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Abstract

Within the primary motor cortex, the activity of local GABAergic interneurons is important in the generation of graded and specific patterns of muscle activation. This intracortical inhibitory tone is therefore an essential aspect of fine motor function. For this reason, abnormal inhibitory tone has often been investigated as a potential contributing factor in situations of altered motor control, such as is seen in healthy older adults. However, despite extensive investigation, studies assessing age-related changes in intracortical inhibition have produced inconsistent findings. The purpose of this thesis was to characterise how the ageing process affects intracortical inhibition, and to identify functional consequences of age-related changes in inhibitory tone. This was achieved by applying single-, paired- and triple-pulse transcranial magnetic stimulation (TMS) in young and old adults under a number of different conditions.

In Chapters 2 and 3, the effects of age-related changes in corticospinal input/output properties on comparisons of short- (SICI) and long-interval intracortical inhibition (LICI) between young and old subjects was assessed. This study found that differences in corticospinal recruitment mainly affect age-related comparisons of inhibition during voluntary activation, with comparisons during relaxation mostly unaffected. Furthermore, significant reductions in post-synaptic GABA_B-mediated inhibition were also observed in old adults.

Subsequently, by investigating interactions between LICI and SICI, Chapters 4 and 5 assessed if these changes in GABA_B-mediated inhibition involved the activity of pre-synaptic receptors. Furthermore, the modulation of SICI, LICI and LICI-SICI interactions during simple (abduction) and complex (precision grip) motor tasks was also compared between young and old subjects. These studies found age-related changes in both pre- and post-
synaptic GABA$_B$-mediated inhibition, as well as a reduced task-dependent modulation of intracortical inhibition in old adults.

In the final experimental chapter (Chapter 6), the modulation of SICI and LICI in young and old adults was investigated during slow shortening and lengthening contractions of a hand muscle controlling the index finger, the performance of which is known to be impaired by ageing. While both groups showed disinhibition during movement, this was significantly greater in old adults for both SICI and LICI. Furthermore, disinhibition of SICI varied between contraction phases for young (but not old) adults, whereas disinhibition of LICI varied between contraction phases for old (but not young) adults. These findings suggest that old adults modulate GABAergic inhibition differently during movement. However, if and how this altered inhibitory modulation contributes to age-related motor deficits during movement remains unclear.

This thesis has provided novel insights into the effects of age on GABAergic intracortical inhibition within primary motor cortex, some of which may contribute to the motor deficiencies that are commonly observed in healthy older adults. Furthermore, our findings have established several lines of investigation for future research, including some with the potential to produce positive clinical outcomes in older individuals. However, our results also demonstrate the need for an increased understanding of the functional relationship between TMS measures of inhibitory neurotransmission and motor output.
Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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1. Literature Review

The human hands ability to achieve fine manipulation is a defining characteristic of our species that has facilitated an unprecedented capacity for environmental interaction and tool use. This ability represents the culmination of finely orchestrated interactions between multiple factors. The neuromuscular system is a physiological facet recognised as being of critical importance in mediating this capacity for complex manipulation. Within this system, activities of both peripheral and central components make important contributions to hand function. However, these components of the neuromuscular system are known to undergo substantial change during the process of healthy ageing. The nature of this change, and how it contributes to age-associated deficits in hand function, is far from completely understood.

The following review is intended to provide an overview of the literature investigating changes within the central nervous system (CNS) during the process of healthy ageing. I will focus on changes in inhibitory processes within primary motor cortex as assessed by studies applying the non-invasive brain stimulation technique of transcranial magnetic stimulation (TMS). As such, I will provide an initial introduction of human motor cortex, intracortical inhibition and the corticospinal system, as well as a brief discussion of some of the factors facilitating human hand function. Subsequently, I will discuss TMS methodology and its importance in investigating human motor control, and consider the few studies that have used this technique to investigate the effect of ageing on intracortical inhibition. Finally, I will highlight the topics of investigation addressed within this thesis that aimed to further identify and understand the ramifications of ageing on intracortical inhibition and motor function.
1.1. Neural control of movement

1.1.1. Human motor cortex

The areas of neocortex specifically concerned with motor control are located on the caudal aspect of the frontal lobe and consist of primary motor, pre-motor (dorsal and ventral), supplementary motor and cingulate motor areas. Primary motor cortex (M1 or Brodmann’s area 4), is specifically located on the precentral gyrus and is the main source of motor output from the cortex. Pre-motor (lateral aspect of Brodmann’s area 6) and supplementary motor (medial aspect of Brodmann’s area 6) cortices are located rostral to M1, and cingulate motor cortex (Brodmann’s area 24) is located on the medial convexity of the hemisphere, on the ventral bank of the cingulate sulcus (Donoghue & Sanes, 1994).

M1 has long been viewed as having an orderly representation of the body mapped on its surface, with neurons controlling the lower extremities, upper extremities and head occurring sequentially along the coronal plane. The definition of this somatotopic map culminated from experiments applying electrical stimuli directly to the exposed cortex of patients undergoing surgical treatment for epilepsy (Penfield & Boldrey, 1937; Penfield & Rasmussen, 1950). Although this work was the first physiological evidence of the ‘homunculus’ in man, the idea of a discrete organisation of muscle representations was first suggested in the late 19th century after convulsions in epileptic patients were observed to travel between neighbouring muscles (Jackson, 1873). This suggestion was supported soon after by research applying electrical stimuli to the canine cortex (Fritsch & Hitzig, 2009). In the early 20th century, the idea of a compartmentalised cortex was furthered by the work of Korbinian Brodmann, who defined distinct areas of cortex based on their unique cellular content, subsequently providing a cytoarchitectural ‘map’ of the human cortex (Brodmann, 1909).
With the progression of technology and understanding, the contemporary line of thinking has deviated from the ideas presented by the seminal works cited above. Although it is recognised that there is a broad lower extremity-upper extremity-head somatotopy within M1, it is now known that, within a region, there is a considerable overlap of muscle representations (Rathelot & Strick, 2006, 2009). Furthermore, it has been demonstrated that a population of M1 neurons located across muscle representations encodes the kinematics of a movement (Georgopoulos et al., 1986; Wessberg et al., 2000), allowing simultaneous activation of functionally related groups of muscles involved in the movement (Buys et al., 1986). This interaction between representations, facilitating the generation of population codes, provides considerable convergence and divergence of information and an integrated approach to the control of movement (Capaday et al., 2013).

Cells of the cortex can be broadly separated into two main types; pyramidal and non-pyramidal. Pyramidal cells demonstrate a triangular shaped soma, use glutamate as their neurotransmitter and project to sites within and between cortices, as well as within subcortical structures (Molnar & Cheung, 2006). These are the main output cells of the cortex. Non-pyramidal cells, also known as stellate cells, demonstrate a star-shaped soma and project locally within cortical areas. These are further divided into spiny and non-spiny, based on dendrite morphology. Spiny stellate cells are excitatory interneurons, using glutamate as their neurotransmitter, whereas non-spiny stellate cells are inhibitory interneurons, using γ-aminobutyric acid (GABA) as their neurotransmitter (DeFelipe, 1997). The distribution of these cells throughout the cortex follows a specific arrangement, with their absence or presence defining 6 distinct horizontal layers (Brodmann, 1909). These are numbered layer I (at the pial surface) to layer VI (at the white matter). Pyramidal cells are primarily located within layers III, V and VI, whereas the smooth stellate cells are ubiquitous throughout the layers, although in varying concentrations. Within M1, smooth stellate cells are most
abundant in layer II, but become increasingly less apparent towards layer VI. The spiny stellate cells are found within layer IV (Jones, 1993).

The specific positioning of different cell types within each cortical layer results in functional specificities between layers. This can be seen when comparing the thickness of cortical lamina between segments of tissue from different functional areas of cortex. For example, when comparing tissue from M1 with tissue from primary visual cortex, M1 demonstrates large layers III and V, but an almost absent layer IV, whereas primary visual cortex shows an extensive layer IV but much reduced layers III and V (Kandel et al., 2000). Considering that M1 is primarily concerned with providing motor commands, whereas visual cortex is more concerned with integrating sensory information, and layers III and V are involved with the output of efferent information while layer IV is involved with the input of thalamic sensory information, it can be seen that these variations in cortical layers are reflecting the functional specificity of each cortical area.

A columnar organisation of cortical neurons has also been demonstrated, with individual columns oriented perpendicularly to the layers described above. These columns vary between 300 – 600 µm in width, contain groups of neurons having similar response properties, and are considered to be the fundamental computational units of the cortex (Kandel et al., 2000). Within M1, the main output from these cellular units arises from the pyramidal neurons of layer V. These outputs form direct projections to the spinal cord, where they synapse with spinal motor neurons in the ventral horn of the grey matter (Lemon, 2008). At the level of both cortex and spinal cord, the neurons of individual columns form complex connections within and between units. For example, within a column, 40% of these direct pyramidal connections will synapse with the spinal motor neuron pool of a specific muscle. The remaining neurons, however, do not innervate the same pool of neurons, instead projecting to motor neuron pools of different muscles that are involved in mediating similar movements.
The nature of the connectivity within this circuitry generates a powerful scope for integrative control of muscle groups. Furthermore, the divergence of connections between columns at the cortical level also provides the cellular basis for the highly plastic nature of M1.

1.1.2. Intracortical inhibition and \(\gamma\)-aminobutyric acid (GABA)

The brain’s major inhibitory neurotransmitter, \(\gamma\)-aminobutyric acid (GABA), is present in 25 – 30% of primate cortex (Jones, 1993) and serves as the primary inhibitory neurotransmitter at as many as 44% of cortical synapses (DeFelipe, 1993). GABAergic interneurons play important roles in the synchronisation of neural networks, the generation of cortical oscillations and the timing of pyramidal cell firing, thus facilitating the flow of information through the brain (Rudy et al., 2011). This diversity of function is mediated by a large range of cellular sub-types, each of which demonstrates unique morphology and molecular and physiological characteristics (Druga, 2009). These cells can be further defined based on the complex synaptic connections they make, with different sub-types targeting the soma, axon or dendrites of pyramidal cells within or between cortical layers and columns (Markram et al., 2004).

GABA is generated from glutamate by glutamic acid decarboxylase (GAD) and broken down to form succinate by the actions of both GABA-transaminase (GABA-T) and succinic semialdehyde dehydrogenase (SSADH; Petroff, 2002). The actions of this important amino acid are primarily mediated by two receptor classes, designated GABA\(_A\) and GABA\(_B\). A third receptor class, referred to as GABA\(_C\), has also been described previously (Cutting et al., 1991). However, more recent research has recognised GABA\(_C\) to be a specialised subtype of GABA\(_A\) and, subsequently, the International Union of Pharmacology has suggested it be referred to as GABA\(_{\alpha}\) (Barnard et al., 1998). GABA\(_A\) and GABA\(_B\) both demonstrate
extensive distributions throughout the mammalian brain. GABA_{\alpha_0} was initially thought to occur exclusively within the retina; however its presence has now been demonstrated more widely throughout the brain, as well as within parts of the peripheral and enteric nervous systems (Martinez-Delgado et al., 2010).

Structurally, GABA_A and GABA_B demonstrate different compositions. GABA_A is a ligand-gated ionotropic chloride channel having five transmembrane domains clustered around a central ion pore. These domains demonstrate multiple subtypes, designated \( \alpha, \beta, \gamma, \delta, \varepsilon, \pi \) and \( \rho \) (Macdonald & Botzolakis, 2009). GABA_A receptors are located postsynaptically, as well as on extrasynaptic membranes (Farrant & Nusser, 2005; Errington, 2014). At both locations, the influx of chloride ions after binding of GABA causes hyperpolarisation of the affected cell, generating an inhibitory post-synaptic potential (IPSP). GABA_B receptors, however, are metabotropic G-protein coupled in nature and consist of a heterodimeric structure. GABA_B also shows significantly more structural conservation than GABA_A, with only two variants known, referred to as GABA_{BR1} and GABA_{BR2} (Jones et al., 1998). Furthermore, GABA_B receptors are found both pre- and postsynaptically, as well as on extrasynaptic membranes (Benarroch, 2012). The influences of the GABA_B receptor are mediated by increased activation of potassium channels postsynaptically, reduced activation of voltage-dependent calcium channels presynaptically and inhibition of adenylyl cyclases (Benarroch, 2012).

Further distinctions can be made between these receptors based on their response characteristics. The ionotropic nature of GABA_A results in an ability to rapidly modulate membrane excitability, with peak activity levels occurring approximately 3 ms after receptor activation (Davies et al., 1990). However, the inhibitory potentials generated by activation of this receptor are rapid and short lived (Farrant & Nusser, 2005). The metabotropic nature of GABA_B results in a much more delayed change in the polarisation of the affected cell, with peak activity levels occurring 100 ms and 150 ms after activation of pre- and postsynaptic
receptors, respectively (Davies et al., 1990; Deisz, 1999). Furthermore, IPSP’s generated by GABA$_B$ activation also tend to be more prolonged in duration (Bettler et al., 2004).

As was mentioned above, GABAergic cells form extensive connections with pyramidal output cells. Within M1, this close apposition is thought to play a crucial role in producing effective and accurate motor control, a concept that has been extensively demonstrated in animal research. Using a monkey model, pharmacological disinhibition of motor cortex using drugs targeting GABA receptor function resulted in significant deficits during a precision grip task, and increased variability in a reaction time task (Matsumura et al., 1991). Furthermore, application of the same modulating agents to individual task-related cells resulted in altered specificity of discharge patterns during task progression, as well as activation of additional cells (Matsumura et al., 1992). These studies demonstrate that GABAergic cells are important in controlling populations of task-related cells, facilitating more specific cortical control. The functional relevance of GABAergic inhibition has also been demonstrated in humans using non-invasive transcranial magnetic stimulation (TMS; see section 1.2.).

