and non-dominant hand using two Ag-AgCl surface electrodes. The electrodes were placed approximately
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2 cm apart, with one electrode over the belly of the FDI muscle and the other over the tendon insertion. A ground electrode common to both recording electrodes was placed over the wrist bone. The EMG signal was amplified (1000 x) and band-pass filtered (high pass = 20 Hz, low pass = 1000 Hz) using a CED1902 (Cambridge Electronic Design, Cambridge, UK), before being digitized at a sampling rate of 2 kHz using a CED 1401 interface (Cambridge Electronic Design).

**Electroencephalography**

EEG data were recorded using a HydroCel Geodesic Sensor Net with 128 electrodes arranged in a 1.0 montage (Electrical Geodesics, Inc., Eugene, USA). EEG data were acquired using an EGI Geodesic EEG System 300 (Electrical Geodesics, Inc.). Signals were amplified (10,000 x), filtered (0.05 – 500 Hz), and digitized at a sampling rate of 1000 Hz before being recorded on a computer for offline analysis. Impedance was checked approximately every 5 minutes throughout the experiment and was adjusted when necessary to be below 50 kΩ (Ferree, Luu, Russell, & Tucker, 2001).

**Transcranial Magnetic Stimulation**

TMS was delivered while the participants were wearing the EEG net. Single- and paired-pulse TMS (monophasic pulse waveform) was administered using a figure-of-eight coil (90 mm diameter) attached to a Magstim BiStim 200² stimulator (Magstim, Whitland, UK). The coil was held so it was lightly resting on the EEG net above the scalp, with the handle pointing backwards and at a 45-degree angle away from the mid-sagittal line. This coil orientation induces a posterior-anterior current flow in the underlying cortex, and is optimal for stimulating the neurons of the motor cortex (Kammer, Beck, Thielscher, Laubis-Herrmann, & Topka, 2001). The coil was placed over C3, and then systematically moved around M1 until the site of optimal stimulation
was found, producing large and consistent responses in the FDI contralateral to stimulation. This location was marked over the EEG net to ensure the same site of stimulation was used throughout the experiment. Resting motor threshold (RMT) was determined with stimuli delivered at the optimal stimulation site, and was defined as the TMS intensity required to evoke MEPs with an amplitude of 50 μV or greater in at least three of five consecutive trials (Rossini et al., 2015), expressed as a percentage of maximum stimulator output (%MSO). The optimal stimulation site and RMT were determined separately for each hemisphere. In addition to those who completed the experiment in full, data collection could not be completed in 11 experimental sessions because participants had a RMT >85% MSO; the decision was made to terminate these sessions as the TMS coil rapidly overheats at intensities over 85% MSO.

**Sham Stimulation**

Sham TMS was included in the present study to allow the identification and removal of artefacts that can contaminate the TMS-EEG recordings (Rogasch et al., 2014). Sham TMS was administered by holding the TMS coil perpendicular to the head so the edge of the wing rested on the EEG net above the optimal stimulation site. In this position, the click of the TMS coil can be heard and some somatosensory from the coil discharge can still be felt, however, it is suggested that the TMS pulse does not induce electrical current flow in the underlying cortex (Rogasch et al., 2014).

**Procedure**

After determining the optimal site of stimulation and RMT, a total of 5 blocks of TMS, each comprising 50 stimuli, were delivered to each hemisphere. These included 2 blocks of single-pulse TMS, 2 blocks of paired-pulse TMS, and 1 block of sham TMS to each hemisphere. The hemisphere that was stimulated first was counterbalanced across participants, and the order of the blocks was randomised within each hemisphere.
Paired-pulse TMS involved delivering a conditioning pulse 100ms before the test pulse. Previous studies have shown this interstimulus interval is optimal for reliable inhibition of the MEP, and TEP in the motor cortex (Farzan et al., 2010). RMT was used as the intensity for all blocks of stimulation; and therefore limited MEPs were elicited during the TMS-EEG recordings. This helped to minimise re-afferent somatosensory feedback from muscle twitches, which can contaminate the EEG signal (Paus, Sipila, & Strafella, 2001). The inter-trial interval between TMS randomly varied from 4 to 6 seconds to reduce anticipation of the next trial.

Each block lasted for approximately 4 minutes, and participants were given a 2-3 minute break between each block of TMS. During the blocks of TMS, participants listened to white noise played through inserted headphones to minimise the auditory evoked potentials that result from the click of the TMS coil (Massimini et al., 2005).

**Manual Dexterity**

Manual dexterity was assessed using the Purdue Pegboard task with standardised instructions for each subtest (Lafayette Instrument). During the peg-moving subtest participants were required to pick up small pegs from a well with one hand, and place them one at a time in a vertical line of small holes on the pegboard (from top to bottom) as quickly as possible. The number of pegs a participant placed in the holes in 30 seconds was recorded. Participants performed the task with the left and right hand, and the hand that was measured first was counterbalanced across participants. During the assembly subtest participants had to place a peg in a hole with their right hand, put a washer on the peg with their left hand, put a collar on the peg with their right hand, and put another washer on the peg with their left hand. This was defined as one assembly, and the participants were asked to make as many assemblies as possible in 60 seconds, keeping both hands moving simultaneously. The number of
individual items placed on the pegboard in 60 seconds was recorded, i.e. one assembly counted as 4 items.

