Direct measurements of the brain mechanisms of motor control in ageing

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Abstract

This research investigates the modulatory effects of cortico-cortical paired associative stimulation (ccPAS) on interhemispheric inhibition (IHI) and cortical excitability within the human motor cortex (M1), focusing on variations due to stimulation direction and participant age. The study comprises two distinct age groups: young adults and older adults, aiming to understand the age-dependent characteristics of neuroplasticity induced by ccPAS. The young adult group was divided into three subgroups to compare left-to-right M1-M1 ccPAS, right-to-left M1-M1 ccPAS, and a control group receiving sham stimulation. The older adult group received only left-to-right M1-M1 ccPAS and a sham condition to mitigate fatigue concerns.

The research adopts a postpositivist epistemological foundation, utilising empirical, observable evidence and quantitative methods to gather data. Through rigorous experimental design, including randomisation and blinding procedures, the study ensures the validity and reliability of its findings. A systematic review of the existing ccPAS literature was conducted to inform the study design and methodology. This review revealed that ccPAS has consistently demonstrated the capacity to modulate corticospinal excitability and interhemispheric inhibition in targeted brain regions. Findings from prior studies underscore the importance of stimulation directionality, the temporal specificity of effects, and the potential clinical applications of ccPAS in neurorehabilitation. The review also identified research gaps, including the limited understanding of age-related differences and temporal dynamics, which guided the formulation of the study's hypotheses and experimental protocols.

The findings from both experiments revealed that M1-M1 ccPAS, particularly in the right-to-left direction, significantly modulates corticospinal excitability and interhemispheric inhibition in young adults. In older adults, left-to-right ccPAS affects cortical excitability in the motor cortex but does not modulate interhemispheric inhibition. Moreover, there was no

significant age-dependent difference in the modulation of cortical excitability by left-to-right ccPAS, indicating that the effects of this intervention are consistent across different age groups. These effects develop over time, indicating temporal dynamics in cortical modulation.

Overall, the study underscores the complexity of cortico-cortical communication and the factors influencing neuroplasticity. It highlights the potential of ccPAS as a non-invasive intervention to modulate cortical excitability and interhemispheric inhibition while also pointing out the limitations and variations in effectiveness. These insights are crucial for developing more effective, age-specific neurorehabilitation strategies and advancing our understanding of the neural mechanisms underlying plasticity and cortical communication.

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Chapter 1: Introduction

Chapter Overview

This chapter examines the global trend towards an ageing population and its health implications, focusing on ageing's effects on the brain and motor control. It highlights the worldwide increase in older adults due to advancements in healthcare and lifestyle, leading to longer life expectancies and raising challenges in managing age-related chronic conditions and healthcare costs. Motor control deterioration, a significant aspect of ageing, results from changes in the nervous system, emphasising the need for interventions to maintain older adults' motor function and independence. It introduces Transcranial Magnetic Stimulation (TMS) and paired associative stimulation (PAS), specifically cortico-cortical PAS (ccPAS), as innovative tools for studying and potentially enhancing neural connectivity and plasticity in the ageing brain.

The Global Demographic Shift Towards an Older Population

The world is witnessing a significant demographic transformation, with a rapid increase in the proportion of older adults (Leeson et al., 2016; Leis & Gijsbers, 2011). Ageing is an inevitable part of life characterised by a gradual decline in the body's ability to repair and maintain itself. Global data indicates that life expectancy is increasing (World Health Organisation, 2021). In the United Kingdom, the number of people aged 65 and above has significantly risen and is expected to continue (Harper, 2016). This group is predicted to increase to 88.5 million in the United States by 2050 (Seidler et al., 2010). The global population aged 65 and older is forecasted to increase from 10 per cent in 2022 to 16 per cent by 2050 (UN, 2022). It is anticipated that those aged 65 years and above will exceed twice the number of children under the age of 5 and approximately equal the number of those under 12 (Richards, 2007; UN, 2022). This population change poses considerable challenges and opportunities for societies worldwide, emphasising the importance of understanding the ageing process and its impact on human health and well-being.

Understanding the nuanced ways ageing affects our brains is an area of considerable interest in neurological and clinical research.

Historically, life expectancy increased mainly due to lower infant and child mortality rates (Lutz et al., 2008; UN, 2022), but recent growth is mainly from reduced mortality among those 65 and older (Harper, 2016). The World Health Organization (WHO) highlights significant factors behind this trend, including advancements in healthcare technology, pharmaceuticals, and systems; breakthroughs in disease prevention, diagnosis, and treatments; and improvements in public health measures like sanitation, nutrition, and health education (World Health Organisation, 2021). These advancements, alongside societal improvements such as better education, economic stability, and technology, have contributed to longer life spans. However, challenges remain in ensuring that extended life expectancy also means improved health and quality of life (Kaeberlein et al., 2015; Weinkove & Goljanek-Whysall, 2017).

Socioeconomic Implications of Ageing

The ageing population has profound socioeconomic implications, including increased healthcare costs, a greater need for long-term care facilities, and a shift in workforce demographics (Leeson et al., 2016). Age-related chronic illnesses pose a significant public health challenge worldwide, reducing the period of good health (health span) and straining individuals, families, public services, and the economy due to increased healthcare costs (Bhagwat & Deodhe, 2023; Harper, 2016; Kaeberlein et al., 2015; Naja et al., 2017).

Chronic illnesses often result in disability and diminished ability to live independently, increasing the demand for care homes, home assistance, and personal care. Families face challenges in providing care for older relatives who live for many years, or even decades, with a diminished quality of life (Leis & Gijsbers, 2011). Simultaneously, countries allocate a growing share of their limited resources to healthcare services for the older population (Kaeberlein et al., 2015). Consequently, gaining deeper insights into the effects of ageing on

cognitive and physical abilities is essential for improved planning and support for an increasingly older population.

Age-related conditions, particularly those affecting motor functions, significantly contribute to disability, loss of independence, and decreased quality of life among older adults. Consequently, there is a growing demand for effective interventions and healthcare strategies to support healthy ageing, maintain functional independence, and reduce the burden on healthcare systems (Leis & Gijsbers, 2011)

Motor Control in Ageing

The primary motor cortex (M1) is the critical region for learning and executing motor activities. It is intrinsically connected to cognitive functions through critical processes such as attention, learning, consolidation, movement inhibition, and emotional, linguistic, and motivational mechanisms (Bhattacharjee et al., 2021; Tomasino & Gremese, 2016). The cognitive impacts of ageing are a significant study area, illustrating a decline in various cognitive functions as individuals age. Research by Seidler et al. (2010) provides a summary of how ageing affects cognition. As individuals age, notable reductions are observed in processing speed, working memory, inhibitory functions, long-term memory, brain structure volume, and the integrity of white matter, as discussed by Park and Reuter-Lorenz (2009) and Perry et al. (2017). Studies have explored the relationship between everyday activities and cognitive abilities (Aartsen et al., 2002), the association of physical frailty with cognitive decline (Buchman et al., 2010), and the advantages of engaging in cognitive training and mental stimulation to mitigate ageing effects (Kelly et al., 2014).

Motor control, crucial for movement regulation, experiences significant age-related changes. These changes result from peripheral and central nervous system alterations, leading to slower and more variable movements (Ketcham & Stelmach, 2004). These brain alterations translate into observable behavioural changes in older adults, including longer reaction times and diminished coordination, fine motor skills and motor anticipation and execution (Bhagwat & Deodhe, 2023; Levin et al., 2014; Li et al., 2023). Additionally, older

adults face decreased motor coordination, strength, gait, and speed, alongside reduced motor learning capabilities (Harada et al., 2009). These are attributed to changes in muscle composition, neuromuscular function, and central nervous system processing. One of the significant challenges in ageing is the decreased ability to inhibit actions, which is crucial for adapting to sudden changes and avoiding hazards. This decreased inhibitory response impacts fall prevention, community navigation, and driving (Chambers et al., 2009). The regulation of cortical excitability, which plays a vital role in motor control, is impaired in older adults, leading to decreased fine motor skills and increased fall risk, a significant concern in this population (Calautti et al., 2001; Liu et al., 2014; Wang et al., 2024).

Studies on inhibitory response across different age groups show increased reaction times during childhood, a plateau during adulthood, and a decline in older age (Bedard et al., 2002; Oliviero et al., 2006; Williams et al., 1999). The decline in inhibitory response is linked to changes in specific neural networks and cortical regions involved in motor control (Aron & Poldrack, 2006; Cai et al., 2014; Fujiyama et al., 2022). Interestingly, older adults display increased brain activation in response to motor tasks, utilising compensatory neural mechanisms to maintain performance (Ward & Frackowiak, 2003). Understanding these changes is vital for devising targeted interventions to preserve motor function and enhance the quality of life among older adults.

Functional Alterations with Ageing

Ageing brings about a decline in brain size, impacting the grey and white matter areas (Seidler et al., 2010). This decrease in brain volume results in the loss of neurons and a reduction in synaptic plasticity, notably in the frontal cortex, prefrontal cortex, striatum, cerebellar cortex, hippocampus, and occipital cortex (Liu et al., 2014; Perry et al., 2017; Peters, 2006). Significant regions, such as the prefrontal cortex and the striatum, play crucial roles in executive functions and motor control and experience considerable changes (Vink et al., 2005). Ageing is associated with structural and chemical changes in the brain that present as deficits in motor abilities, including slower movements and loss of fine motor skills

(Mattay et al., 2002). To adjust for these changes, the ageing brain undergoes functional reorganisation and employs compensatory mechanisms to maintain motor performance (Mattay et al., 2002; Ward & Frackowiak, 2003). Ward and Frackowiak (2003) studied the impact of ageing on brain activation during hand grip tasks, hypothesising age-related differences in a frontoparietal network due to the complex effects of ageing on motor performance. In their study, 26 volunteers aged 21–80 performed hand grip tasks, with functional MRI data used to explore brain activation patterns. They found that hand grip activated several brain regions, including the contralateral sensorimotor cortex and ipsilateral superior cerebellum, with increased bilateral activations in older subjects. This indicates that older subjects activated more areas of the motor network to maintain performance. This non-linear activation pattern suggested complex ageing effects and potential for motor network adaptive plasticity. Increased activation in older subjects was observed in the left hemisphere, hinting at hemispheric dominance in motor control and greater neural resource utilisation with age. The study's findings suggest that the normal ageing process involves compensatory changes in the motor system's functional organisation. While healthy ageing has been shown to activate compensation mechanisms to counterbalance motor function deterioration, conditions like Mild Cognitive Impairment (MCI), Alzheimer’s Disease (AD), and Parkinson's disease (PD) motor functions deteriorate beyond the compensatory capabilities observed in healthy ageing (Kaasinen & Rinne, 2002; Poirier et al., 2021).

One of the most notable changes in the ageing brain is a reduction in neural connectivity, which refers to the efficiency and effectiveness of communication between different brain areas (Park & Reuter-Lorenz, 2009; Perry et al., 2017). In Alzheimer's Disease (AD) and Mild Cognitive Impairment (MCI), synapse dysfunction plays a crucial role in the observed deterioration of fine motor control. Synapses are the key points of communication between neurons, and their health and functionality are essential for all aspects of brain activity, including motor control. Research continues to explore potential

interventions that can protect synapses or restore synaptic function to mitigate the effects of these neurodegenerative conditions.

It is suggested that difficulties in adjusting cortical excitability may be at the root of various motor deficits encountered by healthy older adults in daily activities, including the decline of fine motor skills (Bhandari et al., 2016). Ageing affects the brain's neurotransmitter systems, including those involving dopamine, serotonin, and acetylcholine, which play critical roles in cognitive functions, mood regulation, and overall brain health (Seidler et al., 2010). Changes in these neurotransmitter systems can contribute to various age-related conditions, including depression, cognitive decline, and increased vulnerability to stress. Parkinson's disease is a neurodegenerative disorder marked by symptoms such as tremors, stiffness, and balance issues, primarily caused by the loss of dopamine-producing neurons in the brain's substantia nigra region (Kaasinen & Rinne, 2002).

Gamma-aminobutyric acid (GABA) is the brain's primary inhibitory neurotransmitter, which regulates cortical excitability. Cortical pyramidal cells' activity is influenced by excitatory and inhibitory inputs, known as cortical inhibition. Recent research indicates a significant link between the diminished capacity to control cortical inhibition and the occurrence of motor delays in healthy older adults (Bhandari et al., 2016; Di Lazzaro et al., 2000). GABAergic neurotransmission plays a crucial role in neuroplasticity, the brain's capacity to adjust and form new neural connections. This adaptability relies on activity-dependent synaptic strength changes, with long-term potentiation (LTP) and long-term depression (LTD) being the most thoroughly examined mechanisms of neural plasticity. LTP augments the connections between neurons, resulting in enhanced synaptic firing, while LTD weakens the connections, decreasing the likelihood of synaptic firing. The decline in LTP-like plasticity with age might explain the motor learning deficits in healthy older adults (Bhandari et al., 2016).

The use of brain stimulation to study the effects of healthy ageing on brain and behaviour

The brain's functionality relies on networks that facilitate information exchange, both at the microscopic level between neurons and synapses and at a macroscopic level across different regions. Advances in neuroscience and brain imaging techniques have enabled more sophisticated ways of exploring brain function and neurotomy. Transcranial Magnetic Stimulation (TMS) is a non-invasive tool extensively used to study brain function and treat certain neurological and psychiatric conditions (Guidali et al., 2021; Hallett et al., 2017). The technique is pain-free, non-invasive, portable, and requires no direct contact with the skin (Barker et al., 1985; Oliviero et al., 2006). By applying strong, brief magnetic fields, TMS induces electric currents in specific brain areas, enabling the study of fundamental neurobiological mechanisms crucial for developing strategies to aid recovery after brain injury, such as motor cortex plasticity (Fiori et al., 2018; Stefan et al., 2000). TMS, including its variations like single-pulse, repetitive (rTMS), and patterned forms (theta-burst TMS, quadripulse TMS), plays a pivotal role in this research area (Hallett et al., 2017). TMS can disrupt or enhance brain functions, providing insights into local and distant brain region connectivity through induced neural plasticity. Combining TMS with other techniques, including Electroencephalography (EEG), Functional Magnetic Resonance Imaging (fMRI), and paired associative stimulation (PAS), has enabled more precise and direct measures of neural activity and neural stimulation. Researchers have shown that it is possible to increase or decrease the excitability of specific cortical areas and target specific neural pathways (Rizzo et al., 2009). TMS has been used to observe and manipulate behaviours in the motor cortex, including hand and finger movements (Aron & Poldrack, 2006; Fiori et al., 2018).

Combining TMS with EEG offers a unique advantage in tracking brain activity (Hallett et al., 2017). This method provides insights into the temporal order of activations within connected cortical areas, identifying excitatory or inhibitory causal interactions. EEG is used to monitor excitability in specific cortical pathways. It records the brain's electrical activity

through electrodes placed on the scalp and provides a continuous measurement of the overall brain activity, including the brain's response to TMS and the functioning of various brain regions during rest and cognitive tasks. While TMS-EEG has faced some challenges like artefact management and the need for protocol standardisation (see Farzan et al. (2016) for a review) it is considered a highly effective method for exploring the connection between brain areas (Hallett et al., 2017).

TMS can target two locations, an approach referred to as “twin coil” TMS. This approach frequently investigates how various brain regions impact the primary motor cortex (M1) both at rest and throughout movement. By administering a stimulus to one brain region before targeting M1, researchers can observe the effects on motor-evoked potential (MEP) amplitudes, comparing them against baseline amplitudes from a single-pulse MEP. These comparisons help determine whether the connectivity effect is either facilitatory, inhibitory, or neutral (Di Lazzaro et al., 1999; Hallett et al., 2017). A review by Di Lazzaro et al. (1999) emphasises the value of MEPs as an effective method for identifying hidden involvement of the central motor pathway in diseases such as motor neuron diseases, multiple sclerosis, muscle disorders, and spinal cord diseases, as well as underlining its diagnostic potential. TMS generates MEPs by stimulating the brain's motor cortex and recording muscle electrical activity. When TMS stimulates the motor cortex, it sends an electrical signal down the motor pathways to a target muscle. This process effectively "jumps starts" the motor pathway, allowing researchers or clinicians to study how well the brain communicates with the muscles. Electrodes are placed on the skin directly over a target muscle to capture the muscle's response to this stimulation. For example, when studying hand movement, electrodes might be placed over the First Dorsal Interosseous (FDI) muscle, located between the thumb and index finger, which plays a key role in moving the index finger. Once the motor cortex is stimulated, the electrodes record any electrical activity in the target muscle. These MEPs are the muscle's direct response to being stimulated. The recorded MEPs are analysed for their amplitude (how strong the response is) and latency (how quickly

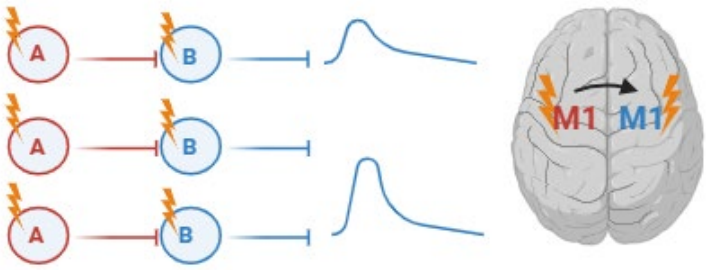
the response occurs after stimulation). These measurements provide valuable information about the integrity and functionality of the motor pathways. A healthy, well-functioning motor pathway will typically show robust and prompt responses, whereas delays or weakened responses may indicate potential issues or damage within the motor pathways.

Combining the detailed information about motor pathway functionality from MEPs with the broader context of brain activity provided by EEG, researchers and clinicians can gain a more comprehensive understanding of the neurophysiological basis of motor control and the effects of neurological disorders on both brain and muscle activity.

Paired associative stimulation (PAS) is a neuroscientific technique in neuroscience and neurorehabilitation (Stefan et al., 2000). It involves pairing electrical stimulation of a peripheral nerve with TMS to the corresponding area in the brain. This process mirrors spike-timing-dependent plasticity (STDP) (Guidali et al., 2021). This is illustrated in Figure 1. The peripheral nerve stimulation is the "conditioning" stimulus, while TMS to the corresponding brain region is the "test" stimulus. By pairing these stimuli in a specific temporal pattern, researchers have demonstrated the ability to modulate synaptic strength in a way that can lead to changes in the neural circuits and potentially enhance or suppress specific motor or sensory functions (Guidali et al., 2021; V. Rizzo et al., 2009; Stefan et al., 2000). By altering the time gap (interstimulus interval or ISI) between paired stimuli, PAS has been shown to induce long-term potentiation (LTP) or long-term depression (LTD) in the synapses between neurons. LTP and LTD are mechanisms through which the strength of synaptic connections between neurons can be modified, and they are believed to play a role in higher-order cognitive functioning and motor control (Guidali et al., 2021).

Figure 1

Illustration of Spike Timing-Dependent Plasticity (STDP) and Its Application in Motor Cortex Stimulation



Note. This image illustrates the concept of spike timing-dependent plasticity (STDP). On the left, neurons A (red, pre-synaptic) and B (blue, post-synaptic) demonstrate the importance of spike timing in modulating synaptic strength. When the pre-synaptic neuron (A) fires shortly before the post-synaptic neuron (B), it leads to long-term potentiation (LTP), strengthening the synapse. Conversely, when the post-synaptic neuron fires before the pre-synaptic neuron, it results in long-term depression (LTD), weakening the synapse. The waveforms in the middle highlight the temporal relationship of neural spikes, which determines the direction of plasticity. On the right, the brain diagram situates this principle in the motor cortex (M1)

PAS protocols have been applied to study neuroplasticity in conditions like major depressive disorder, Alzheimer's disease, and the impact of substance use (e.g., alcohol). These studies highlight the utility of PAS in enhancing our understanding of frontal cortex functionality, working memory, and the neurophysiological basis of cognitive and clinical conditions.

PAS has been investigated for its potential applications in neurorehabilitation, particularly in the recovery of motor functions after stroke or other neurological injuries. TMS-induced MEP measurements have shown that PAS induces effects similar to long-term

depression (LTD) in fast-transmitting corticospinal output neurons (Stefan et al., 2000). PAS has been found to increase motor cortical neural excitability (Classen et al., 2004) and has successfully modulated excitability between two interconnected neural pathways.

Cortico-Cortical Paired Associative Stimulation (ccPAS)

Cortico-cortical paired associative transcranial magnetic stimulation (ccPAS) is a technique pioneered by Rizzo et al. (2009), which involves applying two magnetic pulses in rapid succession to two interconnected cortical regions, for example, the primary motor cortex (M1-M1), with a fixed interstimulus interval (ISI). The pulses are timed to coincide with the peak of excitability in the targeted areas, causing them to be paired, leading to changes in neural plasticity. An initial TMS pulse is administered to one brain area, followed shortly by a subsequent TMS pulse to another interconnected area. This technique is grounded on a principle known as Hebb's rule, coined by the Canadian psychologist Donald Hebb in 1949 (Hebb, 1949). It is encapsulated in the succinct phrase, "Cells that fire together, wire together." Hebb's rule posits that the simultaneous activation of two neurons (or cells) strengthens their connection. This idea is crucial to synaptic plasticity, which refers to the capacity of synapses to modify their strength over time. Synaptic plasticity is a cornerstone of the brain's ability to learn and retain information. If the timing between the pulses is optimal, it can strengthen the synaptic connections between these areas, enhancing their functional connectivity. ccPAS has been used in research settings to investigate the connections and interactions between different brain areas and to explore the potential for inducing long-term changes in neural activity. Research has explored connections within the same hemisphere (intrahemispheric) or between hemispheres (interhemispheric) (Hernandez-Pavon, San Agustín, et al., 2023).

Cortico-cortical paired associative stimulation in Studying Motor Control

Previous reviews have explored the use of ccPAS, highlighting its use in both fundamental research and clinical contexts (Chiappini et al., 2022; Guidali et al., 2021; Hernandez-Pavon, San Agustín, et al., 2023; Koch, 2020; Sel et al., 2021; Tarasi et al.,

2024; Trajkovic et al., 2023; Tremblay et al., 2019). These reviews have demonstrated the pivotal role of cortico-cortical paired associative stimulation (ccPAS) in deepening our understanding of brain connectivity and plasticity. They highlight ccPAS's effectiveness in probing higher-order cognitive functions such as memory, attention, and decision-making and its application in studying the visual system and motor functioning. Furthermore, these discussions underscore ccPAS's potential therapeutic applications, showcasing its versatility as a tool for both research and clinical interventions.

By targeting specific motor and premotor areas, researchers have induced plastic changes that mimic learning-induced plasticity observed in motor skill acquisition. For instance, applying ccPAS to the primary motor cortex (M1) and a corresponding sensory or associative area has been shown to improve motor function, suggesting a potential avenue for enhancing motor learning and rehabilitation. Studies have shown that the effects of ccPAS can include increased MEP amplitude, indicating enhanced cortical excitability and potentially leading to improved motor performance (Rizzo et al., 2009; Rizzo et al., 2011; Sel et al., 2021; S Turrini et al., 2023; S. Turrini, F. Fiori, et al., 2023). Rizzo et al. (2009) original study aimed to identify whether interhemispheric inhibition could be modulated using ccPAS in a group of young adults (mean age 30 years). Using paired TMS applied over the two motor cortexes, they demonstrated that inhibitory responses could be modulated.

Identifying the Research Gap

Despite substantial advancements in understanding the ageing process and its effects on motor control, significant gaps remain in our knowledge, particularly around how interconnected brain areas communicate and the changes that ageing brings. Ageing research has extensively documented the decline in motor function and neuroplasticity, yet the precise neural pathways and processes are not fully understood. Furthermore, there is a pressing need for innovative approaches that can directly measure and modulate these brain mechanisms to mitigate age-related motor decline. Understanding cortico-cortical protocols is essential for investigating how different brain regions communicate and work together to

support various cognitive and behavioural processes. Dysfunction in cortico-cortical communication has been implicated in various neurological and psychiatric disorders. ccPAS has the potential to explore and augment neural connectivity; however, it is still an experimental technique, and more research is needed to understand its effects and potential applications fully.

The significance of targeting the motor cortex in interventions aimed at older adults cannot be overstated. The primary motor cortex (M1) is crucial for learning and executing motor activities and is intricately connected to cognitive functions. As age-related changes in the central and peripheral nervous systems lead to decreased motor control, understanding and mitigating these changes become crucial for promoting healthy ageing.

Previous reviews have played a crucial role in synthesising the existing knowledge on ccPAS, offering insights into its efficacy and mechanisms. My systematic review aims to collate evidence on the applications of ccPAS, including the most up-to-date literature, providing an overview of how ccPAS has been employed to modulate synaptic efficiency and excitability in pathways connecting different brain areas. This review traces the development of the method, studies its impact, and pinpoints areas that merit further investigation.

Chapter 2: Systematic Review

Chapter Overview

The chapter includes a systematic review of the existing ccPAS literature. The review aims to provide an in-depth overview of the various applications of ccPAS. I will trace the method's development, study its impact on corticocortical plasticity and connectivity, and seek to pinpoint areas that merit further research. I will consider the protocol's potential as a tool for neurorehabilitation, particularly for conditions involving disrupted connectivity between different brain regions. Its potential as an intervention within a clinical setting will also be considered.

Methods

A systematic review was conducted to ascertain the efficacy of cortico-cortical paired associative stimulation (ccPAS) as an effective methodology for modulating neural pathways.

The methodology and research approach followed the PRISMA 2020 protocol (Page et al., 2021) , which provided a defined structure for the systematic review regarding the research methods, data analysis and processing, and reporting of the obtained results.

Data sources

Studies were retrieved manually from three online databases: PubMed, Scopus and Web of Science. These were selected due to their broad compendium of peer-reviewed papers, good reputation, and advanced search capacities. All were accessed through the University of Essex online server and used independently of each other, with the planned dates of coverage ranging from 2008 to 2023. A hand search of bibliographies further supported this search.

Inclusion criteria

To qualify for this review, studies needed to meet the following criteria:

- Studies objectively assess and measure corticocortical connectivity between brain regions using ccPAS as a technique.
- Investigations should aim to explore functional and structural connectivity and electrophysical communication in the brain using two or more TMS coils for stimulation.
- Studies have been peer-reviewed, basic or clinical studies involving human participants exclusively.
- All studies meeting these criteria were included in the analysis, irrespective of participant age, gender, or clinical status.

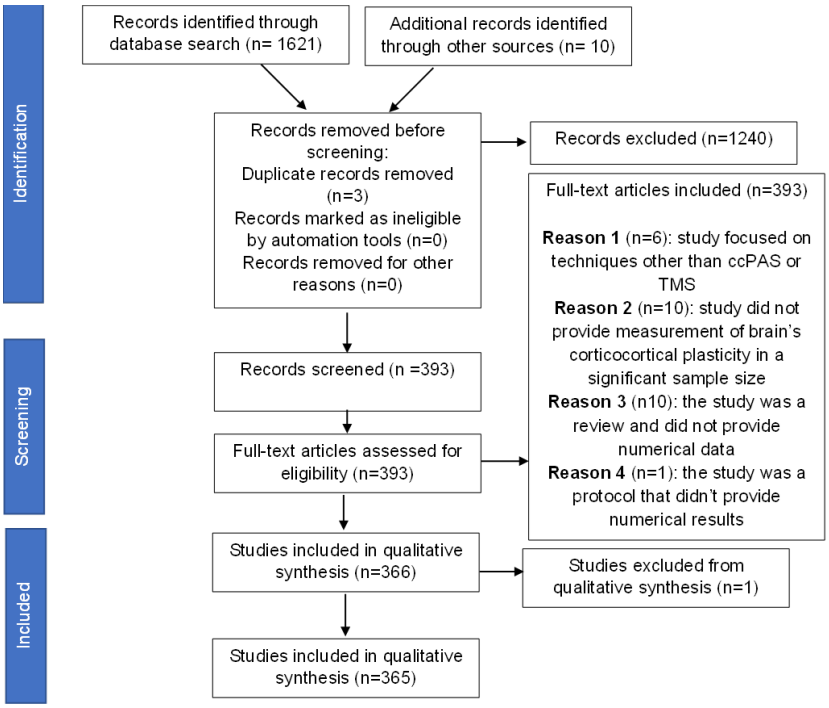
Exclusion criteria

The following exclusion criteria were:

- Studies had to be published in English.
- Refer to Figure 1 for the PRISMA flow diagram.

Figure 2

PRISMA-based chart showing the process of systematic literature review



Literature research strategy

The advanced search feature of each of the databases searches was used to introduce the following keywords that relate to corticocortical connectivity in brain regions using the ccPAS technique: ccPAS, ccPAS, c-PAS, PAS, cortico-cortical paired associative stimulation, corticocortical paired associative stimulation, paired associative stimulation, repetitive dual coil transcranial magnetic stimulation, repetitive dual-coil transcranial magnetic stimulation, repetitive dual coil TMS, repetitive dual-coil TMS, repetitive paired-pulse transcranial magnetic stimulation, repetitive paired-pulse transcranial magnetic stimulation, repetitive paired-pulse TMS, repetitive paired-pulse TMS, and repetitive ppTMS. Every mentioned keyword was accompanied by the term “human” and quotation marks to retrieve the most relevant studies for this review, as shown in Table 1. Additionally, specific fields were selected to eliminate non-related subjects and narrow the number of studies to papers related to neuroscience and psychology. These excluded economics, business, agriculture, biology, biochemistry, immunology, and engineering, which are not relevant and, therefore, were selected as excluding fields in all databases.

Table 1

Systematic review searched keywords.

Keyword number	Keyword	Filter	Limiters
1	“cc-PAS”	Human AND Psychology AND Neuroscience NOT Economics NOT Business NOT Agriculture NOT Biology NOT Biochemistry NOT Immunology NOT Engineering	Articles and Reviews
2	“cortico-cortical paired associative stimulation”	Human AND Psychology AND Neuroscience NOT Economics NOT Business NOT Agriculture NOT Biology NOT Biochemistry NOT	Articles and Reviews

3	“corticocortical paired associative stimulation”	Immunology NOT Engineering Human AND Psychology AND Neuroscience NOT Economics NOT Business NOT Agriculture NOT Biology NOT Biochemistry NOT Immunology NOT Engineering Human AND Psychology AND Neuroscience NOT	Articles and Reviews
4	“paired associative stimulation”	Economics NOT Business NOT Agriculture NOT Biology NOT Biochemistry NOT Immunology NOT Engineering Human AND Psychology AND Neuroscience NOT	Articles and Reviews
5	“repetitive dual coil transcranial magnetic stimulation”	Economics NOT Business NOT Agriculture NOT Biology NOT Biochemistry NOT Immunology NOT Engineering Human AND Psychology AND Neuroscience NOT	Articles and Reviews
6	“repetitive dual-coil transcranial magnetic stimulation”	Economics NOT Business NOT Agriculture NOT Biology NOT Biochemistry NOT Immunology NOT Engineering Human AND Psychology AND Neuroscience NOT	Articles and Reviews
7	“repetitive dual coil TMS”	Economics NOT Business NOT Agriculture NOT Biology NOT Biochemistry NOT Immunology NOT Engineering Human AND Psychology AND Neuroscience NOT	Articles and Reviews
8	“repetitive dual-coil TMS”	Economics NOT Business NOT Agriculture NOT Biology NOT Biochemistry NOT Immunology NOT Engineering Human AND Psychology AND Neuroscience NOT	Articles and Reviews

		Economics NOT Business NOT Agriculture NOT Biology NOT Biochemistry NOT Immunology NOT Engineering Human AND Psychology AND Neuroscience NOT	
9	“repetitive paired pulse transcranial magnetic stimulation”	Economics NOT Business NOT Agriculture NOT Biology NOT Biochemistry NOT Immunology NOT Engineering Human AND Psychology AND Neuroscience NOT	Articles and Reviews
10	“repetitive paired-pulse transcranial magnetic stimulation”	Economics NOT Business NOT Agriculture NOT Biology NOT Biochemistry NOT Immunology NOT Engineering Human AND Psychology AND Neuroscience NOT	Articles and Reviews
11	“repetitive paired pulse TMS”	Economics NOT Business NOT Agriculture NOT Biology NOT Biochemistry NOT Immunology NOT Engineering Human AND Psychology AND Neuroscience NOT	Articles and Reviews
12	“repetitive paired-pulse TMS”	Economics NOT Business NOT Agriculture NOT Biology NOT Biochemistry NOT Immunology NOT Engineering Human AND Psychology AND Neuroscience NOT	Articles and Reviews
13	“repetitive ppTMS”	Economics NOT Business NOT Agriculture NOT Biology NOT Biochemistry NOT	Articles and Reviews

	Immunology NOT Engineering

Concerning the choice of referencing software to handle the collected studies, key considerations included prioritising ease of use, effectiveness, and features for organising and cataloguing the acquired studies. Consequently, EndNote was identified as the most suitable software, given its ability to categorise references, compatibility with the export formats for citing references in the three chosen databases, and its capability to identify and manage duplicate references through the "Duplicate search" option in the created library.

The total number of studies found in PubMed was 155, 277 for Scopus, and 16 for Web of Science, for a total of 398 studies relating to corticocortical connectivity in brain regions using ccPAS and TMS. These searches were further supported by a hand search of bibliographies and backward and forward citation searches.

Data management

The primary tools for managing records and data throughout the systematic review were the citation manager EndNote version 21 and Microsoft Office Excel version 2311. Each study underwent a comprehensive analysis to identify relevant data, which was then systematically organised within an Excel spreadsheet for further examination. A critical assessment of each paper's methodology and results sections was conducted to verify that the reported data met the criteria established for this systematic review. The review focused on obtaining an objective measure of corticocortical connectivity in brain regions using the ccPAS technique.

Results

Overall, 44 studies that used the ccPAS technique to investigate its potential to elicit alterations in neural plasticity in the human brain were identified. Table 2 and Figure 3 summarise all the studies, including their ccPAS parameters and findings. The results of the 44 studies are grouped and reviewed by the targeted brain regions and presented

chronologically. This approach highlights the protocol's continued development and expansion into different cortical regions, research, and clinical contexts.