1.1.3. Descending pathways in the control of movement

Several distinct neuronal pathways exist that are solely concerned with the control of skeletal muscle. These pathways originate in the cortex or brainstem and project to pools of motor neurons within the spinal cord. These pools are organised into two anatomically and functionally specific nuclei within spinal cord grey matter; those within the medial column that primarily innervate axial and proximal limb musculature, and those within the lateral column that primarily innervate distal limb musculature (Romanes, 1964). The pathways originating from the brainstem can be similarly divided into two groups having origins within either medial or lateral motor nuclei. Those with a medial origin (the tectospinal, reticulospinal and vestibulospinal tracts) project to the medial column motor nuclei of the
spinal cord (therefore controlling axial and proximal limb musculature) whereas those having a lateral origin (the rubrospinal tract) project to lateral column motor nuclei of the spinal cord (therefore controlling distal limb musculature; Kuypers, 1964).

The descending pathways that originate in the cortex, referred to as the corticospinal system, have contributions from several different functional areas, including primary motor, premotor, supplementary motor and cingulate motor cortices, as well as sensory and posterior parietal cortices (Lemon, 1997). The corticospinal system is formed mainly from the axons of pyramidal neurons in layer V (Murray & Coulter, 1981). These cells project out of the cortex via the internal capsule and form the medullary pyramids within the brainstem. At this level, approximately 75% of the fibres within the tract cross to the contralateral side of the brainstem, forming the pyramidal decussation (Chouinard & Paus, 2006). These fibres continue into the dorsal area of the lateral spinal cord column, forming the lateral corticospinal tract, and innervate dorsolateral motor nuclei. The fibres that fail to cross the midline at the medulla continue ipsilaterally into the medial column, forming the ventral corticospinal tract, and innervate ventromedial motor nuclei bilaterally. Subsequently, in humans, the ventral corticospinal tract is primarily concerned with the control of axial and proximal limb musculature, whereas the lateral corticospinal tract is primarily concerned with the control of distal limb musculature.

The projections made by the corticospinal tract can have a variety of terminations within the spinal cord (Lemon, 2008). Some of these projections affect the activity of motor neurons indirectly by first targeting interneurons located within the intermediate zone of the spinal cord grey matter (Lemon et al., 2004). The nature of motor output able to be generated by this oligosynaptic chain is inherently limited in diversity, due to a reliance on spinal circuitry. However, other corticospinal neurons demonstrate direct termination onto spinal motor neurons located within the ventral horn. These neurons, referred to as corticomotoneuronal
cells, mediate a higher degree of input from the cortex in controlling complex movements (Lemon et al., 2004). These monosynaptic connections between cortex and spinal motor neurons innervating intrinsic hand muscles are thought to be of particular importance in facilitating relatively independent finger movements, as well as fractionation of muscle activation (Lemon & Griffiths, 2005), activities which are fundamental in producing skilled hand function. For example, as the degree of dexterity increases from new world monkeys, to old world monkeys and great apes, to humans, parallel changes in the size and number of corticomotoneuronal projections are seen. These differences correlate with indices of manual dexterity (Courtine et al., 2007). Furthermore, experiments involving artificially induced lesions of the corticospinal tract within multiple species have supported its role in dextrous movement (Lemon, 2008).

1.1.4. Hand function and Grip-Lift tasks

The ability of the human hand to manipulate objects using discrete movements of individual digits underpins many essential activities of daily life and is facilitated by certain structural and functional characteristics. The most obvious of these can be seen when comparing the thumb and fingers. The fingers demonstrate relatively conserved structural form that allows the greatest degree of movement within the flexion-extension plane, facilitating the application of gross force. This includes a close positioning of carpometacarpal joints and a minimal rotational ability of metacarpophalangeal and interphalangeal joints (Taylor & Schwarz, 1955). The thumb, however, has a much higher range of motion mediated by its rotated first metacarpal and unimpeded carpometacarpal joint (van Duinen & Gandevia, 2011), subsequently allowing complex opposition with the fingers, and mediating the application of fine, graded forces during more delicate tasks. Further contributions to the skilled use of our hands can be seen when considering the hand as a whole. For example, multiple muscles acting around single joints, and many degrees-of-mechanical-freedom
conferred by multiple joints, allow a certain degree of independence between the hand’s structural components. This degree of independence is another important factor in achieving fine manipulations (Schieber & Santello, 2004).

The cortical processes involved in controlling hand muscles are also an important consideration in understanding human hand function. These processes are complex and not completely understood. However, key to skilled manipulation is the ability to move one digit independently of the others. While structural components play a role in mediating this (see above), neural control of muscles is also fundamentally important. Within the body of research assessing human hand function, the process of grasping and lifting an object between thumb and index finger (referred to as a precision grip) has been extensively researched and quantified. Johansson and Westling (1984) were the first to relate this grip-lift process to specific phases of finger force coordination essential to achieving tasks. Briefly, these are the preload phase (digit contact, initial force application), loading phase (scaling of grip and lift forces), transitional phase (object movement to target zone, compensation for inertial forces) and static phase (force stabilisation, maintenance of target position). This study also investigated how grip and lift forces relate to one another, demonstrating a precise temporal interaction. Subsequent research has used the specificities of these phases, and their temporal relationship, as indices of performance for assessing hand function. This approach was adopted in Chapters 2 and 3 to assess hand function in young and old adults.

The indices of performance derived from this grip-lift task have demonstrated sensitivities to even slight abnormalities in manual dexterity (Nowak, 2006). They have also been shown to reflect an extensive amount of information regarding the neurological control of dextrous movement (for review, see Johansson & Flanagan 2009). For example, when considering absolute grip force, initial magnitude is a product of higher order cognitive input, scaling reflects the ability to integrate multimodal sensory feedback into ongoing motor output, and
the rapid response to perturbation arises from the ability to generate and assess error codes based on predicted-versus-actual sensory response. Furthermore, application of forces slightly exceeding those required to maintain grip represent a safety margin partially reflecting integrity within sensory processes (Johansson & Westling, 1984). These factors demonstrate the relevance of using a grip-lift task in assessing human hand function.

1.2. Brain stimulation and Transcranial Magnetic Stimulation

1.2.1. Development of non-invasive brain stimulation (NIBS) techniques

Initial investigations into the cortical mechanisms of motor control in humans were restricted to the application of electrical current directly to the cortical surface (see section 1.1.1.). These experiments provided fundamental information upon which the modern understanding of the neural control of movement has been built. For obvious reasons though, the practicality of these investigations was limited. However, in 1980 Merton and Morton demonstrated that it was possible to activate the brain of intact, awake human subjects by applying high voltage electrical currents to the scalp (Merton & Morton, 1980). Although previous research using trains of electrical stimuli had demonstrated limited success (Gualtierotti & Paterson, 1954), Merton and Morton’s application of brief, individual, high-voltage pulses to the scalp over M1 demonstrated an ability to cause muscle twitches in the contralateral hand and foot. Furthermore, application of the same stimulus over primary visual cortex was also able to generate responses within the subject’s visual field, referred to as ‘phosphenes’. However, the high resistance of scalp and skull tissue to this transcranial electrical stimulation (TES) resulted in very little of the applied current penetrating to the brain. Instead, the current would activate superficial excitable tissues, generating contraction of scalp muscles, and activation of pain receptors. This resulted in subject discomfort and presented a significant limitation to the potential application of this technique. However, in 1985 experiments by Barker and colleagues provided a solution to these issues of resistance faced by Merton and Morton. This
group applied strong magnetic pulses to M1 by discharging high capacitance devices into coils of copper windings able to be held on the head over motor cortex (Barker & Jalinous, 1985). As the typically high resistance scalp and skull do not impede magnetic fields, the pulse was easily able to penetrate into the brain, subsequently generating an electrical current within the excitable neural tissue underlying the coil. The induced current resulted in the activation of corticospinal cells and generation of observable and recordable responses within contralateral hand and leg muscles. Furthermore, these responses were elicited without notable subject discomfort. This technique, referred to as transcranial magnetic stimulation (TMS), has now become common practice within many clinical and research settings worldwide, and has significantly facilitated our understanding of motor control in humans. As a method of functional brain imaging, TMS provides a very high temporal acuity and a relatively good spatial acuity. Furthermore, it has the ability to distinguish between excitatory and inhibitory activity within M1, a function that is not achievable by other neuroimaging techniques.

1.2.2. Transcranial Magnetic Stimulation (TMS)

Our understanding of how TMS activates the corticospinal system was facilitated by early studies in animal models. These studies demonstrated that application of an electrical stimulus directly to exposed motor cortex caused high frequency discharges within corticospinal neurons, which was seen as a complex descending volley. The characteristics of the first wave in this volley of activity were different to those of the later waves (Patton & Amassian, 1954). It was suggested that the first wave, referred to as the D-wave, occurred due to direct activation of corticospinal neurons by the stimulus, whereas the later waves, termed I-waves and appearing at a periodicity of approximately 1.5 ms, originated from interneurons causing trans-synaptic depolarising of the same corticospinal neurons. These findings have been replicated by numerous studies and significant evidence for this D/I-wave hypothesis of
corticospinal activation has been provided (Phillips, 1956; Kernell & Chien-ping, 1967). In the late 1980’s, this body of work in animal models was expanded to include results from human subjects by research using TMS. Day and colleagues investigated the response of single motor units to the application of cortical stimuli and, using post-stimulus time histograms, were able to indirectly demonstrate that activation of human corticospinal neurons also occurred according to a D/I-wave system (Day et al., 1989).

Direct evidence of this D/I-wave hypothesis occurring in humans has been provided by more recent studies recording corticospinal volleys directly from electrodes implanted in the cervical epidural space of patients undergoing treatment for intractable pain (Nakamura et al., 1996; Di Lazzaro et al., 1998b). These studies compared the corticospinal responses to both TES and TMS, demonstrating that the two methods of non-invasive brain stimulation (NIBS) activated the corticospinal tract differently. The first wave in the volley generated by application of TES was a D-wave, as was expected based on the previous animal studies. However, when the volley generated by TMS was compared to this, it was apparent that the onset latency of the first wave was approximately 1.5 ms later than was seen after TES, corresponding to the latency of the first I-wave (I1). Furthermore, as the intensity of TMS was increased to moderate and then to high levels, later I-waves became apparent, followed by a D-wave. These findings suggested that TMS preferentially activates corticospinal neurons within M1 trans-synaptically, with direct activation only occurring at high intensities, concepts that are now commonly accepted.

These differences in activation are thought to stem from the way in which the current from each technique interacts with the brain. While TES produces a current that travels radially from its source and penetrates into the brain, TMS induces a current that travels parallel to the surface of the brain (Rothwell, 1997). This results in cells oriented perpendicularly to the brain surface, such as the pyramidal neurons that contribute to the corticospinal tract, having a
higher threshold to TMS than TES (Rothwell et al., 1999), whereas the cells oriented parallel to the brains surface, such as the interneurons that synapse with corticospinal projections (Day et al., 1989), have a lower threshold to TMS. These suggestions were supported by studies altering the direction of TMS-induced current within the brain by changing the orientation of the stimulating coil. This manipulation of coil orientation changes the structures that are preferentially activated by the stimulus. When the current was directed anteriorly and perpendicular to the central sulcus (activation of elements parallel to the brains surface), responses from single motor unit recordings and surface electromyography (EMG) were delayed by approximately 1.5 ms, relative to currents directed latero-medially (activation of elements perpendicular to the brains surface; Werhahn et al., 1994). Furthermore, onset latencies of responses generated by application of latero-medially directed current were consistent with the latencies of responses generated by TES.

In healthy subjects, it is not possible to directly record the response of human corticospinal neurons to application of TMS. However, if components of the corticospinal descending volley are sufficiently large, their summation within the spinal cord can lead to depolarisation of spinal alpha motor neurons, subsequently generating a volley within peripheral neurons. This produces contraction of the target muscle and an electromyographic (EMG) potential referred to as a motor evoked potential (MEP). The amplitude of the MEP reflects the excitability of the neural elements activated by the pulse at the time of stimulus application (see section 1.2.3.2.). Generally, TMS is applied in one of three ways; as single pulses, as a set of two or three pulses (paired- and triple-pulse TMS) or as long trains of stimuli (repetitive TMS or rTMS). rTMS is used to induce neuroplastic changes within the cortex (Fitzgerald et al., 2006) but is beyond the scope of the current discussion and will therefore not be addressed further. In the following sections, single-, paired- and triple-pulse TMS techniques
will be described, and some of the measurements commonly recorded when applying these patterns of stimulation will be discussed.

1.2.3. Single-pulse TMS

Single-pulse TMS can be used to assess both excitatory and inhibitory processes within M1. Cortical excitability can be assessed using at least four different methods. The first is by mapping the cortical representation of the target muscle. This is achieved by systematically applying constant-intensity stimuli at neighbouring scalp sites until no MEP response is recorded (Wassermann et al., 1992). Graphical representations of the MEP amplitudes recorded at each location are then used to produce a cortical map of the target muscle. The second method is to apply stimuli of gradually increasing intensity to the cortical location that produces an optimum response in the target muscle, referred to as the cortical ‘hot spot’. Plotting the generated MEP amplitudes relative to the applied stimulation intensities produces a sigmoidal curve that represents corticospinal input-output properties (Devanne et al., 1997). The last two methods also apply stimuli at the cortical hotspot and are described in more detail below. A method for assessing cortical inhibition using single-pulse TMS is also described.