**Data Analysis**

Four of the young participants’ EEG data were contaminated with large amplitude 50Hz noise, and were subsequently removed from analysis. Analysis of EEG data was completed using EEGLAB (Delorme & Makeig, 2004) and the TMS-EEG signal analyser (Rogasch et al., 2017) on the MATLAB platform (R2015b, The Mathworks, USA). Only left (dominant) hemisphere EEG data was analysed in the current study, as the aim of the study was to investigate differences in TEPs in young and middle-aged adults. Data from the right hemisphere contributed to another study investigating hemispheric differences in TEPs in young adults. Data from all left hemisphere blocks were merged into a single file, and specific time-windows (epochs) were extracted from the continuous EEG signal. The epochs consisted of the EEG data that spanned 1000 ms before and after the TMS pulse. The data were baseline corrected -650 to -200 ms before the TMS pulse.

The first step of pre-processing the data was to remove large amplitude artefacts associated with the TMS pulse from each epoch. TMS induces a large artefact in the EEG data, as the high-current pulse from the TMS saturates the EEG electrodes that are designed to record electrical activity, precluding the recording of the true neural signal (Rogasch et al., 2014). In single-pulse trials, the data were cut from -1.5 to 20 ms around the TMS pulse, and in paired-pulse trials the data were cut from -1.5 to 20 ms around the first TMS pulse, and -110 to -50 ms to remove the second TMS pulse artefact. This was performed as the removal of large amplitude artefacts in EEG data is necessary for effective decomposition by independent component analysis (Hernandez-Pavon et al., 2012; Rogasch et al., 2017), which is used in a later stage of pre-processing. The missing sections of data in each epoch were replaced using cubic
interpolation. Data were band-pass filtered (1 – 100 Hz), and notch filtered (50 Hz) to remove electrical interference from the main power supply. An independent component analysis was then performed to identify components relating to TMS pulse decay, blinks and eye movements, auditory-evoked potentials, and muscle activity (Rogasch et al., 2014). The identified components were removed.

In paired-pulse TMS trials, the conditioning (first) pulse generates a TEP, because the conditioning pulse is the same intensity as the test (second) pulse, which also generates a TEP. Therefore, a correction procedure was carried out on paired-pulse trials to remove the TEP that is generated by the conditioning stimulus from the TEP that is generated by the test stimulus. This was achieved by ‘time-shifting’ the TEP generated by single-pulse trials to coincide with the application of the conditioning TMS pulse in the paired-pulse trials, and subtracting the TEP generated by single-pulse trials from the paired-pulse data (Opie et al., 2018). This ‘time-shifting’ and subtraction procedure resulted in a ‘corrected paired-pulse TEP’, as demonstrated by an example from Premoli, Rivolta, et al. (2014) in Figure 2. For all analyses, the corrected paired-pulse TEP was compared to the single-pulse TEP.
Figure 2. Determination of the corrected paired-pulse TEP. The single-pulse TEP (A) was subtracted from the paired-pulse TEP (B) aligned to the time of the conditioning stimulus at -0.1 seconds, resulting in the corrected paired-pulse TEP (C). TS = test stimulus, CS = conditioning stimulus. The dotted vertical lines indicate when the TMS was applied. Reprinted from “Characterization of GABAB-receptor mediated neurotransmission in the human cortex by paired-pulse TMS–EEG”, by I. Premoli et al., 2014, *NeuroImage*, 103, p. 152-162. Copyright 2018 by Elsevier. Reprinted with permission.

The amplitude of five commonly reported TEP components was assessed from the single-pulse and corrected paired-pulse TEP: N100, P30, N45, P60, and P180. The N100 (the primary dependent variable) was identified as the maximum negative peak between 70 and 145 ms following the TMS pulse. The other components were identified by assessing the maximum positive peaks between 20 - 30 ms (P30), 50 - 70 ms (P60) and 160 - 270 ms (P180), and the maximum negative peak between 40 - 60 ms.
(N45). The region of interest from which the TEP components were assessed was C1, C3, FC1 and FC3, which are known electrodes over motor regions in the left hemisphere.

The size of the single-pulse TEP was quantified as the difference between the largest positive peak and the largest negative peak occurring within 25 - 300 ms after the TMS pulse (TEP_{MAX} – TEP_{MIN}) (Opie et al., 2018). LICI was then quantified by expressing the corrected paired-pulse TEP as a percentage of single-pulse TEP size (Opie et al., 2018). For positive peaks (P35, P60 and P180) this was calculated by the following equation:

$$LICI_{Positive \ Peaks} = \frac{(Peak_{Single} – Peak_{Paired})}{(TEP_{MAX} – TEP_{MIN})} \times 100$$

For negative peaks (N45 and N100), this was calculated by the following equation:

$$LICI_{Negative \ Peaks} = \frac{(Peak_{Single} – Peak_{Paired})}{(TEP_{MIN} – TEP_{MAX})} \times 100$$

It is important to note that for negative peaks, the single-pulse TEP size was expressed as TEP_{MIN} – TEP_{MAX} to allow for the positive and negative peaks to be compared on the same scale. For all components, a larger percentage represented greater inhibition (Opie et al., 2017; Opie et al., 2018; Rogasch, Daskalakis, & Fitzgerald, 2013, 2015).

Throughout this study, LICI TEP will refer to the inhibition of the corrected paired-pulse TEP, and LICI_{COMPONENT} will refer to the inhibition of each component of the corrected paired-pulse TEP.