Table 2 – *Summary Of Cortico-Cortical Paired Associative Stimulation Studies*

Study ID	Author	Participant size	Mean Age in years	Targeted pathway	ISI (ms)	Measures	Outcome
1	Arai et al. (2011)	29	28.2	SMA-M1 M1-> SMA	3.2 6 10 15	EMG MEP	↑MEP SMA->M1 with ISI of 6ms ↓MEP SMA-> with ISI of 15ms
2	Buch et al. (2011)	35	26.09	PMv -> M1 pre-SMA -> M1 M1 -> PMv	8	EMG MEP	↑MEP PMv->M1 with ISI 8ms ↓MEP M1->PMv with ISI of 8ms
3	Casarotto, Dolfini, Cardellicchio, et al. (2023)	39	26	PMv -> M1 M1-> PMv	1 3	MEP, LICI, SICI, ICF, SICF	↑MEP PMv→M1 PA post 30 ↓MEP PMv→M1 AP POST30
4	Casula et al. (2016)	14	29.9	L DLPFC -> L PPC & M1	10	EEG TEP	↑ P3 TEP spTMS over LDLPFC & RDLPCF LPPC→LDLPFC with ISI 10ms ↓ P3 TEP spTMS over DLPFC LDLPFC→LPPC with ISI 10ms
5	C. C. Chao et al. (2015)	35	29.7	PPC -> M1	4 6 8	MEP, RMT, I/O	↑ MEP PPC -> M1 with ISI of 8ms POST60 & POST120

6	Chiappini et al. (2020)	28	24.9	PMv -> M1 SMA -> M1 SHAM	40	MEP, rMT	↓ MEP LPMv→LM1 with ISI of 40ms POST0 ↑ MEP SMA→M1 with ISI of 40ms POST20
7	Chiappini et al. (2018)	16	25.3	V5/MT+ -> V1/V2 V1/V2 -> V5/MT+	20	PT	↑ Perceptual sensitivity V5 -> V1/V2 with ISI 20ms at 80% PT
8	Chiappini et al. (2022)	71	23.6	lV5->rV5 rV5->lV5 V5&V5 Lv5-rV5 Sham	25 0	Parity Ratio	↓ Parity ratio LV5 -> RV5 POST0, POST60, & POST90 ↓ Parity ratio RV5 -> LV5 POST30 ↑ Rightward perceptual Bias RV5 -> LV5 POST0
9	Di Lorenzo et al. (2017)	12		LPPC-> LM1 LM1 -> LPPC	5	MEP SAI	Control ↑MEP LM1 ->LPPC POST10 & POST20 ↓MEP LPPC ->LM1 POST10 & POST20
10	Di Luzio et al. (2022)	51	24	V5/MT+-to-V1/V2 IPS/LIP-to-V1/V2 IPS/LIP-to-V1/V2	20 30 0		↑Perceptual sensitivity V5 -> V1/V2 with ISI of 20ms ↑Metacognitive Efficiency IPS -> V1/V2 with ISI 30ms

11	Fiori et al. (2018)	54	23.1	PMv-to-M1 M1-> PMv PMv->M1 Sham	8	MEP	↑ MEP LPMv →L M1
12	Fricke et al. (2019)	20	58.5	PMd -> M1 PMd -> M1 Sham	25	EMG MEP	No effects
13	Johnen et al. (2015)	30	24	PMv-> M1 PMv -> M1	8 500	fMRI BOLD MEP EMG	↑Functional Connectivity PMv -> M1 with ISI 8ms ↑ responsiveness of M1 to PMv PMv -> M1 with ISI 8ms
14	Koch et al. (2013)	29	21-38	PPC -> M1	5 20 50	MEP	↓MEP PPC -> M1 PA with ISI 5ms POST15 & POST20 With ISI 20ms POST10 & POST15 ↑MEP M1→PPC PA With ISI 5ms & 20ms POST5, POST10, POST20 & POST25 With ISI 20ms at POST5, POST10, POST15, POST20 & POST25
15	Koganemaru et al. (2009)	23	27.7	M1-> M1	5 10 15 25	EMG MEP rMT SP	↑MEP RM1→LM1 with ISI 15ms POST0 & POST20

16	Kohl et al. (2019)	25	26.77	IFC -> preSMA preSMA -> IFC	10 4	RT MEP EMG	↓SSRT Pre-SMA→IFC with ISI of 10ms
17	Lazari et al. (2022)	37	18-32	RPMv -> LM1	6	MRI MEP	↑ RPMv ->LM1 POST 24hr
18	Lu et al. (2012)	19	29.8	CB -> M1	2 6 10 random	MEP SICI ICF CBI	↑ MEP RCB -> LM1 with ISI of 2ms POST0, POST30 & POST60 ↓ MEP RCB -> LM1 with ISI 6ms POST0, POST30 & POST60 ↓ MEP RCB -> LM1 with ISI 10ms POST0 & POST30
19	Mandali et al. (2021)	37	22-59	preSMA -> rIFC	4	RMT MEP	↑ preSMA -> rIFC with ISI of 4ms
20	Momi et al. (2020)	29	25.43	PFC -> M1	10 0	MRI BOLD RMT	↑ in Logical reasoning accuracy LIPL -> LMFG ↓ in Relational reasoning accuracy LMFG -> LIPL
21	Nord et al. (2019)	30	35.9	RLPFC -> RIPS RIPS -> RLPFC Control	10 100	Decision Making Task	Change in goal-based decision making. RIPS-> RLPFC with ISI 10ms

						Working Memory Task	
22	Ribolsi et al. (2017)	24	38 (31 - 45)	PPC -> M1	5	EMG MEP	Control ↑MEP LM1 -> LPPC ↑MEP RM1 -> RPPC POST0, POST10 & POST20 Schizophrenia ↑MEP RM1 -> RPPC POST10 & POST20
23	Rizzo et al. (2011)	10	24-46	M1-> M1	8 9 10	MEP IHI	↑ MEP LM1→RM1 with ISI 8ms POST30
24	V. Rizzo et al. (2009)	12	32 (24-39)	M1-> M1	8	MEP SICI ICF CSP RT IHI	↑ LM1 -> RM1 with ISI of 8ms ↓RM1 -> LM1 with ISI of 8ms POST0 & POST20
25	Romei et al. (2016)	32	22.31 (18 - 26)	V1 ->V5 V5 -> V1	20	MST	↑Perceptual sensitivity V5 -> V1 with ISI of 20ms POST30 & POST60
26	Santarnecchi et al. (2018)	17	24.2 (20 - 28)	DMN <-> TPN (M1-> PFC)	0 10	MEG fMRI	↓negative correlation between DMN and TPN DMN -> TPN with ISI 10ms ↑ positive connectivity between DMN and TPN TPN -> DMN with ISI 10ms

27	Sel et al. (2021)	12	22.7	PMv -> M1 M1 -> PMv	8	EEG MEP RT	↑ PMv -> M1 with ISI of 8ms ↓ M1-> PMv with ISI of 8ms
28	Turrini et al. (2022)	109	22.8	PMv <-> M1	8	rMT MEP SICI	↑ MEP PMv→M1 with ISI of 8ms
29	S. Turrini, F. Fiori, et al. (2023)	60	23	PMv <-> M1	8	MEP ISCI ICF rMT	↑ MEP PMv -> M1 with ISI of 8ms ↓ SICI PMv -> M1 with ISI of 8ms
30	Sonia Turrini et al. (2023)	28	21-78	PMv <> M1	8	9HPT cRT MEP	↑ 9HPT PMv-> M1 with ISI of 8ms for Young POST 0 & POST30
31	D. Veniero et al. (2013)	13	27.6 (24-32)	PPC <-> M1	5	EEG MEP	↓MEP PPC -> M1 PA With ISI of 5ms POST
32	Zibman et al. (2019)	30	24.2 (22-26)	LPFC-RPFC RPFC-LPFC	8	EEG	↑ ISP L ->R ↓ ISP R->L LLPFC-> RLPFC with ISI of 8ms ↑ISP R->L ↓ISP L-R RLPFC -> LLPFC with ISI 8ms
33	Hernandez-Pavon, Schneider-Garces, et al. (2023)	11	19 - 47 (33)	M1 -> M1	4 9 14	ccEP MEP MRI EEG	↓Effective connectivity LM1 -> RM1 with ISI 4ms No changes in effective connectivity LM1 -> RM1 with ISI 9ms

							↑ Effective connectivity LM1 -> RM1 with ISI 14ms
34	Carson et al. (2021)	14	21-27 (24)	M1-> M1	6	EMG MEP	↑MEP RM1→LM1 with ISI 6ms At POST10, POST20 & POST30
35	Pauly et al. (2021)	38		pMD -> M1 CB -> M1	6	MEP EEG	↓ MEP RCB -> LM1 with ISI 6ms
36	Hooyman et al. (2022)	11	(26.4 +/- 5.6)	PFC -> M1	5 500	MEP rs-EEG	↑Functional connectivity RPFC -> RM1 with ISI 5ms No changes in functional connectivity RPFC -> RM1 with ISI 500ms
37	Wang et al. (2022)	50	18-60	dlPFC PPC dlPFC ->PPC	100	HAM-A HAM-D PSQI	↓in all measures POST0 & POST10 days and POST1 month
38	Lin et al. (2020)	29		RDLPFC -> RPPC	10 20 50	HRSA HRSD PSQI	HRSA ↓at POST0 & POST2 weeks and POST1 month
39	Casarotto, Dolfini, Fadiga, et al. (2023)	31	23.33	PMv -> M1 (AP) PMv -> M1 (PA)	6	MEP	AP ↑ PMv -> M1
40	Bevilacqua et al. (2023)	16	27	V1-> MT MT -> V1	20	EEG	Forward ccPAS ↑ MT Backward ccPAS ↑ MT->V1 Backward ccPAS ↑ V1 Backward ccPAS ↑ motor detection & awareness

41	Guidali et al. (2023)	25	24.9	FP-ccPAS FEF-> IPL PF-ccPAS IPL-> FEF	10 100		FP-ccPAS with ISI 10 ↑ pseudoneglect PF-ccPAS with ISI 10 no effect FP-ccPAS with ISI 100 no effect
42	S. Turrini, N. Bevacqua, et al. (2023)	28	21-77	PMv -> M1	8	MEP RT Accuracy	YA ↑ 9HTP OA 9HTP no change
43	Borgomaneri et al. (2023)	155	22.1	pSTS -> V1/V2 V1/V2 -> pSTS	200	EEG ERP	ERP ↑ pSTS -> V1/V2 with ISI 200ms No effect V1/V2 -> pSTS with ISI 200ms
44	Trajkovic et al. (2023)	36	23.61	PMv -> M1 M1 -> PMv	6 8	EEG	PMv-to-M1 ccPAS ↑ alpha & beta phase synchrony M1-to-PMv ccPAS ↓ theta phase synchrony

Cortico-cortical paired associative transcranial magnetic stimulation of the primary motor cortex

This review identified five papers focusing on modulating motor cortex neuronal activity. Rizzo et al. (2009) were the pioneers in the development of ccPAS TMS targeting the human motor cortex. They set out to investigate whether ccPAS could modulate interhemispheric inhibition (IHI) as observed in animal research (Stephan, 2000). To record the effect of ccPAS, two transcranial stimuli were consistently paired over the left and right M1 hand regions to induce motor-evoked potentials (MEPs). Each pair of stimuli involved a single TMS pulse delivered to the ipsilateral M1 hand, followed by a single TMS pulse applied to the contralateral M1 hand, maintaining a fixed interstimulus interval (ISI) of 8ms. It was hypothesised that ccPAS would induce associative spike-timing-dependent plasticity (STDP)---like changes in the conditioned M1 hand by stimulating the ipsilateral side. Results demonstrated that ccPAS pulses at a frequency of 0.05Hz were sufficient to reduce the inhibitory impact of M1 on the contralateral region. Left-to-right ccPAS protocol enhanced response times, specifically for the left index finger, as measured using a simple reaction time task, whereas right-to-left ccPAS caused a reduction. This demonstrated that ccPAS could induce associative plasticity in the connections between targeted cortical regions with an ISI of 8ms. Subsequent trials demonstrated no observable alterations in left-to-right IHI or corticospinal excitability when left-to-right ccPAS was administered with an interstimulus interval (ISI) of 1 ms or various ISIs presented in random order. Rizzo et al. (2011) went on to explore the effects of using the same ccPAS protocol on specific hand movements, confirming their previous finding. This novel discovery showed that ccPAS influenced the performance of basic finger movements, specifically in changing the duration of Touch Duration (TD) and Inter Tapping Interval (ITI).

Koganemaru et al. (2009) built upon Rizzo et al. (2009) study and implemented paired bihemispheric stimulation (PBS) to induce STDP-like changes in the motor cortex. Twice as many pulses and twice as fast a frequency (0.1Hz) than Rizzo were used: 180

paired pulses with a specific timing interval of 15ms, preceding left M1 stimulation with right M1 stimulation. The aim was to investigate how the timing interval could influence the propagation of neuronal signals between the two hemispheres of M1. ISI was based on a previous animal study, which demonstrated that intervals of less than 20 ms could induce associative plasticity (Bi & Poo, 1998). Using varying ISI in 10ms increments, ranging from -25ms to 25ms, they hoped to identify whether the effects of PBS depended on a specific timing. To determine the duration of the effects, outcomes were recorded at different time intervals after stimulation: T0, T20, and T40. To discern any behavioural alterations, response times were measured during tasks involving a nine-hole peg and choice reaction, revealing notable enhancements in performance. Like Rizzo et al. (2009), a significant reduction in IHI was observed when PBS with ISIs of 10ms and 40ms, contrasting with the initial protocol, which employed an ISI of 8ms.

Carson et al. (2021) adopted an ISI of 6ms to modulate M1-to-M1 connectivity and observed a decrease in the normal inhibitory influence, with the effect lasting for between 20 and 30 minutes. Hernandez-Pavon, Schneider-Garces, et al. (2023) varied the ISI between stimulations in three sessions: one where the ISI was 5ms shorter than the normal delay between these two regions, one where it was equal, and one where it was 5ms longer. The findings indicated that ccPAS has a unidirectional influence, suggesting that the stimulation explicitly increased the flow of information from one neural region to another.

In summary, these studies collectively suggest that ccPAS modulates neural plasticity and interhemispheric inhibition, with stimulation's specific directionality and timing playing crucial roles in determining the observed outcomes. These findings advance the understanding of associative plasticity by investigating its mechanisms, experimental applications, and potential clinical relevance. While they provide a robust foundation, they share common limitations, including small sample sizes and short-term observations.

Cerebellum and M1

The study by Lu et al. (2012) set out to investigate if 120 ccPAS pulses at a frequency of 0.25Hz could alter the excitability of M1 by targeting the cerebellum, a region of the brain essential for motor control and adaptation. The aim was to induce bidirectional modifications (increasing and decreasing excitability) in the motor cortex (M1). Conditioning stimulation was applied over the right lateral cerebellum of 19 healthy volunteers, followed by TMS on the left M1 area at different intervals: 2ms, 6ms, 10ms, or a mix of 2ms and 10ms. The effects of PAS on the excitability of M1 were measured using several metrics: MEP amplitude, short-interval intracortical inhibition (SICI), intracortical facilitation (ICF), and cerebellar-motor cortex inhibition (CBI). The different ISIs between the cerebellum and M1 stimulations had varying effects on M1 excitability: A 2ms interval (RCB→LM1 PAS 2ms) increased MEP. Intervals of 6ms and 10ms (RCB→LM1 PAS 6ms & RCB→LM1 PAS 10ms) decreased MEP. Randomly alternating intervals (RCB→LM1 PAS Control) resulted in no changes. The effects persisted for 30-60 minutes. The SICI and CBI measurements decreased across all ccPAS protocols, suggesting a non-specific effect, while ICF remained unchanged.

These results further support ccPAS's usefulness in mimicking STDP within the motor cortex. It highlights the importance of targeting specific pathways and timing intervals to achieve desired plasticity outcomes, a principle that is directly relevant to refining ccPAS protocols. The distinct MEP changes observed at different ISIs highlight the role of temporal specificity in inducing STDP-like plasticity.

Supplementary motor area (SMA) and M1

The supplementary motor area (SMA) and M1 are two key brain regions associated with voluntary movement (Arai et al., 2011). Arai et al. (2011) deployed 150 ccPAS pulses with a frequency of 0.2 Hz to explore changes in the excitability of the SMA-M1 network; SMA -M1 and pre-supplementary motor area (pre-SMA). 29 healthy participants took part in the study, with different subjects of participants used for specific experiments, Changes in

the electrical activity in the First Dorsal Interosseous muscle, as measured by MEP amplitude, were recorded before and after the application of ccPAS. Stimulation of the left primary motor cortex (M1) consistently occurred before the stimulation of the right primary motor cortex (M1). When the SMA was stimulated 6ms before M1, the MEP amplitude increased. Conversely, the MEP amplitude decreased when the SMA was stimulated 15ms after M1. Stimulation of the pre-SMA did not result in any observable changes.

The study highlights the bidirectional modulation of excitability within the SMA-M1 network and the critical dependence of these effects on the interstimulus interval (ISI). Stimulation of the pre-SMA did not produce significant changes in excitability, indicating that the SMA-M1 network has distinct anatomical and functional connectivity that governs its response to paired stimulation. The results suggest that the observed bidirectional modulation follows the principles of STDP, which align with existing ccPAS research. The observed effects of plasticity being short-lived (lasting up to 30 minutes). Once again, the study's small and variable sample size limits the statistical power and generalisability of its findings. The lack of exploration into the long-term durability of these effects leaves questions about their potential for therapeutic application. Nonetheless, the study provides valuable insights into timing-dependent associative plasticity and its implications for targeted neural modulation.

Ventral motor area

The brain's ventral motor area, also called the ventral premotor cortex, is involved in planning and executing motor movements. It's located in the frontal lobe, adjacent to the primary motor cortex. Eleven studies used ccPAS to modulate connectivity between the ventral premotor cortex (PMv) and primary motor cortex (M1).

Buch et al. (2011) conducted a study to examine the effects of continuously pairing PMv and M1 TMS on the strength of functional connectivity of the PMv–M1 pathway. In addition, they explored whether the alterations in PMv–M1 connectivity were pathway-specific by seeing if similar changes arose from the consistent pairing of pre-SMA and M1

TMS. Ninety ccPAS pulses at a frequency of 0.1Hz with an ISI of 8ms were used. Stimulating both the pre-SMA and M1 did not alter PMv–M1 connectivity. The outcome depended on the stimulation order: stimulating PMv before M1 enhanced the PMv–M1 pathway, while the opposite weakened it. Changes were observed to be dependent on the participant's cognitive state during testing; paired PMv–M1 stimulation increased PMv inhibitory effect on M1 at rest but facilitated M1 during a visuomotor task. The researchers were able to evidence pathway-specific plasticity between the two cortical regions. The plasticity developed quickly and lasted at least an hour, diminishing over 3 hours post-intervention.

Buch et al. (2011) established that PMv demonstrates an inhibitory functional impact on M1 in a resting state, and that this inhibition transforms into a facilitatory influence during grasping. Comparable results were observed by Johnen et al. (2015) where ccPAS with an ISI of 8ms led to increased functional connectivity between PMv and M1 only during task performance. Functional magnetic resonance imaging (fMRI) scans were conducted to evaluate functional connectivity at baseline and post ccPAS. The increased functional connectivity was associated with improved motor performance and was specific to the stimulated pathway. As no faciliatory changes was observed at rest in both studies, It suggests that behavioural engagement is necessary to manifest plasticity-induced connectivity changes. The effects of ccPAS on functional connectivity and behaviour were long-lasting and persisted for at least an hour after the stimulation. To ensure that the functional connectivity changes observed were attributed to the induction of plasticity, the researchers used a control condition where an ISI of 500ms. This resulted in no observable changes, suggesting that the changes induced with the ISI of 8ms were indicative of plasticity. Together, these studies highlight the dynamic interplay between resting and active states, revealing how cognitive and motor engagement determines the functional expression of plasticity-induced changes in the corticocortical PMv-M1 network.

Fiori et al. (2018) utilised ccPAS to ascertain whether improving action performance in the PMv-to-M1 pathway is possible. Within this pathway, PMv translates visual information concerning objects into motor commands, which are then passed to M1, facilitating precise control over finger movements. For the 9-HPT (Mathiowetz et al., 1985), participants were required to pick up nine pegs one by one and place them into corresponding holes on a board as quickly as possible, and then remove them. This task taps into the fine motor control and dexterity facilitated by the PMv-M1 pathway. The control task (cRT) involved responding as quickly and accurately as possible to visual stimuli (numbers "1" or "2") by releasing a key with either the index or middle finger. This was designed to assess simple visuomotor mapping without the dexterity component. The participants who received 90 ccPAS pulses at a frequency of 0.1Hz and an ISI of 8ms for the PMv-to-M1 pathway demonstrated enhanced motor responsiveness and improved scores on the 9-Hole Peg Test (9-HPT). The enhancements were pathway-specific, as no improvements were observed when ccPAS was administered in the opposing direction (M1-to-PMv) or by sham. No enhancements were recorded for the visuomotor control task, suggesting that the impact of ccPAS was functionally specific.

These studies reported plasticity changes from ccPAS with an 8ms ISI when administered to the PMv and M1 pathway. This ISI was chosen as it has consistently yielded replicable results in assessing functional connectivity between PMv and M1 and is aligned with the timing regulations of short-latency connections (Buch et al., 2011; Fiori et al., 2018). As with the previous studies, state-dependant effects were observed; while stimulation occurred at rest, behavioural enhancements emerged only during active task performance. This supports the view that enhanced connectivity becomes functionally relevant when the network is actively engaged for motor tasks. These findings provide strong support for the role of STDP in ccPAS-induced plasticity within the PMv-M1 network, reinforcing the concept that precise timing and directionality of neural activation govern cortical plasticity outcomes.

Chiappini et al. (2020) explored whether 90 ccPAS pulses at a frequency of 0.1Hz employing long-latency interactions (ll-ccPAS) could produce associative plasticity within the PMv-M1 pathway with an ISI of 40ms. The same ll-ccPAS protocol was used to target the SMA-to-M1 pathway to test for specificity, while a sham ll-ccPAS group was used as a control. No significant changes were observed in the sham group after the ll-ccPAS stimulation. Both the PMv-M1 and SMA-M1 groups resulted in enhanced inhibition, with PMv-to-M1 stimulation being most noticeable, specifically at T0. Unlike short-latency ccPAS, the effects on the PMv-to-M1 neural pathway were temporary, and no effects were recorded at T20 or T40. At T0, changes were specific to pathways; SMA-to-M1 stimulation resulted in no change. However, at T20, the SMA-to-M1 stimulation increased the inhibitory interactions in the PMv-to-M1 pathway. In contrast, short-latency ccPAS did not have a notable effect on unstimulated pathways—it either left them unchanged or weakened. Overall, the effects of ll-ccPAS varied depending on the type and timing of the stimulation, with some results being temporary and others lasting longer. The findings align with STDP principles, where precisely timed stimulation strengthens synaptic efficiency, even in indirect pathways. The study only assessed resting-state effects, so it remains unclear whether ll-ccPAS-induced plasticity would have functional relevance during active task performance. The indirect effects observed with SMA-to-M1 stimulation highlight the complexity of long-latency networks. Compared to short-latency ccPAS, the effects of ll-ccPAS were short-lived. While short-latency ccPAS enhanced connectivity for extended periods, ll-ccPAS effects dissipated quickly, indicating that long-latency pathways may have lower plastic potential or require more sustained stimulation. Further research is needed to determine the precise mechanisms underlying these interactions.

ccPAS has been used to test theories of oscillatory cortical communication. Oscillatory activity in the brain refers to the rhythmic or repetitive neural activity in the central nervous system (Sauseng & Klimesch, 2008). This activity can be measured using electroencephalography (EEG) and magnetoencephalography (MEG). Oscillations are

believed to play a role in various cognitive processes, information transfer, and the coordination of neuronal activity across different brain regions (Sauseng & Klimesch, 2008). It is theorised that oscillations (rhythmic neural activities) reflect the communication or interaction between distinct brain parts. Sel et al. (2021) used ccPAS to increase or decrease the influence of PMv over M1 to understand if the strength of coupling between the two brain regions impacts the observable oscillatory activity in the motor system. Activity was measured in the alpha, beta, and theta frequency bands of the EEG during a motor task. Participants were asked to make a preplanned action (Go) or to refrain from doing so (No-Go) during a motor task. When the coupling between PMv and M1 was increased, the oscillatory rhythms in both beta and theta frequency bands were observed. Effects of ccPAS on beta and theta rhythms were task-state dependent; beta effects emerged during movement, while theta effects appeared during action inhibition. This study provides robust evidence that ccPAS can modulate interregional brain connectivity and drive frequency-specific oscillatory changes in the PMv-M1 network. These results align with STDP principles and highlight ccPAS as a powerful tool for studying oscillatory dynamics in motor control networks. No significant changes in reaction times or accuracy were observed, which contrasts with previous ccPAS studies. The researchers attributed this to the simplicity of the tasks. This suggests that behavioural enhancements emerge only during more taxing active tasks.

It has been hypothesised that abnormal oscillations in the subthalamic nucleus (STN) may be related to the movement problems seen in Parkinson's disease (PD) (Levy et al., 2000). Fricke et al. (2019) speculated that TMS when applied to specific areas of the brain like the M1 and the dorsal premotor cortex (PMd), might influence specific groups of neurons within the STN. It was proposed that ccPAS could improve PD symptoms by disrupting the abnormal connections between STN neurons. In a blinded, placebo-controlled cross-over design, 20 PD patients were administered associative dual-site rTMS targeting the PMd to M1 network. The ISI was set at 25ms, which was identified as the optimum frequency for

STN oscillations. While ccPAS did not significantly improve motor symptoms, it was speculated that this may have been attributed to the ISI of 25ms being less effective in desynchronising STN neurons.

Lazari et al. (2022) aimed to discover whether myelin plasticity followed Hebbian rules. Researchers applied ccPAS to the right PMv and left M1 pathway, which led to changes in M1 excitability, which was detectable 24 hours later. Specifically, the researchers observed increased white matter in the corticospinal tract specific to the hemisphere targeted by ccPAS. The increase in white matter depended on the timing of the TMS, with a more significant increase observed when TMS was applied during task execution rather than during rest, once again providing evidence for task-specific effects. Compensatory connectivity changes were also observed, where ccPAS stimulation led to decreased functional connectivity between the PMv-M1 pathway and non-stimulated visuomotor areas. Turrini et al. (2022) reported that 90 ccPAS pulses at a frequency of 0.1Hz led to a gradual increase in corticomotor excitability, as measured by MEPs during a visuomotor dexterity and choice reaction task. The researchers compared forward ccPAS (PMv-to-M1) with reverse ccPAS (M1-to-PMv). An increase in excitability was observed during forward PMv-M1 ccPAS, and was dependant on ccPAS timing with an ISI of 8ms. Reverse ccPAS showed a trend towards inhibition, but this effect was not statistically significant. Resting motor threshold (rMT) significantly predicted responsiveness to forward ccPAS, and participants with lower rMT (greater baseline excitability) exhibited greater MEP increases. This highlights motor excitability as a predictor of plasticity induction. Using two groups, the researchers could control for effects attributed to repeated stimulation of PMv-M1. As a result, it was concluded that the pattern of corticomotor excitability did not solely arise from the repetitive stimulation of PMv or M1 but was fundamentally contingent on the sequence in which each pair of pulses was administered across these two regions (Turrini et al., 2022). The findings also suggest that extending ccPAS duration beyond 90 pulses could maximize plasticity effects, highlighting potential implications for rehabilitation protocols.

A solid evidence base of studies shows how ccPAS can strengthen the connection between PMv and M1 by employing Hebbian spike-timing-dependent plasticity (STDP). S. Turrini, F. Fiori, et al. (2023) sought to understand whether ccPAS could directly influence M1's local neural activity by manipulating the connectivity between PMv and M1 in 60 right-handed young, healthy individuals. A combination of dual-coil TMS and single- and paired-pulse TMS was administered to study the neural activity specifically within M1. The study found that MEPs increased when PMv and M1 were stimulated in tandem during the ccPAS. A decrease in short-interval intracortical inhibition (SICI) was observed, suggesting a suppression of specific inhibitory mechanisms within M1. These effects were specific to strengthening PMv-to-M1 connectivity through the Hebbian principle, demonstrating that ccPAS also induces local changes in the targeted region (M1) where plasticity is induced.

A further study by S. Turrini, N. Bevacqua, et al. (2023) explored the impact of ageing on motor control, neural plasticity, and how these changes correlate with motor abilities in young and older adults. The mean age of the older adults was 72 years, the first time that older adults have been realistically represented. The study focused on plasticity within premotor-motor circuits, specifically the connectivity between the ventral premotor cortex (PMv) and primary motor cortex (M1), and its prediction of motor ability. Using ccPAS with an ISI of 8ms, the study modulated PMv-to-M1 connectivity in young and older adults. Motor-evoked potential (MEP) changes were used as a measure of corticomotor excitability and behavourial changes were measured by using the time taken to complete the 9-hole timed peg task. Older adults showed lower motor performance and reduced PMv-M1 network plasticity than younger adults, indicating age-related declines in motor abilities and neural plasticity. The findings emphasise the decrease in neural plasticity with age, reflected in the diminished effectiveness of ccPAS in elderly adults compared to young adults. It was concluded that the reduction in plasticity may contribute to the observed decline in motor performance with ageing.

Casarotto, Dolfini, Cardellicchio, et al. (2023) studied the influence of 100 ccPAS pulses at a frequency of 0.25Hz on producing I-waves within the PMv-M1 pathway. I-waves are a series of neural responses that follow TMS's direct activation of the corticospinal tract and reflect the complex interaction of excitatory and inhibitory circuits in the motor cortex. The pattern and number of I-waves generated by TMS can explain the excitability of the motor cortex and the balance of excitatory and inhibitory influences. This information is particularly valuable in studies of neurological and psychiatric conditions and in designing TMS protocols for therapeutic purposes (Casarotto, Dolfini, Cardellicchio, et al., 2023). Researchers predicted that unique interactions would be observed with the I2-wave interneurons. Short intracortical facilitation protocol (SICF) was used to measure these interactions. As in previous studies PMv-M1 ccPAS induced both LTP and LTD like effects on M1 neuronal activity. The changes in M1 activity were associated with a bidirectional shift in I2-wave activity, measured at the SICF timing of 2.5 ms inter-stimulus interval (ISI). It was concluded that plastic changes observed in the PMv-M1 circuit appeared to be influenced by a specific mechanism whereby PMv impacts M1 by targeting I2-wave interneurons. In another study by Casarotto, Dolfini, Fadiga, et al. (2023), PMv-M1 100 ccPAS pulses at a frequency of 0.25Hz stimulation in an anterior-posterior (AP) direction resulted in a distinct alteration of motor control for precision grip. Notably, when MEPs were assessed following AP stimulation, there was a discernible enhancement in corticospinal excitability associated with precision grip movements.

Trajkovic et al. (2023) were interested in investigating how altering the connection strength between the ventral premotor cortex (PMv) and the primary motor cortex (M1) affects neural communication. They measured EEG activity from the brain's motor and prefrontal regions before and after applying ccPAS to either increase or decrease the connectivity strength between PMv and M1. The study involved two groups of participants. In Group 1, the ccPAS protocol was applied to stimulate PMv before M1, with a short inter-pulse interval (IPI) of 6 or 8 milliseconds. It was hypothesised to increase PMv-M1

connectivity strength. In Group 2, the order was reversed (M1-to-PMv), which was hypothesised to decrease the connectivity strength between these regions. By recording EEG activity at rest before and after these interventions, the researchers aimed to identify if strengthening the PMv-M1 pathway enhanced synchronisation (phase synchrony increase) and if weakening this pathway reduced synchronisation (phase synchrony decrease) between the brain areas. In addition, the study hypothesised that the manipulations of connectivity strength would not only affect interregional phase synchrony but also influence the amplitude of oscillatory activity relevant to movement control, suggesting that changes in connectivity could have functional implications for motor behaviour. PMv-to-M1 ccPAS was found to increase phase synchrony in the alpha and beta frequency bands, which was predictive of changes in oscillatory power during movement execution. M1-to-PMv ccPAS resulted in decreased theta phase synchrony, predictive of changes in oscillatory power during movement inhibition. These results suggest a functional link between synaptic efficacy in the PMv-M1 pathway and the oscillatory frequencies mediating their interaction.

The reviewed studies demonstrate that ccPAS can effectively modulates PMv-M1 connectivity through timing- and pathway-specific mechanisms, adhering to Hebbian spike-timing-dependent plasticity (STDP) principles. Short-latency ccPAS with an 8 ms ISI consistently enhances PMv-to-M1 connectivity, as shown by Buch et al. (2011) and Johnen et al. (2015), with effects lasting at least an hour and translating into improved motor performance during visuomotor tasks. Functional outcomes, such as enhanced fine motor control, were reported by Fiori et al. (2018) and Turrini et al. (2022), highlighting the practical relevance of ccPAS in improving dexterity.

Studies like Sel et al. (2021) and Trajkovic et al. (2023) linked ccPAS-induced changes to oscillatory activity, revealing its impact on beta and theta rhythms associated with motor control. Long-latency ccPAS (ll-ccPAS) explored by Chiappini et al. (2020) demonstrated transient, pathway-specific effects, further underscoring the role of ISI in

shaping plasticity. Lazari et al. (2022) provided evidence of structural plasticity in the corticospinal tract, suggesting ccPAS's potential for inducing long-term neurorehabilitation.

However, it should be noted that ccPAS effects were often transient, with limited exploration of long-term impacts in clinical populations. Once more, small sample sizes and variability in methodologies further limit the generalisability of these findings. Despite these limitations, ccPAS shows significant promise as a tool for studying neural plasticity and improving motor function, paving the way for future research and therapeutic applications.

Cortico-cortical paired associative transcranial magnetic stimulation of parietal and motor networks

The review identified six studies that have explored the use of ccPAS within the parietal and motor areas. The parietal lobe plays a crucial role in various sensory and cognitive functions. Its role overlaps with those of other lobes to support complex cognitive processes. It works in conjunction with other areas of the brain, including the frontal, temporal, and occipital lobes, as well as the motor and somatosensory cortex. The parietal regions work closely with the motor cortex and are involved with the planning and executing of movements by providing sensory feedback and spatial information to guide motor actions. However, little is known about how this process works. As a result, the interaction of these areas has been a focus of several ccPAS studies.

Koch et al. (2013) used 100 ccPAS pulses at a frequency of 0.2Hz to investigate STDP in cortico-cortical connections within the human brain. They aimed to determine whether the Hebbian and anti-Hebbian STDP principles observed in animal studies could be extended to human cortical circuits. In Hebbian STDP, the presynaptic neuron fires a few milliseconds before the postsynaptic neuron, resulting in long-term potentiation (LTP). In anti-Hebbian STDP, the order is reversed, resulting in long-term depression (LTD). The study involved applying TMS to the M1 and Posterior parietal cortex (PPC) and recording the resulting MEPs from the FDI muscle of the hand. Changes in MEP amplitudes were taken to measure the plasticity induced by ccPAS. Researchers found evidence of bidirectional

changes in the strength of cortical connections based on ccPAS timing. When the ISI timing followed the principles of Hebbian STDP, the strength of synaptic connections between cortical areas was potentiated. Conversely, synaptic connections were weakened when the timing followed anti-Hebbian STDP principles. Koch et al. (2013) demonstrated that the temporal window of STDP varies between proximal and distal synapses, resulting in varying degrees of potentiation or depression. Additionally, it was found that the location of dendritic interaction with the incoming signals plays a crucial role in determining the direction of plastic changes. These findings suggested that the mechanisms of Hebbian and anti-Hebbian STDP, known to play a role in synaptic plasticity and learning in animal models, were also present in the human cortex. Furthermore, ccPAS could induce bidirectional changes in the strength of cortical connections. In essence, Koch et al. (2013) identified that ccPAS could selectively strengthen or weaken brain connections in humans based on the timing of stimulation, offering insights into how the brain adapts and learns.

The impact of repetitive PPC-M1 activation and the observed changes in MEP amplitude were dependent on when TMS was delivered and the specific groups of neurons being stimulated (Koch et al., 2013). To ascertain whether the effects of ccPAS were restricted solely to the primary motor cortex (M1) or if they extended to influence the activity of other targeted areas, Domenica Veniero et al. (2013); D. Veniero et al. (2013) investigated the effects of 100 ccPAS pulses at a frequency of 0.2Hz on both the posterior parietal cortex (PPC) and the primary motor cortex (M1). Researchers applied different PAS conditions to deliver PPC stimulation 5ms before or after M1. The physiological signals TMS generated at the cortical level were measured using electroencephalography (EEG). The researchers were able to provide evidence that opposite forms of STDP were linked to unique patterns of activity at the cortical level. These plastic after-effects resulted in changes in the activity of cells in M1. In addition, the researchers found that information was transmitted between brain regions through different cortical rhythms. This provided evidence of using ccPAS to manipulate the functional connectivity between two cortical areas selectively. The study

provides strong evidence that ccPAS can selectively modulate connectivity between cortical regions, specifically the PPC and M1, by leveraging timing-dependent plasticity. The inclusion of EEG to measure cortical activity is a methodological strength, offering insights into how ccPAS operates at the neural level. However, the absence of behavioural data and long-term follow-up limits the practical relevance of the findings.

In a study of 35 healthy right-handed individuals C.-C. Chao et al. (2015) investigated whether applying 180 ccPAS pulses at a frequency of 0.2Hz to the ipsilateral posterior parietal cortex (PPC) and M1 could modulate the excitability of M1 and the connectivity between PPC and M1. Parietal ccPAS applied to the left hemisphere resulted in increased excitability of the conditioned left M1, as measured by MEPs. When conditioning stimuli were applied to the left PPC with an ISI of 8ms, an increase in MEPs in the left M1 was observed. However, this interaction significantly weakened after 60 minutes of undergoing left ccPAS. Further experiments demonstrated that the plasticity induced by parietal ccPAS depended on the timing of stimuli and was not observed when the ISI was increased to 100ms. Interestingly, similar effects were observed in the right hemisphere. This study once again identified the usefulness of ccPAS in modulating M1 excitability and PPC–M1 connectivity, suggesting that parietal ccPAS could serve as a new approach for modulating motor excitability and the interaction between sensory and motor systems, a finding that could have implications for motor rehabilitation. The study clearly demonstrates that the plasticity induced by ccPAS depends on precise timing between PPC and M1 stimulation. The use of an 8-millisecond interstimulus interval (ISI) increased M1 excitability, while a longer ISI of 100 milliseconds failed to induce similar changes. It provides evidence that ccPAS can modulate motor excitability in a hemisphere-specific but symmetrical manner, a valuable insight for potential clinical applications. It is unclear whether these effects persist over time.

Casula et al. (2016) utilised the combination of ccPAS with EEG to explore STDP mechanisms within the dorsolateral prefrontal cortex (DLPFC), two areas known to play a significant role in memory and attention. The study aimed to understand the neural

connections between PPC and DLPFC. PAS and EEG were combined to capture the post-synaptic potentials generated by the neuronal depolarisation evoked by TMS-evoked potentials (TEPs). It was discovered that repeatedly applying ccPAS with an ISI of 10ms to both pre-and post-synaptic inputs in the PPC and the DLPFC led to bidirectional STDP effects in the DLPFC. As in previous research (Koch et al., 2013) it was identified that STDP followed a timing pattern opposite to the conventional Hebbian STDP, aligning more closely with anti-Hebbian rules. Two explanations were discussed to explain the bidirectional STDP effects. The shift in timing from Hebbian to anti-Hebbian rules DLPFC may be facilitated by specific GABAergic interneurons receiving input from the presynaptic site (PPC) and exerting control over the output of pyramidal neurons within the postsynaptic site (DLPFC). Hence, the polarity of bidirectional STDP in the DLPFC could be determined by modulating the GABAergic tone. Alternatively, the distance between the two synaptic inputs may have led to a gradual shift in the timing requirements necessary to induce LTP or LTD, being significant enough to reverse the conventional Hebbian rules completely. The findings are specific to the DLPFC-PPC network and may not generalise to other cortical circuits. The DLPFC is a key region involved in working memory, decision-making, and cognitive control, processes that are often impaired in clinical conditions such as schizophrenia, depressive disorders and age-related cognitive decline. By demonstrating that ccPAS can modulate DLPFC excitability and oscillatory activity (e.g., beta and gamma rhythms), the findings suggest that similar protocols could be applied to restore prefrontal function in these disorders. Enhancing LTP-like plasticity through PAS may improve cognitive performance, such as working memory and executive function. The shift to anti-Hebbian plasticity observed here could reflect unique properties of this network rather than a general feature of ccPAS-induced plasticity. While the hypotheses about GABAergic modulation and synaptic distance are intriguing, they remain speculative. The study does not directly test these mechanisms, leaving important gaps in understanding the precise drivers of anti-Hebbian plasticity in the DLPFC. Once again, no evidence supports whether the findings persist long enough to be relevant to clinical applications.