1.2.3.1. Motor threshold (resting and active)

Motor threshold is the lowest TMS intensity able to produce a reliable MEP within the target muscle. This measurement is ubiquitous across TMS studies and reflects the membrane excitability of corticospinal neurons innervating the target muscle, as well as the excitability of associated corticocortical interneurons (Rothwell et al., 1991; Paulus et al., 2008). It is also dependent on the excitability of synapses at cortical and peripheral levels (Devanne et al., 1997). Motor threshold is further divided into measurements recorded during complete relaxation of the target muscle (resting motor threshold or RMT) or while the muscle is
tonically active in holding a low intensity (usually 5 – 10% of maximum) isometric contraction (active motor threshold or AMT). Each of these measurements is defined based on specific requirements. RMT is the minimum intensity at which MEP’s ≥ 50 µV peak-to-peak amplitude are produced by 3 out of 5 (Carroll et al., 2001) or at least 5 out of 10 (Rossini et al., 2015) consecutive stimuli. AMT is similarly defined, but MEP’s are required to be ≥ 200 – 300 µV (Rothwell et al., 1999). Changes in cortical and spinal excitability during muscle activation means that the stimulation intensity required for AMT is lower than that for RMT, usually by approximately 20% (Garry & Thomson, 2009).

1.2.3.2. MEP amplitude
For most TMS experiments, changes in MEP amplitude (evoked using a standard intensity) in response to interventions or pathology are used as one of the main experimental outcomes. As suggested above, the amplitude of a MEP (peak-to-peak or area) reflects the excitability of the neural elements innervating the target muscle (Rothwell, 1997). However, it is also an indirect measure of the number of corticospinal neurons activated by the TMS pulse. Other factors which affect MEP amplitude include coil orientation and placement (Brasil-Neto et al., 1992), spinal motor neuron recruitment, synchronisation and firing and background activity (Kiers et al., 1993; Rösler et al., 2008). For experiments using changes in MEP amplitude as an experimental outcome, stimulus parameters are usually defined according to either the intensity required to produce a specific amplitude (usually 1 mV, peak-to-peak) or as a percentage of individual subject motor threshold (usually 120% - 130% RMT). Although some controversy exists as to which is the more appropriate method, both are currently accepted practice.

1.2.3.2. EMG silent period (SP)
The EMG silent period (SP) is a period of EMG silence following an MEP generated by suprathreshold single-pulse TMS during tonic activation of the target muscle (Fuhr et al.,
1991; Inghilleri et al., 1993). This suppression of activity has complex inhibitory contributions; the first 50 ms are thought to arise from spinal sites (after hyperpolarisation and recurrent inhibition of spinal motor neurons), while the latter part of the suppression is thought to be cortical in origin (Inghilleri et al., 1993; Brasil-Neto et al., 1995; Chen et al., 1999). Investigations into the cortical mechanisms mediating the latter part of the SP have used pharmacological interventions to suggest an involvement of the GABA$_B$ receptor (Siebner et al., 1998; Werhahn et al., 1999), although the exact mechanism is not clear. SP duration has been shown to be modulated by task complexity (Sale & Semmler, 2005) and to be different in patients with movement disorders (Edwards et al., 2008; Ni & Chen, 2012), suggesting possible functional importance.

1.2.4. Paired-pulse TMS

Paired-pulse TMS can be used to assess the influence of excitatory and inhibitory corticocortical projections onto corticospinal neurons. As the name suggests, this stimulation paradigm consists of two magnetic stimuli applied at short interstimulus intervals. The first stimulus, referred to as the conditioning pulse, activates the intracortical circuit to be assessed. The second stimulus, referred to as the test pulse, generates a MEP response (referred to as the ‘test MEP’) in the target muscle. When the conditioning pulse precedes the test pulse, the effects of each stimulus temporally summate at the corticospinal neuron, and changes in the amplitude of the test MEP are seen, compared to the test MEP generated by an unconditioned test pulse given alone. Activation of inhibitory circuits reduces the amplitude of the test MEP, whereas activation of excitatory circuits has the opposite effect. The nature of the circuit activated (inhibitory or excitatory) is determined by the intensity of the conditioning and test stimuli, as well as the time interval between stimuli (interstimulus interval or ISI). For example, when a conditioning stimulus below RMT (subthreshold) is applied 10 - 20 ms prior to a test stimulus above RMT (suprathreshold), facilitation of the test MEP is observed.
(Ziemann et al., 1996c) which is thought to be mediated by activation of glutamatergic interneurons and is referred to as intracortical facilitation (ICF; Paulus et al., 2008). The following sections will focus on two paired-pulse paradigms that assess the influence of different subsets of inhibitory interneurons on the activity of corticospinal output from M1.

1.2.4.1. Short-interval intracortical inhibition (SICI)

Short-interval intracortical inhibition (SICI) is a paired-pulse TMS technique that involves a subthreshold conditioning pulse being applied prior to a suprathreshold test pulse. Inhibition of the test MEP is seen if the interstimulus interval is less than 5 ms (Kujirai et al., 1993). Research comparing the descending volley generated by application of SICI, to the volley generated by the test pulse alone, has shown specific reductions in the I3 wave, while the early I-waves and D-wave are unchanged (Di Lazzaro et al., 1998c). These observations suggest that the reduction in test MEP amplitude observed during SICI has cortical contributions. Furthermore, the subthreshold nature of the conditioning pulse makes it unlikely that modifications to spinal excitability contribute to reductions in the amplitude of the test MEP. This is supported by studies demonstrating that this paradigm does not modify the H-reflex (Kujirai et al., 1993; Ziemann et al., 1996c). Studies investigating changes in the magnitude of inhibition in response to ingestion of pharmacological substances have demonstrated that inhibition of the test MEP most likely reflects a synaptic GABAergic mechanism mediated by activation of the GABA_A receptor subtype (Ziemann et al., 1996b; Di Lazzaro et al., 2000; Ilic et al., 2002). However, some evidence suggests that SICI measurements using an interstimulus interval of 1 ms may produce inhibition of the test MEP via alternative mechanisms (Fisher et al., 2002; Roshan et al., 2003; Vucic et al., 2011). Although the exact mechanism is not clear, previous work has suggested axonal refractoriness (Fisher et al., 2002), synaptic processes (Roshan et al., 2003; Vucic et al., 2011) and activation of extrasynaptic GABA_A receptors (Stagg et al., 2011) as possible contributing factors.
Several lines of evidence have demonstrated the importance of the inhibition reflected by SICI in mediating motor control. For example, the magnitude of SICI acting on a target muscle is reduced when that muscle is activated to perform a contraction (Ridding et al., 1995) suggesting that changes in SICI contribute to the generation of efferent output to target muscles. These changes in inhibition have been shown to occur prior to the onset and offset of EMG associated with voluntary contraction (Reynolds & Ashby, 1999; Buccolieri et al., 2004), providing good evidence that they occur prior to the changes in spinal excitability associated with voluntary contraction. Furthermore, when activation in neighbouring muscles is consciously limited, the decrease in inhibition is specific to the task-related muscle (Stinear & Byblow, 2003; Zoghi et al., 2003), suggesting that SICI may also play a role in the fractionation of muscle activation. Further evidence of SICI’s role in motor function can be seen in interventions that change both inhibition and motor performance (Zanette et al., 2004) and in motor control pathologies that demonstrate altered inhibition (for review, see; Berardelli et al., 2008). This last point is particularly interesting as recent work using somatosensory stimulation to normalise aberrant patterns of SICI in patients with focal hand dystonia have shown concomitant improvements in motor performance (Rosenkranz et al., 2009).

1.2.4.2. Long-interval intracortical inhibition (LICI)

A second paired-pulse paradigm involves suprathreshold conditioning and test stimuli separated by an ISI of 100 – 150 ms (Valls-Sole et al., 1992). This technique, referred to as long-interval intracortical inhibition (LICI), also produces inhibition of test MEP amplitude. As with SICI, experiments investigating the effects of LICI on the descending volley have demonstrated specific reductions in I-waves. In contrast to SICI though, LICI is associated with reductions in both I₂ and I₃, while I₁ and the D-wave are unaffected (Di Lazzaro et al., 2002b). Although these observations suggest that inhibition observed during LICI has cortical
contributions, the extent of these is currently unclear. The suprathreshold conditioning stimulus used in this paradigm generates a descending volley within corticospinal neurons, leading to activation and contraction of target muscles and modifications to spinal excitability. Although earlier work suggested that using ISI’s greater than 50 ms allows recovery of spinal excitability (Nakamura et al., 1997; Di Lazzaro et al., 2002b), more recent research using cervicomedullary stimulation to directly activate corticospinal axons have demonstrated that this may not be the case (McNeil et al., 2009, 2011). Despite this, studies recording the response to TMS using concurrent EEG recordings have shown significant correlations between changes in the TMS evoked cortical potential (TEP) and the amplitude of the MEP following application of LICI, providing strong evidence for a substantial cortical contribution to LICI (Rogasch et al., 2013). Furthermore, pharmacological investigations have demonstrated an ability to modulate the magnitude of inhibition recorded during LICI by applying drugs that affect GABAergic function through the GABA$_B$ receptor subtype (McDonnell et al., 2006).

Relative to SICI, LICI’s functional relevance has received considerably less investigation. However, changes in the magnitude of LICI have been observed during muscle activation (Hammond & Vallence, 2007; McNeil et al., 2011) and after a period of muscle fatigue (Benwell et al., 2007). Furthermore, interactions between SICI and LICI assessed using triple-pulse TMS (see section 1.2.5.) also imply an importance of LICI in motor control. In addition, complex interactions between SICI and LICI in different hand muscles have been demonstrated using somatosensory stimulation. Rosenkranz and Rothwell (2003) showed that vibrational stimulation applied to a target muscle reduces SICI and increases LICI for that muscle, but produces opposite effects on inhibition in neighbouring hand muscles not targeted by stimulation. These observations extended previous observations that intracortical inhibition
may facilitate muscle fractionation (Zoghi et al., 2003) by suggesting that LICI may mediate changes in SICI (Rosenkranz & Rothwell, 2003, 2004).

1.2.5. Triple-pulse TMS

While paired-pulse TMS allows an assessment of activity within particular inhibitory and excitatory circuits, triple-pulse TMS allows an assessment of interactions between these circuits. Within this technique, the patterns of stimulation used to assess individual paired-pulse measurements are combined into a single stimulation paradigm containing 3 or 4 TMS stimuli at very close intervals (Ni et al., 2011b; Fig. 1.1.). Using this technique, interactions between many paired pulse measurements have been investigated, including LICI and ICF (Sanger et al., 2001), LICI and short-interval intracortical facilitation (SICF, a low threshold excitatory circuit; Cash et al., 2011) and interhemispheric inhibition (IHI) and SICI, LICI and ICF (Daskalakis et al., 2002; Udupa et al., 2010). Furthermore, interactions between different brain regions have also been studied, such as between the cerebellum and intracortical circuits of the motor cortex (Daskalakis et al., 2004). However, one of the most investigated interactions has been between the circuits mediating SICI and LICI (Fig. 1.1.), which is seen as a reduction of SICI when assessed in the presence of LICI (Sanger et al., 2001). Several lines of evidence have suggested that this disinhibition represents an autoinhibition of inhibitory interneurons responsible for SICI via activation of pre-synaptic GABA_B receptors by collateral branches of LICI neurons (Werhahn et al., 1999; Sanger et al., 2001; McDonnell et al., 2006; Muller-Dahlhaus et al., 2008; Ni et al., 2011a).

Some evidence supporting a relevance of these interactions to motor control has been reported. As mentioned above (see section 1.2.4.2.), a role for LICI-SICI interactions in somatosensory function has been previously suggested (Rosenkranz & Rothwell, 2003, 2004). Furthermore, these interactions are modulated by muscle activation (Ni et al., 2007) and are
different in subjects with Parkinson’s disease (Chu et al., 2009). However, the functional importance of these interactions required further investigation. This will be one focus of the current thesis, with the healthy ageing process used as a model for changes in motor function (Chapter 5).

**Figure 1.1** Schematic showing how the individual paired-pulse TMS paradigms used to assess LICI (A) and SICI (B) are combined during triple-pulse TMS into a single pattern of stimulation (C). Arrows indicate the stimulus artefact associated with the application of each TMS pulse.

### 1.3. Healthy ageing, intracortical inhibition & TMS

The healthy ageing process is accompanied by significant changes in anatomy and physiology that culminate in altered functionality. As life expectancy is increasing, the impact of these
anatomical and physiological changes is becoming more apparent, both medically and psychosocially. Subsequently, understanding the changes occurring during this process will facilitate better treatment, resulting in a more comfortable transition into senescence, and a reduction in the burden on medical and welfare infrastructure. The following sections will provide a brief overview of some of the neural and muscular changes specific to motor control that are known to occur during the ageing process, as well as a summary of the few studies that have used TMS to assess intracortical inhibition in older adults.

1.3.1. Age-related changes in form and function

1.3.1.1. Brain Morphology, neural activation and neurochemistry

The healthy ageing process is known to be accompanied by extensive changes to all areas of the brain. Some of the most apparent changes can be seen on a gross anatomical level as increases in the volume of the ventricles (and other cerebrospinal fluid cavities), widening of the sulci, gyral atrophy and general reductions in brain weight and volume (Anderton, 1997, 2002). Early investigations into age-related reductions in brain volume suggested a key role of neuronal loss in facilitating the observed changes (Brody, 1955). However, limitations in stereological methodology may have confounded these findings, with more recent studies suggesting that neuronal loss is not as extensive as first thought (Haug & Eggers, 1991; Pakkenberg & Gundersen, 1997). Rather, these changes are more likely related to reductions in synaptic density with age (Masliah et al., 1993).

Investigations into the distribution of age-related structural changes have produced a range of sometimes conflicting findings (for review, see; Raz & Rodrigue, 2006). This variation in results most likely stems from methodological differences, including the imaging technique, characteristics of the subject cohort and study design (cross-sectional or longitudinal). Despite this, there is a general consensus that the brain demonstrates regionally specific sensitivities to
the effects of ageing, with different effects of age seen when comparing different cortical areas (Resnick et al., 2003; DeCarli et al., 2005), subcortical structures (Raz et al., 2005; Walhovd et al., 2011) and white and grey matter (Ge et al., 2002; Salat et al., 2004; Salat et al., 2005). Several studies have also demonstrated variations in the rate of volumetric change between different brain areas at different points in the lifespan (Walhovd et al., 2011). Some structures, such as the cerebral cortex, demonstrate linear reductions from a young age, whereas other structures, such as the hippocampus and cerebral white matter, show initial increases in volume in the younger years of life, a plateau in the middle years of life and a rapid decrease in later years. The physiological processes underpinning these linear and non-linear affects are not completely understood. However, it has been suggested that aspects of these trends occurring during the early years of life reflect maturational processes, whereas those occurring during later life represent brain ageing (Ge et al., 2002; Raz et al., 2005).