To ensure that any specific TEP differences between young and middle-aged participants were not driven by global differences in cortical excitability, measures of global mean field amplitude (GMFA) were compared between groups (Opie et al., 2018). GMFA is an index of global cortical excitability, and was calculated as the standard deviation of EEG data across electrodes (Komssi, Kähkönen, & Ilmoniemi, 2004). It was then quantified by assessing the area under the GMFA curve for the first 300 ms after the TMS pulse (Opie et al., 2018).
**Statistical Analysis**

To test for age-related differences in RMT, an independent samples *t* test was performed on the left hemisphere RMT of the young and middle-aged participants. To test for age-related differences in global cortical excitability, a mixed model ANOVA was performed on the GMFA area under the curve data, with the within-subjects factor being Condition (Sham, Single-pulse, and Paired-pulse) and the between-subjects factor being Age (young and middle-aged).

To test for differences between the sham and single-pulse TEPs, the sham TMS condition was compared to the single-pulse TMS condition using a two-way repeated measures ANOVA, with within-subjects factors of Condition (sham and single-pulse) and Component (P35, N45, P60, N100, P180). The ANOVA was run separately on young and middle-aged participants.

To test for age-related differences in the single-pulse TEP, a mixed model ANOVA was performed, with the within-subjects factor being Component (P35, N45, P60, N100, P180) and the between-subjects factor being Age (young and middle-aged). To test for age-related differences in LICI, a mixed model ANOVA was performed on the LICI TEP, with the within-subjects factor being LICI Component (LICI\textsubscript{P35}, LICI\textsubscript{N45}, LICI\textsubscript{P60}, LICI\textsubscript{N100}, LICI\textsubscript{P180}) and the between-subjects factor being Age (young and middle-aged).

To test for age-related differences in manual dexterity, a mixed model ANOVA was performed on the unimanual peg-moving subtest scores, with the within-subjects factor being Hand (left and right) and the between-subjects factor being Age (young and middle-aged). An independent samples *t* test was performed on the assembly subtest scores of the young and middle-aged participants. A bivariate Pearson’s product-moment correlation coefficient (*r*) was used to investigate linear relationships between
LICI TEP and manual dexterity. Each LICI component was correlated with the assembly subtest scores, and the right hand peg-moving subtest scores.

All statistical analysis was performed using IBM SPSS Statistics for macOS, Version 23.0 (IBM Corp, Armonk, NY).

**Results**

**Resting Motor Threshold**

Figure 3 presents left hemisphere RMT for young and middle-aged participants. It is clear from the figure that RMT was similar across the age groups. An independent samples t test was used to compare the left hemisphere RMT of the young participants to the left hemisphere RMT of the middle-aged participants. Neither Shapiro-Wilk statistic was significant; indicating the assumption of normality was not violated. Levene’s test was also non-significant, thus equal variances can be assumed. There was no significant difference in RMT between young and middle-aged participants, \( t(27) = 1.17, p = .252, \) two-tailed, \( d = 0.45, 95\% \) CI [-12.16, 3.33].

![Figure 3](image.png)

*Figure 3*. A column scatter graph showing left hemisphere resting motor threshold (RMT) for young and middle-aged participants. Each data point represents an individual participant, and the horizontal lines represent the mean. MSO = maximum stimulator output.

**Global Mean Field Amplitude**
Measures of GMFA were similar between young and middle-aged participants, with greater GMFA in the single- and paired-pulse TMS conditions compared to the sham condition. A mixed model ANOVA was used to investigate the effect of age on the GMFA data in the sham, single-, and paired-pulse TMS conditions. There was a minor violation of normality within the middle-aged paired-pulse GMFA condition, \( W(14) = .838, p = .016 \). Despite this, repeated measures ANOVA is known to be robust enough to withstand such violations in normality and was consequently allowed (Field, 2013). All other conditions were normally distributed. \( F_{\text{max}} = 4.25; \) demonstrating homogeneity of variances, and Mauchly’s test indicated that the assumption of sphericity was not violated.

The ANOVA showed a significant main effect of condition, \( F(2, 46) = 15.34, p < .001, \) partial \( \eta^2 = .400 \). There was no significant main effect of age, \( F(1, 23) = .550, p = .466, \) partial \( \eta^2 = .023 \), and there was no significant interaction between condition and age, \( F(2, 46) = 1.017, p = .370, \) partial \( \eta^2 = .042 \). Pairwise comparisons with a Bonferroni correction revealed that GMFA was significantly higher in the single-pulse TMS condition (\( M = 380.07, SD = 172.77 \)) and the paired-pulse TMS condition (\( M = 315.09, SD = 147.70 \)) compared to the sham condition (\( M = 244.81, SD = 103.15 \)), all \( p < .017 \). There were no significant differences in GMFA between the single- and paired-pulse TMS conditions, \( p = .061 \).

**Sham TEP Compared to Single-Pulse TEP**

Table 1 shows the amplitude of the TEP components generated by sham and single-pulse TMS for young participants. The TEPs generated by the two TMS conditions did not differ for young participants. A two-way repeated measures ANOVA was used to investigate the effect of condition (sham and single-pulse TMS) on the components of the TEP. This test was run separately for the young and middle-aged participants.
Table 1
Mean Amplitude ($\mu V$) of Sham and Single-Pulse TEP Components for Young Participants

<table>
<thead>
<tr>
<th>TEP Component</th>
<th>Sham TEP</th>
<th>Single-Pulse TEP</th>
</tr>
</thead>
<tbody>
<tr>
<td>N100</td>
<td>-1.48 (0.96)</td>
<td>-2.51 (2.23)</td>
</tr>
<tr>
<td>P35</td>
<td>0.06 (0.62)</td>
<td>1.07 (1.59)</td>
</tr>
<tr>
<td>N45</td>
<td>-0.18 (0.68)</td>
<td>-0.66 (1.41)</td>
</tr>
<tr>
<td>P60</td>
<td>0.08 (1.03)</td>
<td>0.16 (0.93)</td>
</tr>
<tr>
<td>P180</td>
<td>1.65 (1.29)</td>
<td>2.75 (2.45)</td>
</tr>
</tbody>
</table>

Note. SD in brackets.