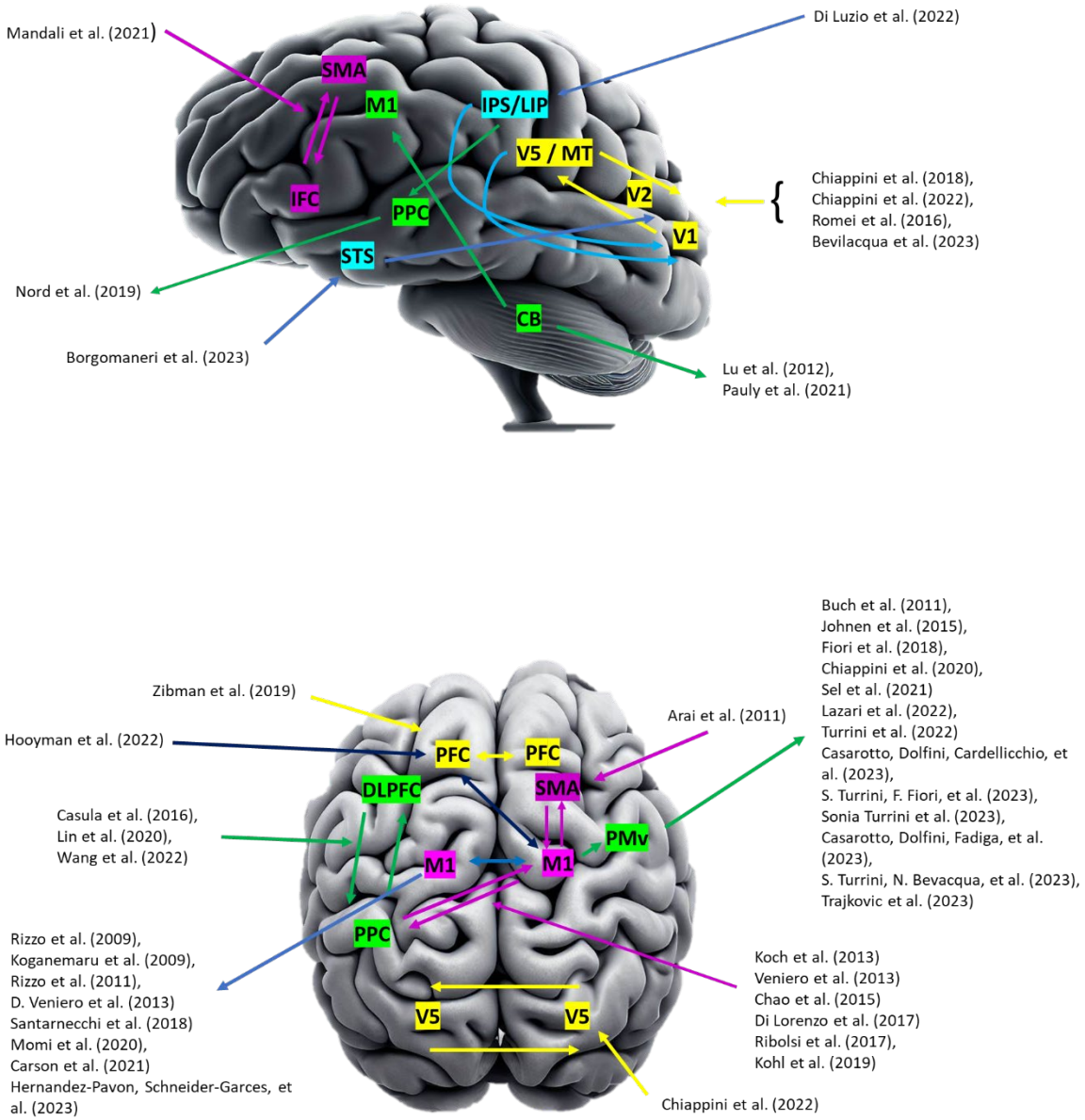
ccPAS protocols have been used to explore the mechanisms of cortical plasticity in patients with Alzheimer's disease (Di Lorenzo et al., 2017) and schizophrenia (Ribolsi et al., 2017). These studies revealed a noticeable impairment in LTP. Di Lorenzo et al. (2017) identified that ccPAS could be used to restore levels of central cholinergic activity within the PPC and M1 pathway when ccPAS was applied with an ISI of 5ms, providing evidence of the role of M1 in modulating the inhibitory intracortical circuit associated with cholinergic transmission. Furthermore, by stimulating the connections between PPC and M1, it was possible to modulate central cholinergic transmission. The findings from Ribolsi et al. (2017) provided evidence of a decline in corticocortical associative plasticity within the left PPC to M1 network in individuals with schizophrenia compared to healthy subjects. However, similar effects were not observed in the right PPC to M1 network, suggesting an apparent asymmetry of the functional connections between the two hemispheres. Both studies provide robust evidence of impaired cortical plasticity in Alzheimer's disease and schizophrenia, respectively. Both identify PPC–M1 connectivity as a critical pathway, highlighting the role of cholinergic deficits in AD and NMDA receptor dysfunction with hemispheric asymmetry in schizophrenia.

All the studies reviewed demonstrate the critical role of timing in STDP, consistent with Hebbian principles. Pre-synaptic stimulation preceding post-synaptic stimulation induces LTP-like plasticity, with the reverse leading to LTD-like plasticity. Effective interstimulus intervals (ISIs) range between 5–10 ms, depending on the cortical pathway and network being targeted (PPC–M1, PMv–M1, DLPFC). ccPAS highlights pathway specific plasticity, and how impaired PPC-M1 plasticity may be a significant contributor to AD and schizophrenia. Studies demonstrated modulation of both inhibitory (GABAergic) and excitatory (glutamatergic) circuits, and that plasticity is state-dependent. Active motor sates appeared to amplify plastic effects where resting states showed less pronounced effects. Limitations include small participant groups, particularly in clinical populations. While most studies report robust neurophysiological effects (e.g., MEPs, TMS-evoked potentials), they

often fail to link these to functional or behavioural improvements. Many studies assess plasticity changes over a short time window (e.g., 30–120 minutes post-stimulation). This limits understanding of the long-term persistence of ccPAS-induced effects, which is crucial for clinical translation.

Figure 3

Cortical Regions Explored In ccPAS Studies



Prefrontal Cortex and cognitive processing

Three studies were identified that explored the use of ccPAS in high-order cognitive processing. In one study, Santarnecchi et al. (2018) explored whether extended ccPAS targeting two fMRI network nodes in the frontal and parietal lobes could result in the selective alteration of their natural connectivity. This was assessed through functional connectivity (FC) analysis of Functional magnetic resonance imaging (fMRI) data. ccPAS manipulated the connections between the default mode network (DMN) and the task-positive network (TPN). These networks typically show opposite activity patterns during rest and task conditions and are negatively correlated (Santarnecchi et al., 2018). fMRI was used to identify these specific nodes in individual participants, with ccPAS modulating the connectivity between the two networks. Results showed that for some participants, the usual negative correlation between these networks was reversed to a more positive correlation. Participants with weaker initial connectivity showed improvements after ccPAS. Changes were also observed in fMRI activity patterns during an attention task. Participants either switched between rest and task states faster or slower, depending on the timing of the TMS pulses. These results suggest that ccPAS has the potential to manipulate DMN and TPN connectivity, which has links with neurodegenerative disorders, including frontotemporal dementia (FTD) and Alzheimer's disease (AD).

Kohl et al. (2019) utilised 100 ccPAS pulses at a frequency of 0.2Hz to explore decision-making and response inhibition. Twenty-five healthy participants were subjected to four distinct ccPAS sessions. Two specific brain regions, the right inferior frontal cortex (IFC) and the right pre-supplementary motor area (pre-SMA), were stimulated by TMS with different ISI between the paired pulses. Participants underwent tests to measure their response inhibition (using the stop signal task) and a control test (the delay discounting task) before and after each ccPAS session. A significant change in the stop signal reaction time was observed, which was also shown to be influenced by age. Younger individuals showed reduced ability to inhibit responses when pre-SMA was stimulated before IFC with a 10 ms

interval. Conversely, older participants showed improved response inhibition when IFC was stimulated before pre-SMA with a 4ms gap. A similar result was obtained by Mandali et al. (2021) who identified that ccPAS could improve response inhibition in both younger and older adults, with a greater effect observed in older adults.

Activation in the right and left lateral prefrontal cortex (LPFC) has been associated with different emotional reactions (Harmon-Jones et al., 2010). While a link between abnormal asymmetry and cognitive impairments has been identified, a direct causal relationship has not been established. Zibman et al. (2019) investigated changes in connectivity between the right and left LPFC using ccPAS by analysing the direction of information flow using TEPs. Two hundred ten ccPAS pulses at a frequency of 0.25Hz were administered to 30 participants across two conditions. The results of this protocol were evaluated behaviourally through an emotional reactivity task and neurophysiologically by measuring TEPs in the PFC. Results showed that LPFC ccPAS effects depended on the stimulation direction: left-to-right prefrontal stimulation increased the attentional bias in the emotional reactivity task, suggesting the induction of depressive effects. Conversely, right-to-left hemisphere ccPAS decreased attentional bias. Furthermore, both ccPAS increased interhemispheric signal propagation in the direction of the paired stimulations. It was concluded that ccPAS could modulate emotional processing in the LPFC.

Previous research has attempted to alter decision-making strategies using neurostimulation targeted at a single brain location but was unsuccessful (Smittenaar et al., 2014). Nord et al. (2019) aimed to use 100 ccPAS pulses at a frequency of 0.2Hz to target both the lateral prefrontal cortex (LPFC) and the intraparietal sulcus (IPS) to see if decision-making could be more goal-directed and less habitual (Nord et al., 2019). Thirty participants participated in the study, receiving three active stimulations, each separated by at least six days. These simulations involved sending 100 paired pulses to the IPS and LPFC with varied time intervals between them: two were close together in time (10 msec) and seen as potentially intervention-relevant, and one was much farther apart (100 msec) and used as a

control. After each stimulation, participants undertook a task to measure their reliance on goal-directed vs. habitual decision-making and a separate working memory task. The stimulation that targeted IPS before LPFC (with a short 10 msec interval) made decision-making more goal-directed than the control stimulation. Stimulating LPFC before IPS had not effect, and no noticeable effects on participants' working memory were observed. These findings highlight the potential of utilising ccPAS in decision-making, but more research on healthy individuals and those with decision-making disorders is required.

Momi et al. (2020) used 180 ccPAS pulses at a frequency of 0.2Hz to explore whether it can enhance fluid intelligence. Pairs of TMS pulses, separated by a 10-ms interval, were directed at two frontoparietal nodes of the gf network, the prefrontal and parietal lobes, believed to be crucial for gf based on cognitive models (Momi et al., 2020). It was hypothesised that ccPAS would improve network connectivity. When the parietal lobe was stimulated before the frontal lobe, improvements were noted in logical reasoning. Conversely, enhancements in relational reasoning were observed when the frontal lobe was stimulated before the parietal lobe.

The reviewed studies collectively explore the potential of cortico-cortical paired associative stimulation (ccPAS) to modulate high-order cognitive processing by targeting key brain networks involved in attention, decision-making, emotional reactivity, and fluid intelligence. These findings highlight the versatility of ccPAS in investigating and potentially influencing complex cognitive functions, but they also underscore methodological limitations and raise questions about its long-term applicability.

The studies demonstrate ccPAS's ability to target and selectively manipulate specific brain networks. Santarnecchi et al. (2018) underscores ccPAS's potential to probe and modify functional connectivity in large-scale brain networks, which has implications for neurodegenerative conditions like Alzheimer’s disease. Across the studies, ccPAS effects were shown to depend critically on the timing of stimulation. Several studies provided evidence of ccPAS-induced changes translating into measurable behavioural outcomes

(Kohl et al., 2019; Mandali et al., 2021; Zibman et al., 2019). Once again studies showed little or no exploration of long-term plasticity or behavourial change. While the studies attribute observed effects to STDP principles, the underlying neural mechanisms are not well-explored. For example, Zibman et al. (2019) hypothesised changes in signal propagation and attentional bias but did not investigate the specific neurotransmitter systems or synaptic dynamics involved. Many studies relied on small sample sizes of healthy participants, limiting the generalisability of the findings to clinical populations. The design of control conditions varied across studies, potentially affecting the robustness of conclusions. For example, Nord et al. (2019) used a 100-ms ISI as a control but did not employ sham stimulation, which could better isolate the active effects of ccPAS.

Cortico-cortical paired associative transcranial magnetic stimulation of the Visual cortex

Seven studies were identified that investigated the use of ccPAS in exploring the visual cortex. The parietal and occipital areas are regions of the brain that play important roles in sensory processing, perception, and attention. In 2016, ccPAS was used for the first time to study the processing of motion perception in the visual system (Romei et al., 2016). The study hypothesised that ccPAS could strengthen the connections (re-entrant projections) between areas V5 and V1 in the visual cortex by enhancing the pathways' sensitivity to motion stimuli. Participants completed a motion coherence discrimination task to measure an individual's ability to perceive and distinguish coherent motion from random motion. Participants were presented with a display of moving dots, some of which moved coherently in a particular direction, while others moved randomly in different directions. They were instructed to judge whether the overall motion of the dots was in a particular direction. Results identified that employing 90 ccPAS pulses at a frequency of 0.2Hz to strengthen connectivity specifically from V5 to V1 (but not vice versa) improved human visual perception of coherent motion, which lasted up to 60 minutes. This enhancement followed a temporal profile consistent with Hebbian plasticity and was observed only with an optimal timing of

20ms between pulses. The inter-pulse interval (IPI) of 20ms was chosen as it was the most effective in inducing LTP-like phenomena in cells, replicated by the ccPAS protocol. The plastic changes in perception were found to be dependent on both the direction and timing of the connectivity modifications.

Chiappini et al. (2018) introduced a novel approach called "function tuning ccPAS to improve the synaptic efficiency of distinct yet interconnected pathways. The focus was on re-entrant projections from V5/MT+ to V1/V2 related to transporting visual motion information. Participants completed a motion coherence discrimination task with a slight difference; stimuli were presented moving in a specific direction to engage direction-specific neurons while activating the V5/MT+ and V1/V2 pathways, inducing functional specific Hebbian plasticity. As in Romei et al. (2016) study, 90 ccPAS pulses at a frequency of 0.1Hz were applied over V5/MT+ followed 20ms later over V1/V2. TMS was also applied in three additional conditions: TMS applied over V5/MT+ at 80% phosphene threshold or 100% and a control. Phosphenes are visual sensations that TMS can induce, and their intensity is directly related to the intensity of the TMS pulse. Using different phosphene levels, the researchers determined the individual phosphene threshold for each participant. Once the individual phosphene threshold was determined, the researchers could adjust the TMS intensity to ensure the protocol was applied at an intensity strong enough to induce synaptic plasticity. When TMS is applied at lower intensities, only pre-activated neurons (i.e., those stimulated by the presented motion direction) would be sufficiently impacted, resulting in limited plastic changes restricted to these neurons. Conversely, all neurons would likely be activated at higher intensities, potentially diminishing the direction-selective effects. An ISI of 20ms was anticipated to be the most effective in inducing LTP-like phenomena (Chiappini et al., 2018).

Using the knowledge acquired in previous research Chiappini et al. (2022) investigated interhemispheric connectivity of the visual motion regions by studying conscious perception. It was hypothesised that the process of conscious perception involves a

hierarchical mechanism that combines low-level sensory encoding with higher-order sensory selection, and this takes place at various levels of brain function, including the integration of information between the hemispheres. In four separate experiments, one of which was a sham, the researchers used 90 ccPAS pluses at a frequency of 0.1Hz to manipulate the connectivity between the left and right visual fields for horizontal motion perception. The ISI of 25 ms was chosen based on previous interhemispheric research (need reference here). The effects of ccPAS were measured at different time intervals: immediately after ccPAS, then 30-, 60-, and 90 minutes post protocol. The findings indicated that enhancing the strength of connections from the left to the right V5/MT+ through ccPAS immediately increased sensitivity to horizontal motion, which lasted up to 90 minutes. Limited perceptual changes were observed when strengthening connections from the right to the left V5/MT+, suggesting that left-to-right hemisphere projection plays a crucial role in integrating local sensory input (Chiappini et al., 2022). This asymmetrical modulation of perceptual bias in the direction of horizontal motion mirrored the asymmetrical nature of the projections connecting left and right V5/MT+ in the human visual cortex.

Di Luzio et al. (2022) used 90 ccPAS pulses at a frequency of 0.1Hz to induce plastic changes in the V5/MT+ to V1/V2 pathway to examine how this impacted perceptual sensitivity and metacognitive ability. Perceptual decision-making involves selecting the most appropriate option from a set of alternatives based on available sensory information and learnt understanding. The ability to make such decisions relies on the perceptual sensitivity of the individual and a level of confidence that the choice made is correct. This confidence represents a metacognitive process. Based on existing research suggesting a function dissociation between decision-making and accuracy (Maniscalco et al., 2016; Rahnev et al., 2012), Di Luzio aimed to explore if there was a functional dissociation between performance accuracy and confidence using a motion discrimination task. Previous studies suggested boosting IPS/LIP-to-V1/V2 back-projections increases metacognitive efficiency without affecting motion sensitivity. It was hoped that ccPAS would further explore the specific

function of the pathway and provide insight into how it relates to the level of certainty in the decision. To achieve this, participants were instructed to identify the horizontal direction of a pattern of dots and subsequently provide a rating of their confidence level in their response. The results showed that stimulation to enhance connections between the V5/MT+ and V1/V2 brain regions improved motion sensitivity without affecting metacognition. In contrast, stimulation that enhanced connections between the IPS/LIP and V1/V2 brain regions improved metacognitive efficiency without affecting motion sensitivity. The results support the view of a double dissociation, providing evidence that distinct neural networks are involved in perceptual sensitivity and metacognitive ability in humans.

Across five experiments, Borgomaneri et al. (2023) demonstrated that enhancing associative plasticity in temporo-occipital back-projections via ccPAS can selectively improve the visual perception of emotions. This improvement is contingent on specific temporal and directional stimulation parameters and is associated with electrophysiological changes indicative of enhanced processing within the visual cortex. The findings underline the functional malleability of the pSTS-to-V1/V2 pathway and its critical role in the perception of emotion from facial stimuli.

The study by Bevilacqua et al. (2023) involved 16 healthy subjects in a double-blinded, crossover design focusing on the effects of cortico-cortical paired associative stimulation (ccPAS) on the visual motion processing network. The ccPAS protocol was applied in two forms: Forward-ccPAS, enhancing V1-to-MT connections, and Backward-ccPAS, strengthening MT-to-V1 connections. Sessions were separated by at least one month, in randomised order. The experimental approach included EEG monitoring to capture baseline and post-ccPAS brain activity alongside performance in a visual discrimination task. Forward-ccPAS increased local MT activity, while Backward-ccPAS led to increased V1 activity. Forward-ccPAS caused a significant increase in the bottom-up V1-to-intraparietal sulcus (IPS) connectivity when V1 was stimulated. Conversely, Backward-ccPAS significantly increased direct bottom-up inputs when V1 was stimulated, and notably, the re-

entrant MT-to-V1 pathway also increased. Behavioural outcomes indicated a significant enhancement of motion direction discrimination only after Backward-ccPAS and not Forward-ccPAS. Backward-ccPAS also significantly improved motion awareness, as indicated by increased metacognitive confidence ratings. These results highlight the pathway and directional specificity of ccPAS in modulating the cortical visual motion processing network. The study demonstrated that enhancing backward projections (MT-to-V1) leads to significant behavioural improvements in motion direction discrimination and awareness, underscoring the potential for modulating specific neural pathways to influence visual perception.

Guidali et al. (2023) ccPAS study focused on investigating pseudoneglect—a phenomenon where individuals show a preference for stimuli in the left visual field over the right, typically associated with right hemisphere dominance in visuospatial processing. The experiment aimed to modulate pseudoneglect in healthy participants using ccPAS. Participants were subjected to two ccPAS protocols; frontal-to-parietal (FP) and parietal-to-frontal (PF), with an inter-stimulus interval (ISI) of 10 ms. For FP, the first pulse targeted the right frontal eye field (FEF), and the second the right inferior parietal lobule (IPL). PF had the pulse order reversed. A control experiment tested the FP-ccPAS with a longer ISI of 100 ms. Pseudoneglect was assessed before and after stimulation using a landmark and manual line bisection tasks. The FP-ccPAS with a 10 ms ISI significantly increased the leftward bias in the landmark task, indicating an enhancement of pseudoneglect; the natural tendency of healthy individuals to favour the left side of space in visuospatial tasks was amplified by the stimulation protocol. This effect was not observed in the manual line bisection task, nor with the PF-ccPAS protocol or the FP-ccPAS with a 100 ms ISI. These results suggest that ccPAS, particularly the FP-ccPAS with a 10 ms ISI, can modulate pseudoneglect by enhancing connectivity between the right FEF and IPL. This enhancement potentially operates through increased top-down attentional control. The specificity of the effect to the

FP protocol and the 10 ms ISI underscores the role of precise intra- and inter-hemispheric connectivity in visuospatial biases.

In accordance with other ccPAS findings, these studies demonstrate that ccPAS is a powerful tool for selectively modulating visual cortical networks, enabling researchers to probe the mechanisms underlying motion perception, attention, and visuospatial processing. While the findings are robust and highlight the pathway-specific and timing-dependent nature of ccPAS effects, limitations such as short-term impacts, small sample sizes, and mechanistic ambiguity remain.

Comparing cortico-cortical paired associative stimulation with other stimulation methods

A study by Carson et al. (2021) aimed to test the efficacy of ccPAS by comparing it with another method, Transcranial Alternating Current Stimulation (tACS). The researchers wanted to know if starting with a tACS stimulus or a sub-threshold TMS (meaning a TMS below the intensity needed to produce a motor response) would produce associative effects like traditional PAS methods. tACS is a form of non-invasive brain stimulation that involves applying a weak electric current to the scalp in an alternating manner (Carson et al., 2021). Instead of just traditional statistics, they used Bayes factors. This provides a ratio that can show the strength of evidence for one hypothesis over another. In this case, it was used to assess how well the two novel PAS methods worked in increasing corticospinal excitability. The researchers designed three conditions to investigate M1 to M1 connectivity. In the first condition, tACS was applied, ending 6ms before a TMS pulse was given to the opposite M1. In the second condition 180 ccPAS pulses at a frequency of 011.Hz was given to the right M1 6ms before a full-strength TMS was applied to the left M1. The third condition was a sham, which acted as a control. The tACS-TMS and ccPAS increased corticospinal excitability for about 20-30 minutes. However, the evidence from the Bayes factors showed that the ccPAS was more effective or convincing than the tACS-TMS pairing.

Pauly et al. (2021) aimed to investigate the effects of various non-invasive brain stimulation techniques on the cerebellum to understand the cerebellum's interactions with other parts of the brain related to movement. Four different techniques were tested: rTMS (repetitive transcranial magnetic stimulation), cTBS (continuous theta-burst stimulation), ccPAS and tDCS (transcranial direct current stimulation). The four techniques were applied to the cerebellum of 20 participants, and only rTMS and ccPAS were compared to their respective sham (placebo or control) conditions in another group of 20 healthy participants. Stimulation of the cerebellum using 1 Hz rTMS and 360 ccPAS pulses at a frequency of 0.2Hz induced changes in brain pathways involving the cerebellum, premotor cortex, and motor cortex. These results were more apparent in the larger samples, suggesting that stimulation effects may be clearer in the future with larger groups of participants.

Hooyman et al. (2022) provided further evidence that ccPAS can be used to increase the resting-state connectivity between the prefrontal and motor regions of the brain. Using a method to measure brain activity when a person is at rest called Resting-state electroencephalography (rs-EEG) the researchers targeted High Beta coherence (hBc), a specific type of synchronisation between brain regions in the high beta frequency range of brain waves. Prior research has suggested that high Beta coherence between the prefrontal and motor cortices might be linked to motor skill learning and stroke recovery (Wu et al., 2018; Wu et al., 2016). 100 ccPAS pulses with an ISI of 5ms significantly increased the connectivity between the right prefrontal and motor cortex compared to the control and baseline conditions.

The reviewed studies underscore the efficacy of ccPAS in modulating neural pathways across diverse regions of the brain. Carson et al. (2021) demonstrated its superiority over tACS-TMS in enhancing corticospinal excitability, while Pauly et al. (2021) showed its potential for cerebellar modulation. Hooyman et al. (2022) provided evidence of its ability to enhance resting-state connectivity between prefrontal and motor cortices.

Despite these promising findings, limitations related to short-term effects, small sample sizes, and a lack of functional outcomes remain.

Cortico-cortical paired associative stimulation and mental health

The review identified two papers that have focused on the use of ccPAS in mental health disorders. Lin et al. (2020) aimed to explore whether 100 ccPAS pulses at a frequency of 1Hz could be used as a therapeutic intervention for patients with a diagnosis of generalised anxiety disorder (GAD). They implemented an augmented ccPAS, referred to as repetitive dual-site paired associative stimulation (rDS-PAS) specifically targeting the right dorsolateral prefrontal cortex (DLPFC) and the right inferior parietal lobe (IPL). These regions are critical for emotion and attention control. All subjects underwent ccPAS over the right DLPFC and right IPL using two coils at three interstimulus intervals (ISI) of rDS-PAS: 10ms, 20ms, and 50ms. Stimulation was administered every day for ten consecutive days. Self-reports of anxiety and depression symptoms and sleep were measured using the Hamilton Rating Scale for Anxiety and Depression (HRSA, HRSD). Pittsburgh Sleep Quality Index (PSQI) was used to record sleep. These measures were recorded at baseline, posttreatment, two-week follow-up, and one-month follow-up. Results showed that rDS-PAS at 1 Hz frequency (with an interstimulus interval (ISI) of 20ms and 50ms) reduced anxiety post-treatment and during follow-ups. No changes in depression of sleep were reported. The researchers hypothesised that the low-frequency rDS-PAS suppressed the excessive activity of the right DLPFC and IPL which are believed to regulate emotional control.

Wang et al. (2022) conducted a similar with GAD patients. The researchers used rDS-PAS to stimulate two brain sites, the dorsolateral prefrontal cortex. Utilising a randomised, double-blind clinical trial, the researchers divided patients with GAD into four groups: two ccPAS groups, which received either 1500 paired pulses or 750 paired pulses, and two single site groups, dlPFC with 1500 paired pulses and PPC with 1500 paired pulses. Results obtained showed that all treatment methods reduced clinical scores for anxiety, depression, and insomnia. The most pronounced improvement for anxiety was within the

ccPAS groups which received 1500 paired pulses. Stimulating the DLPFC and the PPC simultaneously was more effective than stimulating just one site (DLPFC or PPC).

The studies by Lin et al. (2020) and Wang et al. (2022) provide promising evidence for ccPAS as a non-invasive intervention for generalised anxiety disorder, with dual-site stimulation showing greater efficacy than single-site approaches. Both studies made attempts to address the limitations identified previously. For example Wang et al. (2022) employed a rigorous randomised, double-blind clinical trial design with multiple treatment groups, providing strong comparative evidence for ccPAS's efficacy. Both studies also included follow-up assessments, with Lin et al. (2020) tracking outcomes up to one month post-treatment, allowing for an evaluation of ccPAS's longer-term effects. However, limitations relating to small sample sizes and variability in ccPAS protocols remain.

Discussion

This review identified 44 studies that employed the ccPAS technique to explore its efficacy in inducing changes in neural plasticity in the human brain. The studies have covered a broad range of cortical areas, building on initial research that focused on the primary motor cortex and expanded to areas including peripheral motor areas, the parietal areas, and the visual cortex. More recently, ccPAS has been used to explore neural networks involved in learning, memory, and decision making. Its clinical utility has been investigated across various client populations, encompassing neurological conditions including Alzheimer's, Parkinson's, and Schizophrenia, alongside instances of anxiety and depression. Studies have included active and rest conditions, compared ccPAS with other non-invasive brain stimulation techniques, and reported the impact on various physical, behavioural, neurological, and psychological conditions.

Despite its promise as a versatile tool for understanding and modulating brain function, several limitations were identified, shaping the design and focus of my research. By addressing these gaps, my study aims to refine ccPAS methodologies and contribute to developing best practices in its application.

Limitations Identified in the Systematic Review and Proposed Solutions

Interstimulus interval (ISI)

The systematic review highlights that, while the ISI is a crucial parameter for the success of ccPAS, its variability and the lack of standardisation present significant challenges. The selection of ISI in ccPAS protocols is influenced by the functional and anatomical properties of the targeted brain regions, the desired direction and nature of plastic changes, and the specific temporal dynamics of the neural processes involved. This selection is critical for aligning the stimulation protocol with the physiological mechanisms underlying synaptic plasticity and functional connectivity, ensuring that ccPAS effectively modulates the brain networks of interest.

Studies consistently demonstrate that certain ISI ranges (e.g., 6–15 ms for motor cortex connectivity) are optimal for promoting synaptic plasticity. However, different brain regions and pathways require distinct ISIs to achieve effective modulation. Research by Rizzo et al. (2009) and Buch et al. (2011) established 8 ms as an effective ISI for modulating interhemispheric inhibition (IHI) and enhancing corticospinal excitability in the primary motor cortex. This timing corresponds to the approximate conduction time for signals to travel between the motor cortices, aligning with the principles of spike-timing dependent plasticity (STDP).

My study focuses on the primary motor cortex, where the use of an 8 ms ISI has been shown to reliably induce changes in M1-M1 connectivity. This makes it a validated choice for investigating interhemispheric motor interactions and provides a robust, replicable framework for exploring ccPAS effects. Although 8 ms is a widely validated ISI for young adults, potential variability in conduction speed and neuroplasticity among older adults may influence its effectiveness. This limitation is acknowledged, and findings will be interpreted within the context of these potential differences.

Small Sample Sizes

Small participant numbers in many studies limit statistical power and generalisability. For example, foundational work by Rizzo et al. (2009, 2011) and Koganemaru et al. (2009) involved small cohorts, making it challenging to extrapolate findings. To address this, my study will recruit a larger and more diverse sample, guided by power analysis, and will optimise experimental design to maximise the utility of data collected from each participant.

Short-Term Follow-Up

Most studies focused on the immediate or short-term effects of ccPAS, typically lasting between 20 and 90 minutes post-stimulation. While studies such as Turrini et al. (2022) and Carson et al. (2021) demonstrate that ccPAS can induce transient plasticity, its long-term effects remain unexplored.

My study will not address this limitation directly due to practical constraints, but I hope to emphasise the need for future research to include follow-ups over days, weeks, or months. Understanding the durability of ccPAS-induced plasticity is essential for translating its benefits into clinical applications.

Resting vs. Active State

The review highlighted that the state of cortical activity (resting vs. active) during ccPAS application plays a pivotal role in determining outcomes. My study will focus on the resting state for several reasons. The resting state provides a standardised and controlled baseline condition, minimising confounding factors associated with task-specific neural activity. This approach ensures that changes in cortical excitability and interhemispheric inhibition (IHI) are directly attributable to ccPAS-induced plasticity. By focusing on the resting state, my study captures intrinsic plasticity mechanisms, offering clearer insights into how ccPAS modulates interhemispheric dynamics. These findings are necessary for understanding baseline IHI and are critical for developing ccPAS for application in clinical contexts, such as neurorehabilitation for stroke or age-related motor decline.

Furthermore, the resting-state approach enhances reproducibility and comparability with existing literature, including studies by Rizzo et al. (2009) and Koganemaru et al. (2009), which investigated ccPAS-induced plasticity in resting conditions. The insights from resting-state investigations provide a robust basis for future studies exploring task-specific effects or dynamic motor learning applications.

In summary, prioritising the resting state allows my study to directly address the limitations of prior research identified in the systematic review, offering a focused exploration of ccPAS effects on IHI under controlled conditions.

Temporal Dynamics of ccPAS Effects

The systematic review identified that ccPAS-induced changes often develop over time rather than manifesting immediately. For instance, Rizzo et al. (2009) reported delayed effects, suggesting that capturing outcomes immediately post-stimulation may miss key dynamics. To address this, my study will segment motor-evoked potential (MEP) data into early (first 20 MEPs) and late (last 20 MEPs) phases to explore how ccPAS effects evolve over time. This approach aims to clarify temporal dynamics and improve understanding of the mechanisms underlying ccPAS-induced plasticity.

Directional Specificity of Stimulation

Directional specificity is a key determinant of ccPAS outcomes, as highlighted by the review. For instance, Buch et al. (2011) found that stimulating PMv before M1 enhanced connectivity, while reversing the order weakened it. Similarly, the systematic review revealed a lack of consistent findings on directional effects in older adults, where age-related declines in neural plasticity complicate the picture.

To address this, my study will:

- Compare left-to-right and right-to-left ccPAS protocols in younger adults to investigate baseline plasticity and directional specificity.

- Focus exclusively on left-to-right ccPAS in older adults to maximise the likelihood of significant results, as this direction aligns with the dominant-to-nondominant hemisphere connection, which is typically more robust and responsive to plasticity-inducing interventions in right-handed individuals.

This dual approach aims to clarify how directional specificity varies with age and contributes to understanding age-dependent plasticity in interhemispheric dynamics.

Handedness and Lateralisation

The systematic review highlighted the importance of handedness and lateralisation of function. Handedness plays a significant role in motor cortex organisation and interhemispheric connectivity. In right-handed individuals, the left hemisphere typically dominates motor control, resulting in stronger excitatory and inhibitory connections from the left primary motor cortex (M1) to the right M1. This lateralisation creates asymmetries in interhemispheric inhibition (IHI), with left-to-right pathways generally more robust than the reverse. Younger adults exhibit higher levels of neural plasticity, allowing both left-to-right and right-to-left pathways to be effectively engaged. Testing ccPAS in both directions within this group facilitates the study of baseline plasticity and directional specificity, while also highlighting the role of handedness in interhemispheric dynamics. This dual approach establishes a foundation for understanding how these dynamics differ in older adults.

In older adults, this study will focus exclusively on the left-to-right pathway for both practical and strategic reasons. The left-to-right pathway represents the dominant-to-nondominant hemisphere connection, which is typically stronger and more responsive to plasticity-inducing interventions in right-handed individuals. Targeting this pathway maximises the likelihood of detecting significant modulation of IHI and cortical excitability while addressing declines in the nondominant hemisphere, which often exhibits weaker connectivity and compensatory inefficiencies. This focused approach also enhances experimental efficiency and reduces variability caused by fatigue, a key factor in ageing populations.

This study will incorporate:

- Handedness Screening: Only right-handed participants will be included, ensuring consistency in lateralisation effects. This
- Baseline Measurements: Individual MEP thresholds for the first dorsal interosseous (FDI) muscle will be established, tailoring stimulation intensity to each participant's cortical excitability.

Laterality Differences in the Context of ageing

Laterality differences become particularly pronounced with ageing, as older adults often experience a reduction in hemispheric asymmetry. This is due in part to declines in the efficiency and plasticity of the dominant hemisphere, coupled with compensatory recruitment of both hemispheres during motor tasks—a phenomenon known as the hemispheric asymmetry reduction in older adults (HAROLD) (Cabeza, 2002). While this compensatory activation may help maintain motor performance, it also underscores the importance of selecting stimulation targets carefully in older populations. Left-to-right ccPAS is likely to be more effective in older adults because it supports interhemispheric communication while reinforcing the stronger, more efficient hemisphere. In contrast, right-to-left ccPAS, while valuable in younger adults, may be less effective in older adults due to age-related declines in the nondominant hemisphere.

Fatigue further complicates laterality dynamics in older adults. Testing both directional pathways would require longer sessions and greater effort, increasing the risk of fatigue-related variability. Fatigue may also interact with age-related declines in plasticity, masking ccPAS effects by inducing general reductions in cortical excitability or heightened IHI. Focusing solely on the left-to-right pathway mitigates these risks, ensuring reliable results and maximising the impact of ccPAS in this group.

Noise and Participant Experience

The noise generated by TMS devices can startle participants and confound responses. To address this, participants will undergo a taster session to familiarise themselves with the equipment and noise levels. This approach enhances participant comfort and ensures informed consent.

Aim of the Study

The review highlighted the ccPAS ability to induce plastic changes in the motor cortex, suggesting its utility in enhancing motor learning and rehabilitation. Given the ageing population's growing need for effective interventions to support motor function, the insights provided by the systematic review are invaluable. They not only clarify the current state of research on ccPAS but also inform the research objectives of this thesis, which seeks to explore the potential of ccPAS in enhancing motor cortex connectivity and function in older adults. Based on the gaps identified by the systematic review, the primary aim of this research is to explore how cortical communication changes with healthy ageing.

This study aims to investigate the modulatory effects of corticocortical paired associative stimulation (ccPAS) on interhemispheric inhibition (IHI) within the human motor cortex. Specifically, I intend to ascertain whether the modulation of IHI varies with the direction of ccPAS—either from left to right or right to left—and how such effects might be influenced by the age of participants. To this end, participants will be categorised into two age groups: young adults and older adults.

Core Aims

The core aims of this research are as follows:

Exploring the Modulatory Effects of ccPAS on IHI

I aim to assess whether M1-M1 ccPAS induces changes in interhemispheric inhibition (IHI) within the motor cortex. Specifically, I will compare the effects of left-to-right

ccPAS with right-to-left ccPAS to determine how stimulation direction influences the modulation of IHI.

Investigating Age-Dependent Characteristics of ccPAS-Induced Modulation

A key objective is to determine whether the modulatory effects of M1-M1 ccPAS on IHI differ between young adults and older adults. I intend to explore whether older adults exhibit reduced neuroplasticity or altered susceptibility to ccPAS compared to younger participants.

Examining the Directional Specificity of ccPAS

I will evaluate whether the direction of ccPAS (left-to-right vs. right-to-left) has a significant impact on the nature of excitatory or inhibitory modulation of IHI. To address this, I have designed the study to include multiple conditions for the young adult group:

- Left-to-right M1-M1 ccPAS
- Right-to-left M1-M1 ccPAS
- Sham stimulation (control).

For the older adult group, I will streamline the protocol to focus on left-to-right ccPAS and a sham condition to address concerns regarding testing fatigue.

Determining the Temporal Development of ccPAS Effects

Building on previous findings (e.g., Rizzo et al., 2009), I aim to investigate whether the effects of ccPAS develop over time. I will compare the first 20 motor evoked potentials (MEPs) recorded post-stimulation with the last 20 MEPs to assess whether cortical excitability changes progressively.

Assessing Corticospinal Excitability in the Motor Cortex

I aim to quantify changes in corticospinal excitability pre- and post-ccPAS intervention using motor-evoked potentials (MEPs). This will allow me to determine whether ccPAS induces excitatory or inhibitory effects on MEP amplitudes.

Hypotheses

Primary Hypotheses

Hypothesis 1: M1-M1 ccPAS Regulates Corticospinal Excitability in the Motor Cortex as measured by MEP

- H_0 (Null Hypothesis): M1-M1 ccPAS does not significantly affect the excitability of the human motor cortex.
- H_1 (Alternative Hypothesis): M1-M1 ccPAS (left-to-right vs. right-to-left) significantly affects the excitability of the human motor cortex.

Hypothesis 2: Directional Effect of M1-M1 ccPAS on Interhemispheric Inhibition (IHI)

- H_0 (Null Hypothesis): The direction of M1-M1 ccPAS (left-to-right vs. right-to-left) does not significantly affect the modulation of IHI in the human motor cortex.
- H_1 (Alternative Hypothesis): The direction of M1-M1 ccPAS significantly affects the modulation of IHI, with either left-to-right or right-to-left ccPAS producing distinct modulatory effects on the human motor cortex.

Hypothesis 3: The Effects of M1-M1 ccPAS Develop Over Time

- H_0 (Null Hypothesis): There is no significant difference in cortical excitability over time.
- H_1 (Alternative Hypothesis): There is a significant difference in cortical excitability over time.

Secondary Hypotheses

Hypothesis 4: Age-Dependent Modulation by M1-M1 ccPAS

- H_0 (Null Hypothesis): There is no significant difference in the modulation of cortical excitability by M1-M1 ccPAS between young and older adults.

- H_1 (Alternative Hypothesis): There is a significant difference in how M1-M1 ccPAS modulates cortical excitability between young and older adults.

Focus of My Research

Directionality of ccPAS: I aim to establish whether the direction of stimulation determines the excitatory or inhibitory effects on IHI.

Age-Related Differences: I will explore how age influences neuroplasticity and responsiveness to ccPAS.

Temporal Dynamics: I intend to determine whether the effects of ccPAS emerge gradually over time, providing further insight into the temporal nature of plasticity.