Interestingly, reports of reduced cortical thickness in precentral gyrus (M1; Salat et al., 2004) and degradation in areas of corticospinal tract white matter, including the internal capsule (Salat et al., 2005) demonstrate specific effects of ageing on brain areas important in motor control.

Along with changes in brain structure, the ageing process has also been shown to affect patterns of neural activity in older individuals. These changes in activation, typically captured using positron emission tomography (PET) or functional magnetic resonance imaging (fMRI) whilst subjects engage in specific tasks, are typified by larger, less lateralised areas of activation, with prominent bilateral activation in prefrontal areas (Cabeza, 2001). This specific pattern of increase in neural activity was termed hemispheric asymmetry reduction in older adults (HAROLD; Cabeza, 2002). Two alternative hypotheses have been put forward to explain this pattern of activity; compensation and dedifferentiation. The compensation model suggests that the increased pattern of activity in older subjects represents a form of neural
plasticity that compensates for the structural and functional deficits inherent within the ageing process (Cabeza et al., 1997). Contrary to this, the dedifferentiation model suggests that HAROLD results from a breakdown in neural machinery with age, leading to non-specific and inefficient activation of brain areas in the elderly (Logan et al., 2002). Similar to effects of ageing on neural activity patterns during cognitive tasks, older adults also exhibit increased prefrontal activation during motor tasks. Furthermore, bilateral activity has also been reported in primary sensory and motor areas during motor tasks (Mattay et al., 2002), and altered activity profiles have also been reported within cortical-subcortical neural loops, including cortico-cerebellar (for review, see; Bernard et al., 2013) and cortico-basal ganglia (Taniwaki et al., 2007; Marchand et al., 2011) connections. However, studies investigating neural activation levels and motor control have mainly found the increased levels of activity in older subjects to be beneficial to performance (for opposing view, see; Bernard & Seidler, 2012), supporting the compensatory hypothesis (Seidler et al., 2010).

Ageing is also known to be associated with alterations in neurochemistry. Reductions in acetylcholine, serotonin, noradrenalin and dopamine in various brain regions have all been reported to accompany the healthy ageing process (Gottfries, 1990). These changes in neurotransmitters are also thought to be involved with the progression of cognitive and motor pathologies commonly found in the elderly, such as Alzheimer’s disease, depression and Parkinson’s disease (Seidler et al., 2010). From animal models, it is also apparent that glutamate, the major excitatory neurotransmitter within the CNS, is reduced with age (Segovia et al., 2001). In support of this finding, a recent study using magnetic resonance spectroscopy (MRS) in humans has demonstrated age-related reductions in glutamate within M1 (Kaiser et al., 2005).
1.3.1.2. The motor unit

Age-related neurophysiological changes occur not just centrally, but also in peripheral structures such as the spinal cord and within skeletal muscle. An important example of these changes can be found in the way that motor units are remodelled in elderly people. At the core of this remodelling is a loss of alpha motor neurons via apoptosis (Brown, 1972), a process resulting in denervation of muscle fibres (Roubenoff & Hughes, 2000). As a result, the denervated fibres either die (referred to as sarcopenia) or are reinnervated by surviving motor neurons via axonal sprouting (Lang et al., 2010). If reinnervated, the newly added muscle fibre changes its properties (contraction speed, fatigability etc.) to those of the recruiting motor unit. These changes result in reduced cross sectional area (CSA) and contraction speed of the muscle, leading to declines in strength. This is reflected by a 20 – 40% reduction in strength in individuals within their seventh and eighth decade of life, relative to younger individuals (Doherty, 2003). Furthermore, motor unit remodelling also results in an increased motor unit innervation ratio (IR), leading to larger twitch forces at lower contraction intensities and reduced steadiness of force application at low contraction intensities (Galganski et al., 1993).

1.3.1.3. The Hand

The human hands ability to finely manipulate objects is instrumental in achieving fundamental daily activities such as dressing, using cutlery and opening containers. Age-related changes in the structure and function of the hand have been shown to degrade this ability, with the resulting functional deficits predicting hospitalisation or admittance into aged care institutions (Siu et al., 1993). Subsequently, age-related loss of hand motor skills has important ramifications for the quality of life of elderly citizens (Scherder et al., 2008). Some of the anatomical changes contributing to this functional deficit include altered tendon structure, as well as bone and joint deformation associated with osteoarthritis and rheumatoid
arthritis. An in-depth discussion of these factors is beyond the scope of the current review. However, see Carmeli et al. (2003) for a more comprehensive discussion of this topic.

In terms of functional alterations within the ageing hand, previous studies have produced an extensive investigation of age-related changes in the ability to coordinate finger force and use anticipatory control to achieve fine manipulation (for review, see; Diermayr et al., 2011), the importance of which was described previously (see section 1.1.4.). This body of research has shown that the ability to predict and scale forces required to lift an object (anticipatory control) is largely intact. However, one of the most consistently observed deficits in older subjects is the application of excessive grip forces and use of an elevated safety margin (Kinoshita & Francis, 1996; Cole et al., 1999; Cole & Rotella, 2001). Furthermore, the temporal aspects of force application and lift (preload and load phases) are delayed with age. Also, when task complexity is increased, or a dual-task paradigm is used, older subjects demonstrate increased force variability (Voelcker-Rehage et al., 2006).

1.3.2. Age-related changes in GABA

An extensive literature has investigated age-related changes to multiple aspects of GABAergic function within many brain regions, including both cortical and subcortical locations. However, the clear majority of studies have utilised animal models. While this research has suggested that the ageing process does not seem to modify the ability of agonists to activate GABA receptors (for review, see; Rissman & Mobley, 2011), it does appear to cause reductions in the number of GABAergic neurons (Miettinen et al., 1993; Hua et al., 2008; Ouellet & de Villers-Sidani, 2014), subunit-specific alterations to the structure of GABA receptors (Rissman et al., 2006; Caspary et al., 2013) and reductions in levels and activity of glutamic acid decarboxylase (GAD; Allen et al., 1983; Raza et al., 1994; Ling et al., 2005; Burianova et al., 2009), but not GABA transaminase (GABA-T; Raza et al., 1994).
Although several studies have reported conflicting effects of age within this literature (Allen et al., 1983; Wenk et al., 1991; Milbrandt et al., 1994; Turgeon & Albin, 1994), the majority appear to suggest that ageing results in reduced GABAergic inhibitory activity. In addition, the functional importance of this reduced inhibitory tone has been demonstrated by studies showing that normalising GABAergic activity profiles in old animals can improve visual (Leventhal et al., 2003) and cognitive (El Idrissi, 2008) performance.

Direct evidence for age-related alterations in human GABAergic function is significantly more limited than that in animal models. Despite this, analysis of post-mortem tissue has demonstrated reduced levels of GABA (Spokes et al., 1980) and reduced activity of GAD (Cote & Kremzner, 1974; McGeer & McGeer, 1976) in older adults, observations that may support an age-related decrease in GABAergic function. However, more recent investigations have utilised non-invasive means to further investigate effects of age on GABA. One example of this is the use of MRS to demonstrate that GABA concentrations are reduced within several different brain regions as a function of age (Gao et al., 2013; Rowland et al., 2015), supporting findings in post-mortem tissue. However, due to its ability to very easily assess the activity of intracortical inhibitory circuits within primary motor cortex, TMS has been the most commonly utilised method to non-invasively measure age-related changes in GABAergic function. In the following sections, I will compare and contrast the findings from work using TMS to investigate age-related changes in intracortical inhibition within the brains cortical motor areas, highlighting where possible how effects of age on GABAergic processes could relate to deficits in motor function in the elderly.

1.3.2.1. Age-related changes in SICI

Age-associated changes in intracortical inhibition have most often been studied using the SICI paradigm. The findings, however, have demonstrated significant inter-study variability, with reported decreases (Peinemann et al., 2001; Hinder et al., 2011; Marneweck et al., 2011;
Beynel et al., 2014), increases (Kossev et al., 2002; McGinley et al., 2010) or no change (Wassermann, 2002; Oliviero et al., 2006; Rogasch et al., 2009; Smith et al., 2009; Cirillo et al., 2010; Cirillo et al., 2011; Fujiyama et al., 2011; Smith et al., 2011; Fujiyama et al., 2012b; Stevens-Lapsley et al., 2013) in the magnitude of inhibition with age. Although the cause of this variation is not clear, a large methodological heterogeneity almost certainly plays a role.

Previous investigations have shown that several factors affect the magnitude of inhibition recorded during SICI. Of these factors, the intensity of the conditioning stimulus is an important consideration (Orth et al., 2003). When examining the body of work assessing changes in SICI with age, a large range of conditioning intensities have been used. The intensities used by some of these studies (Peinemann et al., 2001; Wassermann, 2002; Oliviero et al., 2006) are known to produce measurements confounded by the influences of low threshold excitatory circuits within M1 (Peurala et al., 2008), suggesting that these reports may not be truly reflective of age-related changes in SICI. Interestingly, two of the three studies reporting an increase in inhibition with age used conditioning parameters very likely to be contaminated by the influence of excitatory processes (Peinemann et al., 2001; Marneweck et al., 2011).

The best method for assessing SICI is thought to be the application of a range of different conditioning stimulus intensities. This produces what is referred to as a SICI recruitment curve, which demonstrates a complete profile of SICI over its recruitment range. This technique allows a more comprehensive assessment of SICI characteristics when comparing different populations with unknown intracortical inhibition recruitment profiles (Rosenkranz et al., 2007). Within the TMS studies assessing age-related changes in SICI, four studies have used this technique. Three of these applied three conditioning intensities of 70, 80 and 90% AMT (Rogasch et al., 2009; Cirillo et al., 2010; Sale et al., 2015) while the fourth applied 7
Intensities ranging from 60% - 120% AMT in 10% increments (Smith et al., 2009). While three of these studies reported no age-related changes in inhibition at any point in their recruitment curves (Rogasch et al., 2009; Smith et al., 2009; Cirillo et al., 2010), the fourth reported an increase in inhibition in old adults (Sale et al., 2015). However, this was only observed using an AP stimulus direction, producing a posteriorly directed cortical current that preferentially recruits I3-waves (Zoghi et al., 2003). This observation could suggest that some of the inconsistent effects of age on SICI may relate to the limitations of PA stimulation to assess changes in the late I-waves.

Test TMS characteristics can also influence the magnitude of inhibition recorded during SICI. Modulation of inhibition has been demonstrated for variations in both test MEP amplitude (Sanger et al., 2001) and test TMS intensity (Garry & Thomson, 2009), and these observations have caused contention as to which is the best method for setting test TMS parameters; relative to threshold or as a target amplitude. Within the ageing and inhibition literature, both techniques have been used. However, the process of targeting specific test MEP amplitudes (usually 1 mV, peak-to-peak) has been the preferred method. Despite this, studies using the same technique for setting test TMS parameters have still produced inconsistent effects of age on intracortical inhibition (Peinemann et al., 2001; McGinley et al., 2010; Cirillo et al., 2011). At first glance, this may suggest that test TMS parameters may not be a crucial factor in explaining the reported inter-study variation. However, this conclusion may be premature, and further investigation of this factor will be one focus of the current thesis (Chapter 3).

Interstimulus interval (ISI) and target muscle are two other factors that have varied between studies investigating effects of age on SICI, although to a lesser extent than the factors mentioned above. For ISI, values of 1 - 5 ms have all been used, spanning the range known to produce inhibition (Kujirai et al., 1993). However, 2 and 3 ms ISI’s have been used by the
majority of studies, with only a single study (Peinemann et al., 2001) having investigated the 1 ms ISI that is thought to be mediated by alternative mechanisms (see section 1.2.4.1.). In regards to the target muscle, the large majority of studies have targeted intrinsic hand muscles (first dorsal interosseous, abductor pollicus brevis, flexor pollicus brevis and abductor digiti minimi). Other studies, however, have targeted extrinsic hand muscles located on the forearm (flexor and extensor carpi radialis; Kossev et al., 2002; McGinley et al., 2010) as well as the quadriceps muscles of the leg (Stevens-Lapsley et al., 2013). As measurements of inhibition are thought to vary between hand and forearm muscles (Wu et al., 2002), comparisons between them may not be appropriate.

In addition to measurements in resting muscle, a limited number of previous studies have investigated age-related changes in the activity-dependent modulation of SICI. Surprisingly, only one of these assessed effects of age during a tonic contraction. Using a 2 mV test MEP and targeting muscles of the forearm, McGinley and colleagues reported that SICI in active muscle was not affected by age (McGinley et al., 2010). Despite this, other investigations of age-related changes in the activity-dependent modulation of SICI have focussed on changes in inhibition in the time leading up to contraction. As mentioned above (see section 1.2.4.1.), reductions in the magnitude of SICI during muscle activation have been shown to occur prior to the onset of EMG in young subjects, suggesting a role of SICI in facilitating contraction. In two studies, Fujiyama and colleagues investigated age-related changes in this inhibitory modulation by comparing changes in SICI during a go/no-go reaction time task between young and old subjects (Fujiyama et al., 2011; Fujiyama et al., 2012b). While both of these studies suggested that the ability to modulate SICI leading up to contraction is maintained with age, significant correlations between premotor time (time between signal to respond and onset of EMG), and the magnitude of SICI in the time leading up to the response signal, suggested that greater inhibition in older but not younger adults may be associated with
reduced reaction time (Fujiyama et al., 2012b). In contrast, a more recent study by Heise et al. (2013) has also used a reaction-time paradigm to show a significant reduction in the ability of old adults to modulate SICI prior to contraction. Furthermore, deficits in SICI modulation correlated with reductions in fine motor performance (Heise et al., 2013). These observations suggest that the ageing process results in an altered ability to modulate inhibitory tone, and that this has ramifications for motor control.