There was a minor violation of normality assessed by the Shapiro-Wilk test within the young single-pulse N100 condition, $W(11) = .853, p = .047$. All other young conditions were normally distributed. $F_{max}$ was 15.62 for the young participants, indicating a minor violation of the homogeneity of variance assumption, however, the outcome of a repeated measures ANOVA is not sensitive to small-to-moderate violations of the homogeneity of variance assumption and was consequently allowed (Allen, Bennett, & Heritage, 2014). Mauchly’s test was significant for the young participants’ main effect of component, $W(9) = .021, p < .001$, indicating a violation of the assumption of sphericity. As such, the Huynh-Feldt Epsilon adjusted test was used to interpret this effect (Tabachnick & Fidell, 2007).

There was no significant main effect of condition for the young participants, $F(1, 10) = .486, p = .502$, partial $\eta^2 = .046$. There was a significant main effect of component, $F(2.11, 21.07) = 15.13, p < .001$, partial $\eta^2 = .608$, and a significant interaction between condition and component, $F(4, 40) = 3.825, p = .01$, partial $\eta^2 = .277$. When correcting for multiple comparisons with a Bonferroni correction, post-hoc paired samples $t$ tests revealed no significant differences between the sham and single-pulse TEP components for the young participants, all $t(10) < 2.50$, all $p > .03$. 
Table 2 shows the amplitude of the TEP components generated by sham and single-pulse TMS for middle-aged participants. The P35 and P180 amplitudes were greater in the single-pulse condition compared to the sham condition.

Table 2

Mean Amplitude (µV) of Sham and Single-Pulse TEP Components for Middle-Aged Participants

<table>
<thead>
<tr>
<th>TEP Component</th>
<th>Sham TEP</th>
<th>Single-Pulse TEP</th>
</tr>
</thead>
<tbody>
<tr>
<td>N100</td>
<td>-1.99 (1.25)</td>
<td>-2.88 (2.88)</td>
</tr>
<tr>
<td>P35</td>
<td>0.32 (0.63)</td>
<td>1.90 (1.95)</td>
</tr>
<tr>
<td>N45</td>
<td>-0.14 (0.33)</td>
<td>-0.46 (1.96)</td>
</tr>
<tr>
<td>P60</td>
<td>0.52 (0.83)</td>
<td>0.05 (2.50)</td>
</tr>
<tr>
<td>P180</td>
<td>2.74 (1.82)</td>
<td>3.99 (2.67)</td>
</tr>
</tbody>
</table>

Note. SD in brackets.

There were minor violations of normality assessed by the Shapiro-Wilk test within the middle-aged sham P35 condition, \( W (14) = .735, p = .001 \), and the single-pulse P35 condition, \( W (14) = .869, p = .04 \). All other middle-aged conditions were normally distributed. \( F_{\text{max}} \) was 78.25, indicating a violation of the homogeneity of variance assumption for the middle-aged participants. Mauchly’s test was significant for the middle-aged participants’ main effect of component, \( W (9) = .027, p < .001 \), and interaction effect of condition and component, \( W (9) = .135, p = .007 \), indicating a violation of the assumption of sphericity. As such, the Huynh-Feldt Epsilon adjusted test was used to interpret these effects (Tabachnick & Fidell, 2007).

There was no significant main effect of condition for the middle-aged participants, \( F (1, 13) = .674, p = .426 \), partial \( \eta^2 = .049 \). There was a significant main effect of component, \( F (1.97, 25.61) = 25.65, p < .001 \), partial \( \eta^2 = .664 \), and a significant interaction between condition and component, \( F (2.90, 37.66) = 4.375, p = .01 \), partial \( \eta^2 = .252 \). Post-hoc paired samples \( t \) tests with a Bonferroni correction
showed a significant difference between the sham and single-pulse P35 component, $t(13) = 3.584$, $p = .003$, $d = 1.09$, and the sham and single-pulse P180 component, $t(13) = 3.743$, $p = .002$, $d = 0.55$, in the middle-aged participants. No significant differences were found between sham and single-pulse TMS for the other components, all $t(13) < 1.32$, all $p > .55$

Single-Pulse TEP

Figure 4 shows the single-pulse TEP from left hemisphere stimulation for young and middle-aged participants. It is clear from the figure that the TEPs generated by single-pulse TMS were similar for young and middle-aged participants.

![Figure 4](image)

Figure 4. Plots showing the TEPs generated by single-pulse TMS for young (YA) and middle-aged (MA) adults. The dotted vertical line (0 ms) shows the time at which TMS was applied. The shaded box demonstrates the section of data that was removed due to the TMS artefact, and replaced with interpolated data.

A mixed model ANOVA was used to investigate the effect of age on the components of the single-pulse TEP. There was a minor violation of normality assessed by the Shapiro-Wilk test within the young single-pulse N100 condition, $W(11) = .853$, $p = .047$, and the middle-aged single-pulse P35 condition, $W(14) = .869$, $p = .04$. All other conditions were normally distributed. Levene’s test was not significant for any of the TEP components, indicating the assumption of homogeneity of variance was not violated. Mauchly’s test was significant for the within-subjects effect of component, $W$
(9) = .106, \( p < .001 \), indicating a violation of the assumption of sphericity. As such, the Huynh-Feldt Epsilon adjusted test was used to interpret this effect (Tabachnick & Fidell, 2007). The ANOVA showed a significant main effect of component, \( F(2.68, 61.54) = 26.56, p < .001 \), partial \( \eta^2 = .536 \). There was no significant main effect of age, \( F(1, 23) = .889, p = .356 \), partial \( \eta^2 = .047 \), and there was no significant interaction between component and age, \( F(4, 92) = .580, p = .678 \), partial \( \eta^2 = .025 \).