Chapter 3: Methods

Epistemological position

Materialism is an oncology that posits mental states, including beliefs, desires, and consciousness itself, are grounded in the brain's physical structure and processes (Churchland, 2002). Modern scientific research, particularly in the field of neuroscience, supports materialism by providing clear evidence of how mental states and consciousness are closely linked to the physical workings of the brain. Studying how the motor cortex communicates within a materialistic ontology framework links the physical and observable processes in the brain to motor functions and behaviours. An epistemology that fits well within a materialistic ontology is post-positivism. In the post-positivist paradigm, research objectives include predicting outcomes, testing hypotheses, and identifying and exploring relationships among variables (Habib, 2020). Post-positivism is evident in neurological research, which employs repeatable processes, falsification, and continuously refining hypotheses based on new data. It recognises the complexities of brain functioning and the limitations of scientific methods. It accommodates this ongoing process of discovery and refinement, recognising that each research finding contributes to a progressively clearer picture of brain functionality. This research will adopt post-positivism as the epistemological foundation, reflecting Thomas Kuhn's ideas of the evolving nature of scientific paradigms (Kuhn, 1962) and building on Karl Popper's emphasis on falsifiability as a cornerstone of scientific inquiry (Popper, 1963; Popper, 1959). Post-positivism values empirical, observable evidence and often uses quantitative methods to gather data (Crossan, 2003).

This research comprises two studies. In study one, participants are young adults, and in study two, participants are older adults. Transcranial magnetic stimulation (TMS) will be employed to modulate interhemispheric inhibition, and the differences between young and old will be compared.

These studies rely on TMS and the ccPAS protocol to investigate motor cortical excitability, using neuroscience practices to collect data to explore communication within cortical pathways and, therefore, fit within this empirical framework. These techniques allow for the quantitative measurement of brain activity and its modulation.

These studies, grounded in a postpositivist epistemology, employ statistical analysis to test hypotheses regarding cortical communication as influenced by ccPAS. This perspective reflects a postpositivist one, acknowledging that scientific knowledge progresses through revising and refining theories based on new evidence rather than reaching absolute truths. Statistical methods are, therefore, valuable in identifying patterns and relationships while acknowledging their limitations and the iterative nature of scientific discovery.

In these studies, I aim to determine how the direction of ccPAS—either from left to right or right to left - differs on IHI and how participants' age is influenced by such effects. This research is set against the backdrop of the ageing brain, a subject that, from a post-positivist perspective, highlights our evolving understanding of brain function across a lifetime.

Through empirical research utilising protocols such as ccPAS, we continually refine our knowledge of how ageing impacts cortical communication and overall brain health. This encourages ongoing enquiry and the adaptation of theories around neuroplasticity, cognitive decline, and the possibilities for interventions to mitigate age-related changes, mirroring the iterative process of scientific discovery in grasping the complexities of the ageing brain.

Operational Definitions

To ensure clarity and consistency in the research, I have provided the following operational definitions for key terms and variables for the study:

Transcranial magnetic stimulation (TMS)

Transcranial Magnetic Stimulation (TMS) is a non-invasive method used to stimulate small regions of the brain. This technique involves placing a magnetic coil near the scalp,

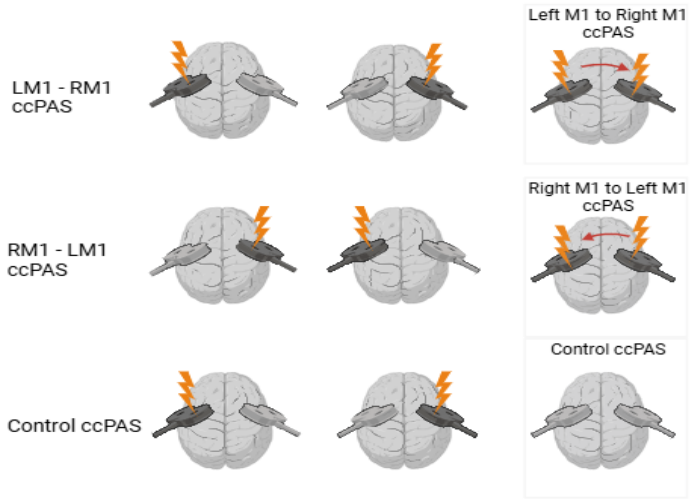
which generates a magnetic field that induces electric currents in the brain tissue, which can induce a motor-evoked potential (MEP) when targeting the motor cortex. In this study, two types of TMS were used: single-pulse and paired-pulse. Single-pulse TMS involves delivering a single magnetic pulse to the brain. Paired-pulse involves delivering two magnetic pulses in quick succession. The first pulse (conditioning pulse) is followed by a second pulse (test pulse) after a short interval.

Corticocortical Paired Associative Stimulation (ccPAS)

ccPAS is a neurostimulation technique involving the delivery of paired magnetic pulses to the primary motor cortices (M1) of both hemispheres. These pulses are administered in a specific sequence and with precise timing, interstimulus interval of 8 milliseconds (Rizzo et al., 2009) to induce changes in cortical excitability and interhemispheric inhibition. This study will use two sequences: left M1 to right M1 and right M1 to left M1. There will also be a control group which will have sham ccPAS, with an interstimulus interval of 1 millisecond. Figure 4 illustrates how ccPAS will be administered across the groups.

Figure 4

Corticocortical Paired Associative Stimulation (ccPAS) across the experimental groups



Note. This image illustrates the principle of Corticocortical Paired Associative Stimulation (ccPAS). In the first row, stimulation is applied to the left motor cortex (LM1) first, followed by stimulation of the right motor cortex (RM1) followed by ccPAS at an ISI of 8m. This specific timing is designed to enhance directional plasticity from LM1 to RM1. In the second row, the order is reversed, with the right motor cortex being stimulated before the left, promoting plasticity in the opposite direction. The third row represents a control condition where an ISI of 1ms is used, serving as a baseline for comparison. The lightning bolts signify the stimulation sites, while the arrows indicate the direction of paired stimulation. In the older adult study ccPAS is not administered in the RM1-LM1 direction.

Motor-Evoked Potentials (MEPs)

MEPs are the electrical responses recorded from muscles following stimulation of the motor cortex using TMS. In this study, MEPs are measured from the first dorsal interosseous (FDI) muscle of the hand. The amplitude of the MEPs, measured in microvolts (μV), serves as an indicator of corticospinal excitability.

Interhemispheric Inhibition (IHI)

IHI refers to the process by which one hemisphere of the brain inhibits the motor activity of the contralateral hemisphere. This is quantified by comparing the MEP amplitudes from conditioned (ccPAS-induced) and unconditioned TMS pulses. Higher IHI values indicate stronger inhibition, whereas lower values suggest weaker inhibition.

Resting Motor Threshold (rMT)

The rMT is defined as the minimum TMS intensity required to elicit a motor response (MEP of at least 50 μV) in the target muscle in at least 50% of trials when the muscle is at rest. This threshold is used to calibrate the TMS intensity for subsequent stimulation, ensuring consistent and comparable stimulation levels across participants.

Young Adult Group

Participants in the young adult group are individuals aged 18 to 52, recruited through the SONA platform. They are right-handed, have normal or corrected-to-normal vision and normal hearing, and meet the specified inclusion and exclusion criteria.

Older Adult Group

Participants in the older adult group are individuals aged 63 to 83 years, recruited from a pool of regular research volunteers at the University of Essex. They are also right-handed, with normal or corrected-to-normal vision and normal hearing and meet the same inclusion and exclusion criteria as the young adult group.

Baseline Block

The baseline block refers to the initial phase of the experimental procedure, during which MEPs are recorded without any prior ccPAS intervention. This serves as a reference point for comparing the effects of ccPAS on cortical excitability.

Expression Block

The expression block is the final phase of the experimental procedure, following the ccPAS intervention. MEPs are recorded again to assess the changes in cortical excitability induced by the ccPAS.

Pseudorandom and Counterbalanced Design

A pseudorandom and counterbalanced design refers to the method used to present the experimental trials in a non-repeating, mixed order that is balanced across participants to control for potential order effects. This ensures that each condition is equally likely to precede or follow any other condition, minimising systematic biases.

Justification of Sample Size

To ensure the study was sufficiently powered to detect meaningful effects, I conducted a priori power analysis using G*Power software (Faul et al., 2007). For the young adult study, I aimed to detect a medium effect size (f = 0.25) with an alpha level of 0.05 and

a power of 0.80. This analysis indicated that a total sample size of 60 participants (20 per group) is required. This sample size allows for adequate detection of motor cortical excitability differences across the experimental conditions, ensuring robust statistical power to support the study's hypotheses.

For the older adult study, due to anticipated challenges in recruitment and the exploratory nature of this investigation, I targeted a smaller sample size of 10 participants. This decision was informed by previous studies in the field (S. Turrini, N. Bevacqua, et al., 2023; S Turrini et al., 2023), which successfully demonstrated significant findings with similar sample sizes. Moreover, the within-subject design of this part of the study enhances statistical power by reducing variability associated with individual differences.

In both studies, a slightly larger sample was recruited initially to account for potential dropouts and data exclusions. The inclusion and exclusion criteria, along with rigorous screening processes, further ensure that the final sample size is adequate and appropriate for the planned statistical analyses.

By adhering to these guidelines, I aimed to achieve a balance between statistical power, practical feasibility, and ethical considerations, ensuring that the findings would be both reliable and generalisable.

Study 1

Participants

Sixty participants were recruited for the young adult study (41 female; mean age 24.88 ± 5.21, range 18 – 52). Participants were split across three experimental groups. 20 participants in the left M1 to right M1 group (9 female; mean age 26.35 ± 5.21); 20 participants in the right M1 to left M1 group (16 females; mean age 22.70 ± 4.18) and 20 participants in the Control ccPAS group (16 females; mean age 25.60 ± 6.96). Participants were recruited through SONA; a comprehensive research and study management platform that universities and research institutions widely use. Participants were reimbursed for their time and chose either 3.5 SONA credits or £30. Following standard protocols for electrical stimulation as a precautionary measure, individuals completed neurostimulation safety screening questionnaires (Appendix A). This was to identify and exclude those at risk from participating in the study. In addition, the following inclusion and exclusion criteria were used to screen participants:

Exclusion

- Personal or family history of seizures or syncope, brain lesions or head trauma
- History of or current heart conditions
- Neurological or psychiatric disorders
- Epilepsy, migraines, sleep deprivation, alcohol dependency, or being pregnant.
- Individuals with implanted medical devices (such as pacemakers or cochlear implants)
- ferromagnetic implants
- Participants who were taking tricyclic antidepressants, neuroleptic drugs, or any medication that could lower the seizure threshold,
- holders of HGV/bus licenses

- Those with wounds or skin conditions in or around the stimulation area

Inclusion

- Right-handed
- Self-reported normal or corrected to normal vision
- Normal hearing

To ensure the inclusion of only right-handed participants in this study, the Edinburgh Handedness Inventory (EHI) Oldfield (1971) was employed. This validated and widely used tool measures handedness based on participants' self-reported hand preference across various everyday tasks, such as writing, throwing, and using scissors. Participants completed the EHI before the study, and only those with a laterality quotient indicating strong right-handedness (Laterality Quotient +40 to +100) were included.

Measures

The primary measures captured during the experiment include:

Motor-Evoked Potentials (MEPs): Single pulse and paired pulse TMS MEPs from the first dorsal interosseous (FDI) muscle were captured to explore the influence of ipsilateral M1 stimulation on contralateral M1 effects.

Interhemispheric Inhibition (IHI): IHI was calculated for each participant to gauge the inhibitory effects exerted by one hemisphere on the other, with the ratio between the conditioned (ccPAS induced) and unconditioned MEP amplitudes serving as the metric.

These measures aimed to elucidate cortical excitability and inhibition dynamics, enhancing the understanding of neural plasticity and interhemispheric communication.

Experimental design

The procedure for the three experiments began with an initial Baseline block, followed by a ccPAS block, and concluded with an Expression block (refer to Figure 4).

During the Baseline and Expression blocks, TMS was administered at rest, targeting both the right and left primary motor cortex (M1). The objective was to examine the influence of stimulating ipsilateral M1 on the stimulation effects of the contralateral M1. This was assessed by comparing the MEPs from the first dorsal interosseous (FDI) muscle on the ipsilateral side. These comparisons were made under three conditions: when single-pulse TMS was applied to contralateral M1 (60 trials each for right M1 and left M1) and when dual-site paired-pulse TMS (combining right M1 and left M1 simultaneous stimulation) was employed (60 trials). An ISI of 8 milliseconds was adopted, as established by previous research (Rizzo et al., 2009; Rizzo et al., 2011), which established that the right and left primary motor cortices (M1) can communicate with each other with such short intervals during rest periods.

In Experiment 1, stimulation began with a TMS pulse to left M1 followed by a pulse to right M1, following a left-to-right M1 ccPAS sequence. Experiment 2 applied the reverse sequence, starting with the right M1 and moving to the left M1, right-to-left M1 ccPAS. Experiment 3 divided participants equally, with one-half experiencing the left-to-right M1 ccPAS and the other half experiencing the right-to-left M1 ccPAS. For every participant across all experiments, the trials involving single-pulse TMS (spTMS) and paired-pulse TMS (ppTMS) were mixed in a pseudorandom and counterbalanced manner within each block.

In each of the three experiments, the ccPAS block consisted of a 15-minute session of ccPAS targeting both the right M1 and left M1 at a frequency of 0.1 Hz, resulting in a total of 90 stimulus pairings, with an ISI of 8ms. In Experiment 1, the stimulation sequence always commenced with the left M1, followed by the right M1, whilst the reverse order was employed in Experiment 2. For Experiment 3, the stimulations to the left M1 and right M1 were delivered almost simultaneously, with an ISI of 1ms, acting as a control (Rizzo et al., 2009).

TMS and electromyography recordings

TMS was administered via two DuoMAG MP devices, each linked to a pair of 50 mm figure-of-eight coils. The resting motor threshold (rMT) technique was followed to identify the optimal scalp positions for stimulating the right and left primary M1. The precise scalp locations for targeting the right and left M1 with TMS were pinpointed on the EEG-cap. These locations were determined by eliciting a 50 μV response in the opposite hand's first dorsal interosseus (FDI) muscle, observed in 5 out of 10 consecutive trials at the lowest necessary intensity to produce such a response. While this measure was obtained, the participants were instructed to keep the contralateral FDI muscle relaxed. Throughout the protocol, participants were regularly asked for feedback on their comfort, with explicit instructions that they could request to stop if needed.

Mirroring the approach of earlier ccPAS research (Rizzo et al., 2009; Rizzo et al., 2011), the conditioning and test pulses in the ccPAS protocol were calibrated to 110% of the resting motor threshold (rMT), to produce single-pulse MEPs of approximately ±1 mV for each M1. Given the natural variability of MEPs, the intensity of the TMS was fine-tuned during the initial 20 trials of the Expression block to keep the MEPs within the targeted range of ±1 mV. The TMS coils were held tangentially against the skull, aligning the coil aimed at M1 at an angle of about 45°, with its handle oriented posteriorly (Ferbert et al., 1992).

Silver-silver chloride (Ag-AgCl) surface electrodes were applied to the belly and tendon of the first dorsal interosseous (FDI) muscle to capture electromyography (EMG) readings from both right and left FDIs. Before attaching the electrodes, the area was cleansed with alcohol wipes to enhance the EMG recording's accuracy, safety, and data quality. Additionally, three electrodes were placed on each hand for comprehensive monitoring. EMG signals were collected at a 10,000 Hz sampling rate, underwent bandpass filtering between 0.10 and 1,000 Hz with additional 50 Hz notch filtering, and were captured using Cambridge Electronic Design equipment; CED D440-4 amplifier, a CED micro1401 Mk.II A/D converter. A separate PC with Signal equipment and software was also used.

MEPs were determined by their peak-to-peak amplitude. Any trial producing MEPs below 0.1mV or above 6mV was discarded, as were trials where FDI muscle activity before stimulation exceeded a set threshold, leading to an average of approximately 19 excluded trials per participant. Data were initially processed using Signal software and further analysed in Matlab. Interhemispheric inhibition (IHI) was assessed by comparing MEP amplitudes from conditioned and unconditioned TMS pulses, indicating the inhibitory effects of one hemisphere on the other, with higher IHI values suggesting weaker inhibition and lower values indicating stronger inhibition.

Analysis

Hypotheses – Young Adult

The following statistical analyses were identified to explore the hypotheses for the Young Adult group.

Hypothesis 1: M1-M1 ccPAS Regulates Corticospinal Excitability in the Motor Cortex as measured by MEP

ccPAS can induce interhemispheric inhibition in the contralateral M1 region, which is identified by a reduction in MEP.

Statistical analysis

A repeated measures ANOVA will be used to explore the effects of TMS Pulse and Block on MEP across all the experimental groups. The within-subject factors will be TMS Pulse (Single-pulse vs Paired-Pulse) and Block (Baseline vs Expression), and the between-subjects factor will be Group (RM1-LM1 ccPAS, LM1-RM1 ccPAS, Control ccPAS).

Hypothesis 2: Directional Effect of M1-M1 ccPAS on Interhemispheric Inhibition (IHI)

The effectiveness of ccPAS in modulating interhemispheric inhibition (IHI) depends on the direction of corticocortical paired associative stimulation (ccPAS).

Statistical analysis

A repeated-measures ANOVA will be used to explore the directional effect of M1-M1 ccPAS on interhemispheric inhibition (IHI). The within-subject factor will be Block (Baseline vs Experimental), and the between-subjects factor will be Group (RM1-LM1 ccPAS, LM1-RM1 ccPAS, Control ccPAS)

Hypothesis 3: The Effects of M1-M1 ccPAS Develop Over Time

The effects of ccPAS on cortical excitability evolve over time.

Statistical analysis

A Repeated-measures ANOVA will be run to explore how the effects of M1-M1 ccPAS develop over time. The analysis will require two within-subject factors of Block (Baseline vs Expression) and TMS pulse (single-pulse TMS vs paired-pulse TMS), conducted for each experimental group (RM1-LM1 ccPAS, LM1-RM1 ccPAS, Control ccPAS).

Study 2

Participants

Ten participants were recruited for the older adult study (8 female; mean age 70.10 ± 5.66, range 63 – 83). Participants were tested across both conditions, ccPAS and control. The participants were older adults who belonged to a pool of volunteers who regularly took part in research studies at the University of Essex. The same inclusion and exclusion criteria used in Study 1 were applied. Participants were reimbursed for their time and paid £30. Following standard protocols for electrical stimulation as a precautionary measure, individuals completed neurostimulation safety screening questionnaires (Appendix X). This was to identify and exclude those at risk from participating in the study. The older adults also completed a capacity to consent form.

Experimental design

The procedure for testing the older adult group was the same as the younger adult group, with the exception that the older adults adopted a within-subjects design.

In Experiment 1, stimulation began with a TMS pulse to Right M1 followed by a pulse to Left M1, following a right to left M1 ccPAS sequence. In experiment 2, the participants received left-to-right M1 control ccPAS with an ISI of 1ms. For every participant across both experiments, the trials involving single-pulse TMS (spTMS) and paired-pulse TMS (ppTMS) were mixed in a pseudorandom and counterbalanced manner within each block.

Analysis

Hypotheses – Older Adult

The following statistical analyses were identified to explore the hypotheses for the Older Adult group.

Hypothesis 4: M1-M1 ccPAS Regulates Corticospinal Excitability in the Older Adult Motor Cortex as measured by MEP

ccPAS can induce interhemispheric inhibition in the contralateral M1 region, which is identified by a reduction in MEP amplitude.

Statistical analysis

A repeated measures ANOVA will be used to examine the effects of TMS Pulse and Block on MEP across two groups (ccPAS and Control). The within-subject factors will be TMS Pulse (Single-pulse vs Paired-Pulse) and Block (Baseline vs Expression), and the between-subject factor will be Group (ccPAS and Control).

Hypothesis 5: Effect of M1-M1 ccPAS on Interhemispheric Inhibition (IHI)

The effectiveness of ccPAS in modulating interhemispheric inhibition (IHI) depends on the direction of corticocortical paired associative stimulation (ccPAS)

Statistical analysis

A repeated measures ANOVA will be conducted to examine the effects of Block on IHI across two groups (ccPAS and Control). The within-subjects factor will be Block (Baseline vs Expression), and the between-subjects factor will be Group (ccPAS and Control).

Hypothesis 6: The Effects of M1-M1 ccPAS Develops Over Time in the Older Adult Motor Cortex

The effects of ccPAS on cortical excitability evolve over time within the older adult M1 region.

Statistical analysis

Repeated measures ANOVAs will be conducted to evaluate the effect of Block, TMS Pulse, and Group on cortical excitability for the first twenty trials of the protocol, followed by the last twenty trials. The within-subject factors will be Block (Baseline vs Expression) and TMS Pulse (Single-pulse vs Paired-Pulse), and the between-subjects factors will be Group

(ccPAS and Control). The same ANOVA will be run to assess IHI. However, the within-subject factor will be Block (Baseline vs Expression), and the between-subject factor will be Group (ccPAS and Control).

Hypothesis 7: Age-Dependent Modulation by LM1-RM1 ccPAS

M1-M1 ccPAS will be effective in modulating cortical excitability, with the young adult group responding more strongly.

This final hypothesis compares young and older adult data together. Two repeated-measures ANOVA will be required to evaluate the age-dependent modulation of cortical excitability by M1-M1 ccPAS. For the MEP data, this will be achieved with the within-subjects factors of Block (Baseline vs Expression) and TMS Pulse (Single-pulse vs Paired-pulse) and the between-subject factor of Age Group (Young vs Old). For the IHI data, the same ANOVA procedure will be run. The within-subject factor will be Block (Baseline vs Expression), and the between-subject factor will be Age Group (Young vs Old).

Rigour and validity

To ensure the rigour and validity of the experimental findings, control conditions and randomisation procedures were implemented where possible throughout the study. Each experiment incorporated control conditions to account for potential confounding variables. In both the young and older adult studies, a control group was included and subjected to a sham ccPAS protocol, which mimics the sensory experience of the actual ccPAS without inducing cortical plasticity. This control group allowed the specific effects of the ccPAS from non-specific effects to be isolated, such as participant expectation or the physical sensation of TMS. Additionally, within each experiment, a counterbalanced design was utilised to mitigate order effects. Participants were randomly assigned to different sequences of the experimental and control conditions. This counterbalancing ensures that any potential order effects do not systematically bias the results.

Participants were randomly assigned to their respective groups to minimise selection bias. For the young adult study, participants were allocated to one of three groups (left M1 to right M1, right M1 to left M1, and control ccPAS) using a computer-generated randomisation schedule. This approach ensures an unbiased distribution of participants across the experimental conditions.

In the older adult study, a within-subjects design was employed. Each participant underwent both the experimental ccPAS conditions and the control condition on separate days, with the order of conditions randomised and counterbalanced across participants. This design reduces inter-individual variability and enhances the power to detect within-subject differences. To further enhance the study's rigour, blinding procedures were implemented. Participants were blinded to the specific hypotheses being tested, which helped to mitigate demand characteristics.

Ethical Considerations

Ethical approval for my project was granted by the University of Essex through its Ethics Review and Management System (ERAMS) under reference number ETH2223-0175 (Appendix A). This approval was obtained as part of a larger research project designed to explore brain mechanisms of motor control.

The larger project involved administering several validated questionnaires, including the Edinburgh Laterality Questionnaire (ELQ), Beck's Depression Inventory (BDI), Mood Disorder Questionnaire (MDQ), Spielberger's State-Trait Anxiety Inventory (State STAI and Trait STAI), Mood and Anxiety Symptom Questionnaire (MASQ), and the Altman Self-Rating Mania Scale.

For the current study, only data collected from the ELQ were utilised, while the remaining questionnaires were part of the broader research framework but were not directly analysed in this study.

Before agreeing to participate, participants were given an Information Sheet (Appendix A) detailing the study's aims, TMS and EEG procedures, and potential risks. This recruitment strategy ensured that individuals had the necessary information and time to make a well-informed choice about their participation. The specific approach of not having the researcher present during the decision-making process ensured the integrity of the participants' informed consent. Those who decided to participate were then given a Consent Form to sign and had the opportunity to raise any questions regarding the study. In addition, the older participants were also asked for their capacity to consent.

Participants were informed that their data would be stored anonymously; only the researcher could access personal information, and no personal details would be included in the experimental data. Consent forms were securely stored in a locked filing cabinet in the Principal Investigator's (PI's) office. All other identifiable information was kept on the institutional servers and was password protected. It was also communicated that the collected data might, at some point, be presented at a conference or published in a scientific journal. However, participants were assured that should this occur, their anonymity would be strictly maintained. Participants were informed of their right to withdraw from the study without giving a reason and how to remove their data.

TMS sessions can be physically and mentally taxing, particularly for older adults. Regular breaks were provided to account for this. Older adult participants were solely involved in ccPAS testing that targeted the right-to-left M1 (primary motor cortex) and a control condition on another day to minimise fatigue and avoid unnecessary retesting. This approach was adopted to ensure the well-being of the participants, recognising that older adults might have a lower threshold for physical and cognitive fatigue, especially in research settings that involve neurostimulation. By focusing on this specific aspect of the study I aimed to gather meaningful data while prioritising the comfort and safety of the older participants.

Dissemination

I hope that this research will significantly advance the understanding of the effects of cortico-cortical paired associative stimulation (ccPAS) on neural plasticity and motor function, specifically in relation to interhemispheric inhibition and corticospinal excitability across different age groups. To ensure these findings reach a wide audience of researchers, clinicians, and stakeholders, I have devised a comprehensive dissemination strategy.

I will begin by submitting my study for publication in a leading, peer-reviewed neuroscience journal to establish a strong academic foundation and credibility. Following publication, I will explore ways to present the findings at conferences focused on neuroscience, neuroplasticity, neuropsychology, clinical psychology and non-invasive brain stimulation, ensuring I engage directly with the scientific community.

Additionally, I plan to leverage digital platforms. This includes creating a detailed video abstract of the study to be shared across academic networking sites like ResearchGate and Google Scholar and social media platforms such as LinkedIn and X (formerly Twitter). This approach will help reach a broader audience, including those in the academic, clinical, and possibly even patient advocacy groups interested in the latest developments in brain stimulation therapies.

To further influence clinical practice and policy, I will aim to engage in direct outreach to healthcare professionals through workshops and seminars that detail the research outcomes and discuss potential clinical applications. These events will be designed to foster dialogue on integrating ccPAS into therapeutic protocols, especially for age-related motor function impairments and traumatic brain injury.

This multidimensional approach will hopefully ensure that my research contributes to the academic discourse and influences practical applications and public knowledge, making a meaningful impact across various sectors of society.

Chapter 4: Results

Chapter Overview

In this chapter, I present the results of two experiments investigating the effects of cortico-cortical paired associative stimulation (ccPAS) on motor function. The focus is on corticospinal excitability and interhemispheric inhibition (IHI) in young and older adults. The chapter is structured to first highlight the key findings, followed by statistical evidence and hypothesis-linked interpretations.

Data Treatment

Prior to analysis, the data underwent thorough examination and preparation to ensure its validity and adherence to statistical assumptions. Normality was assessed by evaluating skewness and kurtosis for all variables. Where deviations from normality were detected, a Log10 transformation was applied to approximate normal distributions, which is essential for parametric testing. To address negative values in the data, a constant of 1 was added to all motor-evoked potential (MEP) values before transformation, ensuring all data points remained within a positive range suitable for logarithmic operations. The dataset contained no missing values across the 60 participants, and each variable was carefully reviewed for completeness and accuracy. This meticulous preprocessing ensured the robustness and interpretability of the statistical analyses while minimising the impact of outliers and non-normal distributions.

In this chapter, I present the results of two experiments investigating the effects of cortico-cortical paired associative stimulation (ccPAS) on motor function. The focus is on corticospinal excitability and interhemispheric inhibition (IHI) in young and older adults. The chapter is structured to first highlight the key findings, followed by statistical evidence and hypothesis-linked interpretations.

Key Findings

- Corticospinal Excitability: ccPAS enhanced corticospinal excitability, with direction-specific effects. RM1-LM1 ccPAS showed the most significant facilitation, particularly during the expression block.
- Interhemispheric Inhibition (IHI): Direction-specific effects of ccPAS were observed on IHI, with RM1-LM1 ccPAS reducing inhibition and LM1-RM1 ccPAS enhancing inhibition.
- Temporal Effects: The effects of ccPAS developed over time, with the RM1-LM1 direction showing sustained facilitation during the last 20 trials of the expression block.

Effects on Corticospinal Excitability

Motor-Evoked Potentials (MEPs)

The analysis of motor-evoked potentials (MEPs) provided critical insights into corticospinal excitability changes induced by ccPAS. Single-pulse MEPs demonstrated an increase from baseline to the expression block in both the LM1-RM1 and RM1-LM1 groups, indicating enhanced excitability. Paired-pulse MEPs also exhibited notable differences, reflecting shifts in excitability and inhibition mechanisms due to ccPAS interventions.

For single-pulse MEPs, the LM1-RM1 group showed a mean increase from 1.237 at baseline to 1.477 during the expression block, with corresponding shifts in skewness and kurtosis indicating distributional changes. The RM1-LM1 group exhibited similar trends, with a mean increase from 1.204 to 1.502. These patterns were absent in the control group, which displayed only minimal changes.

Paired-pulse MEPs further illustrated the differential effects of ccPAS. The LM1-RM1 group demonstrated a mean increase from 0.873 to 0.988, while the RM1-LM1 group showed an increase from 0.822 to 1.146. These findings underscore the effectiveness of ccPAS in modulating corticospinal excitability, particularly in experimental groups.

Table 6

Mean Motor-Evoked Potential (MEP) Values for Single- and Paired-Pulse TMS

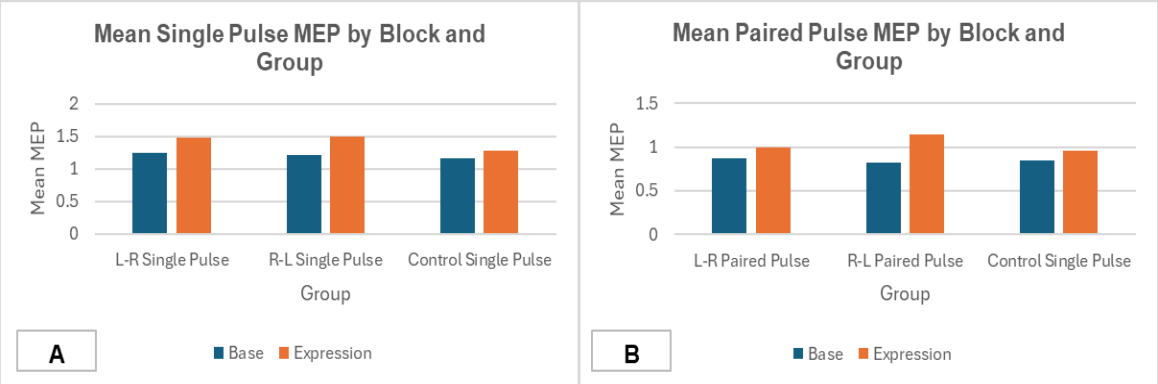
	LM1-RM1 Single-pulse	RM1-LM1 Single-pulse	Control Single-pulse
Base	0.061	0.045	0.008
Expression	0.128	0.119	0.034

	LM1-RM1 Paired-pulse	RM1-LM1 Paired-pulse	Control Single-pulse
Base	-0.145	-0.137	-0.144
Expression	-0.021	-0.117	-0.107

Note. Values represent the mean motor-evoked potentials (MEPs) for single- and paired-pulse TMS at baseline and expression blocks for each experimental group. These data highlight the changes in corticospinal excitability induced by ccPAS.

Figure 7

MEP Values For Single-Pulse TMS At Base And Expression Blocks By Direction



Note. **Image A represents the single-pulse outcomes for each group indicating that both base and expression block values show enhanced MEP compared to the control. Image B represents the paired-pulse outcomes for each group, also demonstrating enhanced MEP**

Participant Demographics

Each group received cortico-cortical paired associative stimulation (ccPAS) using different protocols:

- LM1-RM1 Group: ccPAS was applied from the left motor cortex (M1) to the right motor cortex (M1) with an interstimulus interval (ISI) of 8 ms.
- RM1-LM1 Group: ccPAS was applied from the right motor cortex (M1) to the left motor cortex (M1) with an ISI of 8 ms.
- Control Group: Sham ccPAS was applied with a short ISI of 1 ms, ensuring no significant associative stimulation effect.

Table 1

Participant Demographics by ccPAS Condition Participant Demographics by ccPAS Condition

Group	Participants (Female)	Mean Age
LM1-to-RM1	20 (9)	26.35 ± 5.21
RM1-to-LM1	20 (16)	22.70 ± 4.18
ccPAS Control	20 (16)	25.60 ± 6.96

Note. **Values are presented as mean ± standard deviation (SD) for age and as count (number of female participants) for participants.**

Descriptive statistics

Baseline and expression block data were examined for normality and transformed using Log10 to address skewness and kurtosis.

Table 2

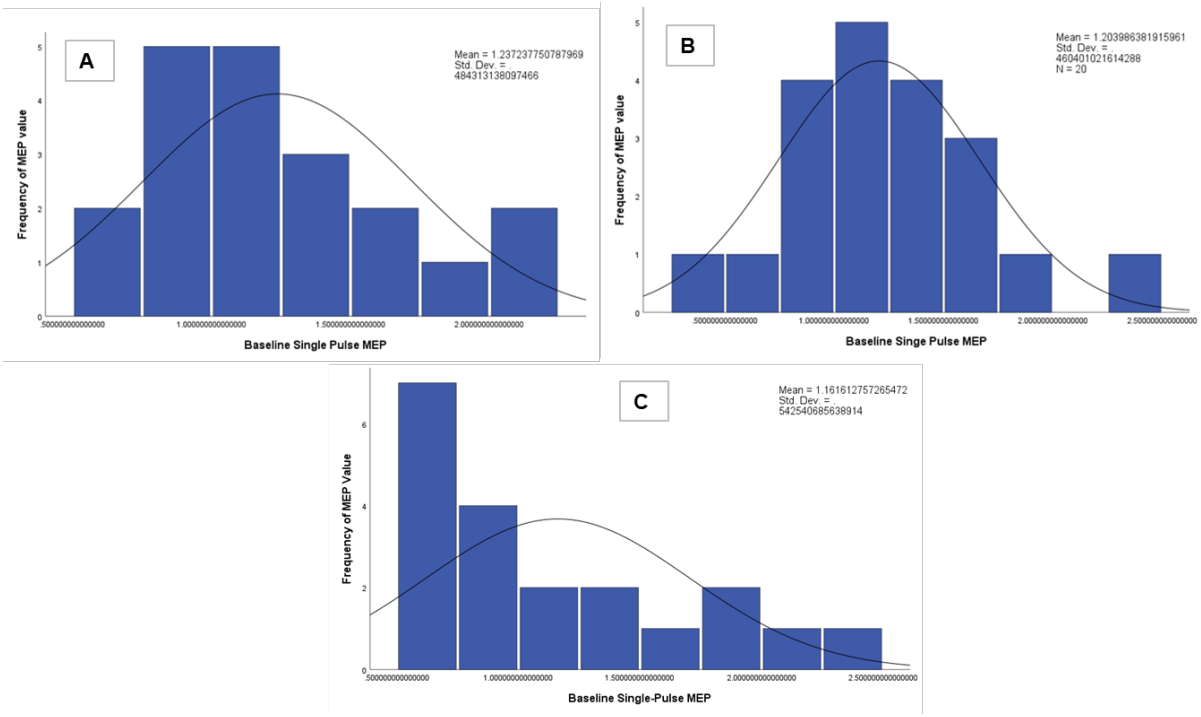
Means, Skewness and Kurtosis for Normality Assessment

Block	LM1-RM1 Single Pulse	RM1-LM1 Single Pulse	Control Single Pulse	LM1-RM1 Paired-Pulse	RM1-LM1 Paired-Pulse	Control Paired-Pulse
Base	M = 1.237, Sk = 0.754, Ku = 0.103	M = 1.204, Sk = 0.311, Ku = 0.389	M = 1.162, Sk = 0.780, Ku = -0.727	M = 0.873, Sk = 1.394, Ku = 1.467	M = 0.822, Sk = 0.489, Ku = -0.535	M = 0.851, Sk = 1.355, Ku = 1.699
Expression	M = 1.477, Sk = 0.240, Ku = -1.096	M = 1.502, Sk = 1.364, Ku = 3.063	M = 1.273, Sk = 1.702, Ku = 2.593	M = 0.988, Sk = 0.930, Ku = -0.711	M = 1.146, Sk = 1.382, Ku = 1.571	M = 0.953, Sk = 1.721, Ku = 2.537

Note. Values for mean (M), skewness (Sk), and kurtosis (Ku) provide an assessment of the normality of the distributions for each ccPAS group. These descriptive statistics offer insight into the changes in corticospinal excitability across the different blocks (baseline vs. expression) and types of TMS (single-pulse vs. paired-pulse) within each experimental group. The data suggest variations in MEPs, Sk, and Ku, reflecting the underlying distributional characteristics of the excitability measures in response to the ccPAS intervention and control conditions.