1.3.2.2. Age-related changes in LICI and the EMG silent period (SP)

In contrast to SICI, only one study has assessed the effects of ageing on LICI. In a cohort of twenty-one young (21.4±0.8 years) and nine old adults (70.9±1.8 years) McGinley et al. (2010) showed that the magnitude of inhibition produced during LICI was increased in older subjects. The stimulus parameters used by this study were in line with those commonly applied; conditioning and test stimuli were separated by an ISI of 100 ms and set at the intensity producing an MEP of 0.5 – 1 mV peak-to-peak amplitude. An ISI of 100 ms has found routine use as it produces maximal inhibition. However, more recent research has suggested that this interval may not be sufficient to allow recovery of spinal excitability after the suprathreshold conditioning stimulus (see section 1.2.4.2.; McNeil et al., 2009; McNeil et al., 2011), suggesting that the findings of McGinley and colleagues may be confounded by spinal factors.

Although age-related changes in LICI have only been assessed in one study, age-related changes in GABA_B receptor mediated inhibition have also been investigated by several studies by comparing the duration of the EMG SP between young and old subjects. As was the case for SICI, these studies have used a range of methodological approaches (different target muscles, contraction intensities and stimulus intensities) and reported discrepant findings. Two reported decreased SP in older subjects (Sale & Semmler, 2005; Oliviero et al., 2006), both of which targeted first dorsal interosseous and applied relatively similar stimulus
intensities (Sale and Semmler, 120% RMT; Oliviero et al., 150% AMT). The contraction intensity, however, differed between studies, with Sale and Semmler requiring subjects to maintain their rectified EMG level at 5% of the maximum EMG level, while Oliviero and colleagues used a higher intensity contraction of 50% MVC. Sale and Semmler also compared differences in SP between hands, and during different prehensile patterns, including power grip, precision grip or scissor grip. Interestingly, results of this study demonstrated greater reductions in SP in old subjects during more functionally demanding prehensile tasks (e.g. scissor grip vs. power grip; Sale & Semmler, 2005), suggesting older subjects require greater modulation of ICI to achieve more complex tasks.

All three of the remaining studies that investigated age-related changes in SP duration targeted extrinsic hand muscles (flexor or extensor carpi radialis). Of these, one reported an increased duration with age (McGinley et al., 2010), while two reported no effect of age (Fujiyama et al., 2009; Fujiyama et al., 2012a). It should be noted that these studies employed various stimulation intensities (McGinley et al., 2010, 130% AMT; Fujiyama et al., 2009, 140% RMT; Fujiyama et al., 2012, 130% RMT). Furthermore, while McGinley et al. (2010) used a 15% MVC contraction intensity, the Fujiyama studies did not define specific contraction levels for their subjects. This was due to the nature of the task employed by these studies, which involved phasic flexion/extension of the hand according to oscillations performed at self-determined amplitudes but at a specific frequency (1 Hz). As SP duration is affected by contraction intensity (Hammond & Vallence, 2007), this lack of specific contraction parameters could, in its self, explain the divergent findings presented by these studies. However, it also seems possible that the higher functional demand of the task utilised by Fujiyama and colleagues may have confounded potential comparisons with other studies investigating age-related changes in the SP.
1.4. Further characterising age-related changes in ICI
From the previous section it is apparent that over the past decade, investigations of age-related changes in intracortical inhibition have developed into a moderate body of literature. However, despite the frequency of investigation, findings from individual studies have often been inconsistent, limiting our ability to profile how normal healthy ageing modulates intracortical inhibition, and whether changes in inhibition contribute to motor deficits. Subsequently, our ability to recognise profiles of unhealthy intracortical inhibition in the elderly, which may be clinically useful, is inadequate. The heterogeneity within this literature is almost certainly multifactorial, with variations in methodology and subject characteristics being important considerations. Furthermore, although several studies have investigated effects of age on inhibition, in some respects, the scope of investigation has been relatively narrow. For example, most studies have been limited to measurements in resting muscle, and all have focussed on the activity of isolated cortical circuits. Because of this, many lines of research remain to be pursued. In the following sections, I will describe the factors investigated within this thesis to further understand if and how the ageing process affects intracortical inhibition in motor cortex.

1.4.1. Corticospinal input-output properties
The response of the corticospinal system to variations in TMS intensity (input-output properties) is highly non-linear, demonstrating a sigmoidal rise in MEP amplitude (Capaday, 1997; Devanne et al., 1997; Carroll et al., 2001). It has been suggested that several factors, involving both central and peripheral components, contribute to this effect (Devanne et al., 1997). For example, the number of waves within the corticospinal descending volley induced by TMS are stimulus dependent (Di Lazzaro et al., 1998a) and have different relative contributions to the evoked MEP (Thickbroom, 2011), and the application of progressively stronger stimuli activates spinal motoneurons with greater motor unit potentials (Henneman,
1957; Devanne et al., 1997). This lack of linearity has also been shown to affect measurements of intracortical inhibition, with previous work showing a sensitivity of both SICI and LICI to test TMS intensity (Garry & Thomson, 2009; McNeil et al., 2011) and test MEP amplitude (Sanger et al., 2001; Roshan et al., 2003). The effect of test TMS intensity on inhibition has been suggested to stem from changes in the relative contribution of the late I-waves (I₃) to the test MEP due to stimulus-dependent variations in the composition of the corticospinal descending volley (Garry & Thomson, 2009). However, effects of test MEP amplitude on inhibition have been suggested to reflect a differential sensitivity of the cortical elements activated by low and high intensity stimulation to the inhibitory circuits mediating SICI and LICI (Sanger et al., 2001).

More recently, it has been suggested that non-linearities introduced by peripheral factors may contribute to these variations in intracortical inhibition. In a study by Lackmy and Marchand-Pauvert (2010), measurements of SICI were recorded using test TMS intensities ranging from threshold levels, to intensities producing a maximum MEP. Regrouping inhibition measurements based on the test alone MEP’s percentage of the maximum muscle response (maximum M-wave; reflects maximum activation of the spinal motoneuron pool, assessed using percutaneous electrical stimulation of the axons of alpha motoneurons) subsequently demonstrated changes in the magnitude of SICI when the test MEP activated different proportions of the motoneuron pool (Lackmy & Marchand-Pauvert, 2010). The authors suggested that this change in inhibition was due to disproportionate contributions of small and large motor units to the MEP, with the inactivation of large motor units when using a large test MEP causing a relatively greater decrease in MEP amplitude, subsequently appearing to cause an increase in inhibition.

Corticospinal input-output properties are thought to be altered in old adults, with previous research suggesting a rightward shift in MEP input-output curves (Pitcher et al., 2003).
Furthermore, the loss of spinal motoneurons and subsequent remodelling in skeletal muscle associated with the ageing process (see section 1.3.1.2.) results in a reduced motoneuron pool that can be seen as a reduced amplitude maximum M-wave (Cirillo et al., 2010; Cirillo et al., 2011). These observations may have significant effects on comparisons of inhibition between young and old subjects. However, given the findings of Lackmy and Marchand-Pauvert, the age-related reduction in the maximum M-wave may be particularly important. Because of this effect, any given MEP amplitude will represent a greater proportion of the maximum M-wave for old, as opposed to young subjects. As the majority of studies investigating ageing and ICI have matched MEP amplitude between young and old subjects (see section 1.3.2.1.), the confounding effects of spinal motor neuron recruitment demonstrated by Lackmy and Marchand-Pauvert may partially explain some of the inconsistent results within this body of work. A clarification of the effects of spinal motoneuron recruitment on comparisons of inhibition between young and old subjects is therefore required. This will be assessed in Chapters 2 and 3 by measuring SICI and LICI in young and old subjects at a range of test TMS intensities (subsequently producing a range of test MEP amplitudes), and then normalising the magnitude of inhibition to the test MEP’s proportion of $M_{max}$.

1.4.2. Activity-related cortical disinhibition

Variations in SICI and LICI as a result of muscle activation partially demonstrate the functional relevance of these measurements (see section 1.2.4.). As was shown above (see section 1.3.2.), assessments of age-associated changes in paired-pulse intracortical inhibition have focussed almost exclusively on measurements recorded in resting muscle. It seems likely that this limitation may have contributed to the inability of previous research to demonstrate effects of age on intracortical inhibition, and relate changes in inhibition to age-related deficits in hand function (Marneweck et al., 2011). The importance of this neglected aspect of investigation is further suggested by a study reporting that increased SICI in the time directly
prior to contraction produces a reduced reaction time in old but not young adults (Fujiyama et al., 2012b), suggesting that age-related changes in the activity-dependent modulation of inhibition may have functional ramifications. These observations necessitate further investigation of age-related changes in inhibitory tone during muscle activation. This will be addressed in Chapters 2 and 3 by applying SICI and LICI paradigms during both rest and active states. Activation of target muscles will be via precision grip, which will serve to increase the functional demand of the task. Furthermore, the practical implications of any changes in inhibition will be assessed by relating them to indices of hand function derived from grip-lift performance measures.

1.4.3. Task-related cortical disinhibition

Several lines of evidence suggest that the modulation of intracortical inhibition during voluntary contraction is not determined purely by the level of activation of the muscle, but also by the way in which the muscle is being used. This task-dependency of intracortical inhibition was first reported by Liepert et al. (1998), who assessed measurements of SICI before and after a series of thumb abductions during which subjects were allowed to either freely abduct the thumb in a self-directed manner, or were required to isolate muscle activation to thumb musculature while relaxing the rest of the hand. This study found that non-isolated thumb abductions did not affect SICI, whereas focussed abduction of the thumb reduced SICI in the abductor pollicis brevis (APB) muscle, but increased SICI in the fourth dorsal interosseous muscle (Liepert et al., 1998). Although measurements within this study were made in resting muscle, a subsequent study by Zoghi et al. (2003) reported a similar differential modulation of SICI in three intrinsic hand muscles when comparing isolated and non-isolated tonic thumb abductions (although this effect was only apparent when the magnetic stimulus produced a posteriorly directed cortical current).
Further evidence for task-dependent variations in intracortical inhibition can be found in studies comparing the response to paired-pulse TMS during isolated and synergistic contractions. A study by Devanne et al. (2002) found that the disinhibition of SICI that was normally observed during isolated activation of a hand muscle was significantly increased when a shoulder muscle was coactivated with the hand during a pointing task. Furthermore, a subsequent study by Kouchtir-Devanne et al. (2012) supported these findings for SICI, and observed similar task-dependent changes in LICI, but did so by comparing isolated index finger abduction with synergistic precision grip between the index finger and thumb. These findings led the authors to suggest that task-dependent changes in both SICI and LICI mediate the functional coactivation of cortical areas innervating task-related muscles (Kouchtir-Devanne et al., 2012). In support of this, animal research has suggested a role of GABA in mediating the generation of muscle synergies. For example, Schneider et al. (2002) showed that application of bicuculline (GABA$_A$-receptor antagonist) to an area of motor cortex allowed microstimulation of a separate cortical area to produce activation of muscles innervated by both the stimulated and pharmacologically disinhibited areas, whereas stimulation prior to disinhibition had only produced activation in muscles innervated by the stimulated area (Schneider et al., 2002).

The studies above suggest that the task-dependent modulation of intracortical inhibition represents a functionally relevant mechanism that may be important in motor control. This could suggest that altered task-dependent inhibitory tone may reflect, or even contribute to, deficits in motor function. Some evidence suggests that the ageing motor system may provide an example of this. As mentioned above (see section 1.3.2.2), a study by Sale and Semmler (2005) used measurements of the SP duration to demonstrate that older subjects require greater attenuation of activity in inhibitory circuits during more complex tasks (Sale & Semmler, 2005). However, no study has investigated age-related changes in the task-
dependent modulation of the response to paired-pulse TMS. In Chapter 4 we will further investigate the task-dependency of intracortical inhibition in young subjects by comparing the modulation of both SICI and LICI between complete relaxation of the hand, isolated index finger abduction and precision grip between the index finger and thumb. In Chapter 5, a cohort of older adults will complete the same motor tasks used in Chapter 4, and the findings will be compared to those found in the young group.

1.4.4. Interactions between inhibitory circuits

As was mentioned previously, many of the circuits assessed using TMS have been shown to interact with each, and these interactions may have a role in motor function (see section 1.2.5.). However, within current literature, no study has specifically investigated age-related changes in the LICI-SICI interaction. However, some evidence suggests that this interaction may be altered by age. In a cohort of older adults (> 60 years old), Chu et al. (2008) showed normal LICI-SICI interactions for measurements using a 100 ms ISI (between LICI’s conditioning stimulus and the test stimulus), but no interaction between the paradigms for measurements using a 150 ms ISI (Chu et al., 2008). As previous work in young subjects has shown LICI-SICI interactions for ISI’s in excess of 190 ms (Cash et al., 2010), these observations may suggest an age-related reduction in the interaction between LICI and SICI. This will be assessed in Chapter 5 by using triple-pulse TMS to compare the effect of LICI on SICI between young and old subjects.

In young subjects, previous research has reported a period of cortical disinhibition at long intervals (~190 ms) following a TMS stimulus, and this was suggested to represent activation of presynaptic GABA_B receptors (Cash et al., 2010). Task-specific changes in the onset of this disinhibitory phase have been suggested to drive task-related variations in SICI (Kouchtir-Devanne et al., 2012; for opposing view, see; Caux-Dedeystère et al., 2014).
suggesting that interactions between LICI and SICI may also be task-dependent. Furthermore, if the task-dependent modulation of SICI and LICI is different in old adults (see previous section), age-related changes in the effects of task on LICI-SICI interactions may be a contributing mechanism. However, effects of task and age on LICI-SICI interactions have not been previously investigated. In Chapter 4, task-related changes in this interaction will be assessed in young subjects by comparing the response to triple-pulse TMS during complete relaxation, isolated index finger abduction and precision grip between the index finger and thumb. In Chapter 5, a cohort of older subjects will complete the same protocol and the results will be compared to that of the young group.