**LICI TEP**

LICI was quantified by expressing the corrected paired-pulse TEP as a percentage of single-pulse TEP size. For all components of the TEP, a larger percentage reflected greater inhibition, and a negative percentage reflected facilitation. As shown in Table 3, the LICI components were similar for young and middle-aged participants.

<table>
<thead>
<tr>
<th>LICI Component</th>
<th>Young</th>
<th>Middle-age</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>LICI_{N100}</td>
<td>1.74 (24.02)</td>
<td>0.12 (27.84)</td>
<td>0.83 (25.71)</td>
</tr>
<tr>
<td>LICI_{P35}</td>
<td>6.67 (36.41)</td>
<td>2.72 (28.85)</td>
<td>4.46 (31.74)</td>
</tr>
<tr>
<td>LICI_{N45}</td>
<td>-10.83 (32.23)</td>
<td>-11.43 (29.01)</td>
<td>-11.17 (29.81)</td>
</tr>
<tr>
<td>LICI_{P60}</td>
<td>1.62 (26.46)</td>
<td>4.33 (30.94)</td>
<td>3.13 (28.50)</td>
</tr>
<tr>
<td>LICI_{P180}</td>
<td>30.25 (20.17)</td>
<td>36.99 (13.05)</td>
<td>34.03 (16.54)</td>
</tr>
</tbody>
</table>

*Note.* SD in brackets.

A mixed model ANOVA was used to investigate the effect of age on the LICI components. There were minor violations of normality assessed by the Shapiro-Wilk test within the young LICI_{N45} condition, \( W(11) = .745, p = .002 \), and the young LICI_{P35} condition, \( W(11) = .795, p = .008 \). All other conditions were normally distributed.
Levene’s test was not significant for any of the LICI components, indicating the assumption of homogeneity of variance was not violated. Mauchly’s test was significant for the within-subjects effect of component, $W(9) = .202, p < .001$, indicating a violation of the assumption of sphericity. As such, the Huynh-Feldt Epsilon adjusted test was used to interpret this effect (Tabachnick & Fidell, 2007).

The ANOVA showed a significant main effect of component, $F(2.83, 65.19) = 8.02, p < .001$, partial $\eta^2 = .259$. There was no significant main effect of age, $F(1, 23) = .031, p = .862$, partial $\eta^2 = .001$, and there was no significant interaction between component and age, $F(4, 92) = .127, p = .927$, partial $\eta^2 = .006$. Pairwise comparisons with a Bonferroni correction revealed that LICI$_{P180}$ was significantly more inhibited than the other LICI components for both young and middle-aged participants, all $p < .01$. There were no significant differences between LICI$_{N100}$, LICI$_{N45}$, LICI$_{P35}$ and LICI$_{P60}$, all $p = 1.00$.

**Manual Dexterity**

Figure 5 shows performance on the peg-moving subtest and the assembly subtest for young and middle-aged participants. Numerically, all participants placed more pegs with their right hand than their left hand during the peg-moving subtest, and young participants placed more items than the middle-aged participants during the assembly subtest.
A mixed model ANOVA was used to investigate the effect of age on the number of pegs placed on the pegboard with the right hand compared to the left hand in the peg-moving subtest. The Shapiro-Wilk, $F_{\text{max}}$ and Levene’s test statistics were used to test the assumptions of normality and homogeneity of variance. The assumptions for a mixed model ANOVA were not violated. The ANOVA showed a significant main effect of hand, $F(1, 27) = 17.36, p < .001$, partial $\eta^2 = .391$, with participants placing more pegs with their right hand compared to their left hand. There was no significant main effect of age $F(1, 27) = 2.69, p = .113$, partial $\eta^2 = .091$, and there was no evidence of an interaction between hand and age $F(1, 27) = 1.66, p = .209$, partial $\eta^2 = .058$.

Even though the interaction between hand and age did not reach significance, there was a theoretical justification for examining post-hoc $t$ tests for the peg-moving subtest, as there is evidence for age-related changes in hemispheric asymmetry that influence handedness and manual dexterity (Hammond, 2002). Paired samples $t$ tests were performed on the peg-moving subtest scores of the left and right hands, with separate tests for young and middle-aged participants. A Bonferroni correction was
applied to account for multiple comparisons, therefore results were significant at $p < .025$. Paired samples $t$ tests showed that performance of the right hand was significantly better than performance of the left hand in middle-aged participants, $t(12) = 3.83, p = .002, d = 0.76, 95\% \text{ CI } [.66, 2.42]$, but not young participants, $t(15) = 2.09, p = .055, d = 0.51, 95\% \text{ CI } [-.02, 1.64]$.

An independent samples $t$ test was used to compare the number of items placed on the pegboard by the young participants to the middle-aged participants during the assembly subtest. Neither Shapiro-Wilk statistic was significant; indicating the assumption of normality was not violated. Levene’s test was also non-significant, thus equal variances can be assumed. The $t$ test was statistically significant, with the young participants placing, on average, 4.5 more items on the pegboard than the middle-aged participants during the assembly subtest, $t(27) = 2.44, p = .02, d = 0.92, 95\% \text{ CI } [.72, 8.3]$.