Figure 3

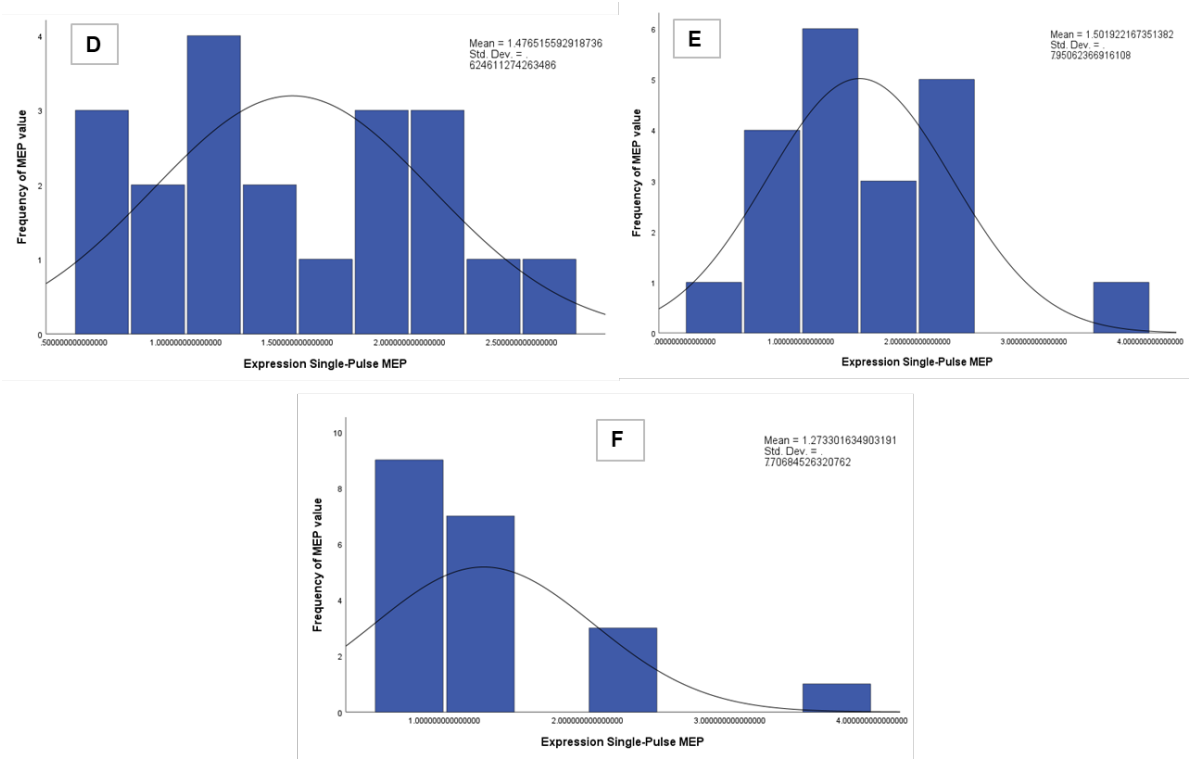
Frequency distribution of Baseline Single-Pulse Motor Evoked Potentials (MEP) for LM1-RM1 and RM1-LM1 ccPAS groups and control ccPAS group



Note. Each graph displays the baseline single-pulse MEP distribution for a specific group. The LM1-RM1 ccPAS (Graph A) and control (Graph C) groups both show right-skewed distributions, while the RM1-LM1 ccPAS group (Graph B) shows a distribution that is closer to normal but slightly right-skewed. The means and standard deviations are relatively similar across all three groups, with the LM1-RM1 ccPAS group having the highest mean MEP value, followed by the RM1-LM1 ccPAS group, and the control group having the lowest. The skewness and kurtosis values provided in the previous tables support these visual observations, indicating that the distributions have some deviation from normality, primarily in the form of positive skewness.

Figure 4

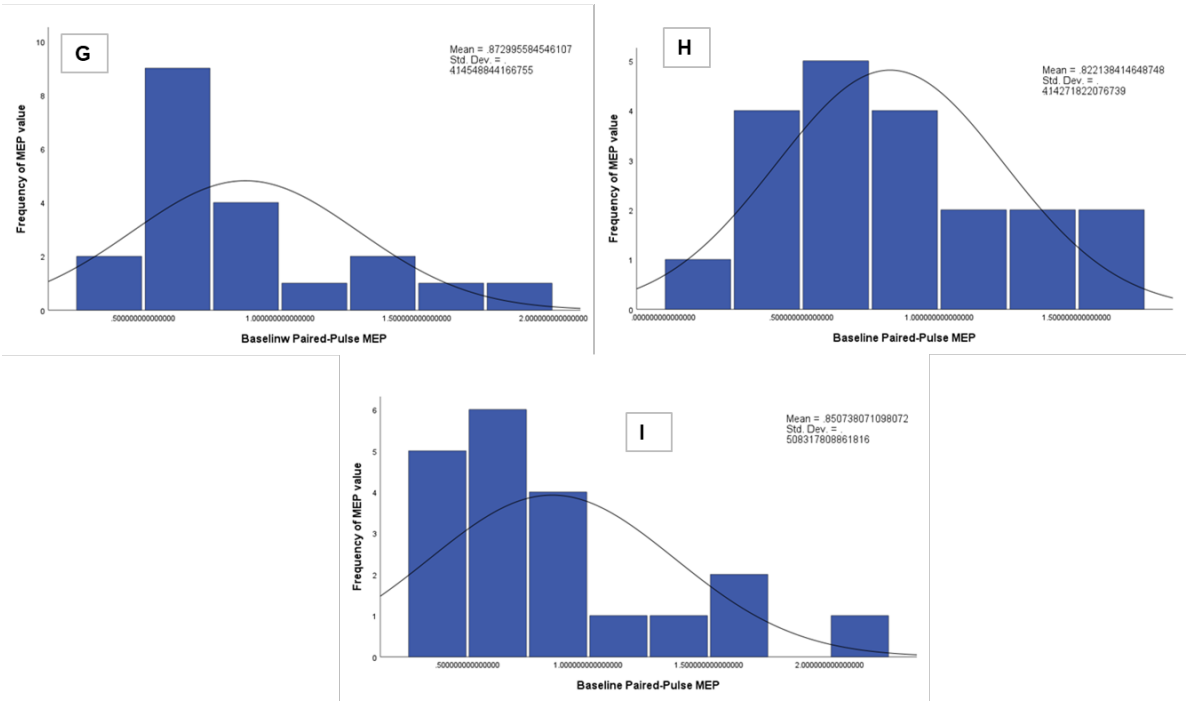
Frequency distribution of Expression Single-Pulse Motor Evoked Potentials (MEP) for the LM1-RM1 and RM1-LM1 ccPAS groups and control ccPAS group



Note. Each graph displays the expression block single-pulse MEP distribution for a specific group. The LM1-RM1 ccPAS (Graph D) and control (Graph F) groups both show right-skewed distributions, indicating more low MEP values and fewer high MEP values. The RM1-LM1 ccPAS group (Graph E) shows a distribution that is closer to normal but still slightly right-skewed. The means and standard deviations indicate an increase in MEP values during the expression block compared to the baseline block for all groups. The skewness and kurtosis values provided earlier (Table 2) support these visual observations.

Figure 5

Frequency distribution of Baseline Paired-Pulse Motor Evoked Potentials (MEP) for LM1-RM1 and RM1-LM1 ccPAS groups and control ccPAS group

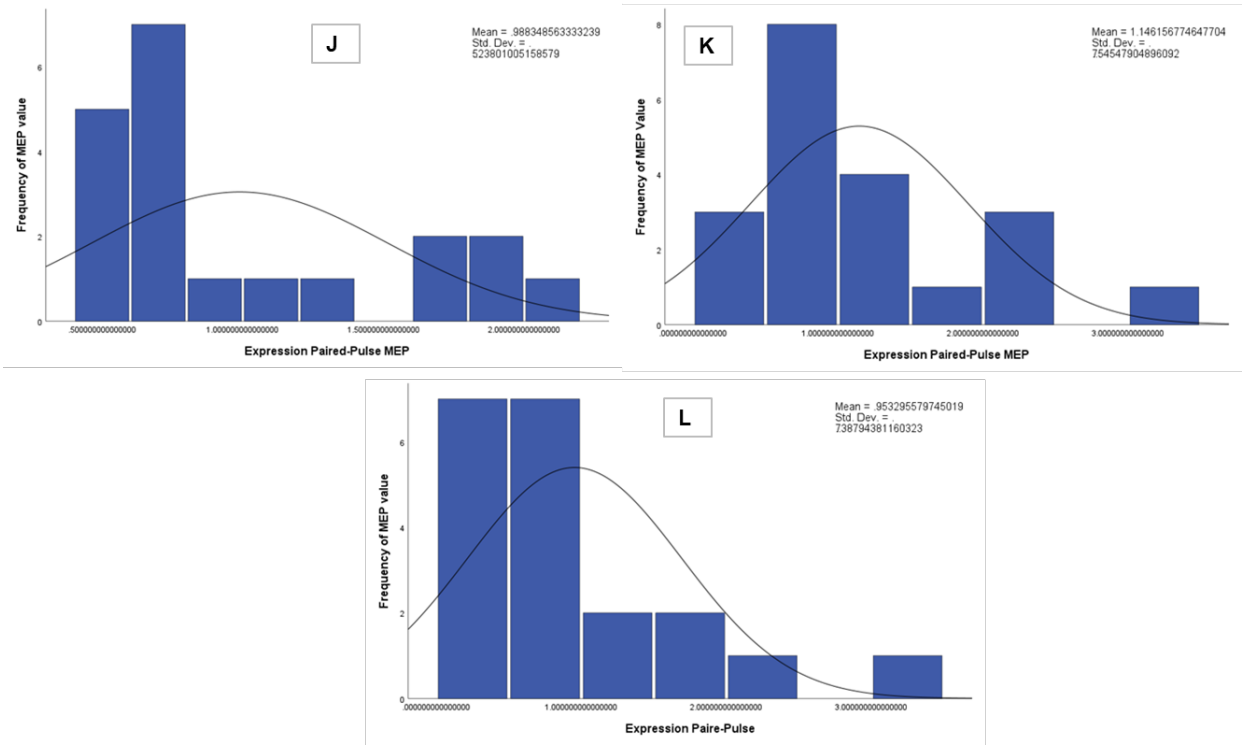


Note. The graphs display the frequency distributions of the baseline block paired-pulse motor evoked potential (MEP) values for three different groups: LM1-RM1 ccPAS, RM1-LM1 ccPAS, and the control group. Each graph includes a histogram with a fitted normal distribution curve. The LM1-RM1 ccPAS (Graph G) and control (Graph I) groups both show right-skewed distributions, indicating more low MEP values and fewer high MEP values. The RM1-LM1 ccPAS group (Graph H) shows a distribution that is closer to normal but still slightly right-skewed. The means and standard deviations are relatively similar across all three groups, with the LM1-RM1 ccPAS group having the highest mean MEP value, followed by the control group, and the RM1-LM1 ccPAS group having the lowest. The skewness and kurtosis values provided in Table 2 support these visual observations, reflecting the underlying distributional characteristics of the

excitability measures in response to the baseline paired-pulse TMS across the different groups.

Figure 6

Frequency distribution of Expression Paired-Pulse Motor Evoked Potentials (MEP) for LM1-RM1 and RM1-LM1 ccPAS groups and control ccPAS group



Note. Each graph displays the expression block paired-pulse MEP distribution for a specific group. The LM1-RM1 ccPAS (Graph J), RM1-LM1 ccPAS (Graph K), and control (Graph L) groups all show right-skewed distributions, indicating more low MEP values and fewer high MEP values. The means and standard deviations indicate variability in MEP values during the expression phase for all groups, with the RM1-LM1 ccPAS group having the highest mean MEP value, followed by the LM1-RM1 ccPAS group, and the control group having the lowest. The skewness and kurtosis values provided earlier (Table 2) support these visual observations, reflecting the underlying distributional characteristics of the excitability measures in response to the expression paired-pulse TMS across the different groups.

Key Observations

Both LM1-RM1 and RM1-LM1 groups exhibited increases in motor-evoked potentials (MEPs) from baseline to the expression block, reflecting enhanced corticospinal excitability as a result of ccPAS. Among the two experimental groups, the RM1-LM1 group demonstrated the most pronounced changes, showing higher mean increases in both single-pulse and paired-pulse MEPs compared to the LM1-RM1 group. In contrast, the control group displayed only minimal changes, further supporting the specificity of the ccPAS intervention. These findings underscore the directional impact of ccPAS on enhancing corticospinal excitability, with RM1-LM1 ccPAS yielding the strongest facilitation effects..

Interhemispheric Inhibition (IHI)

The analysis of interhemispheric inhibition (IHI) provided critical insights into the effects of ccPAS on inhibitory dynamics between the hemispheres. Baseline and expression block data revealed notable variations across the LM1-RM1, RM1-LM1, and control groups. Direction-specific effects were evident, with the RM1-LM1 direction showing a reduction in inhibition, while the LM1-RM1 direction demonstrated an enhancement in inhibition. These findings suggest that ccPAS modulates interhemispheric communication differently depending on the stimulation direction.

For the LM1-RM1 group, IHI values decreased slightly from a baseline mean of 0.711 to 0.683 during the expression block, indicating increased inhibition. This suggests that the left-to-right stimulation strengthened the inhibitory effect of the left hemisphere on the right. Conversely, for the RM1-LM1 group, IHI values increased from 0.678 to 0.751, reflecting a reduction in inhibition. This reduction suggests that the right-to-left stimulation decreased the inhibitory influence of the right hemisphere on the left.

The control group exhibited minimal changes, with IHI values decreasing marginally from 0.725 to 0.713. These findings underscore the hypothesis that ccPAS

exerts direction-specific effects, with RM1-LM1 ccPAS producing the most pronounced modulation of interhemispheric dynamics.

Table 6

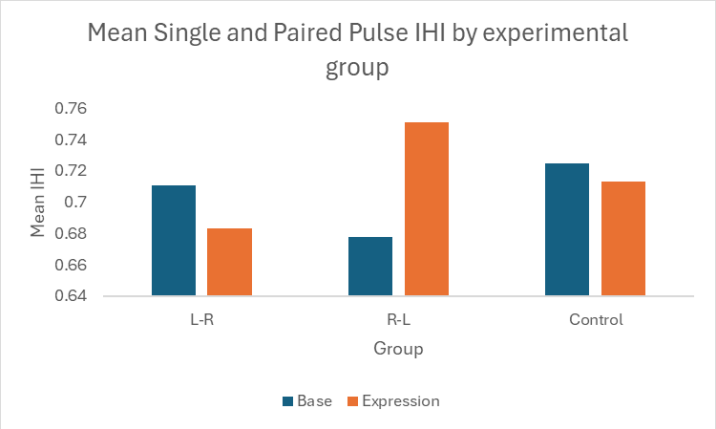
IHI values for Single and Paired-pulse TMS by Block

	LM1-RM1	RM1-LM1	Control
Base	M = 0.711	M= 0.678	M= 0.725
Expression	M = 0.683	M= 0.751	M= 0.713

Note. Values are presented as means (M) for interhemispheric inhibition (IHI).

Figure 8

Means for Single and Paired-pulse IHI at Base and Expression Blocks



Note. Mean IHI Values for Base and Expression Blocks by Group. The increase in IHI demonstrated by the RM1-LM1 ccPAS group indicates that RM1-LM1 ccPAS may enhance the inhibitory effect of the right hemisphere on the left hemisphere

Key Observations

Both LM1-RM1 and RM1-LM1 groups exhibited directional effects on interhemispheric inhibition (IHI) due to ccPAS. The RM1-LM1 group showed the most significant reduction in inhibition, with an increase in mean IHI values from 0.678 to 0.751, indicating a decreased inhibitory effect of the right hemisphere on the left. In contrast, the LM1-RM1 group demonstrated an increase in inhibition, with mean IHI values decreasing slightly from 0.711 to 0.683, reflecting a strengthening of the

inhibitory effect of the left hemisphere on the right. The control group displayed minimal changes, further supporting the directional specificity of ccPAS. These results highlight the role of stimulation direction in modulating interhemispheric dynamics.

Temporal Effects

Motor-Evoked Potentials (MEPs) Results

The temporal dynamics of ccPAS effects on motor-evoked potentials (MEPs) were explored by analysing single-pulse and paired-pulse TMS responses across the first and last 20 trials of baseline and experimental blocks. The data reveal distinct patterns of change, particularly in the experimental blocks.

For single-pulse TMS, both the LM1-RM1 and RM1-LM1 groups showed increases in mean MEP values over time. In the LM1-RM1 group, mean MEPs increased from 0.03418 in the first 20 trials to 0.08829 in the last 20 trials during the baseline block, and from 0.0798 to 0.14251 during the experimental block. Similarly, the RM1-LM1 group demonstrated increases from 0.00667 to 0.05837 during baseline and from 0.07683 to 0.12967 during the experimental block. The control group exhibited smaller changes, with baseline values shifting from -0.01617 to 0.03786 and experimental values increasing from -0.17546 to -0.11309.

For paired-pulse TMS, the LM1-RM1 group showed reductions in inhibition, with values moving from -0.17305 to -0.03049 during baseline and from -0.10672 to -0.05434 during the experimental block. The RM1-LM1 group transitioned from inhibition to facilitation, with baseline values changing from -0.18228 to -0.13117 and experimental values shifting from -0.06852 to 0.00817. The control group displayed minimal changes.

Table 7

MEP mean values for the first and last twenty trials according to the experimental block

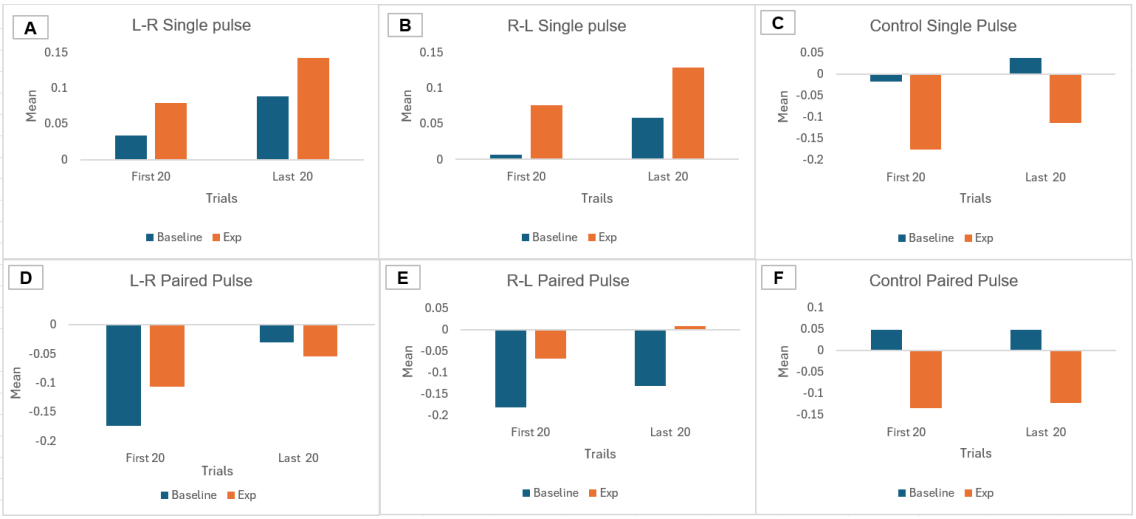
Block	LM1-RM1 Single pulse		RM1-LM1 Single pulse		Control Single Pulse	
	First 20	Last 20	First 20	Last 20	First 20	Last 20
Baseline	0.03418	0.08829	0.00667	0.05837	-0.01617	0.03786
Exp	0.0798	0.14251	0.07683	0.12967	-0.17546	-0.11309

	LM1-RM1 Paired Pulse		RM1-LM1 Paired Pulse		Control Paired Pulse	
	First 20	Last 20	First 20	Last 20	First 20	Last 20
Baseline	-0.17305	-0.03049	-0.18228	-0.13117	0.04836	0.04862
Exp	-0.10672	-0.05434	-0.06852	0.00817	-0.13322	-0.12193

Note. The values represent the mean single and paired-pulse TMS responses for baseline and experimental conditions across the first and last 20 trials for each group. Single-pulse data indicates an increase in mean MEP values across both ccPAS groups between the first and last trials. The paired-pulse shows a decrease in inhibition for both ccPAS groups. This suggests that ccPAS is effective in modulating neural excitability.

Figure 9

Mean Single And Paired-Pulse TMS Responses For Baseline And Experimental Blocks Across The First And Last 20 Trials For Each Group



Note. This figure illustrates the mean responses for baseline and experimental blocks across different pulse types, divided into the first 20 and last 20 trials. Panels A and B show the mean responses for LM1-RM1 Single-Pulse and RM1-LM1 Single-Pulse, respectively. Panels C and F display the mean responses for Control Single-Pulse and Control Paired-Pulse, respectively. Panels D and E show the mean responses for LM1-RM1 Paired-Pulse and RM1-LM1 Paired-Pulse, respectively. For each panel, the blue bars represent the baseline block, and the orange bars represent the experimental (Exp) block. The data indicate that ccPAS generally enhances the response compared to the baseline, with more pronounced improvements in the last 20 trials for both single and paired-pulse types, suggesting that the effects induced by ccPAS take time to develop.

Key Observations

The temporal analysis revealed a consistent increase in single-pulse MEPs across all groups, with the most pronounced changes observed in the RM1-LM1 group. Paired-pulse MEPs showed a reduction in inhibition, with the RM1-LM1 group transitioning to facilitation during the experimental block. These findings suggest that the effects of ccPAS develop over time, particularly in the RM1-LM1 direction, where enhancements in corticospinal excitability were more substantial.

Effect of Time on Interhemispheric Inhibition (IHI)

The data in Table 8 presents a comprehensive view of the mean IHI values from single and paired-pulse TMS measurements taken from the baseline and experimental blocks. The temporal dynamics of interhemispheric inhibition (IHI) were analysed across the first and last 20 trials of baseline and experimental blocks, revealing direction-specific effects.

For the LM1-RM1 group, mean IHI values increased from 0.6425 in the first 20 trials to 0.7935 in the last 20 trials during the baseline block, indicating a reduction in inhibitory IHI over time. In the experimental block, IHI values decreased from 0.721 to 0.662, reflecting enhanced inhibition. For the RM1-LM1 group, baseline IHI values were relatively stable, moving from 0.6895 to 0.685. In the experimental block, IHI values increased from 0.7645 to 0.773, suggesting a reduction in inhibition over time. The control group exhibited minimal changes across all conditions.

Table 8

Mean Interhemispheric Inhibition (IHI) Values by Block and Group

Block	**LM1-RM1**		**RM1-LM1**		**Control**	
	First 20	**Last 20**	**First 20**	**Last 20**	**First 20**	**Last 20**
Base	0.6425	0.7935	0.6895	0.685	0.745	0.7385
Expression	0.721	0.662	0.7645	0.773	0.6865	0.707

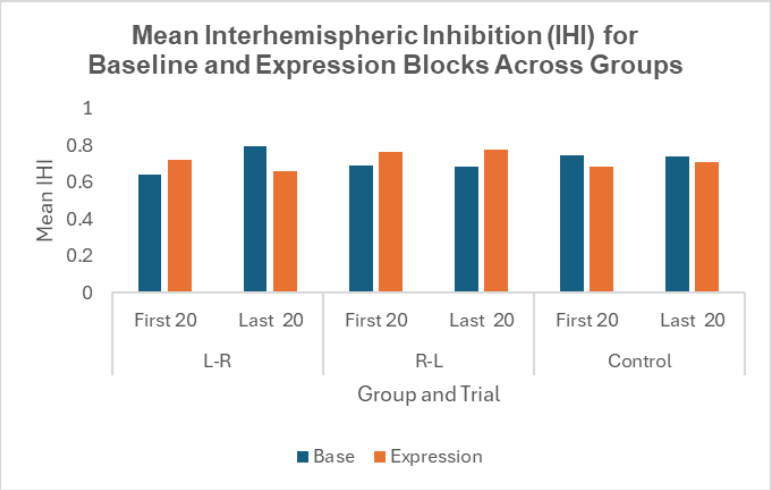
Note. This table presents the mean interhemispheric inhibition (IHI) values for both baseline and expression blocks across the different groups (LM1-RM1 ccPAS, RM1-LM1 ccPAS, Control) and the First 20 and Last 20 trials. The data indicates changes in IHI values over time for each of the blocks and groups, highlighting the impact of the M1-M1 ccPAS compared to the baseline.

The data presented in Table 8 illustrates the mean IHI values for baseline and expression blocks across the different ccPAS groups (LM1-RM1, RM1-LM1, and

Control). The First 20 and Last 20 trials are compared to ascertain the effect of time on IHI.

Figure 10

Mean Interhemispheric Inhibition (IHI) for Baseline and Expression Blocks Across Groups



Note. This figure depicts the mean interhemispheric inhibition (IHI) values for each block across the different groups, measured during the first 20 and last 20 trials. The data show that the LM1-RM1 group under the expression block experiences an increase in IHI over time, indicating a reduction in inhibition. Conversely, the RM1-LM1 group under the expression block shows a decrease in IHI, indicating an increase in inhibition. The Control group exhibited minimal changes, suggesting the effects observed in the expression block are likely due to M1-M1 ccPAS.

Key Observations

The temporal analysis of IHI highlighted distinct directional effects due to ccPAS. The LM1-RM1 group exhibited enhanced inhibition during the experimental block, while the RM1-LM1 group showed a reduction in inhibition over time. Minimal changes in the control group reinforce the specificity of ccPAS. These findings

underscore the importance of stimulation direction in shaping temporal changes in interhemispheric inhibition.

Young Adults Hypothesis Testing and Statistical Analysis

Hypothesis 1: M1-M1 ccPAS Regulates Corticospinal Excitability in the Motor Cortex as measured by MEP

To examine their effects on MEP, a repeated-measures ANOVA using the within-subjects factors of Block (Base vs Expression) and TMS Pulse (Single-pulse v Paired-pulse) for each of the experimental groups was conducted (RM1-LM1 ccPAS, LM1-RM1 ccPAS, Control ccPAS).

In the LM1-RM1 ccPAS group, results indicated that TMS Pulse had a significant effect on MEP, ($F(1, 19) = 50.094$, $p < .001$, $\eta^2 = 0.725$), with a very large partial eta square effect size. Block and the interaction between Block and TMS Pulse did not show significant effects.

Analysis of RM1-LM1 ccPAS indicated that TMS Pulse had a significant effect on MEP, ($F(1, 19) = 33.348$, $p < .001$, $\eta^2 = 0.637$), with a very large partial eta square effect size. There was also a significant effect of Block, ($F(1, 19) = 11.953$, $p = .003$, $\eta^2 = 0.386$), with a large partial eta square effect size. Additionally, there was a significant interaction between Block and TMS Pulse, ($F(1, 19) = 7.068$, $p = .016$, $\eta^2 = 0.271$). These findings suggested that Block and TMS Pulse influence MEP amplitude when M1-M1 ccPAS is delivered RM1-LM1.

In the Control ccPAS group, results indicated that TMS Pulse once again significantly affected MEP, ($F(1, 19) = 50.094$, $p < .001$, $\eta^2 = 0.725$), with a very large partial eta squared and observed power. Block and the interaction between Block and TMS Pulse did not show significant effects.

In summary, these findings strongly support the alternative hypothesis (H_1) that M1-M1 ccPAS significantly modulates corticospinal excitability, particularly in the RM1-LM1 group. Significant effects of Block, TMS Pulse, and their interaction were observed in the RM1-LM1 ccPAS group, suggesting that ccPAS delivered in the right-to-left direction enhances excitability in a manner dependent on the type of TMS pulse used. Conversely, in the LM1-RM1 ccPAS and Control groups, the effects were driven solely by the type of TMS pulse, partially supporting the null hypothesis (H_0) in these groups. Overall, these results underscore the directional specificity of ccPAS, with the right-to-left direction being particularly effective.

Hypothesis 2: Directional Effect of M1-M1 ccPAS on Interhemispheric Inhibition (IHI)

A repeated-measures ANOVA was conducted to investigate the directional effect of M1-M1 ccPAS on interhemispheric inhibition (IHI). The within-subjects factor was Block (Baseline vs Experimental), and the between-subjects factor was Group (RM1-LM1 ccPAS, LM1-RM1 ccPAS, Control ccPAS). No significant main effect of Block or interaction was observed in the LM1-RM1 ccPAS or control groups. Results for the RM1-LM1 ccPAS group indicated a significant main effect of Block, ($F(1, 19) = 7.268$, $p = 0.014$, partial $\eta^2 = 0.277$). These results suggest that RM1-LM1 ccPAS creates an inhibitory effect on the ipsilateral M1, by decreasing the inhibitory effect of Right M1 over Left M1. The direction of ccPAS (specifically RM1-LM1) significantly affects the modulation of IHI, demonstrating a directional specificity in the effects of ccPAS on the human motor cortex.

In summary, the findings provide evidence supporting the alternative hypothesis (H_1) that the direction of M1-M1 ccPAS significantly affects the modulation of IHI. Specifically, the RM1-LM1 ccPAS group demonstrated a significant change in IHI,

indicating that the directionality of ccPAS produces distinct modulatory effects on the human motor cortex.

Conversely, the LM1-RM1 ccPAS and control groups did not show significant changes in IHI, suggesting that these conditions did not produce the same modulatory effects. This indicates a directional specificity in the effects of M1-M1 ccPAS, with the RM1-LM1 direction having a significant impact on interhemispheric inhibition.

Overall, the results suggest that the direction of M1-M1 ccPAS is crucial for modulating IHI, with the right-to-left direction being particularly effective, thus supporting the alternative hypothesis (H_1).

Hypothesis 3: The Effects of M1-M1 ccPAS Develop Over Time

Previous research has identified that the effect of ccPAS was not evident until at least 30 minutes post-stimulation (Rizzo et al., 2009; Rizzo et al., 2011). Given that the measurement block was 15 minutes long, I was able to investigate whether the effect of ccPAS emerged during the later stages of the Expression measurement protocol. To evaluate the effect of time, I recalculated single-pulse TMS, paired-pulse TMS, and IHI by averaging the first twenty and last twenty trials from both the Baseline and Expression blocks.

As with the previous analysis, a repeated-measures ANOVA was utilised, with two within-subject factors of Block (Baseline vs Expression) and TMS pulse (single-pulse TMS vs paired-pulse TMS), conducted for each experimental group. The first twenty trials for the MEP data for the LM1-RM1 and Control groups were analysed. As before, significant effects of Pulse were observed, ($F(1,19) = 40.123$, $p<0.001$, $\eta 2 = 0.679$) and ($F(1,19) = 35.586$, $p<0.001$, $\eta 2=0.652$) respectively. No significant main effects of Block or interaction were observed. For the first twenty trials of the RM1-LM1 group, there was a significant main effect of TMS Pulse ($F(1, 19) = 21.797$, $p < .001$, $\eta^2 = 0.534$) as well as a significant effect of Block ($F(1, 19) = 6.111$, $p = .023$, $\eta^2 = 0.243$).

The analysis of the first twenty trials of IHI for each of the groups revealed no significant results, though there was a near-significant (p = .064) increase in IHI in RM1-LM1 ccPAS group.

Analysis of the last twenty trials of the MEP data for the three groups revealed the following; LM1-RM1 ccPAS revealed a significant main effect of Pulse, (F(1,19) = 41.142, p < .001, η² = 0.684), with a large partial eta square effect size. No main effect for Block; however, there was a significant interaction between Block and Pulse (F(1,19) = 6.291, p = .021, η² = 0.249). A paired-sample t-test was run to explore this interaction further, determining if there were any statistically significant differences. They revealed no statistically significant difference between the baseline and experimental conditions for both the last 20 trials for single-pulse TMS, $t(19)=-1.47, p=.159$, and paired-pulse TMS, $t(19)=0.61, p=.547$. The effect sizes for the differences were small, with Cohen's d values of -0.328 (95% CI [-0.774, 0.127]) for single-pulse and 0.137 (95% CI [-0.305, 0.576]) for paired-pulse.

The Control group reported a significant main effect of TMS Pulse, ($F(1,19) = 34.440$, $p<.001$, η² = .644), with a large partial eta square effect size and no other effects. The RM1-LM1 ccPAS group revealed a significant main effect of TMS Pulse, (F(1, 19) = 33.212, p < .001, η² = .636), a significant main effect of Block, (F(1, 19) = 8.895, p = .008, η² = .319), and interaction, (F(1, 19) = 7.283, p = .014, η² = .277). The partial eta squared values indicated moderate to large effect sizes for the significant within-subjects effects. The observed power for the significant effects was high, indicating a high likelihood of detecting a true effect if it exists. A paired-sample t-test was run as a follow-up test. There was a significant difference in the scores for Base single-pulse TMS (M = 0.058, SD = 0.250) and Expression single-pulse TMS (M = 0.130, SD = 0.239); $t(19)=-2.30, p=.033, d=-0.513$. Similarly, there was a significant difference in the scores for Base paired-pulse TMS (M = -0.131, SD = 0.273) and

Expression paired-pulse TMS (M = 0.008, SD = 0.256); $t(19)=-3.24, p=.004, d=-0.725$. These results suggest that the experimental condition of RM1-LM1 ccPAS had a statistically significant effect on both single-pulse and paired-pulse MEP.

Analysis of the last twenty trials of the IHI data for the three groups revealed the following; Significant effect of Block, ($F(1,19)=7.219$, $p=.015$, $\eta 2=.275$) in the LM1-RM1 ccPAS group and no significant effects in the Control group. The RM1-LM1 ccPAS group revealed a significant effect of Block, ($F(1,19)= 5.095$, $p=.036$, *η2=.211).*

In summary, the results suggest that the effects of M1-M1 ccPAS develop over time, with significant temporal effects observed, particularly in the RM1-LM1 direction. These findings align with previous research highlighting the time-dependent nature of ccPAS-induced cortical modulation and support the alternative hypothesis (H_1).

Experiment 2: Effects of ccPAS in Older Adults

Data Treatment

Before conducting statistical analyses, the data were examined for deviations from normality using the Kolmogorov-Smirnov and Shapiro-Wilk tests. Baseline single-pulse and paired-pulse TMS conditions generally adhered to normality assumptions. However, the expression block data for both single-pulse and paired-pulse conditions, as well as the control group during the expression block, demonstrated significant deviations from normality.

To address these deviations, a Log10 transformation was applied to the data. This transformation effectively reduced skewness and kurtosis, ensuring that subsequent parametric analyses were appropriate. Values were adjusted to avoid negative numbers by adding a constant equal to one unit greater than the absolute value of the most negative number observed. This adjustment shifted all data points into a positive range suitable for logarithmic conversion. The descriptive statistics for skewness and kurtosis post-transformation indicated improved normality across all variables.

Participant Demographics

Seventeen participants were recruited for the older adult study (8 female; mean age = 70.10 ± 5.66 years, range = 63–83). Each participant took part in both experimental conditions: LM1-RM1 ccPAS and Control ccPAS. The LM1-RM1 group received ccPAS with an interstimulus interval (ISI) of 8 ms from the left motor cortex (M1) to the right M1. The control condition used a sham ccPAS with an ISI of 1 ms, ensuring no significant associative stimulation.

Descriptive Statistics

Descriptive statistics were calculated to provide insight into the distribution of motor-evoked potential (MEP) values under different conditions. These include mean,

skewness, and kurtosis for single-pulse and paired-pulse TMS across baseline and expression blocks.

For the baseline LM1-RM1 single-pulse condition, the mean MEP value was 1.161, with a skewness of 0.001, indicating a nearly symmetric distribution, and a kurtosis of -0.843, reflecting a flatter-than-normal distribution. In contrast, during the expression block, the LM1-RM1 single-pulse mean increased to 1.463, accompanied by a skewness of 1.257 and kurtosis of 1.908, suggesting a right-skewed distribution with heavier tails.

Similarly, for paired-pulse TMS, the baseline LM1-RM1 condition exhibited a mean MEP value of 0.873, with a slight right skew (Sk = 0.291) and flat kurtosis (Ku = -1.232). During the expression block, the mean increased to 1.160, while skewness (Sk = 2.235) and kurtosis (Ku = 6.663) values indicated a strongly right-skewed distribution with heavy tails.

The control group displayed smaller mean increases across single-pulse and paired-pulse TMS, with baseline and expression block values also demonstrating right-skewed distributions but less pronounced than the LM1-RM1 group.

Table 9 summarises these statistics, highlighting the distributional characteristics and changes between baseline and expression blocks.

Table 9

Mean, Skewness, and Kurtosis for Single- and Paired-Pulse TMS for Baseline and Expression Blocks

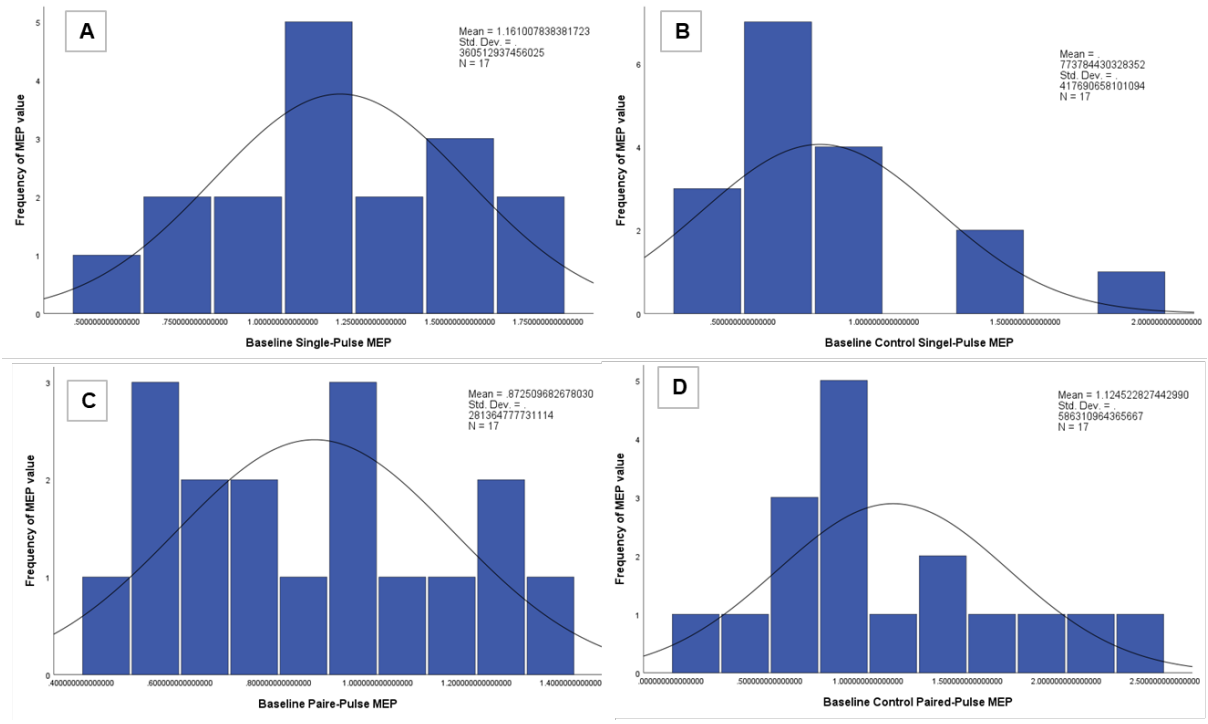
Block	LM1-RM1 Single Pulse	Control Single Pulse	LM1-RM1 Paired Pulse	Control Paired Pulse
Base	M = 1.161, Sk = 0.001, Ku = -0.843	M = 1.125, Sk = 0.704, Ku = -0.291	M = 0.873, Sk = 0.291, Ku = -1.232	M = 0.774, Sk = 1.31, Ku = 1.247
Expression	M = 1.463, Sk = 1.257, Ku = 1.908	M = 1.169, Sk = 1.272, Ku = 1.396	M = 1.160, Sk = 2.235, Ku = 6.663	M = 0.883, Sk = 2.127, Ku = 4.810

Note. Values represent the mean (M), skewness (Sk), and kurtosis (Ku) for each condition. These descriptive statistics assess the normality of MEP distributions and highlight notable changes across blocks.