1.4.5. Movement phase

Dynamic contractions can be divided into two phases based on whether the muscle is shortening (referred to as concentric phase) or lengthening (referred to as eccentric phase). These phases possess unique profiles of force generation and electromyographic characteristics (Enoka, 1996). Furthermore, a rapidly expanding body of literature suggests that these contraction phases are accomplished using different neural strategies (Duchateau & Enoka, 2008; Duchateau & Baudry, 2014). Within this literature, several studies have used TMS to investigate contraction phase dependent variations in corticospinal excitability. For those studies examining the response to single-pulse TMS, the majority have suggested that MEPs are reduced during lengthening contractions (Abbruzzese et al., 1994; Sekiguchi et al., 2001; Sekiguchi et al., 2003; Gruber et al., 2009; Duclay et al., 2011, 2014). However, several have also reported that these effects are accompanied by reductions in the H-reflex (Abbruzzese et al., 1994; Duclay et al., 2011, 2014), smaller cervicomedullary evoked potentials (CMEPs; Gruber et al., 2009) and a reduced response to TES (Abbruzzese et al., 1994), suggesting that changes in MEP amplitude during lengthening contractions likely reflect reductions in spinal excitability.
Recently, phase-dependent changes in intracortical inhibition have been suggested, providing evidence that cortical excitability is altered by contraction phase. This includes reports that SP duration is significantly shorter during lengthening contractions than during either isometric (Duclay et al., 2011) or shortening contractions (Duclay et al., 2011, 2014), and that, in M1 ipsilateral to contraction, SICI is reduced relative to rest during shortening contractions, but reduced relative to both rest and shortening contractions during lengthening contractions (Howatson et al., 2011). Interestingly, Howatson et al. (2011) also observed contraction phase-dependent changes in ipsilateral ICF and IHI from active to non-active cortex, reporting a progressive increase in facilitatory measurements but decrease in interhemispheric inhibitory measurements.

In addition to studies using TMS, results from other neuroimaging techniques have provided further evidence supporting the existence of unique neural control strategies during shortening and lengthening contractions. For example, the movement-related cortical potential derived from electroencephalographic (EEG) recordings was significantly greater during lengthening than shortening contraction of the elbow joint, suggesting that performance of the lengthening contraction required increased preparation and processing of afferent information (Fang et al., 2001, 2004). Furthermore, patterns of cortical activity assessed using fMRI have also been shown to differ between anisometric contractions, with lengthening movements demonstrating increased activity in areas such as the pre-supplementary motor area and anterior cingulate cortex (Kwon & Park, 2011). The increased activity in these areas was suggested to reflect requirements for a higher degree of cognitive control during lengthening movements (Kwon & Park, 2011).

Taken together, the results of TMS, EEG and fMRI studies not only support phase-dependent neural control mechanisms, but also demonstrate the involvement of higher order processing during lengthening contractions. This increased higher order input suggests that, relative to
shortening contractions, lengthening contractions represent a more complex task. In support of this, several studies have reported that the fidelity of motor output, assessed via movement variability and accuracy, is reduced during the performance of lengthening contractions in young subjects (Christou & Carlton, 2002b; Christou et al., 2003; Neto et al., 2012). However, in old subjects, this phase-specific performance deficit has been shown to be significantly enhanced (Burnett et al., 2000; Graves et al., 2000; Laidlaw et al., 2000; Christou & Carlton, 2002a; Christou et al., 2003; Kornatz et al., 2005). Furthermore, performance deficits during lengthening contractions are greater again in older adults with a history of falling (Carville et al., 2007), suggesting that these changes may be important considerations in the increased risk of falling seen in old adults (Rubenstein, 2006). Currently, the factors contributing to this exaggerated phase-specific performance deficit are poorly understood. However, as inhibitory circuits are important in motor control (see section 1.2.4.) and may be differentially modulated by movement phase, age-related changes in phase-specific intracortical inhibitory tone may be a contributing factor. This will be investigated in Chapter 6.

1.5. Summary

The activity of inhibitory GABAergic circuits within the brains cortical motor areas have been well established as an important component of effective motor output. In humans, much of the evidence supporting this suggestion comes from the non-invasive brain stimulation technique of transcranial magnetic stimulation (TMS), which allows an assessment of intracortical inhibitory tone in awake and intact subjects. Using this technique, profiles of inhibitory modulation during different functional states have been developed in healthy subjects and subsequently used to identify abnormal inhibitory function in situations of altered motor control. This approach has been adopted by many previous studies attempting to understand the neurophysiological factors contributing to motor deficits in older adults.
However, the findings of individual studies have often been contradictory, precluding the definitive identification of age-related changes in inhibitory function within the motor areas of the brain.

Despite previous studies having assessed effects of age on inhibitory function, the scope of investigation has been relatively narrow and the impact of several factors on age-related comparisons remain to be explored. The following chapters describe experiments I have undertaken to examine some of these factors, with the aim of providing a more definitive characterisation of age-related changes in inhibitory function. Chapters 2 and 3 examine if age-related changes in corticospinal input-output properties affect comparisons of intracortical inhibition measurements between young and old subjects in both resting and active muscle. Chapters 4 and 5 investigate if task-dependent changes in the activity of inhibitory circuits, and in the interaction between these circuits, are affected by age. Finally, Chapter 6 examines if intracortical inhibition is differentially modulated during different movement phases, and if age-related changes in this modulation contribute to phase-specific deficits in motor output.
CHAPTER II

MODULATION OF SHORT- AND LONG-INTERVAL INTRACORTICAL INHIBITION WITH INCREASING MOTOR EVOKED POTENTIAL AMPLITUDE IN A HUMAN HAND MUSCLE

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Corticospinal I/O Properties & ICI

Chapter 2

STATEMENT OF AUTHORSHIP

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By signing the Statement of Authorship, each author certifies that their stated contribution to the publication is accurate and that permission is granted for the publication to be included in the candidate’s thesis.

George Opie (Candidate)

Contribution: Experimental design, subject recruitment, collection and analysis of data, interpretation of data, wrote manuscript

Signed: ________________________________ Date: 12/6/5

John Semmler

Contribution: Experimental design, supervised development of work, data interpretation, critical manuscript evaluation, acted as corresponding author

Signed: ________________________________ Date: 12/6/5
2. Modulation of short- and long-interval intracortical inhibition with increasing motor evoked potential amplitude in a human hand muscle

2.1. Abstract

The aim of the current study was to investigate the effect of increasing test motor evoked potential (MEP) amplitude on short- (SICI) and long-interval intracortical inhibition (LICI) at rest and during activation of the first dorsal interosseous (FDI) muscle. In 22 young subjects, a conditioning-test transcranial magnetic stimulation (TMS) paradigm was used to assess SICI and LICI at 5 different test TMS intensities (110%–150% motor threshold) in resting and active FDI. In 9 additional subjects, SICI and LICI data were quantified when the test MEP amplitude represented specific proportions of the maximal compound muscle action potential (M_max) in each subject. Test TMS intensity influenced SICI and LICI in rest and active FDI muscle. The normalised test MEP amplitude (%M_max) did not influence SICI at rest, whereas there was a decrease in LICI at rest and an increase in SICI in active FDI with an increased normalised test MEP amplitude (%M_max). Our results demonstrate differential effects of normalised test MEP amplitude (%M_max) on SICI and LICI in resting and active FDI muscle. Estimation of SICI and LICI under some circumstances may be influenced by the normalised test MEP amplitude in subject populations with different M_max characteristics.
2.2. Introduction

Paired-pulse transcranial magnetic stimulation (TMS) is a commonly utilised method of non-invasive brain stimulation that allows a functional assessment of intracortical inhibition within primary motor cortex (M1). Short-interval intracortical inhibition (SICI) consists of a subthreshold conditioning pulse followed 2–5 ms later by a suprathreshold test pulse (Kujirai et al., 1993). In this paradigm, a reduction in the amplitude of a test motor evoked potential (MEP) occurs due to the activation of gamma-aminobutyric acid (GABA)A-mediated inhibitory interneurons in primary motor cortex (M1) by the subthreshold conditioning TMS pulse (Ziemann et al., 1996a; Ziemann et al., 1996b). Another paired-pulse paradigm, known as long-interval intracortical inhibition (LICI), uses a suprathreshold conditioning pulse that reduces the size of a suprathreshold test pulse when delivered 100–150 ms later (Valls-Sole et al., 1992), which is thought to be due to the activation of GABA\(_B\)-related inhibitory interneurons (Werhahn et al., 1999). Several lines of evidence suggest that SICI is functionally important, as it is reduced with muscle activation (Ridding et al., 1995; Zoghi et al., 2003), is abnormal in some movement disorders (Berardelli et al., 2008) and is altered after interventions that change motor performance (e.g., fatigue; see Vucic et al., 2011).

Although the functional relevance of LICI is less well established, changes in this paradigm have also been observed during muscle activation (Hammond & Vallence, 2007; McNeil et al., 2009) and in motor control pathologies (Berardelli et al., 2008).

Along with these functional effects, methodological factors are known to influence estimates of intracortical inhibition with paired-pulse TMS. For example, several studies have shown that increasing the size of the test MEP can influence the magnitude of SICI and LICI (Sanger et al., 2001; Daskalakis et al., 2002; Daskalakis et al., 2004). This effect is thought to be due predominantly to an increase in test TMS intensity generating a larger test MEP, which alters the relative contribution of indirect (I) waves in the corticospinal descending volley (Di
Lazzaro et al., 1998a; Garry & Thomson, 2009; McNeil et al., 2011). However, a recent study has suggested that the amplitude of the test MEP when normalised to the maximum muscle response (maximal compound muscle action potential; $M_{\text{max}}$) may also influence the estimation of SICI in resting first dorsal interosseous muscle (Lackmy & Marchand-Pauvert, 2010). In this previous study, they found that the relationship between the estimate of SICI and the normalised test MEP ($\%M_{\text{max}}$) was non-linear, and suggested that this effect was partly due to properties of the motor neuron pool where small and large motor units have unequal contributions to the MEP (Lackmy & Marchand-Pauvert, 2010). These findings suggest that the more than two-fold difference in $M_{\text{max}}$ that is commonly observed between young healthy subjects (Lee & Carroll, 2005) could confound comparisons of SICI when a similar absolute test MEP amplitude is used between subjects. However, it is not currently known whether the normalised test MEP amplitude ($\%M_{\text{max}}$) influences the assessment of LICI, or whether SICI and LICI are influenced by normalised test MEP amplitude ($\%M_{\text{max}}$) when the muscle is voluntarily activated.

The aim of the current study was therefore to investigate the effect of increasing test MEP amplitude on SICI and LICI at rest and during activation of the FDI muscle. Our approach was to quantify the effect of increasing test TMS intensity on SICI and LICI to produce a range of test MEP amplitudes in each subject, and to compare this with SICI and LICI responses when the test MEP was expressed relative to $M_{\text{max}}$ obtained in each subject. Based on the previous results for SICI in resting FDI (Lackmy & Marchand-Pauvert, 2010), and the sensitivity of both paradigms to changes in test TMS intensity, we expected that the estimation of LICI at rest would also be influenced by the normalised test MEP amplitude ($\%M_{\text{max}}$). Furthermore, as muscle activation changes the magnitude of both SICI (Ridding et al., 1995; Zoghi et al., 2003) and LICI (Hammond & Vallence, 2007; McNeil et al., 2009), we expected that the effect of normalised test MEP amplitude ($\%M_{\text{max}}$) on SICI and LICI...
would be reduced when the muscle was voluntarily activated. The findings from this study will determine whether normalising the test MEP amplitude to the maximum motor response of the muscle ($M_{\text{max}}$) is an important consideration in the estimation of SICI or LICI in resting and active FDI muscle.

2.3. Methods

Thirty one young (mean ± SD; 21.8 ± 2.8 years), healthy subjects were recruited from the university and wider community to participate in the current study. Twenty two (mean ± SD; 22.3 ± 3.1 years) subjects participated in the main experiment (Experimental Series 1), and an additional 9 subjects (mean ± SD; 20.7 ± 1.1 years) were recruited for a second series of experiments (Experimental Series 2). Exclusion criteria included a history of stroke or epilepsy, history of neurological or psychiatric disease, or current use of psychoactive medication (antidepressants, antipsychotics, anxiolytics etc). Hand preference and laterality was assessed using the Edinburgh Handedness Inventory (Oldfield, 1971). Each subject provided written, informed consent prior to participation. All experimentation was approved by the University of Adelaide Human Research Ethics Committee and conducted in accordance with the declaration of Helsinki.

2.3.1. Experimental arrangement

For all experiments, subjects were seated in a comfortable chair with their right arm abducted approximately 45° at the shoulder. This allowed the forearm and hand to sit comfortably on an arm support placed next to them. Surface electromyography (EMG) was used to record responses from the first dorsal interosseous (FDI) muscle of the right hand. Two Ag–AgCl electrodes (3.2 cm diameter) were attached to the skin over the muscle in a belly-tendon montage, with a grounding strap around the wrist acting as a reference. EMG recordings were conditioned using a CED1902 (Cambridge Electronic Design, Cambridge, UK) and sampled
using a CED1401 interface (Cambridge Electronic Design). EMG was amplified (x300), band-pass filtered (20 Hz high pass, 1 kHz low pass) and digitized at 2 kHz before being recorded and stored offline for analysis. To facilitate muscle relaxation when required, real-time EMG signals were displayed under high gain on an oscilloscope placed in front of the subject. During active trials, force was measured with a load cell (model MLP-100; Transducer Techniques, Temecula, CA, USA) that was mounted between two polished brass disks that were 30 mm apart. When activating the target muscle (FDI), subjects grasped the brass discs between the index finger and thumb using a precision grip. Force was amplified (x1000) and sampled at 400 Hz with the CED data acquisition system.