**Manual Dexterity and LICI Correlates**

Figure 6 presents scatterplots of the LICI components and the Purdue pegboard scores. It is clear from the scatterplots that there was no relationship between LICI TEP and Purdue pegboard measures of manual dexterity. A bivariate Pearson’s product-moment correlation coefficient ($r$) was used to investigate linear relationships between the LICI components and the scores from the right hand peg-moving subtest and the assembly subtest. Tests were conducted separately for each LICI component and subtest. The assumptions of linearity and homoscedasticity did not appear to be violated. When correcting for multiple comparisons using a Bonferroni correction, there were no significant correlations between any LICI component and subtest score, all $r (23) < .31$, all $p > .13$. 
Figure 6. Scatterplots showing bivariate Pearson’s product-moment correlations between each LICI component and the peg-moving subtest scores (A – E) and the assembly subtest scores (F – J). The solid line in each scatterplot represents the line of best fit.
Discussion

The current study used paired-pulse TMS-EEG applied to the dominant M1 to determine whether there are differences in intracortical inhibition between young and middle-aged adults. There are four main findings from the current study. First, the sham and single-pulse TEPs did not differ for young participants, however the amplitude of the P35 and P180 components were significantly greater in the single-pulse condition compared to the sham condition for middle-aged participants. Second, the single-pulse TEPs did not differ between young and middle-aged participants. Third, the LICIN100 of the corrected paired-pulse TEP did not differ between young and middle-aged participants. Fourth, no significant correlations were evident between LICIN100 and manual dexterity. The following section will discuss these findings in the context of the existing literature on age-related changes in movement control, and provide suggestions for future research.

Global Mean Field Amplitude

GMFA was significantly greater in the real TMS conditions compared to the sham TMS condition. GMFA is an index of global cortical activity (Komssi et al., 2004). Therefore, the TEPs generated by both single- and paired-pulse TMS have a greater contribution of cortical activity than the TEP generated by the sham condition. There are limited TMS-EEG studies that include both a sham TMS condition and measures of global cortical activity. It is important that both of these measures are included in future TMS-EEG studies in order to accurately investigate cortical activity generated by real TMS. There were no differences in GMFA between young and middle-aged adults, suggesting that any differences in the TEP components between
these groups were unlikely to be confounded by a generalised change in cortical excitability. This is consistent with the TMS-EEG study by Opie et al. (2018), who demonstrated no difference in GMFA between young and older adults. The current study has furthered these findings by demonstrating the same results between young and middle-aged adults, which is important in the context of using TMS-EEG to investigate changes in cortical activity across the lifespan.

**Comparison of Sham and Single-Pulse TEP**

Despite the differences in GMFA between the sham and real TMS conditions, suggesting a greater neural contribution to the TMS TEPs than the sham TEP, there were no differences in the TEP components between the single-pulse condition and the sham condition for the young participants. Middle-aged participants showed greater amplitude of the P35 and P180 components in the single-pulse condition compared to the sham condition, but there were no differences in the amplitude of the other TEP components between the two stimulation conditions.

There are a number of explanations for the similar TEPs generated by the sham and single-pulse TMS conditions in the current study. The main limitation of the current study is the artefacts in the EEG recording induced by the strong magnetic field of the TMS pulse and various physiological responses. Figure 7 demonstrates the effects of common TMS artefacts on the TEP from a study by Rogasch et al. (2014). A long-lasting artefact known as the decay artefact can affect electrodes close to the site of stimulation (Rogasch et al., 2014). The source of the decay artefact likely reflects movement and heating of the electrodes due to a build-up of eddy-currents at the electrodes (Rogasch & Fitzgerald, 2013). If not removed, the decay artefact can significantly alter the amplitude of the N45, P60 and N100 components (Rogasch et al., 2014). TMS often results in a blink startle reflex, which can affect anterior EEG electrodes. The blink artefact has been shown to significantly alter the amplitude of the
N45 and N100 components (Rogasch et al., 2014). Discharge of the TMS coil results in a loud clicking noise, which causes an auditory-evoked potential that coincides with the latency of the N100 and P180 components (Rogasch & Fitzgerald, 2013). Although the click of the TMS coil was masked with white noise, it may not have been sufficient to prevent the auditory-evoked potential. A quiet TMS device known as qTMS is under development, which increases the frequency of the TMS click above the human hearing threshold, and shortens the length of the clicking sound (Peterchev, Murphy, & Goetz, 2015). The qTMS would be beneficial for use in future TMS-EEG studies to prevent auditory-evoked potentials. It is possible that not all artefacts were removed from the EEG signal during data analysis in the current study, which may have distorted the TEP. A published template for artefact removal was used to analyse the EEG data (Rogasch et al., 2017), however this is a new tool that will likely undergo further development.

A further explanation for the similar TEPs generated by sham and single-pulse TMS is the possibility that the sham condition was inducing a current flow in the cortex, as the wings of the TMS coil was were held close to the scalp during this condition.
The TMS current flows around the figure-of-eight TMS coil, and although the induced electric field is greatest under the intersection of the ‘8’, the wings of the coil still generate a weak electric field (Wassermann, 2008). A placebo coil could be used during the sham condition, which provides sensory stimulation and discharge noise similar to a real TMS coil without stimulating the cortex (Bonato et al., 2006).