Visualisations of the frequency distributions for baseline and expression block data further illustrate these patterns (Figures 11 and 12). The baseline data reveal near-normal distributions for LM1-RM1 paired-pulse responses, while the expression data indicate right-skewed distributions with heavier tails.

Figure 11

Frequency distributions of baseline block single-pulse and paired-pulse MEPs for LM1-RM1 and control ccPAS groups.

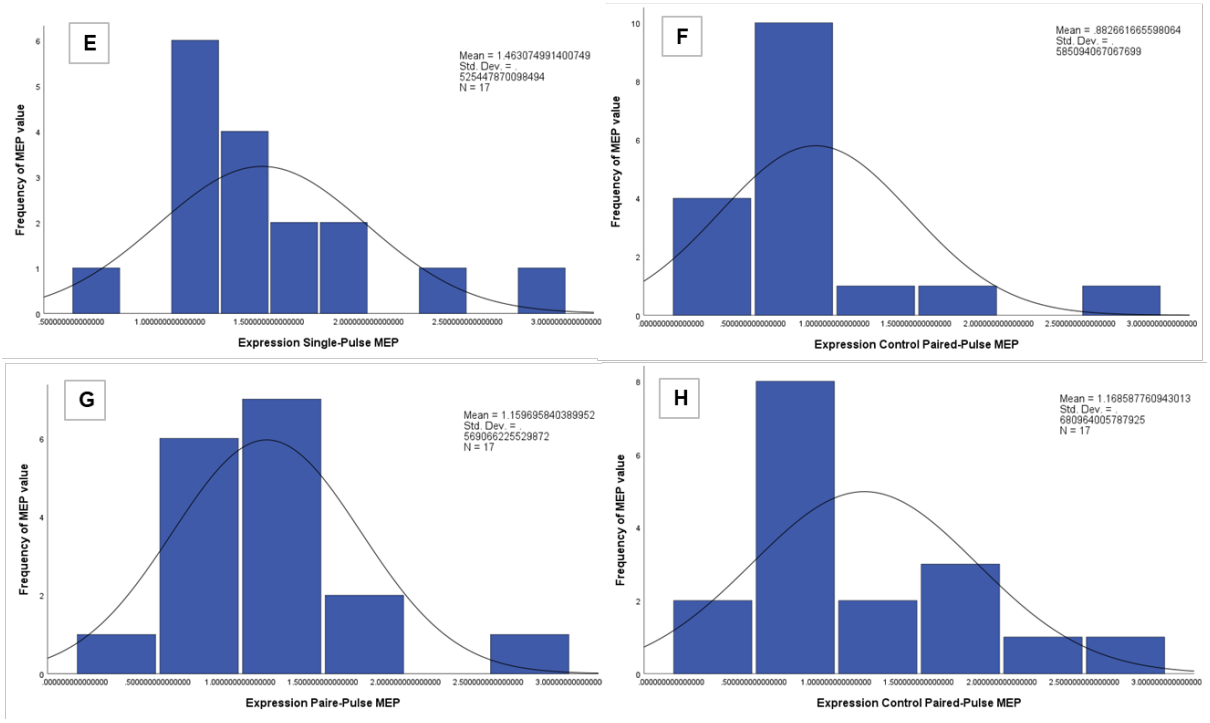


Note. Each graph displays the baseline MEP distribution for single-pulse and paired-pulse TMS for each of the groups. The LM1-RM1 group (Graphs A and C) and control group (Graphs B and D) show different distribution patterns. In Graph A, the LM1-RM1 single-pulse baseline MEP values are relatively symmetric with a slight right skew,

indicating most MEP values cluster around the mean of 1.161 (SD = 0.361), with a few higher values. Graph B shows that the baseline block control single-pulse TMS values have a right-skewed distribution, suggesting a higher frequency of lower MEP values and a tail extending towards higher values, with a mean of 0.774 (SD = 0.418). For the baseline paired-pulse TMS values, Graph C illustrates that the LM1-RM1 group has a symmetric distribution around the mean of 0.873 (SD = 0.281), fitting the normal curve well. Graph D shows the baseline control group's paired-pulse TMS values with greater variability and a moderate right skew, indicating a wider range of MEP values, and a mean of 1.125 (SD

Figure 12

Frequency distributions of expression block single-pulse and paired-pulse MEPs for LM1-RM1 and control ccPAS groups.



Note. Each graph displays the expression block MEP distribution for single-pulse and paired-pulse TMS for the LM1-RM1 and control groups. Graph E shows the LM1-RM1 group's single-pulse TMS values during the expression block, with a mean of 1.463 (SD = 0.525), displaying a right-skewed distribution. Graph F illustrates the control group's

paired-pulse TMS values, with a mean of 0.883 (SD = 0.585), also showing a right-skewed distribution. Graph G presents the LM1-RM1 group's paired-pulse TMS values, with a mean of 1.160 (SD = 0.569), and a right-skewed distribution. Graph H shows the control group's paired-pulse TMS values, with a mean of 1.169 (SD = 0.681), which are similarly right-skewed. These graphs indicate that both groups exhibit higher mean MEP values during the expression block, with the LM1-RM1 group having slightly higher means than the control group. The distributions for both groups are right-skewed.

Motor-Evoked Potentials (MEPs)

The analysis of motor-evoked potentials (MEPs) highlights changes in corticospinal excitability in older adults. Descriptive statistics revealed notable shifts in distribution characteristics between baseline and expression blocks. The LM1-RM1 group consistently demonstrated higher mean MEP values than the control group, particularly during the expression block, reflecting enhanced excitability.

For single-pulse TMS, the LM1-RM1 group's baseline data showed a mean MEP value of 1.161, with a nearly symmetric distribution (Sk = 0.001) and flat kurtosis (Ku = -0.843). During the expression block, the mean increased to 1.463, with a skewness of 1.257 and kurtosis of 1.908, indicating a right-skewed distribution with heavier tails, suggesting increased variability in MEP responses.

For paired-pulse TMS, the LM1-RM1 group exhibited a baseline mean of 0.873, with a slight right skew (Sk = 0.291) and flat kurtosis (Ku = -1.232). In the expression block, the mean rose to 1.160, with pronounced skewness (Sk = 2.235) and kurtosis (Ku = 6.663), highlighting an increase in extreme values.

The control group showed smaller increases, with single-pulse MEP means rising from 1.125 to 1.169, and paired-pulse means increasing from 0.774 to 0.883.

These changes were accompanied by skewness and kurtosis values that also indicated right-skewed distributions but with less pronounced variability than the LM1-RM1 group.

Table 10 summarises these results, and Figure 13 provides a graphical depiction of the mean values for single- and paired-pulse TMS across conditions.

Table 10

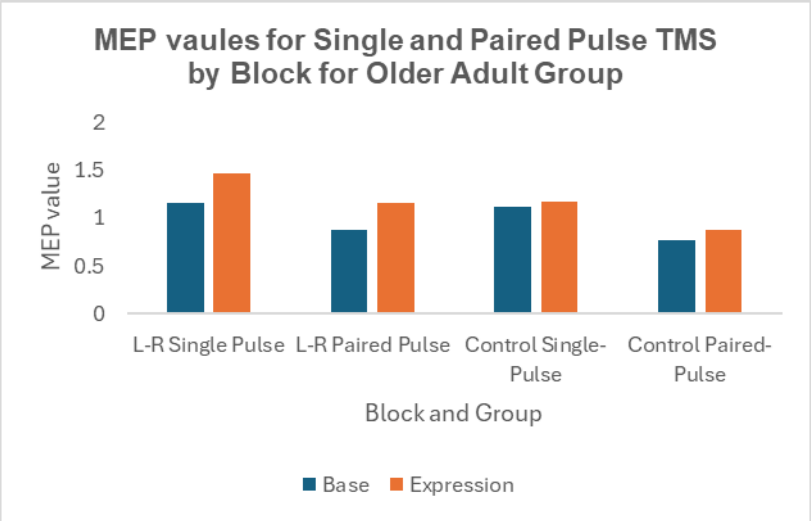
Motor-Evoked Potential Values for Single- and Paired-Pulse TMS by Block and Group for Older Adults

Block	LM1-RM1 Single Pulse	LM1-RM1 Paired Pulse	Control Single Pulse	Control Paired Pulse
Base	1.161	0.873	1.125	0.774
Expression	1.463	1.160	1.169	0.883

Note. MEP values indicate increased corticospinal excitability in the LM1-RM1 group compared to the control. During the expression block, the LM1-RM1 group exhibited greater increases in single- and paired-pulse MEP values compared to the control. These results suggest that LM1-RM1 ccPAS effectively enhances corticospinal excitability.

Figure 13

Mean single- and paired-pulse TMS responses for baseline and expression blocks across groups.



Note. This figure illustrates the mean MEP for older adults across the different blocks (Baseline and Expression) and pulse types (RM1-LM1 Single-Pulse, RM1-LM1 Paired-Pulse, and Control). The blue bars represent the baseline block (Base), while the orange bars represent the experimental block (Expression). The LM1-RM1 Single-Pulse shows a significant increase in mean MEP from baseline to expression, indicating a strong response to ccPAS. The LM1-RM1 Paired-Pulse also shows an improvement, with the mean MEP increasing in value in the expression block. The Control group shows minor changes with a slight increase in mean MEP from baseline to expression. These results suggest that M1-M1 ccPAS effectively enhances corticospinal excitability in older adults, particularly in single and paired-pulse conditions.

Key Observations

The LM1-RM1 group demonstrated significant increases in MEP values from baseline to expression, with single-pulse MEPs rising from 1.161 to 1.463 and paired-pulse MEPs increasing from 0.873 to 1.160. The control group showed smaller changes, with single-pulse MEPs increasing from 1.125 to 1.169 and paired-pulse MEPs rising from 0.774 to 0.883. These results highlight the efficacy of ccPAS in enhancing corticospinal excitability in older adults, particularly in the LM1-RM1 condition.

Interhemispheric Inhibition (IHI)

Table 11 and Figure 14 summarises the IHI results for baseline and expression blocks across the LM1-RM1 and control groups.

Table 11

Mean IHI Values for Baseline and Expression Blocks in Older Adults

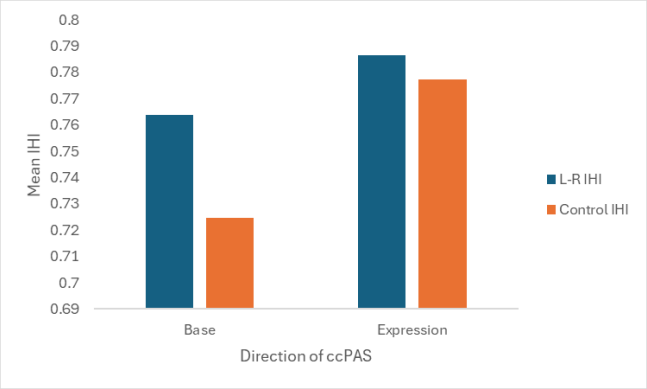
Block	LM1-RM1	Control
Base	0.7639	0.7247
Expression	0.7864	0.7775

Note. IHI values show slight reductions in inhibition during the expression block for both groups.

For the LM1-RM1 group, IHI values increased slightly from 0.7639 to 0.7864, suggesting a reduction in inhibition. In the control group, IHI values rose from 0.7247 to 0.7775, indicating non-specific effects contributing to reduced inhibition.

Figure 14

Mean IHI values for baseline and expression blocks across groups.



Key Observations

LM1-RM1 ccPAS led to a modest reduction in inhibition, as evidenced by increased IHI values during the expression block. Similar changes were observed in the control group, albeit with less pronounced effects. These findings suggest that ccPAS influences interhemispheric inhibition, although non-specific factors may also contribute to the observed changes

Temporal Effects

Motor-Evoked Potentials (MEPs) Results

To evaluate the temporal effects of ccPAS, Table 12 summarises MEP values across the first and last 20 trials for baseline and expression blocks.

Table 12

MEP Mean Values for First and Last Twenty Trials by Experimental Block

Group	LM1-RM1 Single Pulse	Control Single Pulse	LM1-RM1 Paired Pulse	Control Paired Pulse
First 20	0.0025	-0.0338	-0.1103	-0.2014
Last 20	0.0560	0.0052	-0.0756	-0.1586
Exp (First 20)	0.1125	-0.0199	-0.0246	-0.1718
Exp (Last 20)	0.1452	0.0078	0.0068	-0.0932

Note. Temporal changes in MEP values highlight enhanced excitability in LM1-RM1 conditions over time.

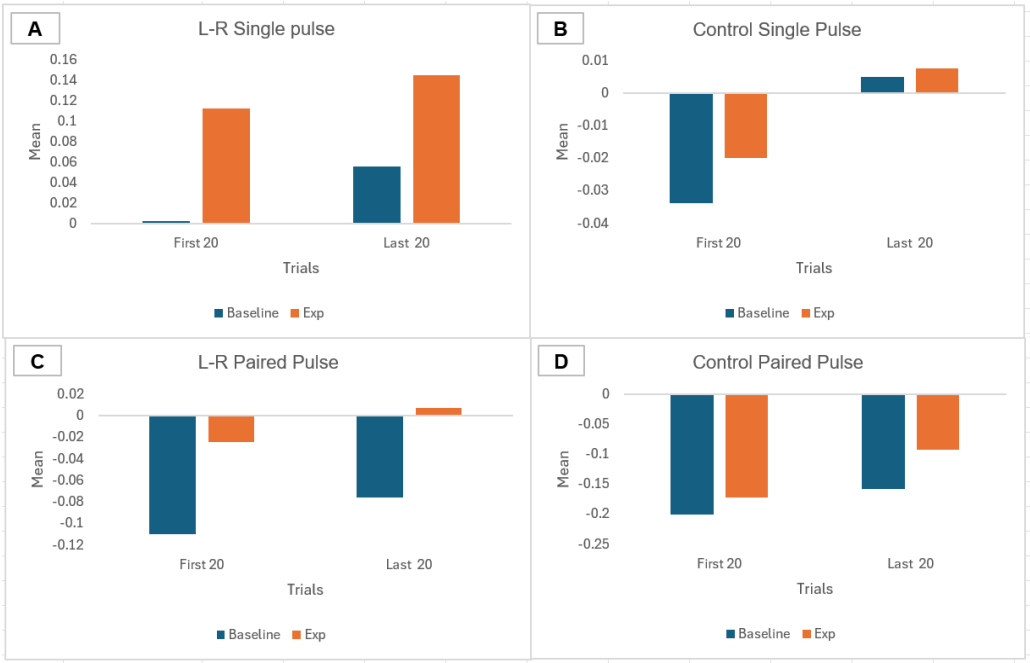
Temporal analysis revealed increasing MEP amplitudes for LM1-RM1 conditions, with single-pulse MEPs rising from 0.1125 to 0.1452 and paired-pulse MEPs shifting from -0.0246 to 0.0068 during the expression block. Control groups exhibited smaller changes, indicating non-specific effects.

Key Observations

LM1-RM1 ccPAS elicited sustained increases in corticospinal excitability over time, particularly in the last 20 trials of the expression block. Control group changes were less consistent, supporting the specific efficacy of ccPAS in modulating cortical excitability.

Figure 15

Mean MEP values for first and last 20 trials across blocks.



Overall, the data indicate that the experimental block consistently outperforms the baseline across all categories and pulse types. LM1-RM1 ccPAS appears to enhance IHI and reduce inhibitory responses more effectively than the baseline, suggesting its potential efficacy in modulating corticospinal excitability. The effects of ccPAS are more pronounced with LM1-RM1 single-pulse and LM1-RM1 paired-pulse TMS, highlighting the intervention's specific impact in these areas. Statistical analysis is necessary to ascertain whether any of these identified changes are significant.

Effects of Time on IHI

Temporal dynamics for IHI were assessed across first and last 20 trials. Table 13 summarises the results and Figure 16 provides a visual illustration.

Table 13

Mean IHI Values for First and Last 20 Trials Across Groups

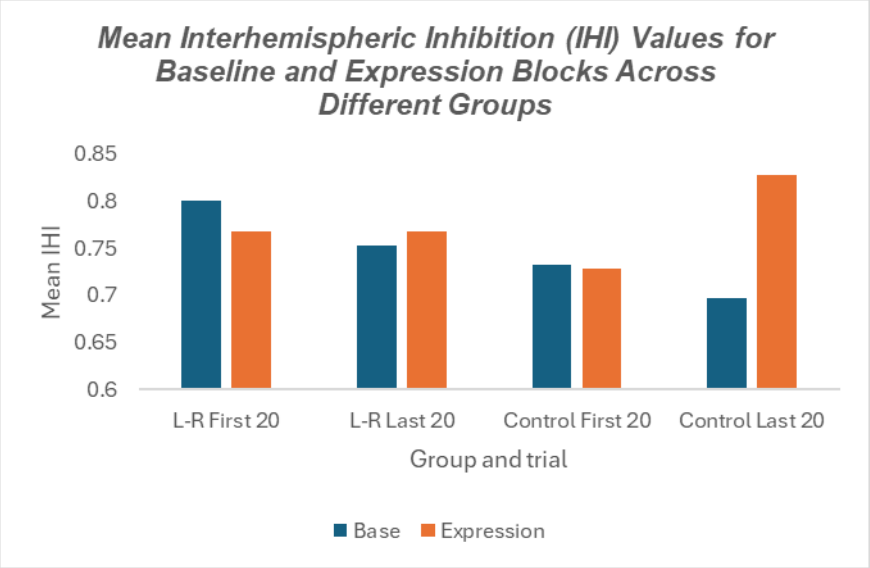
Block	LM1-RM1 First 20	LM1-RM1 Last 20	Control First 20	Control Last 20
Base	0.8014	0.7529	0.7327	0.6967
Expression	0.7677	0.7682	0.7287	0.8280

Note. Temporal IHI changes reflect stability in LM1-RM1 conditions and variability in control conditions.

The LM1-RM1 group exhibited stable IHI values during the expression block, with minimal changes between the first and last 20 trials. The control group, however, showed an increase in IHI values over time, suggesting potential non-specific effects.

Figure 16

Mean IHI values for first and last 20 trials across blocks.



Overall, the data show that LM1-RM1 ccPAS maintains stable IHI, while the control group exhibits a reduction in IHI during the expression block. Statistical analysis is required to ascertain whether any of these observed effects are significant.

Key Observations

LM1-RM1 ccPAS maintained consistent IHI effects during the expression block, with minimal changes over time. The control group exhibited greater variability, likely driven by non-specific factors rather than targeted ccPAS effects.

Older Adults Hypothesis Testing and Statistical Analysis

Hypothesis 4: M1-M1 ccPAS Regulates Corticospinal Excitability in the Older Adult Motor Cortex as measured by MEP

A repeated measures ANOVA was conducted to examine the effects of TMS Pulse and Block on MEP across two groups (ccPAS and Control). The within-subjects factors were TMS Pulse (Single-pulse vs Paired-Pulse), Block (Baseline vs Expression), and Group (ccPAS and Control). A significant main effect of TMS Pulse $F(1, 16) = 43.299$, $p < .001$, $\eta^2 = .730$, reflecting a very strong effect, and a significant main effect of Block, ($F(1, 16) = 9.137$, $p = .008$, $\eta^2 = .363$, indicating a substantial effect of block (baseline vs. expression) on MEP values were found. There was no significant main effect of Group. Additionally, there were no significant interactions between Group and Pulse, Group and Block, Pulse and Block, or Group, Pulse, and Block. The Group and Block interaction approached significance, $F(1, 16) = 2.810$, $p = .113$, $\eta^2 = .149$, suggesting a potential, but not statistically significant combined effect of Group and Block on MEP values.

These findings suggest that LM1-RM1 M1-M1 ccPAS and the type of TMS pulse independently regulate corticospinal excitability in the motor cortex. The lack of significant interaction effects indicates that these factors do not influence each other's impact on MEP amplitude. Overall, the results support the alternative hypothesis (H_1) that LM1-RM1 M1-M1 ccPAS significantly affects motor cortex excitability, as measured by MEP.

Hypothesis 5: Effect of M1-M1 ccPAS on Interhemispheric Inhibition (IHI)

A repeated measures ANOVA was conducted to examine the effects of Block on IHI across two groups (ccPAS and Control). The within-subjects factors were Block (Baseline vs Expression), and Group (ccPAS and Control). The results indicated that there were no significant main effects of Block on IHI, nor were there significant interaction effects between Block and Group. This suggests that the changes in IHI from baseline to experimental conditions were not significantly different between the ccPAS and Control groups. The lack of significant differences implies that LM1-RM1 M1-M1 ccPAS did not differentially affect IHI.

In summary, these findings fail to support the hypothesis that LM1-RM1 M1-M1 ccPAS significantly modulates interhemispheric inhibition in the motor cortex of older adults.

Hypothesis 6: The Effects of M1-M1 ccPAS Develops Over Time in the Older Adult Motor Cortex

A repeated measures ANOVA was conducted to evaluate the effect of Block, TMS Pulse and Group on cortical excitability for the first twenty trials. There was a significant main effect of Group, $F(1,16) = 33.356, p<.001, \eta p2=.676$, suggesting that there are substantial differences between the groups. The partial eta squared value of .676 implies a large effect size, indicating that a significant proportion of the variance in the dependent variable can be attributed to group differences.

Additionally, the Pulse variable also showed a noteworthy effect, though it was not statistically significant at the .05 level, $F(1,16) = 3.858, p =.067, \eta p2=.194$. This result suggests a trend towards significance, with a medium effect size, implying that the pulse condition might have a meaningful impact on the dependent variable. The interaction between Block and Pulse did not yield a significant effect. Similarly, the interaction between Block and Group was also not significant.

The repeated measures ANOVA was conducted a second time to evaluate the effect of Block, Pulse and Group on cortical excitability for the last twenty trials. There was a significant main effect of Block, $F(1, 16) = 8.431$, $p = .010$, $\eta^2 = .345$, indicating that the mean differences across experimental blocks were statistically significant. TMS Pulse also shows a significant main effect, $F(1, 16) = 49.435$, $p < .001$, $\eta^2 = .755$, suggesting substantial differences across different pulse conditions. The Group (LM1-RM1 ccPAS vs Control) similarly yields a significant main effect, $F(1, 16) = 15.037$, $p = .001$, $\eta^2 = .484$, demonstrating notable differences between experimental groups. The Block by Pulse interaction was significant, $F(1, 16) = 13.675$, $p = .002$, $\eta^2 = .461$, indicating that the effect of Block varies depending on the Pulse condition. The Block by Group interaction was highly significant, $F(1, 16) = 96.123$, $p < .001$, $\eta^2 = .857$, as was the Pulse by Group interaction, $F(1, 16) = 128.973$, $p < .001$, $\eta^2 = .890$, showing that the effects of these factors are interdependent. Moreover, the three-way interaction of Block, Pulse, and Group was significant, $F(1, 16) = 80.470$, $p < .001$, $\eta^2 = .834$, suggesting that the combined effect of these factors significantly influences the outcome.

These results provide strong evidence that LM1-RM1 M1-M1 ccPAS leads to significant changes in cortical excitability over time, supporting the alternative hypothesis (H_1).

Hypothesis 7: Age-Dependent Modulation by LM1-RM1 M1-M1 ccPAS

Two repeated measures ANOVA were conducted to evaluate the age-dependent modulation of cortical excitability by M1-M1 ccPAS. From the previous analyses, data from the later trials was more likely to produce an accurate result, so the MEP and IHI data from the last twenty trials for both groups were compared. Also, again, based on the previous finding, only LM1-RM1 ccPAS data was compared.

The MEP data was analysed. The within-subjects factors were Block (Baseline vs Expression) and TMS Pulse (Single-pulse vs Paired-pulse), and the between-

subjects factor was Age Group (Young vs Old). The analysis revealed a significant main effect of Block on MEP, $F(1, 35) = 13.771$, $p < .001$, $\eta^2 = .282$, and a significant main effect of Pulse on MEP, $F(1, 35) = 62.972$, $p < .001$, $\eta^2 = .643$. However, there was no significant interaction between Block and Age Group, nor between Pulse and Age Group. The three-way interaction between Block, Pulse, and Age Group was marginally non-significant, $F(1, 35) = 3.002$, $p = .092$, $\eta^2 = .079$. The main effect of Age Group was also not significant.

The IHI data was analysed. The within-subjects factor was Block (Baseline vs Expression), and the between-subjects factor was Age Group (Young vs Old). The analysis revealed no significant main effect of Block on interhemispheric inhibition (IHI). Additionally, there was no significant interaction effect between Block and Age Group. The main effect of Age Group was also not.

These findings suggest that M1-M1 ccPAS does not modulate cortical excitability differently between young and older adults, failing to reject the null hypothesis (H_0) for age-dependent effects.

Summary of Results

The study consisted of two experiments designed to explore the modulation of cortical excitability and interhemispheric inhibition (IHI) through M1-M1 ccPAS in both young and older adults.

Experiment 1: Young Adults

Sixty young adults participated in this experiment, divided into three groups: LM1-RM1 ccPAS, RM1-LM1 ccPAS, and a control group. Log10 transformations were applied to address deviations from normality in the data, as indicated by significant skewness and kurtosis.

Corticospinal Excitability (MEP)

Analysis of MEP data supported the hypothesis that M1-M1 ccPAS regulates corticospinal excitability. Significant effects of TMS Pulse on MEP values were observed across all groups. Notably, in the RM1-LM1 ccPAS group, significant main effects of Block (Baseline vs Expression) and an interaction between Block and Pulse were found, suggesting that both the type of TMS pulse and the experimental block influenced cortical excitability. In contrast, the LM1-RM1 ccPAS and control groups showed significant effects of Pulse but not Block or their interaction, indicating that changes in excitability were not attributable to ccPAS in these groups.

Interhemispheric Inhibition (IHI)

A significant main effect of Block was observed in the RM1-LM1 ccPAS group, indicating that this direction of ccPAS decreases ipsilateral inhibition. Neither the LM1-RM1 ccPAS nor the control group exhibited significant effects on IHI. These findings suggest that the modulation of IHI is directionally specific, with RM1-LM1 ccPAS having a notable impact.

Temporal Effects

To evaluate the development of ccPAS effects over time, data from the first and last 20 trials of each block were analysed. Significant effects of Block and Pulse, as well as their interaction, were observed in the RM1-LM1 ccPAS group during the last 20 trials, demonstrating temporal dynamics in cortical excitability. In contrast, the LM1-RM1 ccPAS and control groups showed significant effects of Pulse but no effects of Block or interactions. This indicates that the temporal development of ccPAS effects is specific to the RM1-LM1 condition.

Experiment 2: Older Adults

Seventeen older adults, with a mean age of 70.10 years, participated in both LM1-RM1 ccPAS and control conditions. Log10 transformations were again applied to correct for deviations from normality.

Corticospinal Excitability (MEP)

Significant main effects of TMS Pulse and Block were observed, indicating that ccPAS and the type of TMS pulse independently influence cortical excitability in older adults. However, there were no significant interactions between these factors, suggesting that ccPAS effects on excitability are consistent regardless of pulse type or block.

Interhemispheric Inhibition (IHI)

No significant main effects or interactions were found for IHI in older adults. These results suggest that M1-M1 ccPAS does not significantly modulate IHI in this age group.

Temporal Effects

Temporal analyses revealed significant main effects of Group, Block, and Pulse during the last 20 trials, along with significant interactions between Block and Pulse, Block and Group, and Pulse and Group. A significant three-way interaction among Block, Pulse, and Group highlighted the complexity of temporal changes in cortical excitability induced by LM1-RM1 ccPAS. The findings suggest that temporal effects are more pronounced in older adults and vary based on block, pulse type, and experimental group.

Overall Summary of Results

The findings demonstrate that M1-M1 ccPAS modulates corticospinal excitability and interhemispheric inhibition, with effects varying by age group and stimulation direction. In young adults, RM1-LM1 ccPAS significantly influences both cortical

excitability and IHI, with effects developing over time. Conversely, LM1-RM1 ccPAS in older adults affects cortical excitability but does not significantly modulate IHI. Temporal effects were observed in both age groups, with significant interactions highlighting the time-dependent nature of ccPAS-induced changes. Importantly, no significant age-dependent differences were found, suggesting that ccPAS effects are consistent across different age groups.

Chapter Summary

This chapter explored the effects of cortico-cortical paired associative stimulation (ccPAS) on motor function, specifically examining corticospinal excitability and interhemispheric inhibition (IHI) in young and older adults.

In Experiment 1, the RM1-LM1 ccPAS group exhibited significant changes in MEP amplitude and IHI, providing evidence of direction-specific modulation of cortical excitability and inhibition. Temporal analyses revealed that these effects develop over time, particularly in the RM1-LM1 condition.

In Experiment 2, LM1-RM1 ccPAS significantly influenced cortical excitability in older adults, with notable temporal dynamics observed in the last 20 trials. However, there were no significant effects of ccPAS on IHI in this age group. Comparisons between young and older adults revealed no significant age-dependent differences in ccPAS effects, suggesting consistent outcomes across age groups.

Collectively, the findings highlight the efficacy of M1-M1 ccPAS in modulating motor cortical activity, with direction-specific and temporal dynamics playing a crucial role. The results provide valuable insights into the neural mechanisms underlying ccPAS and its potential application across different age groups.

Chapter 5: Discussion

Chapter Overview

This chapter examines the findings from two experiments investigating the modulatory effects of cortico-cortical paired associative stimulation (ccPAS) on corticospinal excitability and interhemispheric inhibition (IHI) within the motor cortex in both young and older adults. Utilising MEP data, the study contrasts changes following the M1-M1 ccPAS intervention against a control group to explore if the effects were specific to ccPAS.

By placing these results within the context of a systematic review of 44 studies, I explore how these findings contribute to our understanding of directionality, age-related plasticity, and the temporal dynamics of ccPAS-induced changes. I also highlight the significance of ccPAS in advancing neuroscientific understanding and clinical practice. The findings reinforce the potential efficacy of ccPAS in modulating cortico-cortical communication.

Revisiting the Research Aims

The overarching aim of this research was to explore the effects of ccPAS on corticospinal excitability and IHI in the motor cortex, with specific emphasis on how these effects are influenced by stimulation direction, temporal dynamics, and age. By comparing young and older adults, this study sought to elucidate differences in cortical plasticity across the lifespan. These objectives guided the experimental design and are revisited here considering the findings.

Systematic review

The systematic literature review identified 44 studies that adopted the ccPAS protocol to explore and modulate neural plasticity within the human brain. The seminal study by Rizzo et al. (2009) established the groundwork for further research into the intricacies of IHI, cortical communication and the broader effects of ccPAS on motor

function and other related neural pathways. Rizzo identified how both LM1-RM1 and RM1-LM1 ccPAS attenuated IHI. In their study, RM1-LM1 ccPAS reduced inhibition without increasing excitability, while LM1-RM1 ccPAS increased excitability in the conditioned hand. Subsequent studies confirmed the ability of ccPAS to influence IHI by targeting the hand regions in both the left and right M1 (Carson et al., 2021; Hernandez-Pavon, Schneider-Garces, et al., 2023; Koganemaru et al., 2009; Rizzo et al., 2011). These studies demonstrated that specific interstimulus intervals (ISIs) are crucial in modulating the inhibitory influence of one M1 hemisphere on the opposite hemisphere. In addition to the motor cortex, the ccPAS protocol has been adopted to explore motor-related areas (Arai et al., 2011; Buch et al., 2011; Fiori et al., 2018; Johnen et al., 2015; Lu et al., 2012; Sel et al., 2021).

My study, based on the protocol developed by Rizzo et al. (2009) further demonstrates that ccPAS is effective in modulating corticospinal excitability and IHI. Specifically, when delivered at a rate of 90 stimulus pairings, with an ISI of 8ms and a frequency of 0.1 Hz, it is possible to modulate the inhibitory effect of M1 on its contralateral counterpart. These findings add further evidence for the effectiveness of TMS to induce associative plasticity. My study also adds important aspects of uniqueness. I offer broader insights with a larger sample size and inclusion of older adults.

Corticospinal Excitability

Young Adults

The results provide strong evidence that ccPAS modulates corticospinal excitability in young adults, with significant direction-specific effects. Initial analysis of mean motor-evoked potential (MEP) values indicated that both LM1-RM1 and RM1-LM1 ccPAS enhanced corticospinal excitability, with the RM1-LM1 direction potentially being slightly more effective in reducing inhibition. The increase in excitability and

reduction in inhibition were more pronounced during the expression block, highlighting the temporal effects of ccPAS.

Statistical analysis confirmed that right-to-left ccPAS (RM1-LM1) significantly influenced excitability, as reflected in increased MEP amplitudes during the expression block. The type of TMS pulse and the experimental block led to notable changes in MEP, indicating that the RM1-LM1 direction of ccPAS plays a pivotal role in modulating cortical excitability. In contrast, left-to-right ccPAS (LM1-RM1) and the sham control condition showed no significant changes in MEP amplitudes, with significant effects limited to the type of TMS pulse.

These findings align with prior research (e.g., (Buch et al., 2011; Rizzo et al., 2009)), which demonstrated that directional specificity plays a critical role in ccPAS efficacy. RM1-LM1 ccPAS appears to leverage the asymmetry of interhemispheric pathways in right-handed individuals, where the right hemisphere exhibits more efficient inhibitory control over the left. This directional influence is particularly evident in young adults, whose neural plasticity is robust.

Older Adults

In older adults, ccPAS also modulated corticospinal excitability, albeit less pronounced than in young adults. LM1-RM1 ccPAS elicited significant but smaller increases in MEP amplitudes compared to the effects observed in young adults. Statistical analysis demonstrated that ccPAS and the type of TMS pulse independently affected motor cortex excitability, with significant main effects observed. However, there were no significant interactions, suggesting that these factors did not influence each other's effects on MEP amplitude.

These findings contribute to the understanding of age-related plasticity, suggesting that while the capacity for excitatory modulation persists, it is diminished by age-related neural changes. Such differences may be attributed to reduced synaptic

efficacy, altered neurotransmitter dynamics, or structural degeneration in corticospinal pathways ((Ketcham & Stelmach, 2004; Mattay et al., 2002; Park & Reuter-Lorenz, 2009; Perry et al., 2017; Seidler et al., 2010)).

The results from older adults support hypothesis 4, that M1-M1 ccPAS regulates corticospinal excitability in the motor cortex, albeit with reduced efficacy compared to younger adults.

Interhemispheric Inhibition (IHI)

Young Adults

The results reveal a clear directional effect of ccPAS on IHI in young adults. RM1-LM1 ccPAS significantly increased IHI, enhancing the inhibitory influence of the right motor cortex over the left. In contrast, LM1-RM1 ccPAS produced a slight reduction in IHI, suggesting a weakening of left-to-right inhibitory pathways. These observations align with prior findings from studies (Buch et al., 2011; Rizzo et al., 2009), which identified a unidirectional nature to ccPAS-induced changes in interhemispheric dynamics.

Older Adults

Unlike their younger counterparts, older adults did not exhibit significant changes in IHI following ccPAS. This outcome does not support the hypothesis that ccPAS modulates IHI in older individuals, suggesting that age-related changes in inhibitory networks may limit the efficacy of this intervention. The observed lack of effect could be attributed to reduced synaptic connectivity or compensatory mechanisms that emerge with age, such as the hemispheric asymmetry reduction in older adults (HAROLD) model proposed by Cabeza (2002).

Temporal Dynamics of ccPAS Effects

Temporal analyses revealed that ccPAS effects develop progressively, with more pronounced changes observed in the later stages of the expression block. This

finding underscores the importance of considering temporal dynamics when designing and interpreting ccPAS protocols.

Young Adults

In young adults, RM1-LM1 ccPAS exhibited significant temporal effects, with marked increases in excitability during the final 20 trials of the expression block. These results align with research by Rizzo et al. (2009) and Chiappini et al. (2020), which demonstrated that ccPAS-induced plasticity evolves over time. The observed delay in peak effects highlights the importance of capturing data across extended timeframes to fully understand the dynamics of ccPAS.

Older Adults

Older adults also demonstrated temporal dynamics, albeit with delayed and attenuated effects compared to young adults. Significant changes in excitability were observed in the latter stages of the expression block, suggesting that ccPAS effects take longer to manifest in ageing populations. These findings point to the need for extended stimulation or observation periods when investigating ccPAS in older adults.

Age-Dependent Effects of ccPAS

A direct comparison of young and older adults revealed notable differences in the magnitude and nature of ccPAS-induced effects. While both groups exhibited changes in corticospinal excitability, older adults demonstrated weaker responses and no significant modulation of IHI. These findings reflect age-related declines in plasticity, necessitating tailored approaches to neuromodulation.

One key consideration when exploring ccPAS in ageing populations is the role of altered hemispheric asymmetry and compensatory recruitment. The hemispheric asymmetry reduction in older adults (HAROLD) model (Cabeza, 2002) suggests that ageing individuals increasingly rely on both hemispheres to perform tasks that younger adults manage using one hemisphere. Older adults might exhibit increased bilateral

brain activation, which could serve as a compensatory mechanism to maintain performance levels (Mattay et al., 2002; Ward & Frackowiak, 2003). This shift may influence the efficacy of ccPAS, which is inherently dependent on precise and directional interhemispheric modulation. For instance, the reliance on bilateral recruitment could dilute the effectiveness of protocols targeting specific interhemispheric pathways, particularly if compensatory activation limits the distinct roles of dominant and non-dominant hemispheres.

The choice of stimulation targets in older adults becomes especially important considering these changes. The unidirectional effects of ccPAS—most evident in protocols like RM1-LM1, which enhance IHI by strengthening inhibitory control from the right to the left hemisphere—may not align with the increasingly bilateral neural recruitment observed in ageing populations (Mattay et al., 2002; Ward & Frackowiak, 2003). While this might initially seem to limit the utility of ccPAS, the observed asymmetry in interhemispheric inhibition (IHI) in both young and older adults suggest an entry point for intervention. Specifically, the targeting of more robust dominant-to-non-dominant pathways (e.g., LM1-RM1 in right-handed individuals) may offer a pragmatic approach to harnessing remaining neural plasticity in older adults.

Moreover, the understanding of hemispheric asymmetry and compensatory recruitment highlights the need for personalised stimulation protocols that account for the unique neural dynamics of each individual. For older adults, this could involve tailoring ccPAS to reinforce pathways that are less reliant on compensatory mechanisms, thereby supporting more efficient cortical communication. Exploring how ccPAS interacts with age-related bilateral recruitment could also reveal novel opportunities to adapt this technique for neurorehabilitation, potentially leveraging compensatory processes rather than attempting to override them.