2.3.2. Experimental Procedures

Maximum Voluntary Contraction. For the assessment of maximum muscle strength, subjects produced a maximal contraction that was held for 3 seconds. This procedure was repeated several times, separated by a 60 second break, until the three largest contractions were within a 10% margin. The largest of these contractions was designated the maximum voluntary contraction (MVC). To optimise force production, feedback was displayed on a computer monitor placed at eye level in front of the subject and verbal encouragement was provided by the experimenter.

Transcranial Magnetic Stimulation. TMS was applied to the left primary motor cortex using a figure-of-eight coil (external wing diameter 9 cms) with two Magstim 200 magnetic stimulators connected through a Bistim unit (Magstim, Dyfed, UK). The coil was held tangentially to the scalp at an angle of 45° to the sagittal plane, with the handle pointed backwards and laterally, producing a current flow in the brain with a posterior to anterior direction. The coil was positioned on the scalp over the location producing an optimum
response in the relaxed FDI muscle. This location was marked on the scalp for reference and continually checked throughout the experiment.

Resting and Active motor thresholds (RMT and AMT, respectively) were obtained in FDI while the TMS coil was placed at the optimal location over primary motor cortex. RMT was defined as the minimum TMS intensity, relative to the maximum stimulator output (%MSO), producing a response amplitude ≥ 50 μV in three out of five trials in resting FDI muscle (Carroll et al., 2001). Active motor threshold (AMT) was defined as the minimum TMS intensity producing a response amplitude ≥ 300 μV in three out of five trials while FDI was active in performing a precision grip held at 5% of MVC force (Rothwell et al., 1999). Force feedback was provided via an oscilloscope placed at eye level in front of the subject, with a target force set on the oscilloscope that was adjusted to 5% of each subjects MVC. TMS was delivered at 0.2 Hz for all conditions.

**Intracortical Inhibition (Experimental Series 1).** In 22 subjects, SICI and LICI were assessed in two activity states; during complete relaxation of FDI (rest state) and while FDI was active in performing a precision grip (active state). SICI used a subthreshold conditioning pulse set at 80% AMT and an ISI of 3 ms (Kujirai et al., 1993), while LICI used a suprathreshold conditioning stimulus set at 120% RMT and an interstimulus interval (ISI) of 150 ms (Valls-Sole et al., 1992). Both paradigms used the same set of test TMS intensities, varied randomly between 110% and 150% of motor threshold in 10% increments. However, during the rest state, these intensities were normalised to RMT, while during the active state they were normalised to AMT. This was performed so that the test MEP amplitudes generated within each state would encompass a range that was representative of those commonly used for the assessment of SICI and LICI. The order of test TMS applied throughout experimental blocks was pseudo-randomised between subjects. Both paired-pulse paradigms (SICI and LICI) were applied in the same experimental block, allowing normalisation of the two conditioned
responses to a common test alone MEP. Using this design, each test block contained 20 paired-pulse trials (10 SICI and 10 LICI) and 10 test-alone trials. As 5 test TMS intensities (110%, 120%, 130%, 140% and 150%) were applied in two activity states (rest, active) 10 experimental blocks were recorded, totalling 300 stimuli. As individual experimental blocks took several minutes to complete, subjects were allowed to rest after active trials, ensuring the muscle was not fatigued.

*Intracortical Inhibition (Experimental Series 2).* In 9 additional subjects, the test TMS intensity was adjusted to produce a test alone MEP amplitude that was equivalent to 0–10%, 10–20% and 20–30% $M_{max}$ in resting and active muscle. SICI and LICI were then assessed in the same stimulus block using conditioning and test TMS intensities as described in Series 1. This process ensured that each subject contributed one measure of SICI and one measure of LICI in each bin relative to the proportion of $M_{max}$.

*Maximal compound muscle action potential ($M_{max}$).* Electrical stimulation applied at the wrist was used to stimulate the ulnar nerve, generating maximal compound muscle action potentials within FDI. Stimuli were applied using a constant-current stimulator (DS7AH,Digitimer, UK) and bipolar surface electrodes with the cathode positioned distally. Each stimulus was a square wave pulse of 100 µs duration and intensity at 120% of that required to produce a maximal response in FDI (i.e. 120% $M_{max}$). $M_{max}$ was obtained by averaging the responses to 5 stimuli delivered at the end of the experiment in Experimental Series 1, and at the start of the experiment in Series 2.

**2.3.3. Data Analysis**

Data analysis was completed manually by visual inspection of offline EMG data. Any trace showing muscle activity 100 ms prior to the stimulus was excluded from analysis in the resting state. MEP and $M_{max}$ amplitudes from each trial were measured peak-to-peak and
expressed in mV. Under all conditions, SICI and LICI were quantified by expressing the average conditioned MEP amplitude as a percentage of the average unconditioned (test TMS) MEP amplitude. For experimental Series 1, the test alone MEP obtained at each test TMS intensity in each subject was normalised to their own $M_{\text{max}}$, and estimates of SICI and LICI for each test alone MEP were grouped into bins of 10% $M_{\text{max}}$. For Experimental Series 2, SICI and LICI were quantified from the normalised test MEP in each bin, which provided an equal number of samples in each bin for all subjects.

2.3.4. Statistical Analysis

The within subject factor of test TMS intensity (110%, 120%, 130%, 140% and 150% RMT) was investigated for its main effects on SICI and LICI using repeated-measures analysis of variance (ANOVA$_{\text{RM}}$). This was repeated separately for the resting and active states. Significant main effects and interactions within the ANOVA$_{\text{RM}}$ were further investigated using Fishers LSD post-hoc test. Within each activity state, the between subject factor of normalised test MEP amplitude (relative to $M_{\text{max}}$) was investigated for its effects on SICI and LICI using one-way ANOVAs. Linear regression of individual subject data was used to further investigate effects of baseline cortical excitability (RMT, AMT), test TMS intensity and test MEP amplitude on SICI and LICI in both activity states. Significance was set at $P < 0.05$ for all comparisons and data are shown as mean ± standard error of the mean (SEM), unless otherwise stated.

2.4. Results

All subjects completed the experiment in full and without adverse reaction. The study cohort for the main experiment (Series 1) consisted of 15 female (21.9 ± 0.5 years) and 7 male (23 ± 0.9 years) subjects. Average RMT was 46.4 ± 1.6% MSO while average AMT was 38.2 ± 1.5% MSO. Results of the Edinburgh Handedness Inventory (Oldfield, 1971) demonstrated
the group was, on average, right-hand dominant (average laterality quotient, 0.88 ± 0.03).

Average test TMS intensities ranged from 51.1 ± 1.7% MSO (110% RMT) to 69.9 ± 2.3% MSO (150% RMT) in the rest state and 42.0 ± 1.7% MSO (110% AMT) to 57.6 ± 2.2% MSO (150% AMT) in the active state. The average $M_{\text{max}}$ for all subjects was 19.1 ± 0.8 mV (range 13.1 to 28.0 mV).

2.4.1. Influence of test TMS intensity on SICI and LICI

A representative example from a single subject showing changes in SICI and LICI in response to low (110% RMT) and high (150% RMT) intensity test TMS in resting FDI is shown in Figure 2.1. For this subject, the average test alone MEP was 0.5 mV at 110% RMT (44% MSO) and 3.9 mV at 150% RMT (60% MSO). Between these two intensities, there was a modest reduction in SICI as the test TMS intensity increased (from 57% to 65%), but a much larger reduction in LICI (from 1% to 61%). The test alone MEPs in this subject represented 4% of $M_{\text{max}}$ at 110% RMT and 28% of $M_{\text{max}}$ at 150% RMT.
Figure 2.1 Representative data from a single subject demonstrating the effect of test TMS intensity on SICI and LICI in resting FDI. Grey traces show the response to test TMS applied alone, whereas the black traces show the conditioned response. Black arrows identify application of the conditioning stimulus, while grey arrows identify application of the test stimulus. The percentage change in MEP amplitude (100% represents no inhibition) between the test alone and conditioned traces represent the magnitude of SICI (upper panel) and LICI (lower panel) in response to low (110% RMT, left panel) and high (150% RMT, right panel) intensity test TMS administered during complete relaxation of FDI. Abbreviations: RMT, resting motor threshold; mV, millivolts; ms, milliseconds; %Mmax, percentage of maximum compound muscle action potential.

For all subjects, increasing test-TMS intensity resulted in greater test-alone MEP amplitudes in both rest and active states ($P < 0.0001$; Fig. 2.2A and 2.2B). In the resting state (Fig. 2.2A), post hoc comparisons demonstrated that test MEPs at 130%, 140% and 150% RMT were significantly larger than those at 110% and 120% RMT ($P < 0.0005$). Furthermore, the absolute test MEP amplitudes at 140% and 150% RMT were significantly larger than at 130% RMT ($P < 0.05$). In the active state (Fig. 2.2B), the progressive increase in absolute test MEP amplitude in response to increasing test TMS intensity was significantly different for all intensities of the test TMS pulse ($P$ values range from 0.03 to 0.0001). Despite these significant effects of test TMS intensity, there was substantial variability in absolute test MEP amplitude between subjects. For example, at 110% RMT in resting muscle, the largest MEP
(1.8 mV) was 22 times greater than the smallest MEP (0.08 mV), whereas at 150% RMT, the largest MEP (7.5 mV) was 10 times greater than the smallest MEP (0.7 mV). This variability between subjects was lower in active muscle, with a 6-fold range at 110% AMT (0.3–1.9 mV) and an 8-fold range at 150% AMT (1.9–14.4 mV).

Increasing test TMS intensity in the rest state resulted in reduced SICI \((P = 0.003)\) and LICI \((P < 0.0001; \text{Fig. 2C and 2E})\). For SICI, varying the test TMS intensity between 110% and 130% RMT produced no significant change in inhibition \((P > 0.2)\). However, 140% and 150% RMT intensities resulted in significantly less inhibition compared with 110% \((P < 0.002)\) and 120% RMT \((P < 0.01)\). For LICI, there was significantly less inhibition at 130%, 140% and 150% RMT compared with 110% RMT \((P < 0.005)\). Furthermore, the magnitude of inhibition at 140% and 150% RMT was significantly less than at 120% and 130% RMT \((P < 0.0004)\) and the decrease in inhibition between 140% and 150% RMT was also significant \((P = 0.008)\). In the active state, increasing test TMS intensity resulted in increased SICI \((P = 0.002; \text{Fig. 2.2D})\) and LICI \((P = 0.01; 2.2F)\). For SICI, post hoc comparisons demonstrated significantly more inhibition at test TMS intensities of 120%, 130%, 140% and 150% AMT compared with 110% AMT \((P < 0.006)\). Similar results were observed for LICI, with increased inhibition at 130%, 140% and 150% AMT compared with 110% AMT \((P < 0.01)\).
Figure 2.2 Effect of test TMS intensity on intracortical inhibition in resting and active FDI muscle. Data show test alone MEP amplitudes (A, B), SICI (C, D) and LICI (E, F) in resting (left panel) and active (right panel) FDI muscle. A, B. Mean test alone MEPs for all subjects (solid horizontal line) along with test alone MEPs in each of the 22 subjects (unfilled circles) at each test TMS intensity. C–F, The dotted horizontal line represents no inhibition, with values below 100% representing inhibition. # P < 0.05 compared with 110% RMT/AMT; * P < 0.05 compared with 110% and 120% RMT/AMT; † P < 0.05 compared with 110%, 120% and 130% RMT/AMT; ‡ P < 0.05 compared with 110%, 120%, 130% and 140% RMT; ¶ P < 0.05 compared with 110% AMT. Abbreviations: MEP, motor evoked potential; RMT, resting motor threshold; AMT, active motor threshold.
2.4.2. Influence of test MEP amplitude on SICI and LICI

To investigate the effect of MEP amplitude on paired-pulse TMS measurements, test TMS intensity data from Experimental Series 1 were categorised based on the amplitude of the test alone MEP, and expressed relative to $M_{\text{max}}$ in each subject (Fig. 2.3). This was performed by pooling the conditioned MEP data into 10% bins (based on the proportion of the test alone MEP relative to $M_{\text{max}}$), which resulted in 3 bins in the resting state (0–10%, 10–20% and > 20% $M_{\text{max}}$) and 4 bins in the active state (0–10%, 10–20%, 20–30% and > 30% $M_{\text{max}}$). For the normalised test-alone MEP ($%M_{\text{max}}$), the average group amplitude for these bins was 4%, 14% and 26% $M_{\text{max}}$ in the rest state (Fig. 2.3A) and 6%, 15%, 24% and 43% $M_{\text{max}}$ in the active state (Fig. 2.3B), and resulted in reduced test MEP variability in each bin compared with test TMS intensity. For both states, all increases in normalised test MEP amplitude between bins were significant ($P < 0.0001$). Furthermore, the average test TMS intensity increased across normalised test MEP ($%M_{\text{max}}$) bins for both rest ($P = 0.03$) and active ($P < 0.0001$) test MEPs. In the rest state, the < 10% $M_{\text{max}}$ bin demonstrated significantly lower test TMS intensity (58% MSO) than either the 10–20% (63% MSO, $P = 0.04$) or > 20% (66% MSO, $P = 0.04$) $M_{\text{max}}$ bins. In the active state, the < 10% $M_{\text{max}}$ bin also demonstrated significantly lower test TMS intensity (44% MSO) compared with either the 10–20% (51% MSO, $P = 0.02$), 20–30% (56% MSO, $P < 0.0001$) or > 30% $M_{\text{max}}$ (53% MSO, $P = 0.0003$) bins.
Figure 2.3 Effect of normalised test MEP amplitude on intracortical inhibition in resting and active FDI muscle (Experimental Series 1). Data show average test alone MEP amplitudes (A, B), SICI (C, D) and LICI (E, F) normalised to Mmax in resting (left panel) and active (right panel) FDI muscle. A, B, Mean normalised (%Mmax) test alone MEPs for all subjects (solid horizontal line) along with normalised test alone MEPs in each of the 22 subjects (unfilled circles) at each normalised test MEP amplitude. C–F, The dotted horizontal line represents no inhibition, with values below 100% representing inhibition. # P < 0.05 compared with 0–10% Mmax; * P < 0.05 compared with 0–10% and 10–20% Mmax; † P < 0.05 compared with 0–10%, 10–20% and > 30% Mmax. Abbreviations: MEP, motor evoked potential; % Mmax, percentage of maximum compound muscle action potential.
When the test alone MEP was normalised to $M_{\text{max}}$ in resting muscle, the magnitude of SICI was unaffected ($P = 0.09$; Fig. 2.3C), whereas LICI was reduced when the test MEP reached larger proportions of $M_{\text{max}}$ ($P = 0.0006$; Fig. 2.3E). Post-hoc analysis demonstrated significantly greater LICI for test MEPs that were 0–10% $M_{\text{max}}$ compared with 10–20% ($P = 0.002$) or > 20% $M_{\text{max}}$ ($P = 0.004$). In the active state, SICI showed increased inhibition with an increase in normalised test MEP amplitude ($P = 0.0001$; Fig. 2.3D), while LICI was not affected by normalised test MEP amplitude ($P = 0.2$; Fig. 2.3F). Post-hoc comparisons for SICI showed that test alone MEP’s greater than 10% $M_{\text{max}}$ all showed significantly more SICI than those less than 10% $M_{\text{max}}$ ($P < 0.03$). Furthermore, test MEP’s of 20–30% $M_{\text{max}}$ were significantly more inhibited than those that were 10–20% $M_{\text{max}}$ ($P = 0.04$).