The similarity of the sham and real stimulation TEP is consistent with a study published this year by Conde et al. (2018), who found the temporal features of the TEP generated by real TMS to closely resemble the TEP generated by sham TMS. In order to overcome these limitations, Conde et al. (2018) suggest future TMS-EEG studies should include a stimulation condition whereby TMS is applied to the shoulder or other peripheral body part in order to better differentiate between transcranial and non-transcranial components of TEPs.

**Comparison of Single-Pulse TEP between Young and Middle-Aged Adults**

Despite the similarities of the sham and single-pulse TEPs, the amplitude and latency of the components of the single-pulse TEP in young adults is consistent with previous TMS-EEG literature (e.g. Bonato et al., 2006; Opie et al., 2017; Premoli, Castellanos, et al., 2014; Premoli, Rivolta, et al., 2014; Rogasch et al., 2013; Rogasch & Fitzgerald, 2013; Rogasch et al., 2014). This consistency, along with the increased global cortical excitability in the real TMS conditions compared to sham TMS, provides justification for examining the TEPs generated by real TMS. The current study is the first to examine TEPs in middle-aged adults. There were no significant differences in the components of the single-pulse TEP between young and middle-aged adults.

The similarity of the P30 amplitude between young and middle-aged adults is consistent with Opie et al. (2018), who found no differences in P30 when comparing single-pulse TEPs between young and older adults. Together, these findings suggest no changes in motor cortical excitability between young, middle-aged, and older adults, as
P30 is thought represent excitability within the stimulated cortex (Rogasch et al., 2013). Opie et al. found an increase in the amplitude of the N45 component in older adults compared to young adults. This result indicates an age-related increase in a subtype of cortical inhibition, as the N45 component is thought to reflect fast-acting GABA_A-mediated inhibition (Premoli, Castellanos, et al., 2014). However, the current study found no difference in N45 amplitude between young and middle-aged adults, suggesting age-related changes in GABA_A inhibition occur after middle age. There is a need for studies that examine single-pulse TEPs across the lifespan to provide data to support these interpretations of when age-related changes in motor cortical excitability and inhibition occur.

Opie et al. also found age-related differences in the N100 and P180 single-pulse components, however, these differences consisted of altered latency and spatial distributions, rather than amplitude. Comparing the latency and spatial distribution of the TEP components in young, middle-age and older adults would therefore be an important extension of the current study, and would provide further information regarding age-related changes in motor control.

Comparison of LICI TEP between Young and Middle-Aged Adults

Age-related comparisons of LICI found that the percentage of inhibition did not differ between young and middle-aged adults for any of the TEP components. In particular, the LICI_{N100} did not differ between young and middle-aged participants. The N100 component is thought to reflect GABA_B-mediated inhibition. Pharmaceutical studies provide two lines of evidence demonstrating that the N100 is GABA_B mediated. First, participants who received a dose of baclofen, a GABA_B receptor agonist, showed an increase in N100 amplitude following single-pulse TMS compared to a placebo (Premoli, Castellanos, et al., 2014). Second, baclofen resulted in a greater suppression of N100 amplitude following paired-pulse TMS compared to a placebo, demonstrating
an increase in LICI of the N100 (Premoli, Rivolta, et al., 2014). This pharmaceutical evidence, together with the current finding that there is no difference in LICIN100 between young and middle-aged adults, suggests that there are no changes in the excitability of GABA_B networks between these age groups.

The previous study on age-related changes in intracortical inhibition using TMS-EEG demonstrated greater LICIN100 in older adults compared to young adults, indicating an increase in the excitability of GABA_B networks in older adults (Opie et al., 2018). This finding, along with the results of the current study, suggest that age-related changes in the N100 component and GABA_B inhibitory networks occur from middle age onwards. Opie et al. (2018) also demonstrated greater LICIP180 in older adults compared to young adults. Although the origin of the P180 component is unclear, pharmaceutical evidence suggests that LICI of the P180 likely involves activation of post-synaptic GABA_B-receptors as a result of paired-pulse stimulation (Premoli, Rivolta, et al., 2014). Opie et al. suggest the age-related increase in LICIP180 could be due to a reduction in GABA reuptake in older adults. The current study found no difference in LICIP180 between young and middle-aged adults, providing further evidence that age-related changes in GABA_B-mediated inhibition occur after middle age. However, there is a need for studies that examine LICI across the lifespan to provide data to support this interpretation, and provide further information about when changes in intracortical inhibition begin.

LICIP180 was greater than the other LICI components in both young and middle-aged adults. The inhibition of the P180 component following paired-pulse TMS is consistent with previous TMS-EEG studies (e.g. Opie et al., 2017; Opie et al., 2018; Premoli, Rivolta, et al., 2014), however, the finding that LICIP180 is greater than the other LICI components is unique. The release of GABA_B peaks between 100 – 150 ms following the conditioning (first) TMS pulse (Fitzgerald, Maller, Hoy, Farzan, &
Daskalakis, 2009), which coincides with the latency of the N100 component. Therefore, it was expected that LICI$_{N100}$ would be similar to LICI$_{P180}$, as demonstrated by previous studies (Opie et al., 2017; Opie et al., 2018; Premoli, Rivolta, et al., 2014). It is possible that the earlier TEP components were more distorted by artefacts compared to the P180, as the latency of many of the artefacts in the EEG recordings occur before P180. This distortion limits the sensitivity of the TEP to accurately measure LICI in earlier components, and potentially limits the sensitivity to detect changes between young and middle-aged adults.