In summary, while the unidirectional effects of ccPAS may appear to restrict its versatility, they simultaneously provide a focused framework for targeting specific neural pathways. The capacity to modulate these pathways—especially when informed by age-related shifts in neural recruitment—positions ccPAS as a tool with considerable potential in ageing research and intervention, provided it is applied with careful consideration of laterality and compensatory dynamics.

Integrating with the Systematic Review

Interstimulus Interval (ISI)

The systematic review emphasised the critical role of ISI in determining ccPAS efficacy. Consistent with Rizzo et al. (2009), this study employed an 8 ms ISI for ccPAS groups, successfully modulating excitability in young adults. The reduced efficacy observed in older adults suggests that age-specific ISIs may enhance outcomes. While my study did not explicitly test varying ISIs, the use of different intervals between the ccPAS and control groups highlighted the importance of timing in influencing plasticity. The 1 ms ISI used in the control group, by contrast, demonstrated minimal modulation, further underscoring the sensitivity of neural responses to ISI.

For clinical applications, especially in treatment settings, understanding the optimal ISI is essential to maximise therapeutic benefits while minimising adverse effects. Establishing robust, evidence-based protocols will be crucial for translating ccPAS research into effective interventions for motor rehabilitation, neurodegenerative conditions, and beyond. Future studies should prioritise experimental designs that systematically explore ISI effects, enabling the development of precise and generalisable protocols for diverse populations.

Directionality and Pathway Specificity

The review emphasised the importance of stimulation directionality, noting that the order of stimulation (e.g., PMv to M1) can enhance or weaken corticospinal

excitability and IHI. The results from the study provide strong evidence for directional specificity, particularly identifying that RM1-LM1 ccPAS significantly modulates both corticospinal excitability and IHI, while LM1-RM1 direction does not produce the same effects. This concurs with previous research (Arai et al., 2011; Bevilacqua et al., 2023; Borgomaneri et al., 2023; Buch et al., 2011; Casarotto, Dolfini, Cardellicchio, et al., 2023) demonstrating that ccPAS can effectively facilitate or inhibit connections based on the timing and direction of the stimulus.

The distinction between stimulating interhemispheric pathways (connecting two hemispheres) versus intrahemispheric pathways (within the same hemisphere) further illustrates the specificity of ccPAS effects. This study focused on interhemispheric inhibition, finding that LM1-RM1 ccPAS reduced the inhibitory effect of left M1 on right M1, while RM1-LM1 ccPAS demonstrated increased IHI, enhancing the inhibitory effect of right M1 on left M1. These findings underscore the importance of aligning ccPAS protocols with the unique directional flow of cortical communication to optimise outcomes.

Practical and Clinical Implications

The demonstrated efficacy of ccPAS in modulating corticospinal excitability and IHI highlights its potential as a therapeutic tool for conditions such as stroke rehabilitation, Parkinson's disease, and age-related motor decline. However, several factors identified in this study and the broader literature highlight the complexities in translating ccPAS into practical and effective interventions.

State-Dependency

State-dependency significantly influences ccPAS outcomes, as shown in previous studies (Arai et al., 2011; Buch et al., 2011). For example, paired PMv-M1 stimulation increased PMv's inhibitory influence over M1 at rest, while facilitating M1 excitability during a visuomotor task. These findings indicate that the cognitive or motor

state at the time of ccPAS delivery critically shapes the outcome of induced plasticity. The systematic review and findings from this study support the use of resting-state ccPAS for standardised exploration of intrinsic plasticity mechanisms, offering reproducibility and comparability across studies. However, the role of active-state interventions, which may evoke dynamic and task-specific changes, remains underexplored.

The resting-state approach used in this study enhances the understanding of baseline interhemispheric inhibition and corticospinal excitability, laying the groundwork for future task-specific investigations. For clinical applications, integrating state-dependent stimulation with behavioural or motor tasks could amplify the functional benefits of ccPAS. This approach is particularly valuable in rehabilitative contexts, where task engagement might enhance the magnitude and specificity of plastic changes.

Skill Set and Practitioner Requirements

The implementation of ccPAS in clinical settings will require a multidisciplinary approach, combining expertise in neurophysiology, clinical psychology, and rehabilitative therapies. Practitioners must possess a strong foundation in brain stimulation techniques, such as TMS, to ensure accurate and safe delivery of ccPAS protocols. An in-depth understanding of neuroanatomy and neuroplasticity will be essential for tailoring interventions to individual patients and conditions.

Specialist technicians will need the skills to operate equipment, manage stimulation parameters, and monitor real-time responses. Neurologists and physiotherapists will provide diagnostic insights and rehabilitation strategies that align with the neurophysiological goals of ccPAS. Clinical psychologists, in particular, will play a pivotal role by addressing the psychological and behavioural dimensions of ccPAS interventions. Their expertise will ensure that treatments are holistic, accounting

for cognitive states, emotional well-being, and patient motivation. They can also support psychoeducation, participant engagement, and ethical oversight, enhancing the overall patient experience.

Current Challenges and Path to Clinical Integration

Despite its promise, ccPAS is not yet ready for widespread clinical use. Several hurdles must be addressed to establish its credibility as a therapeutic intervention. These include the validation of protocols through large-scale trials, the standardisation of stimulation parameters, and the exploration of long-term effects. Questions remain about the optimal interstimulus interval (ISI), stimulation intensity, and protocol duration for various conditions. Addressing these gaps is essential to develop evidence-based guidelines for clinical applications.

For clinical applications, especially in treatment settings, understanding the optimal ISI is crucial to maximise therapeutic benefits while minimising adverse effects. Establishing robust, evidence-based protocols will be instrumental in translating ccPAS research into effective interventions for motor rehabilitation, neurodegenerative conditions, and beyond. Future studies should prioritise experimental designs that systematically explore ISI effects, enabling the development of precise and generalisable protocols for diverse populations.

The temporal dynamics observed in this study suggest that repeated or prolonged stimulation protocols could enhance the therapeutic potential of ccPAS. Capturing both immediate and sustained effects is critical for optimising clinical outcomes. As protocols become more refined, integrating state-dependent stimulation with behavioural or cognitive tasks and implementing multi-session approaches could extend ccPAS's clinical utility further. This would enable clinicians to harness the temporal and directional dynamics of ccPAS for tailored, patient-specific therapies.

Moreover, addressing challenges such as patient fatigue, especially in older populations, will be key in refining ccPAS protocols. Balancing efficacy with patient comfort and ensuring accessible equipment and training for practitioners will also facilitate the transition of ccPAS from experimental use to practical clinical applications. Through collaboration across disciplines and a commitment to ongoing refinement, ccPAS has the potential to transform approaches to motor rehabilitation and neuroplasticity-related treatments.

Study Limitations

Several limitations must be acknowledged in this study, as they may impact the interpretation and generalisability of the findings. The smaller sample size for older adults may restrict the applicability of results to the broader ageing population. Recruiting a larger and more diverse cohort would strengthen future research and provide a more nuanced understanding of age-related plasticity. Additionally, time constraints during testing sessions limited the range of data collected, particularly behavioural measures, which could have offered valuable insights into the functional significance of neurophysiological changes. This absence highlights an important area for future exploration, bridging the gap between observed plasticity and real-world outcomes.

The study exclusively recruited right-handed participants, which limited the exploration of lateralisation effects in left-handed individuals. Hemispheric asymmetry and interhemispheric communication can differ based on handedness, suggesting that future research should incorporate participants with diverse lateralisation profiles to better understand the broader implications of ccPAS.

Furthermore, the study was conducted in a resting-state context, which, while providing controlled and reproducible conditions, does not capture the state-dependent effects that may emerge during active cognitive or motor tasks. Investigating how

ccPAS interacts with task-related neural activity could reveal dynamic, task-specific plastic changes that were not assessed in this study. Similarly, the focus on short-term effects without extended follow-up precluded a detailed analysis of the longevity and sustainability of ccPAS-induced plasticity. Longitudinal designs with multiple sessions and follow-up assessments would address this limitation, providing insights into the cumulative and lasting impacts of ccPAS interventions.

Finally, practical limitations, such as the time-intensive nature of ccPAS protocols, particularly for older participants, pose challenges for its clinical translation. Balancing the need for comprehensive data collection with participant comfort and fatigue is crucial for designing effective yet feasible protocols, especially for populations with varying levels of endurance.

Addressing these limitations in future work will enhance the comprehensiveness of ccPAS research, supporting its development as a robust tool for understanding and modulating cortical plasticity. By incorporating larger, more diverse samples, behavioural measures, active-state protocols, and extended follow-ups, future studies can build on the findings of this research to refine ccPAS applications and improve their clinical utility.

Future Research Directions

Temporal Dynamics and Longitudinal Effects

This study identified temporal dynamics in ccPAS effects, with changes in corticospinal excitability and IHI becoming more pronounced during the latter stages of the expression block. These findings suggest that ccPAS-induced plasticity evolves over time, highlighting the importance of capturing both immediate and delayed outcomes.

Research by Buch et al. (2011) reported rapid plasticity changes that persisted for up to three hours post-stimulation but began to reverse thereafter. Conversely,

Chiappini et al. (2020) found that ccPAS-induced effects were transient and dissipated within 40 minutes. These contrasting findings underscore the variability in the duration of ccPAS effects and their dependence on factors such as interstimulus intervals (ISIs), participant characteristics, and stimulation parameters. While this study focused on capturing immediate post-stimulation changes, the inclusion of extended follow-up periods would provide a more comprehensive understanding of the temporal evolution and sustainability of ccPAS-induced plasticity. Longitudinal studies incorporating repeated stimulation sessions could explore cumulative effects and provide insights into the potential for long-lasting neural and behavioural changes.

Behavioural Measures and Functional Relevance

The lack of behavioural measures in this study was a limitation necessitated by the extended duration of testing sessions, particularly for older participants. Including tasks such as reaction time assessments or motor performance tests in future studies would provide a direct link between ccPAS-induced changes in neural excitability and functional outcomes. This would bridge the gap between neurophysiological findings and their real-world applicability, offering critical insights into the practical value of ccPAS in rehabilitation and cognitive enhancement. Moreover, these measures could help determine the specificity of ccPAS effects for targeted behaviours, such as fine motor skills or executive functions.

State-Dependency and Task-Based Protocols

Another important avenue for future research is the exploration of state-dependency in ccPAS protocols. As highlighted in the systematic review and existing literature, the cognitive or motor state of participants during stimulation significantly influences ccPAS outcomes. Task engagement during ccPAS has been shown to evoke distinct plastic changes compared to resting-state protocols (Arai et al., 2011; Buch et al., 2011). Future studies should investigate how active-state stimulation interacts with ccPAS to modulate excitability and IHI in task-relevant contexts.

Combining ccPAS with behavioural or cognitive tasks could yield novel insights into its potential applications in motor learning, cognitive enhancement, and rehabilitation. Incorporating state-dependent designs may also help refine ccPAS protocols to maximise task-specific plasticity.

Intrinsic Brain Activity and Personalised Protocols

The dynamic nature of intrinsic brain activity, even during so-called "resting" conditions, must be considered. The notion that the brain is never truly at rest suggests that baseline neural activity and ongoing oscillatory dynamics may influence ccPAS efficacy. Future research could utilise advanced imaging techniques such as EEG or fMRI to explore how intrinsic brain activity shapes ccPAS-induced plasticity. These approaches could identify individual differences in baseline activity that might predict responsiveness to ccPAS, enabling the development of personalised protocols tailored to each participant’s neural state. Personalisation could optimise ccPAS outcomes, improving both efficacy and reproducibility in diverse populations.

Refinement of Stimulation Parameters

The study highlighted the need for optimising ccPAS stimulation parameters, particularly in older adults, where diminished plasticity necessitates tailored interventions. Adjusting ISIs, stimulation intensity, and protocol duration for different populations could improve efficacy. Moreover, systematic exploration of parameter variations across demographic groups would help establish evidence-based guidelines for clinical applications. Research should also explore multi-session protocols, as repeated interventions may offer cumulative benefits, especially for individuals with neurological or age-related impairments.

Integration with Neuroimaging and Neuromodulation Techniques

Integrating ccPAS with complementary techniques such as transcranial direct current stimulation (tDCS) or neurofeedback could provide a multifaceted approach to

enhancing cortical plasticity. Combining ccPAS with functional imaging during and after stimulation could offer real-time insights into its neural mechanisms, advancing both theoretical understanding and clinical applications. Exploring these synergies represents a promising direction for advancing ccPAS-based interventions.

By addressing these research priorities, future studies can advance the understanding and application of ccPAS, refining its protocols for both experimental and therapeutic contexts.

Conclusion

This study has addressed its primary aims, providing robust evidence of the modulatory effects of cortico-cortical paired associative stimulation (ccPAS) on corticospinal excitability and interhemispheric inhibition (IHI). The findings contribute significantly to our understanding of directionality, temporal dynamics, state-dependency, and age-related changes in cortical plasticity.

The first aim of exploring whether ccPAS modulates corticospinal excitability and IHI was comprehensively addressed. The results demonstrated that ccPAS induces significant changes in corticospinal excitability, particularly when delivered in the RM1-LM1 direction. This directionality was associated with enhanced excitability and increased IHI in young adults, contrasting with the more limited effects observed in the LM1-RM1 direction and the control condition. In older adults, ccPAS produced measurable changes in corticospinal excitability, affirming its potential in this demographic, albeit with diminished effects and no evidence of IHI modulation. These findings underline the ability of ccPAS to induce plastic changes and emphasise the role of age in shaping these outcomes.

The second aim of examining the influence of stimulation directionality was clearly supported by the results. RM1-LM1 ccPAS emerged as the most effective protocol for modulating excitability and IHI, reinforcing the critical role of direction-

specific stimulation in targeting interhemispheric pathways. The differential effects of RM1-LM1 and LM1-RM1 ccPAS also align with prior literature, which highlights the asymmetric nature of interhemispheric communication and the necessity of precision in protocol design.

In addressing the third aim—assessing age-dependent characteristics of ccPAS-induced modulation—this study provided key insights into how neural plasticity changes across the lifespan. While older adults retained some capacity for plastic changes, their responses were weaker and less consistent compared to younger participants. This attenuated plasticity, evident in the lack of IHI modulation, reflects the structural and functional changes associated with ageing. These findings further reinforce the importance of developing age-appropriate stimulation protocols to maximise the efficacy of ccPAS interventions in older populations.

The fourth aim of exploring the temporal development of ccPAS effects was addressed by analysing MEP data from the early and late stages of the expression block. The results revealed a clear temporal progression, with ccPAS effects becoming more pronounced over time, particularly in young adults. This dynamic was less pronounced in older adults, who exhibited delayed but observable changes. These temporal findings provide valuable evidence for the evolving nature of ccPAS-induced plasticity and highlight the importance of extended observation periods in capturing the full scope of these effects.

The fifth aim, investigating the role of state-dependency, was indirectly informed by the study's resting-state protocol and its comparison to findings in the systematic review. This study provided a standardised baseline for exploring intrinsic plasticity mechanisms, while the literature highlights the potential for task-based protocols to evoke distinct plastic changes. These insights open avenues for integrating behavioural

or cognitive tasks to amplify ccPAS's functional relevance, particularly in rehabilitative contexts.

While this study did not include behavioural measures due to practical constraints, it has laid a foundation for future research to bridge the gap between neurophysiological changes and functional outcomes. The insights gained into the mechanisms of ccPAS, particularly in the context of directionality, state-dependency, and age, offer a strong basis for developing clinically relevant applications in motor rehabilitation and cognitive enhancement. The findings also underscore the need for further work to refine stimulation parameters, such as ISI and intensity, to optimise ccPAS outcomes for diverse populations.

In conclusion, this study has demonstrated the efficacy of ccPAS in modulating cortical plasticity, with its effects shaped by stimulation direction, temporal dynamics, state-dependency, and age-related changes. These findings align closely with the systematic review, further validating ccPAS as a promising tool for investigating and modulating cortical communication. By addressing key gaps in the literature, this research provides a framework for optimising ccPAS protocols and translating them into effective interventions for diverse populations. Future work should prioritise exploring the long-term sustainability of ccPAS effects, integrating behavioural measures, and refining protocols to enhance applicability in both young and ageing populations. The path towards clinical integration will require robust validation studies, multidisciplinary collaboration, and tailored approaches that account for individual differences in neural responsiveness and functional needs.

Chapter 6 Reflection

My life experiences, from growing up in a working-class home in the 1980s and 1990s to achieving a more middle-class existence through education and employment, have shaped me into the person I am today. I have worked in the civil service, assisting vulnerable and disadvantaged people, as well as in healthcare and mental health services. I am now nearing the end of my training to become a clinical psychologist and have developed a keen interest in specialising in neuropsychology, working with individuals with neurological disorders. Currently, I work in neurorehabilitation services and will be transitioning to an older adult dementia service post-qualification. My interest in the ageing process spans personal, professional, and societal levels, as I believe it is crucial to understand how the changing needs of this population impact my role as a clinical psychologist.

My personal motivation for this research is deeply rooted in my passion for understanding how our brains work and why people behave as they do. My love for neuroscience and neuropsychology has been partly influenced by my family's experiences with neurological disorders. Growing up with an older brother who is autistic and has ADHD, I witnessed firsthand the significant social stigma attached to these conditions in the 1980s and 1990s. Autism and ADHD were not commonly spoken about, and families often faced blame and discrimination. I didn't know what autism was as a child; I just knew my brother behaved differently. People often stared at us, which made me curious about why he was different and why people could be so cruel. Tragically, my brother suffered a stroke following a traumatic brain injury in his late teens, leaving him with severe physical and cognitive challenges. Neurorehabilitation has been able to help him regain some abilities but has also been limited in what it can achieve. As my parents have aged, they have grown too old to

care for him, and he now resides in a care facility. I have taken over the role of advocate for him since my parents stepped aside.

My mother, who has vascular dementia, has endured a brain tumour and a mild stroke, significantly impacting her mobility and necessitating residential care. While neurorehabilitation has helped her regain some abilities, it is limited by what she can do herself and the support available from limited services. At times, it feels as though both my brother and mother have been abandoned by services due to their ages and perceived lack of economic contribution. This makes me wonder, as we live longer, how services will cope without more tools in their arsenal to tackle neurological damage and ageing.

My knowledge of neuroscience, neuropsychology, and clinical practice has enabled me to advocate effectively for both my brother and mother with the healthcare professionals responsible for their care. Additionally, my father now requires increasing support, particularly as his ability to act independently is gradually diminishing despite his current mental and physical capability. His motor skills are declining rapidly, and the loss of these will severely impact his independence and freedom.

My professional experience in neurorehabilitation and my forthcoming role in an older adult dementia service have also drawn me to this research study. These roles have provided me with practical insights into the challenges faced by individuals with neurological conditions, reinforcing my commitment to contribute to this field through research. I was particularly attracted to this research because it has the potential to improve or delay the signs and symptoms of ageing, giving us more time. I wonder if one day it will be possible to have a pacemaker for the brain to regulate connectivity or restore function. Transcranial Magnetic Stimulation protocols such as reported here with the ccPAS approach are safe, can be seen to be modulating neural activity, and

are possible ingredients in what would be a complex, not yet designed brain pacemaker. Research such as this study is a step, albeit a tiny one, in this direction.

Balancing my professional responsibilities with the demands of research has been a significant challenge. Managing the time required to produce this research alongside my clinical placements, attending lectures, and caring for my husband, son, mother, and brother has been extremely demanding. I admit to feeling stressed at times and needing to reach out for support. Additionally, the emotional toll of conducting research closely related to my family's experiences has been considerable.

While I have a master's degree in neuroscience and neuropsychology, I found the systematic review challenging, not only wading through the significant number of studies but also reminding myself of the technical terms and academic writing style; it felt like learning a new language. Writing up the methods was a challenging task since I was not directly involved in administering the TMS and ccPAS protocols. To overcome this, I relied on what I had learned from my systematic review, discussions with researchers, and guidance from my supervisor. Additionally, I watched YouTube presentations on the use of TMS and attended a training day to learn how to use the equipment. While I wish I had had more time to immerse myself in the administration of TMS, interact with participants, and attend professional meetings with my colleagues involved in the research, I utilised every available resource to ensure an accurate and comprehensive description of the methods.

Undertaking this research has provided me with valuable insights into the complexities of neuropsychological studies and the impact of neurological disorders. Professionally, I have gained a deeper understanding of the methodologies and challenges involved in neuropsychological research. Personally, the process has been cathartic, allowing me to channel my family's experiences into meaningful academic and clinical contributions.

This research has reinforced my resolve to specialise in clinical neuropsychology, particularly focusing on interventions for individuals with neurological disorders. It has equipped me with the knowledge and skills necessary to contribute effectively to neurorehabilitation and dementia care, aligning with my career aspirations.

Reflecting on my research journey, I am grateful for the opportunity to merge my personal experiences with my professional aspirations. This process has been enlightening and challenging, yet deeply rewarding. As I move forward in my career, I am committed to leveraging these insights to make a meaningful impact in the field of clinical psychology and neuropsychology.

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Appendix A

Ethics ETH2223-0175:

Ethics application

Project overview

Title of project

Direct measurements of the brain mechanisms of motor control in ageing

Proposed start date of research

23 Dec 2022

Expected end date

23 Dec 2027

Will this project be externally funded?

No

Will the research involve human participants?

Yes

Will the research use collected or generated personal data?

Yes

Will the research involve the use of animals?

No

Will any of the research take place outside the UK?

No

Project details

Summary of the project

By 2050, the global population aged 60+ will reach 2 billion, more than double that in 2015. Healthy older adults exhibit a decline in motor skills such as suppressing or stopping actions. Motor control is key for obstacle avoidance during walking and driving, and for promoting independence and safety; its decline leads to accidents and falls: over one-third of adults aged 65+ fall each year. Age-related deficits in motor control have been linked to changes in connectivity between the pre-motor and the primary motor cortex. However, despite the extensive work on ageing and motor control, making an accurate examination of the effects of healthy ageing on the neural connections that control actions has been proven difficult so far. This project aims to investigate if the decline in motor control results from decreases in the efficacy of the connections in the motor control brain network.

It is possible to safety induce transient changes in the neural connections between brain areas. Such a manipulation is possible by stimulating the brain systems using a technique known as transcranial magnetic stimulation (TMS). For example, we can use TMS to transiently manipulate connectivity between two regions in the ageing brain and test whether changes in brain connectivity have a direct influence on motor abilities.

TMS can also be combined with electroencephalography (EEG) and this combination enable us to examine the temporal evolution of signals in the brain. In this way it becomes achievable to measure the impact of the TMS on activity in a brain pathway. In this study, we will therefore record the temporal profile of motor control related activity as well as to subtly change the activity with TMS. This will help us understand the impact of natural ageing on the brain mechanisms of motor control.

Specifically, participants will be asked to perform a simple motor control task where they are expected to perform a simple motor action, such as pressing a response bottom or lifting a small object, when a “go” signal and to refrain from performing the action and do nothing when a “no-go” signal appears. The visual stimuli will mostly be simple geometric shapes but some will have additional information about task performance and probability of points to be won if responses are correct. Some stimuli may be pictures of faces. We will measure response key accuracy and reaction time, eye movements, and electrical activity from the outside of the scalp using the non-invasive electroencephalography (EEG) technique. For the purpose of behavioural piloting, For the purpose of behavioral piloting or testing of participant performance, we also plan to conduct behavioral experiments without the use of TMS to validate our experimental paradigms. These will closely resemble the experiments conducted using TMS. As an additional means of assessing our experimental paradigms during the process of testing and particularly piloting, we plan to conduct participant observations and unstructured interviews. In some cases, participants will be asked to fill in questionnaires that may be of relevance to the particular study. These can be: Edinburgh Laterality Questionnaire; Beck’s Depression Inventory, Mood disorder questionnaire (MDQ), Spilberg’s State Trait Anxiety Inventory (State STAI and Trait STAI), Mood and Anxiety Symptom Questionnaire (MASQ), and The Altman Self- Rating Mania Scale.

Sample and will consist of approximately 300 male and female adult volunteers, between 18 and 85 years old, with normal or corrected to normal vision, with no history neural or mental illness.

Participants will be recruited via Essexlab database or via SONA.

None of the participants that do not meet any of the exclusion criteria are expected to be adversely affected.

Research project proposal

Which department does your research primarily fall within?

Psychology

Will the participants, either the subjects or the investigators, be involved in any activities that could be considered to be unlawful in the UK?

No

If the project is being undertaken outside the UK, will the participants, either the subjects or the investigators, be involved in any activities that could be considered to be unlawful in the country overseas?

Participant details

Who are the potential participants?

The sample will be a convenience sample and will consist of male and female adult volunteers, between 18 and 85 years of age or above recruited via SONA. All participants will need to be ighthanded and have self-reported normal or corrected to normal vision and hearing to avoid these variables affecting the perception of the tasks. Participants will also have no family history of epilepsy

How will they be recruited?

Information Sheet, providing information about the study, will be provided before the study. This method of recruitment will ensure that participants have time and information available to make an informed decision of participation in the study. Moreover, the above method, in particular the absence of the researcher when the decision to participate is made, will also ensure that participants informed consent is not compromised. Participants that agree to participate will be provided with the Consent Form to sign and given an opportunity to ask any questions about the study. It is planned to include approximately 300 participants

Recruiting materials

Will participants be paid or reimbursed?

Yes

If yes, please provide details and justification for this payment.

Participants will receive either course credits or financial reimbursement. Where applicable, participants will be reimbursed at a rate of £10-£12 per hour

How much will the participants be paid?

£ 10

Could potential participants be considered vulnerable?

No

If yes, please explain how the participants could be considered vulnerable and why vulnerable participants are necessary for the research.

Could potential participants be considered to feel obliged to take part in the research?

No

If yes, please explain how the participants could feel obliged and how any possibility for coercion will be addressed.

Will the research involve individuals below the age of 18 or individuals of 18 years and over with a limited capacity to give informed consent?

No

Is a Disclosure and Barring Service (DBS) Check required?

No

If yes, has the DBS check been completed?

If your project involves children or vulnerable adults but does not require a DBS check, please explain why.

Informed consent

How will consent be obtained?

Written

If consent will be obtained in writing, please upload the written consent form for review and approval.

If consent will be obtained orally, please explain why.

Please upload a copy of the script that will be used to obtain oral

consent. If no script is available to upload please explain why.

Who will be obtaining and recording consent?

The experimenters involved in the study

Please indicate at what stage in the data collection process consent will be obtained.

Participants will be provided with the Consent Sheet to sign before commencing the study. Consent Sheet will be kept secure in the office of the researcher for the duration of 10 years

If informed consent will not be obtained, explain why.

Please upload a participant information sheet.

Have you reviewed the information provided by the REO on participant information and consent?

Yes

Confidentiality and anonymity

Will you be maintaining the confidentiality and anonymity of participants whose personal data will be used in your research?

Yes

If yes, describe the arrangements for maintaining anonymity and confidentiality.

Experimental data will be stored anonymously; no-one other than the researchers will have access to personal information, and no personal information will be included in the experimental data.

Because the study involves a multiple visits, we will need to keep some idenfifiable information in a master list so that we can track data / information belonging to each participant. This information will be stored securely in a password protected master list only accesible to the researchers. Consent forms will be stored securely in a locked filling cabinet in the PI's office. All the other identifiable information will be stored on the institutional servers and it will be password protected. Data collected may, at some point, be presented at a conference and/or published in a scientific journal. However, should this happen anonymity will be ensured.

If you are not maintaining anonymity and confidentially, please explain your reasons for not doing so.

Data access, storage and security

Describe the arrangements for storing and maintaining the security of any personal data collected as part of the project.

Experimental data will be stored anonymously and no personal information will be included in the experimental data. Consent forms will be stored securely in a locked filling cabinet in the PI's office.

All the other identifiable information will be stored on the institutional servers and it will be password protected. Data collected may, at some point, be presented at a conference and/or published in a scientific journal. However, should this happen anonymity will be ensured

Please provide details of all those who will have access to the data.

The named researcher(s), researchers directly involved in the project or other researchers under the direction of the named researcher (including undergraduate and postgraduate students supervised by the named researcher(s).

Data sharing

Do you intend to share or archive data generated from this project once it is complete?

Yes

If yes, please describe briefly.

Should data lead to publication it is planned to share the data via OSF or similar

Please indicate the means by which you intend to share/archive your data:

Other

If you chose other, please provide more details.

OSF

If you do not intend to share data please provide specific reasons why the data will not be made available.

Risk and risk management

Risk Assessment documents

Are there any potential risks (e.g. physical, psychological, social, legal or economic) to participants or subjects associated with the proposed research?

Yes

If yes, please provide full details and explain what risk management procedures will be put in place to minimise the risks.

TMS carries a risk of causing seizures (fits) in susceptible individuals. A seizure has been reported in approximately 20 individuals worldwide over the past 15 years. Considering the number of people tested this is an extremely low risk (Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. Rossi S, Hallett M, Rossini

PM, Pascual-Leone A; Safety of TMS Consensus Group. Clin Neurophysiol. 2009

Dec;120(12):2008-

39. doi: 10.1016/j.clinph.2009.08.016. Epub 2009 Oct 14. Review.). In most cases the seizure was associated with a family history of epilepsy, existing neurological disease (e.g. multiple sclerosis) or medication (anti-depressant or dopamine medication). The risk of a provoked seizure occurring in healthy individuals due to TMS is extremely small. As a precaution, it may not be possible to give

TMS to someone with a personal or close family (first-degree relative e.g. parent, sibling, child) history of epilepsy, another significant neurological or psychiatric disorder, or extreme mood fluctuations. If you are taking any medication, you should discuss this with the researcher beforehand. If you suffer with migraine headaches, you should not take part in this study.

The exact number of regular or lifetime non-invasive brain stimulation studies a participant can take part in is unknown. However, many studies use non-invasive brain stimulation to treat disorders (e.g.depression) and administer stimulation daily, as the therapeutic effects are thought to accumulate across sessions. Sessions separated by 48h do not show cumulative effects, however. To minimise the possibility of cumulative effects of brain stimulation for healthy subjects not enrolled

in treatment studies, we recommend that you should participate in sessions on no more than two consecutive days and no more than four sessions in a month. While no guideline has been provided for a “cooling-off” period between stimulation sessions, some have suggested it to be between 48 hours and one week after stimulation. Therefore, to protect subjects from repeatedly being called upon to participate in non-invasive brain stimulation studies, we recommend that the period of abstinence between different brain stimulation studies would be at least one week. However, additionally, if you are participating in any other brain stimulation study while volunteering for ours, please inform us so that we are aware of it and can judge whether there might be any potential interactions.

Participants will be informed that they can withdraw from the experiment at any time, without having to give a reason why, and experiments in which participants are perceived to be uncomfortable but do not report this will also be terminated by the experimenter.

There is no higher risk for older people to take part in a TMS session

Are there any potential risks (e.g. physical, psychological, social, legal or economic) to the researchers working on the proposed research?

Yes

If yes, please provide full details and explain what risk management procedures will be put in place to minimise the risks.

It is our policy not to give TMS to someone who is pregnant. If there is a possibility that the experimenter is pregnant, therefore, the experimenter must not conduct the study

Are there any potential reputational risks to the University as a consequence of undertaking the proposed research?

No

If yes, please provide full details and explain what risk management procedures will be put in place to minimise the risks.

Are there any other ethical issues that have not been addressed which you would wish to bring to the attention of the reviewer(s) of your application?

Other documents

Attached files

Research project

proposal.docx Poster_Advert.docx

Consent_Form.docx

Info_Sheet_Sel.docx

Approval_Sel.pdf

Re Neuromodulation committee TMS study.pdf TMS-EMF-risk-assessment_v1_as.docx

Neurostimulation Safety Screening Form_as.docx

Beck-Depression-Inventory-BDI.pdf

handedness.pdf

MDQ.pdf

Mini-MASQ.pdf

State-Trait-Anxiety-Inventory.pdf tool_asrm.pdf

Description of the project

By 2050, the global population aged 60+ will reach 2 billion, more than double that in 2015. Healthy older adults exhibit a decline in motor skills such as suppressing or stopping actions. Motor control is key for obstacle avoidance during walking and driving, and for promoting independence and safety; its decline leads to accidents and falls: over one-third of adults aged 65+ fall each year. Age-related deficits in motor control have been linked to changes in connectivity between nodes of the brain control network in prefrontal and premotor regions. Integrity within this network is associated with better motor control abilities. For instance, the motor decline with advancing age is accompanied by altered functional connectivity in the pathways connecting the prefrontal areas with the primary motor cortex. These connectivity changes occur in tandem with altered oscillatory electroencephalography (EEG) responses and declined functional connectivity, which are often accompanied with poorer motor performance in older adults. Yet, despite the extensive research work on brain ageing and motor control, accurately examining the causal effect of age-related connectivity changes on the motor control brain pathway has proven challenging thus far. A critical major limitation arises from the inability of conventional non-invasive tools to selectively assess and manipulate brain plasticity and connectivity in the motor neural pathways. This project aims to investigate how age-related changes in brain plasticity affect the neural networks underlying motor control.

In order to properly understand how ageing affects the brain motor control system it is necessary to manipulate the neural connections that underpin this ability. Such a manipulation is possible by stimulating the brain systems using a technique known as transcranial magnetic stimulation (TMS). It is possible with TMS to test the influence that one brain area has over the another anatomically connected area during information processing. These protocols, often referred to as paired-pulse TMS protocol, will allow us to test whether by transiently enhancing a region-specific pathway, we can change the influences onto brain areas that sense information such as the somatosensory cortex or that make movements such as the primary motor cortex. For example, we can use TMS to transiently manipulate connectivity between two

regions in the ageing brain and test whether changes in brain connectivity have a direct influence on motor abilities.
TMS can also be combined with electroencephalography (EEG) and this combination enable us to examine the temporal evolution of signals in the brain. In this way it becomes achievable to understand if a particular brain region is causally involved in the sensory integration process and at what point in time it contributes to the integration process. We also use EEG to measure the impact of the TMS on activity in a brain pathway. We will therefore record the temporal profile of motor control related activity as well as to subtly change the activity with TMS. This will help us understand the impact of natural ageing on the brain mechanisms of motor control.

In this project, we aim to investigate how healthy ageing affects the neural network underpinning motor control performance. To this aim, participants will be asked to perform a simple motor control task where they are expected to perform a simple motor action, such as pressing a response bottom or lifting a small object, when a “go” signal and to refrain from performing the action and do nothing when a “no-go” signal appears. The visual stimuli will mostly be simple geometric shapes but some will have additional information about task performance and probability of points to be won if responses are correct. Some stimuli may be pictures of faces. We will measure response key accuracy and reaction time, eye movements, and electrical activity from the outside of the scalp using the non-invasive electroencephalography (EEG) technique. The average task duration will be 1 hour and 30 minutes plus ~30 minutes for EEG setup. However, EEG setup time varies depending on participant hair type and skin conductance. We also need to set up the coils and localization for the transcranial magnetic stimulation (TMS), which will take another 30 minutes. Furthermore, participants might require some more practice or explanation of the task, which should not be longer than 30 minutes but can sometimes take longer. Therefore, we will advertise the sessions as potentially lasting up to 4 hours, although most participants will more likely take roughly 3 hours. If needed, participants will first be invited to a “tester” session so that they know how the TMS feels if they are unfamiliar with the technique. Then they will be asked to take part in two testing sessions on different days. Participants will be asked to complete

questionnaires on mood and personality and bodily perception, as well as questions on their performance on these questionnaires (i.e. metacogniton). In all cases, with no exception, codes linking performance and/or brain activity data to participants' identities are kept in a secure place accessible only to the researchers.

There will always be one-week period between two TMS sessions. For the purpose of behavioral piloting or testing of participant performance, we also plan to conduct behavioral experiments without the use of TMS to validate our experimental paradigms. These will closely resemble the experiments conducted using.

We will administer questionnaires that might give us information about the participants thoughts during the experimental task, as well as using the below questionnaires in particular. These questionnaires are not intended as a screening tool and we do not aim to identify participants with a high level of distress or those that match a clinical phenotype. Instead, we will investigate a healthy population but are interested in subclinical variation with respect to variations in mood. More specifically, in some cases participants will be asked to fill out a set of validated questionnaires from the literature (Edinburgh Laterality Questionnaire; Beck's Depression Inventory, Mood disorder questionnaire (MDQ), Spilberg's State Trait Anxiety Inventory (State STAI and Trait STAI), Mood and Anxiety Symptom Questionnaire (MASQ), and The Altman Self-Rating Mania Scale). With respect to the mood and personality questionnaires, participants will be provided with details of relevant services they can contact if they have any concerns about their mood. The questionnaires we would like to administer give some indication about participants' mental health, but are not sufficient to establish a clinically significant diagnosis. However, trained clinical psychologists will be consulted if the researcher is worried about unusual participant responses/communication or if the participants themselves express any concerns. We would ask participants to fill these in as part of their last visit, or, alternatively, online after their last visit. If participants are asked to fill in the questionnaires online after their last visit, informed consent will be given again online before the questionnaires are presented. If we ask them to fill in the questionnaire online at a later date after their last

visit, an email will be sent to them including information and instructions as to how to fill in the questionnaire online.

Sample and will consist of approximately 300 male and female adult volunteers, between 18 and 85 years old, with normal or corrected to normal vision, with no history neural or mental illness. Participants will be recruited via Essexlab database or via SONA. Following standard protocols for electrical stimulation, as standard preventative measure participants will complete neurostimulation safety screening forms to identify those at risk so that they can be excluded from participation. Participants will be excluded where the following is applicable (History/family history of seizures or syncope, brain lesions or head trauma, heart diseases, neurological or psychiatric diseases, epilepsy, migraine, sleep deprivation, alcoholism, pregnancy). Also, to be excluded from participation are people with implanted medical devices (e.g. pacemakers, cochlear implants) or ferromagnetic implants, those currently taking tricyclic antidepressants, neuroleptic agents or any drug that might lower seizure threshold; those who hold a HGV/bus licence, and those with wounds or diseases skin at or near the area of stimulation.

None of the participants that do not meet any of the exclusion criteria are expected to be adversely affected.

University of Essex

Participants wanted for brain stimulation study

Study: Direct measurements of the brain mechanisms of motor control in ageing

Ethics code: ETH2223-0175

Researchers at the Department of Psychology, University of Essex are seeking volunteers aged 18-85 for a for a combined Transcranial Magnetic Stimulation (TMS) and Electrophysiological (EEG) recordings study.

The purpose of this study is to examine how the brain understand how the natural changes that occur in the human brain in healthy ageing can affect the ability to control movements. TMS is a noninvasive technique that allows us to stimulate the brain by by rapid switching of a magnetic field in a coil placed over the head. Participants may experience some discomfort during TMS. By using this technique, we hope to find out how healthy ageing affects our ability to control movements.

This study involves two visits to the Department of Psychology for the study sessions. Each entire session will take no more than 4 hours, but usually take less than 2-3 hours. If you are unfamiliar with the techniques then we can also arrange an additional "tester" session so that you know what the experience feels like. Volunteers will be reimbursed for their time taken in all sessions. We are looking for participants between 18 and 85 years old, right handed with normal or corrected to normal vision, with no history fainting, or mental illness and no experience of, or family history of, epilepsy.

If you are interested in participating in this study and/or would like further information, please contact *Experimenter name – xxxxx*@essex.ac.uk

University of Essex

Department of Psychology

CONSENT FORM

Title of the Project: ***Direct measurements of the brain mechanisms of motor control in ageing***

Please initial box

1. I confirm that I have read and understand the Information Sheet dated XXX for the above study. I have had the opportunity to consider the information and ask questions; any questions have been answered satisfactorily. ☐
2. I understand that my participation is voluntary and that I am free to withdraw from the project at any time without giving any reason and without penalty. ☐
3. Risk Statement: I understand that, due to the nature of the stimulation used, TMS sessions may not be suitable to individuals who suffer, or have suffered, from epileptic seizures, that I am aware of the potential risks associated with that, and I confirm that, to the best of my knowledge, I have never had epileptic seizures. ☐
4. I understand that the identifiable data provided will be securely stored and accessible only to the members of the research team directly involved in the project or other researchers under the direction of the Principal Investigator, and that confidentiality will be maintained. ☐
5. I give consent for my data to be published in scientific journal articles, in which case data will remain completely anonymous. ☐
6. I give consent for the data collected about me to be used to support other research in the future, and to be shared anonymously with other researchers. ☐
7. I give consent for the de-identified (anonymised) behavioural, physiological and EEG data that I provide to be shared in permanent, publicly accessible archives accessible from any country. ☐
8. I consent to having my data processed as described in the Information Sheet. ☐
9. I agree to be contacted in the future by the researchers. ☐

10. I agree to take part in the above study.

Participant Name	Date	Participant Signature
_____	_____	_____
Researcher Name	Date	Researcher Signature
_____	_____	_____

University of Essex

Department of

Title

Direct measures of the brain mechanisms of motor control in ageing Ethics code: ETH2223-0175

Date of approval: XXXX

Invitation to our study

We would like to invite you to participate in this research project. You should only participate if you want to; choosing not to take part will not disadvantage you in any way. Before you decide whether you want to take part, it is important for you to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or you would like more information.

The Study

The first purpose of this study is to investigate how healthy ageing affects the brain mechanisms underpinning our ability to control actions. We can investigate this by using Transcranial Magnetic Stimulation (TMS) in combination with electrophysiological brain recordings (electroencephalogram; EEG) and electrocardiogram (ECG). TMS, EEG and ECG are non-invasive which means that we use sensors placed on the scalp.

What is TMS?

TMS is a safe, transient and non-invasive treatment technique that allows us to stimulate the brain by rapid switching of a magnetic field in a coil placed over the head. TMS is delivered via a plastic paddle held against the scalp, which houses a coil of copper wires.

TMS is currently used in both clinical treatment and in research studies with adults and children.

Study Plan Example

Here is an example of what this study might look like

Day 1: Tester Session (allow 30 min)

Venue: Department of Psychology, University of Essex

Day 2: TMS-EEG study Part 1 (allow 3 hours)

Venue: Department of Psychology, University of Essex

We might also call you back for a fourth session that is very similar to day 2, but this will need to be confirmed closer to the time

TMS procedure:

This study includes up to 3 visits to the Department of Psychology in the University of Essex. The first visit will take 30 minutes, the second 2 visits will take no more than 3 hours. We will use TMS to stimulate your brain.

We can measure the effects of this stimulation by recording the activity of muscles (electromyography; EMG). EMG activity of the muscle is measured at the surface of the skin by attaching an electrode (small silver disc). Several electrodes will be taped on the skin over muscles on your hands.

During TMS, a coil is positioned over the scalp and single pulses are used to stimulate the brain. The intensity of stimulation is varied until the EMG recording consistently shows activity in the muscle in response to the stimulation. Once we have established this motor threshold we will use 10% more than this intensity throughout the study for stimulation.

Participants will be asked to put in earplugs to protect ears from the clicking noise from the TMS coil.

During the “tester session”, you will have the opportunity to get a sense of what TMS actually feels like. It will include measuring the motor threshold (as described in the previous paragraph). You will then have a chance to experience how the TMS will feel before deciding whether to continue with the rest of the study.

If the tester session goes well, we will continue with the study. We will use TMS to stimulate your brain in one of the following ways:

1) Single- dual- or triple-pulse stimulation (known as 'multi-pulse TMS')

Single pulses or pairs or triplets of pulses (separated by less than a second) will be applied over the scalp. At the same time, the activity may be measured in your muscles using EMG or you may be asked to complete a task on the computer. You will be told to either contract or relax your muscles.

2) Low-frequency repetitive stimulation (rTMS at or <1Hz)

The TMS will be applied over the scalp at a maximum rate of one pulse per second (0.6 - 1 Hz) for up to 20 minutes.

High-Short trains of up to 5 pulses lasting less than a second will be applied and repeated for a fixed number of pulses.

3) Patterned rTMS: e.g. theta-burst stimulation will involve bursts of high-frequency (50 Hz) triplets applied every 200ms for up to 40 sec total stimulation time; max 600 pulses.

4) Sham TMS: This is ineffective stimulation used a control for specific stimulation protocols.

You might be also asked to complete a couple of simple computerizes tasks before TMS, after TMS or during TMS. In these tasks visual, auditory and occasional auditory stimuli will be presented (lasting around 1 hour and 30 minutes). The visual stimuli will mostly be simple geometric shapes but some will have additional information about task performance and probability of points to be won if responses are correct. Some stimuli may be pictures of faces or emotional stimuli of other kind. During these tasks we will measure response key accuracy and reaction time, eye movements and pupil dilation, and electrical activity from the outside of the scalp using EEG.

EEG procedure:

We would measure brain activity from the scalp throughout the study.

The procedure for measuring brain waves is harmless and painless and carries no significant risk to participants. The researcher would place a snug fitting cap made of an elasticated cloth material and some metal contacts (sensors). We will also put a sensor on your chest to record activity from your heart. To make electrical contact, a gel containing salts that conduct electricity would be placed under each metal contact. In order to make a good electrical connection, it is often necessary to clean and prepare the area of the scalp under the sensors by applying and rubbing an abrasive conductive gel using a cotton swab. We would ask you to let the researcher know if at any time the procedure becomes uncomfortable. In that case, we would terminate the session, without this having any negative consequences for you. The preparation procedure may take an additional thirty minutes. This means that in total the entire session may take up to three hours and thirty minutes but on average the entire session is likely to take about three hours.

The gel used to make the electrical contact is water-based and washes away easily. In the laboratory there is a shower head, shampoo and a hair dryer for removal of the gel after the completion of the study.

Once the set-up is completed, participants will be asked to calmly rest for a couple of minutes with open and closed eyes and / or to perform the computerised task in a darkened small study room, while sitting in a chair. It is possible that none of the very limited set of EEG caps fits your specific head shape perfectly. As we need very good contact and signal strength for the kind of questions we are interested in, we would in that case not proceed with the main study, but instead compensate you for your time so far.

5) frequency repetitive stimulation (rTMS >1Hz)

Potential Risks

TMS carries a risk of causing seizures (fits) in susceptible individuals. A seizure has been reported in approximately 20 individuals worldwide over the past 15 years. Considering the number of people tested this is an extremely low risk (Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. Rossi S, Hallett M, Rossini PM, Pascual-Leone A; Safety of TMS Consensus Group. Clin Neurophysiol. 2009 Dec;120(12):2008-39. doi: 10.1016/j.clinph.2009.08.016.

Epub 2009 Oct 14. Review.). In most cases the seizure was associated with a family history of epilepsy, existing neurological disease (e.g. multiple sclerosis) or medication (anti-depressant or dopamine medication). The risk of a provoked seizure occurring in healthy individuals due to TMS is extremely small. As a precaution, it may not be possible to give TMS to someone with a personal or close family (first-degree relative e.g. parent, sibling, child) history of epilepsy, another significant neurological or psychiatric disorder, or extreme mood fluctuations. If you are taking any medication, you should discuss this with the researcher beforehand. If you suffer with migraine headaches, you should not take part in this study.

The exact number of regular or lifetime non-invasive brain stimulation studies a participant can take part in is unknown. However, many studies use non-invasive brain stimulation to treat disorders (e.g. depression) and administer stimulation daily, as the therapeutic effects are thought to accumulate across sessions. Sessions separated by 48h do not show cumulative effects, however. To minimise the possibility of cumulative effects of brain stimulation for healthy subjects not enrolled in treatment studies, we recommend that you should participate in sessions on no more than two consecutive days and no more than four sessions in a month. While no guideline has been provided for a “cooling-off” period between stimulation sessions, some have suggested it to be between 48 hours and one week after stimulation. Therefore, to protect subjects from repeatedly being called upon to participate in non-invasive brain stimulation studies, we recommend that the period of abstinence between different brain stimulation studies would be at least one week. However, additionally, if you are participating in any other brain stimulation study while volunteering for ours, please inform us so that we are aware of it and can judge whether there might be any potential interactions.

It is our policy not to give TMS to someone who is pregnant. If there is a possibility that you are pregnant, therefore, you must not take part in this study. We do not test for pregnancy as routine so if you think you may be pregnant you should not take part in this study.

What are the side effects of TMS?

Participants may experience some discomfort during TMS. In susceptible individuals, TMS may cause headache, which usually responds well to over-the-counter painkillers (e.g. paracetamol).

Informed consent

Should you agree to take part in this experiment, you will be asked to sign a consent form

Withdrawal

Your participation is voluntary and you will be free to withdraw from the project at any time before you have completed it without giving any reason and without penalty. If you wish to withdraw, you simply need to notify the principal investigator (see contact details below). It will not be possible to withdraw from the study after you have completed it because we will not be able to identify your data.

Data gathered

- We will collect the following data from each participant: EEG data, task performance data, and physiological data. We will also store your name and contact details in a password-protected folder. This folder will be locked in a different place than the above data.
- Your experimental data will be fully anonymous, so that it is not possible to identify you from our stored data.
- We are using your data to understand how the brain perceives bodily signals.
- Your data will be gathered by Alex Sel and researchers under his direction
- Signed consent forms will be kept separately from individual experimental data and locked in a drawer in Dr Sel's office.
- Our legal basis for storing your consent form is that you have consented to it.
- The data controller is the University of Essex.
- Essex University's Data Protection Officer can be contacted on dpo@essex.ac.uk.
- Your anonymous data may be published in scientific journal articles, and shared in permanent, publicly accessible archives accessible from any country.

Funding

The research is funded by Department of Psychology, University of Essex

Ethical approval

This project has been reviewed on behalf of the University of Essex Science and Health Ethics Sub-committee, and had been given approval with the following Application ID: XXXX

Concerns and complaints

If you have any concerns about any aspect of the study or you have a complaint, in the first instance please contact the Principal Investigator of the project (see contact details below). If you are still concerned or you think your complaint has not been addressed to your

satisfaction, please contact the Director of Research in the Principal Investigator's department (see below). If you are still not satisfied, please contact the University's Re

Contact details Principal investigator

[redacted]

Director of Research, Dept of Psychology

[redacted]

University of Essex Research Governance and Planning Manager

Sarah Manning-Press, Research & Enterprise Office, University of Essex, Wivenhoe Park, CO4 3SQ,

Colchester. [redacted]

University of Essex

Department of [redacted]

Psychology

[redacted]

Faculty of Science

and Health [redacted]

[redacted]

University of Essex
Wivenhoe Park
Colchester
Essex CO4 3SQ
United Kingdom

Colchester, 8 December 2022

RE: Project plan approval for brain stimulation study

To whom it might concern,

This letter is to confirm that the project plan entitled 'Direct measurements of the brain mechanisms of motor control in ageing' submitted by [redacted] has been revised and commented by the members of the Neuromodulation Committee (excluding [redacted]). The committee suggested a number of changes that have been successfully incorporated to the project proposal. The updated project proposal has been approved by the Neuromodulation Committee.

Sincerely,

[redacted]

University of Essex

University of Essex

Neurostimulation Safety Screening Form (Confidential)

If you agree to take part in this study, please answer the following questions.

The information you provide is for screening purposes only and will be kept completely confidential

1. Do you have epilepsy or have you ever had a convulsion or a seizure (fit)?
NO
Has anyone in your immediate or distant family suffered from seizures?
NO
If YES please state your relationship to the affected family member.

2. Have you ever had a fainting spell or syncope? If yes, please describe on which occasion(s)? NO

3. Have you ever had a head trauma that was diagnosed as a concussion or was associated with loss of consciousness?
NO

4. Do you have any hearing problems or ringing in your ears? NO

5. Do you have cochlear implants?
NO

6. Are you pregnant or is there any chance that you might be?
NO

7. Do you have metal in the brain, skull or elsewhere in your body (e.g., splinters, fragments, clips, etc.)? NO
If so, specify the type of metal and where it is located.

8. Do you have an implanted neurostimulator (e.g., DBS, epidural/subdural, VNS)? NO

9. Do you have a cardiac pacemaker or intracardiac lines? NO

10. Do you have a medication infusion device? NO

11. Are you taking any prescribed or unprescribed medications (or herbal

remedies)? (please list) NO

12. Did you ever undergo TMS/tES/tVNS in the past?
If YES, please state if there were any problems and describe them. NO

13. When was your last TMS/tES/tVNS session

How many TMS/tES/tVNS sessions have you had in the past month?
How many TMS/tES/tVNS sessions have you had in the past 12 months?

13. Did you ever undergo MRI in the past? If so, were there any problems. NO

14. Have you ever undergone a neurosurgical procedure (including eye surgery)? NO
If YES, please give details

15. Are you currently undergoing anti-malarial treatment? NO

16. Please indicate if any of these are true: Have you drunk more than 3 units of alcohol in the last 24 hours? Have you drunk alcohol already today? Have you used recreational drugs in the last 24 hours?
NO

17. Have you had more than one cup of coffee, or other sources of caffeine, in the last hour? NO

18. Did you have very little sleep last night?
NO

_____
I (please give full name in CAPITALS) confirm that I have completed the above questionnaire.

Signature_____A._E_____Date _____

Please note: All data arising from this study will be held and used in accordance with the Data Protection Act (1984). The results of the study will not be made available in a way that could reveal the identity of individuals.

(Based on Screening13-item Questionnaire for rTMS Candidates recommended by Rossi, Hallett, Rossini and Pascual-Leone 2011; updated 15/02/12)

Beck's Depression Inventory

This depression inventory can be self-scored. The scoring scale is at the end of the questionnaire.

1.
- 0 I do not feel sad.
- 1 I feel sad
- 2 I am sad all the time and I can't snap out of it.
- 3 I am so sad and unhappy that I can't stand it.

2.
- 0 I am not particularly discouraged about the future.
- 1 I feel discouraged about the future.
- 2 I feel I have nothing to look forward to.
- 3 I feel the future is hopeless and that things cannot improve.

3.
- 0 I do not feel like a failure.
- 1 I feel I have failed more than the average person.
- 2 As I look back on my life, all I can see is a lot of failures.
- 3 I feel I am a complete failure as a person.

4.
- 0 I get as much satisfaction out of things as I used to.
- 1 I don't enjoy things the way I used to.
- 2 I don't get real satisfaction out of anything anymore.
- 3 I am dissatisfied or bored with everything.

5.
- 0 I don't feel particularly guilty
- 1 I feel guilty a good part of the time.
- 2 I feel quite guilty most of the time.
- 3 I feel guilty all of the time.

6.
- 0 I don't feel I am being punished.
- 1 I feel I may be punished.
- 2 I expect to be punished.
- 3 I feel I am being punished.

7.
- 0 I don't feel disappointed in myself.
- 1 I am disappointed in myself.
- 2 I am disgusted with myself.
- 3 I hate myself.

8.
- 0 I don't feel I am any worse than anybody else.
- 1 I am critical of myself for my weaknesses or mistakes.
- 2 I blame myself all the time for my faults.
- 3 I blame myself for everything bad that happens.

9.
- 0 I don't have any thoughts of killing myself.
- 1 I have thoughts of killing myself, but I would not carry them out.
- 2 I would like to kill myself.
- 3 I would kill myself if I had the chance.

10.
- 0 I don't cry any more than usual.
- 1 I cry more now than I used to.
- 2 I cry all the time now.
- 3 I used to be able to cry, but now I can't cry even though I want to.

11.

0 I am no more irritated by things than I ever was.
1 I am slightly more irritated now than usual.
2 I am quite annoyed or irritated a good deal of the time.
3 I feel irritated all the time.

12.

0 I have not lost interest in other people.
1 I am less interested in other people than I used to be.
2 I have lost most of my interest in other people.
3 I have lost all of my interest in other people.

13.

0 I make decisions about as well as I ever could.
1 I put off making decisions more than I used to.
2 I have greater difficulty in making decisions more than I used to.
3 I can't make decisions at all anymore.

14.

0 I don't feel that I look any worse than I used to.
1 I am worried that I am looking old or unattractive.
2 I feel there are permanent changes in my appearance that make me look unattractive
3 I believe that I look ugly.

15.

0 I can work about as well as before.
1 It takes an extra effort to get started at doing something.
2 I have to push myself very hard to do anything.
3 I can't do any work at all.

16.

0 I can sleep as well as usual.
1 I don't sleep as well as I used to.
2 I wake up 1-2 hours earlier than usual and find it hard to get back to sleep.
3 I wake up several hours earlier than I used to and cannot get back to sleep.

17.

0 I don't get more tired than usual.
1 I get tired more easily than I used to.
2 I get tired from doing almost anything.
3 I am too tired to do anything.

18.

0 My appetite is no worse than usual.
1 My appetite is not as good as it used to be.
2 My appetite is much worse now.
3 I have no appetite at all anymore.

19.

0 I haven't lost much weight, if any, lately.
1 I have lost more than five pounds.
2 I have lost more than ten pounds.
3 I have lost more than fifteen pounds.

20.

0 I am no more worried about my health than usual.
1 I am worried about physical problems like aches, pains, upset stomach, or constipation.
2 I am very worried about physical problems and it's hard to think of much else.
3 I am so worried about my physical problems that I cannot think of anything else.

21.

0 I have not noticed any recent change in my interest in sex.
1 I am less interested in sex than I used to be.
2 I have almost no interest in sex.
3 I have lost interest in sex completely.

INTERPRETING THE BECK DEPRESSION INVENTORY

Now that you have completed the questionnaire, add up the score for each of the twenty-one questions by counting the number to the right of each question you marked. The highest possible total for the whole test would be sixty-three. This would mean you circled number three on all twenty-one questions. Since the lowest possible score for each question is zero, the lowest possible score for the test would be zero. This would mean you circles zero on each question. You can evaluate your depression according to the Table below.

Total Score	Levels of Depression
1-10	These ups and downs are considered normal
11-16	Mild mood disturbance
17-20	Borderline clinical depression
21-30	Moderate depression
31-40	Severe depression
over 40	Extreme depression

Edinburgh Handedness Inventory

Surname_____ Given Name_____

Date of
Birth_____ Sex_____

Please indicate your preferences in the use of hands in the following activities by *putting + in the appropriate column*. Where the preference is so strong that you would never try to use the other hand unless absolutely forces to, *put* ++. If any case you are really indifferent put + in both columns.

Some of the activities require both hands. In these cases the part of the task, or object, for which hand preference is wanted is indicated in brackets.

Please try to answer all the questions, and only leave a blank if you have no experience at all of the object or task.

	Left	Right
1. Writing		
2. Drawing		
3. Throwing		
4. Scissors		
5. Toothbrush		
6. Knife (without fork)		
7. Spoon		
8. Broom (upper hand)		
9. Striking Match (match)		
10. Opening box (lid)		
i. Which foot do you prefer to kick with?		
ii. Which eye do you use when using only one?		

L.Q.	Leave the spaces blank	DECLE

The Mood Disorder Questionnaire (MDQ) - Overview

The Mood Disorder Questionnaire (MDQ) was developed by a team of psychiatrists, researchers and consumer advocates to address the need for timely and accurate evaluation of bipolar disorder.

Clinical Utility

- The MDQ is a brief self-report instrument that takes about 5 minutes to complete.
- This instrument is designed for *screening purposes only* and is not to be used as a diagnostic tool.
- A positive screen should be followed by a comprehensive evaluation.

Scoring

In order to screen positive for possible bipolar disorder, all three parts of the following criteria must be met:

- "YES" to 7 or more of the 13 items in Question 1 **AND**
- "Yes" to Question number 2 **AND**
- "Moderate Problem" or "Serious Problem" to Question 3

Psychometric Properties

The MDQ is best at screening for bipolar I (depression and mania) disorder and is not as sensitive to bipolar II (depression and hypomania) or bipolar not otherwise specified (NOS) disorder.

Population /type	Sensitivity & Specificity
Out-patient clinic serving primarily a mood disorder population[1]	Sensitivity 0.73 Specificity 0.90
General	Sensitivity 0.28
37 Bipolar Disorder patients 36 Unipolar Depression patients[3]	Overall Sensitivity 0.58 (BDI 0.58-BDII/NOS 0.30) Overall Specificity 0.67

Primary care patients receiving treatment for		Sensitivity 0.58

1. *Hirschfeld RMA. et, al. Development and validation of a screening instrument for bipolar spectrum disorder: The Mood Disorder Questionnaire, Am J of Psychiatry, 2000, 157:1873-1875.*
2. *Hirschfeld RMA. The mood disorder Questionnaire: A simple, patient-rated screening instrument for bi-polar disorder. Journal of Clinical Psychiatry Primary Care Companion 2002; 4: 9-11.*
3. *Miller CJ et al, Sensitivity and specificity of the Mood Disorder Questionnaire for detecting bipolar disorder. J Affect Disorder 2004. 81: 167-171.*
4. *Hirschfeld RMA, et al. Screening for bipolar disorder in patients treated for depression in a family medicine clinic. JABFP 2005, 18: 233-239.*

Mood Disorder Questionnaire

Patient Name _____ Date of Visit _____

Please answer each question to the best of your ability

1. Has there ever been a period of time when you were not your usual self and...	YES	NO
...you felt so good or so hyper that other people thought you were not your normal self or you were so hyper that you got into trouble?	☐	☐
...you were so irritable that you shouted at people or started fights or arguments?	☐	☐
...you felt much more self-confident than usual?	☐	☐
...you got much less sleep than usual and found that you didn't really miss it?	☐	☐
...you were more talkative or spoke much faster than usual?	☐	☐
...thoughts raced through your head or you couldn't slow your mind down?	☐	☐
...you were so easily distracted by things around you that you had trouble concentrating or staying on track?	☐	☐
...you had more energy than usual?	☐	☐
...you were much more active or did many more things than usual?	☐	☐
...you were much more social or outgoing than usual, for example, you telephoned friends in the middle of the night?		
...you were much more interested in sex than usual?		
...you did things that were unusual for you or that other people might have thought were excessive, foolish, or risky?		

...spending money got you or your family in trouble?

---

2. If you checked YES to more than one of the above, have several of these ever happened during the same period of time? ☐ ☐

---

3. How much of a problem did any of these cause you - like being unable to work; having family, money or legal troubles; getting into arguments or fights?

☐ No problems ☐ Minor problem ☐ Moderate problem ☐ Serious problem

This instrument is designed for screening purposes only and not to be used as a diagnostic tool. Permission for use granted by RMA Hirschfeld, MD

ID# _____

Mini-MASQ

Below is a list of feelings, sensations, problems, and experiences that people sometimes have. Read each item and then fill in the blank with the number that best describes how much you have felt or experienced things this way during the past week, including today. Use this scale when answering::

1	2	3	4	5
not at all	a little bit	moderately	quite a bit	extremely

_____ 1. Felt really happy
_____ 2. Felt tense or "high strung"
_____ 3. Felt depressed
_____ 4. Was short of breath
_____ 5. Felt withdrawn from other people
_____ 6. Felt dizzy or lightheaded
_____ 7. Felt hopeless
_____ 8. Hands were cold or sweaty
_____ 9. Felt like I had a lot to look forward to
_____ 10. Hands were shaky
_____ 11. Felt like nothing was very enjoyable
_____ 12. Felt keyed up, "on edge"
_____ 13. Felt worthless
_____ 14. Had trouble swallowing
_____ 15. Felt like I had a lot of interesting things to do
_____ 16. Had hot or cold spells
_____ 17. Felt like a failure
_____ 18. Felt like I was choking
_____ 19. Felt really lively, "up"
_____ 20. Felt uneasy
_____ 21. Felt discouraged
_____ 22. Muscles twitched or trembled
_____ 23. Felt like I had a lot of energy
_____ 24. Was trembling or shaking
_____ 25. Felt like I was having a lot of fun
_____ 26. Had a very dry mouth

Mood and Anxiety Symptom Questionnaire Scoring Key
26-item version (Mini-MASQ)

To score scales, sum the subject's responses for each positively keyed scale item. For each negatively keyed item, add 6 and then subtract the subject's response. All items are positively keyed unless otherwise noted.

General Distress: (GD, 8 items): 2, 3, 7, 12, 13, 17, 20, 21

Anxious Arousal (AA, 10 items): 4, 6, 8, 10, 14, 16, 18, 22, 24, 26

Anhedonic Depression (AD, 8 items)

Positively keyed items: 5, 11
Negative keyed items: 1, 9, 15, 19, 23, 25

Casillas, A. & Clark, L. A. (2000, May). *The Mini Mood and Anxiety Symptom Questionnaire (Mini- MASQ)*. Poster presented at the 72nd Annual Meeting of the Midwestern Psychological Association, Chicago, IL.

Abstract. The Mini Mood and Anxiety Symptom Questionnaire (Mini-MASQ) is a short form of the MASQ which measures anxiety and depression symptoms following Clark and Watson's tripartite model. Results from two samples indicate that the Mini-MASQ holds promise as a quick, reliable, and valid measure of anxiety and depression symptoms.

Problem or Major Purpose. Anxiety and depressive syndromes are highly cormorbid and many measures of depression and anxiety have poor discriminant validity. Clark and Watson (1991) examined the evidence related to these syndromes and developed a tripartite model that explains the overlap as due to a general (nonspecific) distress factor, with each syndrome separately identifiable by specific factors: anxious arousal (specific anxiety) and anhedonia (specific depression), respectively. The original (90-item) Mood and Anxiety Symptom Questionnaire (MASQ) was developed by Watson and Clark (1991) to test the tripartite model. Tests of the MASQ in a variety of samples (student, adult, and patient) met with reasonable success (Watson, Weber, Assenheimer, Clark, Strauss, & McCormick, 1995). However, the MASQ is too long for certain purposes and a shorter measures with good validity and reliability is desirable. The Mini-MASQ is an abbreviated (26-item) form developed to address this problem.

Procedure. The Mini-MASQ was administered to two samples: (a) a set of mostly African-American adults (*N*= 896; mean age = 38.1; range = 23 to 80 years) from the Family and Community Health Study (FACHS; Cutrona, 1997) in an interview format, and (b) a set of college students (*N* = 509; mean age = 19; range = 17 to 22 years) as a self-report questionnaire. Participants indicated to what extent they had experienced each symptom (1 = not at all, 5 = extremely) "during the past week, including today." In addition, the FACHS sample was

administered the Brief Temperament Survey (BTS; Clark, 1995), a short-form of the General Temperament Survey (Clark & Watson, 1991), which measures the broad domains of negative temperament, positive temperament, and disinhibition.

Results. A principal factors analysis with varimax rotation was performed on each sample. Three factors emerged: general distress, anxious arousal, and anhedonia; together these factors accounted for over 90% of the variance in each sample. Correlational analyses yielded good convergent validity between the nonspecific and specific scales, as well as evidence of discriminant validity between the specific scales. Alphas for each of the scales were in the mid .80s.

Conclusions and Implications. The results of these analyses are consistent with the tripartite model proposed by Clark and Watson (1991). Of particular note is the fact that the results were similar despite differences in population samples and mode of administration, indicating that the symptom structure is consistent across samples. Although inspection of individual item loadings suggested some refinements to the measure.the Mini-MASQ holds promise as a quick, reliable, and valid measure of anxiety and depression symptoms of utility in a variety of settings.

MINI-MASQ Descriptive statistics

Sample 1. Primarily African-American, rural, low-income community-dwelling adults in Georgia and Iowa

SCALE	*M*	*SD*
overall (N=890)		
ANHEDONIC DEPRESSION	18.1	6.5
ANXIOUS AROUSAL	12.6	4.8
GENERAL DISTRESS	13.6	5.8
women (N=829)		
ANHEDONIC DEPRESSION	18.2	6.5
ANXIOUS AROUSAL	12.6	4.9
GENERAL DISTRESS	13.7	5.9
men (N=61)		
ANHEDONIC DEPRESSION	16.9	6.5
ANXIOUS AROUSAL	11.6	3.1
GENERAL DISTRESS	12.1	4.4

Internal consistency (coefficient alpha):

ANHEDONIC DEPRESSION (8 items)	.85
ANXIOUS AROUSAL (10 items)	.84
GENERAL DISTRESS (8 items)	.85

Scale intercorrelations:

AD-AR	.27
GD-AD	.51
GD-AR	.48

Sample 2. University of Iowa college students

SCALE	*M*	*SD*
overall (N=509)		
ANHEDONIC DEPRESSION	20.0	6.2
ANXIOUS AROUSAL	15.8	5.9
GENERAL DISTRESS	16.5	5.9

Internal consistency (coefficient alpha):

ANHEDONIC DEPRESSION (8 items)	.88
ANXIOUS AROUSAL (10 items)	.83
GENERAL DISTRESS (8 items)	.87

Scale intercorrelations:

AD-AR	.19
GD-AD	.52
GD-AR	.40

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SELF-EVALUATION QUESTIONNAIRESTAI

Please provide the following information:

Name_____ D e _____ S_____

Age_____ Gender *(Circle)* **M F** **T**___

DIRECTIONS:

A number of statements which people have used to describe themselves are given below. Read each statement and then circle the appropriate number to the right of the statement to indicate how you feel *right* now, that is, *at this moment.* There are no right or wrong answers. Do not spend too much time on any one statement but give the answer which seems to describe your present feelings best.

	NOT AT ALL	SOMEWHAT	MODERATELY SO	VERY MUCH SO
1. I feel calm	1	2	3	4
2. I feel secure	1	2	3	4
3. I am tense	1	2	3	4
4. I feel strained	1	2	3	4
5. I feel at ease	1	2	3	4
6. I feel upset	1	2	3	4
7. I am presently worrying over possible misfortunes	1	2	3	4
8. I feel satisfied	1	2	3	4
9. I feel frightened	1	2	3	4
10. I feel comfortable	1	2	3	4
11. I feel self-confident	1	2	3	4
12. I feel nervous	1	2	3	4
13. I am jittery	1	2	3	4
14. I feel indecisive	1	2	3	4
15. I am relaxed	1	2	3	4

SELF-EVALUATION
STAI Form Y-2

Name_____ Date_____

DIRECTIONS

A number of statements which people have used to describe themselves are given below. Read each statement and then circle the appropriate number to the right of the statement to indicate how you *generally* feel. There are no right or wrong answers. Do not spend too much time on any one statement but give the answer which seems to describe how you generally feel.

	ALMOST NEVER	SOMETIMES	OFTEN	ALMOST ALWAYS
21. I feel pleasant	1	2	3	4
22. I feel nervous and restless	1	2	3	4
23. I feel satisfied with myself	1	2	3	4
24. I wish I could be as happy as others seem to be	1	2	3	4
25. I feel like a failure	1	2	3	4
26. I feel rested	1	2	3	4
27. I am "calm, cool, and collected"	1	2	3	4
28. I feel that difficulties are piling up so that I cannot overcome them	1	2	3	4
29. I worry too much over something that really doesn't matter	1	2	3	4
30. I am happy	1	2	3	4
31. I have disturbing thoughts	1	2	3	4
32. I lack self-confidence	1	2	3	4
33. I feel secure	1	2	3	4
34. I make decisions easily	1	2	3	4
35. I feel inadequate	1	2	3	4
36. I am content	1	2	3	4
37. Some unimportant thought runs through my mind and bothers me	1	2	3	4
38. I take disappointments so keenly that I can't put them out of my mind	1	2	3	4
39. I am a steady person	1	2	3	4
40. I get in a state of tension or turmoil as I think over my recent concerns and interests	1	2	3	4

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STAIP-AD Test Form Y
www.mindgarden.com

State-Trait Anxiety Inventory for Adults Scoring Key (Form Y-

Developed by Charles D. Spielberger in collaboration with R.L. Gorsuch, R. Lushene, P.R. Vagg, and G.A. Jacobs

To use this stencil, fold this sheet in half and line up with the appropriate test side, either Form Y-1 or Form Y-2. Simply total the scoring **weights** shown on the stencil for each response category. For example, for question # 1, if the respondent marked 3, then the **weight** would be **2.** Refer to the manual for appropriate normative data.

Form Y-1					Form Y-2				
1.	**4**	**3**	**2**	**1**	21.	**4**	**3**	**2**	1
2.	**4**	**3**	**2**	**1**	22.	**1**	**2**	**3**	**4**
3.	**1**	**2**	**3**	**4**	23.	**4**	**3**	**2**	1
4.	**1**	**2**	**3**	**4**	24.	**1**	**2**	**3**	**4**
5.	**4**	**3**	**2**	**1**	25.	1	**2**	**3**	**4**
6.	**1**	**2**	**3**	**4**	26.	**4**	**3**	**2**	**1**
7.	**1**	**2**	**3**	**4**	27.	**4**	**3**	**2**	1
8.	**4**	**3**	**2**	**1**	28.	1	**2**	**3**	**4**
9.	1	**2**	**3**	**4**	29.	1	**2**	**3**	**4**
10.	**4**	**3**	**2**	1	30.	**4**	**3**	**2**	**1**
11.	**4**	**3**	**2**	1	31.	**1**	**2**	**3**	**4**
12.	1	**2**	**3**	**4**	32.	**1**	**2**	**3**	**4**
13.	1	**2**	**3**	**4**	33.	**4**	**3**	**2**	1
14.	**1**	**2**	**3**	**4**	34.	**4**	**3**	**2**	**1**
15.	**4**	**3**	**2**	**1**	35.	**1**	**2**	**3**	**4**
16.	**4**	**3**	**2**	1	36.	**4**	**3**	**2**	1
17.	**1**	**2**	**3**	**4**	37.	**1**	**2**	**3**	**4**
18.	**1**	**2**	**3**	**4**	38.	**1**	**2**	**3**	**4**
19.	**4**	**3**	**2**	**1**	39.	**4**	3	**2**	1
20.	**4**	3	**2**	1	40.	1	**2**	3	**4**

Altman Self-Rating Mania Scale (ASRM) - Overview

- The ASRM is a 5-item self rating mania scale, designed to assess the presence and/or severity of manic symptoms.
- The ASRM may be used in an inpatient or outpatient setting to screen for the presence of and/or severity of manic symptoms for clinical or research purposes.
- Because it is compatible with DSM-IV criteria, and correlates significantly with Clinician-Administered Rating Scale for Mania (CARS-M), Young Mania Rating Scale (YMRS), it can be used effectively as a screening instrument to facilitate diagnostic assessment in patients with hypomanic symptoms.

Clinical Utility

- In outpatient settings the ASRM may be used as a psycho-educational tool to help patients recognize and monitor their own symptoms.
- It may be used reliably as a self-report measure of efficacy for patients receiving clinical treatment.
- It may be used in combination with self-rating depression scales to assess mixed states of mania and depression.

Scoring

1. Sum items 1-5
   - A cutoff score of 6 or higher indicates a high probability of a manic or hypomanic condition (based on a sensitivity rating of 85.5% and a specificity rating of 87.3%).
   - A score of 6 or higher may indicate a need for treatment and/or further diagnostic workup to confirm a diagnosis of mania or hypomania.
   - A score of 5 or lower is less likely to be associated with significant symptoms of mania.

2. As a self-report measure of clinical efficacy, items 1-5 should be summed to give a total score, which then may be compared to subsequent total scores during and after treatment.

Psychometric Properties

Specificity of 85.5

Sensitivity of 87.3[1]

1. *Altman EG, Hedeker D, Peterson JL, Davis JM. The Altman self-rating mania scale. Society of Biological Psychiatry 1997; 42:948-955.*

Altman Self-Rating Mania Scale (ASRM)

Name _____ Date

_____

Instructions:

1. There are 5 statements groups on this questionnaire: read each group of statements carefully.
2. Choose the one statement in each group that best describes the way you have been feeling for the past week.
3. Check the box next to the number/statement selected.
4. Please note: The word “occasionally” when used here means once or twice; “often” means several times or more and “frequently” means most of the time.

Question 1

- ☐ 0 I do not feel happier or more cheerful than usual.
- ☐ 1 I occasionally feel happier or more cheerful than usual.
- ☐ 2 I often feel happier or more cheerful than usual.
- ☐ 3 I feel happier or more cheerful than usual most of the time.
- ☐ 4 I feel happier or more cheerful than usual all of the time.

Question 2

- ☐ 0 I do not feel more self-confident than usual.
- ☐ 1 I occasionally feel more self-confident than usual.
- ☐ 2 I often feel more self-confident than usual.
- ☐ 3 I feel more self-confident than usual.
- ☐ 4 I feel extremely self-confident all of the time.

Question 3

- ☐ 0 I do not need less sleep than usual.
- ☐ 1 I occasionally need less sleep than usual.
- ☐ 2 I often need less sleep than usual.
- ☐ 3 I frequently need less sleep than usual.
- ☐ 4 I can go all day and night without any sleep and still not feel tired.

Question 4

- ☐ 0 I do not talk more than usual
- ☐ 1 I occasionally talk more than usual.
- ☐ 2 I often talk more than usual.
- ☐ 3 I frequently talk more than usual.

4 I talk constantly and cannot be interrupted

Question 5

0 I have not been more active (either socially, sexually, at work, home or school) than usual.

1 I have occasionally been more active than usual.

2 I have often been more active than usual

3 I have frequently been more active than usual.

4 I am constantly active or on the go all the time.