**Manual Dexterity**

Results from the Purdue pegboard task indicate that, on average, participants placed more pegs with their right hand compared to their left hand during the unilateral peg-moving subtest. However, the difference in performance between the hands was only significant for middle-aged adults, indicating a greater asymmetry in manual dexterity in middle-aged adults compared to young adults. Using the assembly subtest, results showed a decrease in bimanual dexterity in middle-aged adults compared to young adults, which could be due to the asymmetry seen in middle-aged adults. This is consistent with previous literature showing an age-related decrease in performance on the assembly subtest (Agnew, Bolla-Wilson, Kawas, & Bleecker, 1988). However, a TMS study examining motor control in young and older adults found an age-related decrease in both manual dexterity asymmetry and hemispheric asymmetry of LICI (Vallence et al., 2017). One explanation for this decline in asymmetry in older adults is brain over-activation, known as compensation. Compensatory processes occur as a result of declines in brain function, and include increased activation and engagement of motor areas in older adults during motor tasks (Seidler et al., 2010). Therefore, it could be speculated that ageing is accompanied by a decline in motor control of the non-dominant hand in middle age, reflected by the asymmetry in the current study, before
compensatory processes occur that lead to a reduction in motor control asymmetry in older adults. Future research should examine motor control asymmetry across the lifespan to provide data to support this interpretation.

**Correlations between Manual Dexterity and LICI TEP**

There were no significant correlations between the Purdue pegboard scores and LICI<sub>N100</sub>, or any of the other LICI components in young and middle-aged adults. This is consistent with previous TMS-alone studies that examined relationships between LICI and performance on the pegboard tasks, failing to show any significant correlations between the behavioural and neurophysiological measurements (Opie, Evans, Ridding, & Semmler, 2016; Vallence et al., 2017). The current study is the first to examine relationships between TEPs and manual dexterity. There are two main explanations for the lack of significant correlations.

First, although GABA<sub>B</sub> inhibitory networks play an important role in the control of manual dexterity (Hammond & Vallence, 2007; Kouchtir-Devanne et al., 2012), there are other neural processes in M1 that contribute to manual dexterity and motor control. For example, short-interval intracortical inhibition can be measured using paired-pulse TMS with an ISI of 1 – 6 ms, and results in inhibited MEPs (Kujirai et al., 1993). It is mediated by fast-acting GABA<sub>A</sub> inhibitory networks, and contributes to motor control through selective suppression of unwanted muscle activity (Stinear & Byblow, 2003). Short-interval intracortical facilitation is another process that can be measured with paired-pulse TMS using ISIs of 1.5, 2.5 and 4.5 ms, and results in increased MEPs (Tokimura, Ridding, Tokimura, Amassian, & Rothwell, 1996).

Facilitation has been shown to correlate with Purdue pegboard performance, suggesting this measure contributes to the execution of manual tasks (Clark, Loftus, & Hammond, 2011). There is evidence for differences in both short-interval intracortical inhibition and facilitation between young and older adults (Marneweck, Loftus, & Hammond,
2011), and therefore future studies should examine these processes across the lifespan, in addition to LICI, to determine when changes in the neural control of manual dexterity occur.

Second, the Purdue pegboard tasks might not be sensitive enough to accurately measure manual dexterity. For example, there are different phases of movement required for the pegboard tasks, such as grasping, manipulating and releasing the pegs (Gonzalez, Rowson, & Yoxall, 2017). The GABA\textsubscript{B} inhibitory network may have a greater contribution to a subset of these movements compared to others. These phases of movement may also be differentially affected by age; however, the movements are not measured independently. In addition, a study using kinematic analysis, which involves motion capture to measure movement, found that finger movements during the pegboard tasks do not compare well with the range of finger movements that account for hand performance during daily tasks, such as unscrewing a bottle cap or picking up a coin (Gonzalez et al., 2017). Using kinematic motion capture to measure different phases of manual dexterity, and including movement tasks that better reflect activities of daily living, would therefore be an important extension of the current study to more accurately measure age-related declines in movement control.

**Future Research**

Although beyond the scope of the current study, future research examining age-related changes in motor control should include participants whose ages cover the entire adult lifespan. This would provide detailed information regarding the age at which motor control starts to decline, and help to inform the age at which interventions should be implemented to counteract the decline. Longitudinal studies would be informative to measure changes in movement control across the participants’ lifespan, however these have their own associated weaknesses (e.g. Ployhart & Vandenberg, 2010). Future research should also combine the varying methods of assessing GABA\textsubscript{B} mediated
intracortical inhibition in order to increase the evidence base and consistency of results. These methods include TMS-alone, TMS-EEG, and imaging methods such as Magnetic Resonance Spectroscopy which can measure levels of GABA neurotransmitters in cortical tissue (e.g. Hermans, Leunissen, et al., 2018).

**Conclusion**

Intracortical inhibition is an important neural process for motor control. Age-related changes in the inhibitory networks of M1 are thought to contribute to the decline in manual dexterity that is associated with ageing. The purpose of the current study was to investigate differences in LICI between young and middle-aged adults using TMS-EEG. The components of the LICI TEP, specifically the LIC$_{N100}$, were not different between young and middle-aged adults. Therefore, there is no evidence from the current study to suggest differences in intracortical inhibition between young and middle-aged adults. However, the similarity of the LIC$_{N100}$ between young and middle-aged adults is speculative, as artefacts heavily influenced the TEPs. It is critical to be able to minimise the artefacts during TMS-EEG recording, and to reliably identify and remove artefacts from the EEG data. The current study provides a number of important practical implications for future research regarding methods to better record TEPs. The current study also emphasises the need to include participants whose ages cover the entire adult lifespan. These suggestions will help to determine the age at which the decline in the neural control of manual dexterity begins, and thus inform the age at which interventions should be implemented.
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