2.4.3. Influence of test MEP amplitude on SICI and LICI (Experimental Series 2)

When the MEPs were normalized to $M_{\text{max}}$ in Experimental Series 1 there were a greater number of samples in the smaller bins, with some subjects contributing no samples to the larger bins. This occurred because of between-subject differences in MEP amplitudes at each TMS intensity, and a large difference in $M_{\text{max}}$ amplitudes in different subjects. To address this issue, we recruited 9 additional subjects to assess SICI and LICI when test MEP amplitudes were specifically targeted at 0–10%, 10–20% and 20–30% $M_{\text{max}}$ in resting and active muscle. Using this approach, we were able to obtain estimates of SICI and LICI in all 9 subjects in the active state, but could only obtain the required MEP amplitudes in the rest state in 4 subjects, as MEPs above 20% $M_{\text{max}}$ at rest were not achievable in some subjects. In addition, from the original sample (Experimental Series 1) we were able to identify 6 subjects in the rest state and 7 subjects in the active state that contributed one sample to each bin up to 30% $M_{\text{max}}$. This resulted in 10 subjects at rest and 16 subjects in the active state that contributed SICI and LICI data to each bin. These data are shown in Figure 2.4. For SICI, the modulation of inhibition was consistent with that seen within Experimental Series 1, with no change in
resting muscle ($P = 0.06$; Fig. 2.4C) and an increase in inhibition in response to larger normalised test MEPs in active muscle ($P = 0.002$). For LICI at rest, the responses were also consistent with Experimental Series 1, with reduced inhibition in response to larger amplitude normalised test MEPs ($P = 0.0003$; Fig. 2.4E). However, in contrast to Experimental Series 1, larger amplitude test MEPs resulted in an increase in LICI in active muscle ($P = 0.02$; Fig. 2.4F).

**Figure 2.4** Effect of normalised test MEP amplitude on SICI and LICI in resting and active FDI muscle (Experimental Series 2). Data shown are from 10 subjects in the resting state and 16 subjects in the active state who have contributed a sample to each bin. Data layout is the same as described in Figure 2.3. # $P < 0.05$ compared with 0–10% Mmax; * $P < 0.05$ compared with 0–10% and 10–20% Mmax. Abbreviations as in Figure 2.3.
2.4.4. Linear regression

Linear regression analysis of individual subject data from Experimental Series 1 was performed to examine the association between several TMS measures (RMT, AMT, test TMS intensity, normalised test MEP amplitude) and the magnitude of intracortical inhibition (SICI and LICI) in resting and active muscle. At rest, linear regression showed statistically significant associations between RMT and SICI ($r^2 = 0.12$, $P = 0.0002$) and AMT and SICI ($r^2 = 0.21$, $P < 0.0001$), whereas there were no significant associations between RMT and LICI ($P = 0.6$) or AMT and LICI ($P = 0.6$). The associations between the test TMS parameters (test TMS intensity and normalised test MEP amplitude) and intracortical inhibition (SICI and LICI) at rest are shown in Figure 2.5. These data show that there were no significant associations between test TMS intensity and SICI (Fig. 2.5A) or normalised test MEP amplitude and SICI ($P > 0.1$; Fig. 2.5C) in individual subjects. In contrast, weak but significant positive relationships were found between test TMS intensity and LICI ($r^2 = 0.11$, $P = 0.0003$; Fig. 2.5B) and normalised test MEP amplitude and LICI ($r^2 = 0.16$, $P < 0.0001$; Fig. 2.5D), indicating that larger test TMS intensities and normalised MEP amplitudes were associated with reduced LICI in individual subjects when the muscle was at rest.
Figure 2.5 Regression plots showing the effects of test TMS intensity and test MEP amplitude on SICI and LICI with FDI at rest. For SICI (black circles), no significant relationships were demonstrated (A, C). For LICI (white circles), reductions in inhibition were significantly related to test intensity (B) and normalised test MEP amplitude (D). Abbreviations: MEP, motor evoked potential; MSO, maximum stimulator output; mV, millivolts; % Mmax, percentage of maximum compound muscle action potential.

In the active state, linear regression analysis failed to show a significant association between RMT and SICI ($P = 0.1$), whereas significant associations were found for AMT and SICI ($r^2 = 0.14$, $P < 0.0001$), RMT and LICI ($r^2 = 0.09$, $P = 0.002$), and AMT and LICI ($r^2 = 0.10$, $P < 0.0008$). Linear regression analysis between test TMS parameters and intracortical inhibition (SICI and LICI) during activation of FDI are shown in Figure 2.6. These analyses showed weak but statistically significant associations between test TMS intensity and SICI ($r^2 = 0.19$, $P < 0.0001$; Fig 2.6A), normalised test MEP amplitude and SICI ($r^2 = 0.09$, $P = 0.002$; Fig 2.6C), test TMS intensity and LICI ($r^2 = 0.13$, $P = 0.0001$; Fig 2.6B) and normalised test MEP amplitude and LICI ($r^2 = 0.06$, $P = 0.01$; Fig 2.6D), indicating that both SICI and LICI were
reduced with increasing test TMS intensity and normalised test MEP amplitude in individual subjects when the muscle was active.

![Regression plots showing the effects of test TMS intensity and test MEP amplitude on SICI and LICI during activation of FDI. For both SICI (black circles) and LICI (white circles), increases in inhibition were significantly related to test intensity (A, B) and normalised test MEP amplitude (C, D). Abbreviations: MEP, motor evoked potential; MSO, maximum stimulator output; mV, millivolts; % Mmax, percentage of maximum compound muscle action potential.](image)

**Figure 2.6** Regression plots showing the effects of test TMS intensity and test MEP amplitude on SICI and LICI during activation of FDI. For both SICI (black circles) and LICI (white circles), increases in inhibition were significantly related to test intensity (A, B) and normalised test MEP amplitude (C, D). Abbreviations: MEP, motor evoked potential; MSO, maximum stimulator output; mV, millivolts; % Mmax, percentage of maximum compound muscle action potential.

### 2.5. Discussion

As a methodological control, it is common practice to normalise submaximal muscle responses to the maximum output of the muscle (M\text{max}) in neurophysiological studies involving H-reflexes, motor unit number estimations, and when assessing the neural activation of muscle with electromyography (Calder et al., 2005). In contrast, TMS studies typically rely on the absolute amplitude of the MEP without consideration of the maximal muscle response in each subject. Because the absolute amplitude of M\text{max} varies considerably
within a healthy population (Lee & Carroll, 2005), the same absolute MEP amplitude may represent a different proportion of the $M_{\text{max}}$ response in each subject. Using two different approaches (Experimental Series 1 and 2), we show that SICI in active FDI muscle and LICI in resting FDI muscle vary with the amplitude of the normalised test MEP ($\%M_{\text{max}}$). These findings suggest that there are differential effects of normalised test MEP amplitude ($\%M_{\text{max}}$) on the estimates of SICI and LICI, and that these effects may depend on the activity state of the muscle.

### 2.5.1. Factors influencing SICI and LICI

When TMS is applied to M1, it generates a high frequency discharge of corticospinal neurons that consists of multiple waves, referred to as the descending volley. The earliest of these waves, known as the direct (D) wave, is thought to result from direct activation of corticospinal neurons, and occurs only at high TMS intensities. The later waves, referred to as I waves, are thought to arise from trans-synaptic activation of corticospinal neurons, and are described as early (known as $I_1$) and late (known as $I_3$) waves based on their latency (Rothwell, 1997; Ziemann & Rothwell, 2000). Direct recordings of corticospinal volleys from the epidural space in man (Di Lazzaro et al., 1998b; Di Lazzaro et al., 2002b) and single motor unit recordings (Hanajima et al., 1998) have shown that the intracortical inhibitory interneurons responsible for SICI and LICI act by specifically inhibiting interneurons responsible for the later $I_3$ waves, while those producing the early $I_1$ wave are not affected. This differential targeting of late I-waves by the circuitry mediating SICI and LICI suggest that factors changing the contribution of early and late I-waves to the MEP, such as test TMS intensity and muscle activation, have the potential to influence the estimation of intracortical inhibition.
Several previous studies have shown that increasing the test TMS intensity in resting muscle influences both SICI and LICI. For example, Sanger et al. (2001) compared changes in the magnitude of SICI and LICI when targeting absolute test MEP amplitudes of 0.2, 1 and 4 mV in resting FDI muscle. They found that larger amplitude test MEPs (produced by a higher TMS intensity) resulted in an increase in SICI and a decrease in LICI, leading to the interpretation that different cell populations mediate SICI and LICI (Sanger et al., 2001). However, when comparing similar absolute test MEP amplitudes between studies (produced by test TMS intensities of 110, 120 and 150% RMT in our study), we found a decrease in both SICI and LICI with increased absolute test MEP amplitude (Fig. 2.2). Although our SICI results do not support those of Sanger et al. (2001), more recent studies using specific test TMS intensities (as opposed to targeting absolute test MEP amplitudes) have shown that both SICI (Garry & Thomson, 2009) and LICI (McNeil et al., 2011) are reduced with increasing test TMS intensity, supporting our data in resting FDI muscle. However, we show no correlation between test TMS intensity and SICI (Fig. 2.5A), and only a weak correlation between test TMS intensity and LICI (Fig. 2.5B), suggesting that test TMS intensity alone is not a major contributor to the magnitude of SICI or LICI in individual subjects.

Along with test TMS intensity, voluntary activation of target muscles has also been shown to alter both SICI (Ridding et al., 1995; Stinear & Byblow, 2003; Zoghi et al., 2003; Garry & Thomson, 2009) and LICI (Hammond & Vallence, 2007; McNeil et al., 2009). For SICI, this reduced inhibition has been suggested to promote the activation of task related muscles as part of the voluntary command for movement (Ridding et al., 1995), and is thought to occur because there is a reduced contribution of I\textsubscript{3} waves to the MEP in the active state (Hanajima et al., 1998; Zoghi et al., 2003). Although less well established, similar reductions in inhibition have also been reported for LICI (Hammond & Vallence, 2007; McNeil et al., 2011), but the interpretation of this is likely to be more complicated as LICI has been shown
to be greatest at low contraction intensities (10–25% MVC) and least at rest or during MVCs (McNeil et al., 2011). Nonetheless, in an active muscle we found that increasing test TMS intensity resulted in increased SICI and LICI (Fig. 2.2), mainly because facilitation (rather than inhibition) was observed at low TMS intensities, and inhibition was only observed at higher test TMS intensities (> 120% RMT for SICI and > 130% RMT for LICI). This pattern of response is likely to occur because the early components of the descending volley are able to generate an MEP due to an increase in the relative contribution of the I_1 wave (Di Lazzaro et al., 1998b) and greater motor neuron excitability in the active muscle (Noordhout et al., 1992), which reduces the contribution of I_3 waves to the MEP. As the TMS intensity increases to moderate levels (below that required for D-wave recruitment), there is an increased contribution of I_3 waves to the MEP (Di Lazzaro et al., 1998c), resulting in greater intracortical inhibition. Correlations between test TMS intensity and intracortical inhibition (Fig. 2.6) showed that the amount of variability explained by test TMS intensity was 19% for SICI and 13% for LICI, suggesting that test TMS intensity has only a small influence on the estimate of SICI and LICI in active muscle.

2.5.2. Influence of normalised test MEP amplitude on SICI

Although the physiological mechanism that contributes to SICI is thought to be cortical in origin (Kujirai et al., 1993; Di Lazzaro et al., 1998c), the estimation of SICI is based on the orderly recruitment of motor units that generate a test MEP that is recorded from the target muscle (i.e. it is not recorded in M1). However, the maximal responses in the target muscle can vary considerably, as shown by the two-fold range in M_max obtained in a healthy subject population in the current study. When normalising the test MEP to the M_max obtained in each subject, Lackmy and Marchand-Pauvert (2010) showed a U-shaped influence of the normalised test MEP on SICI in resting FDI, with the greatest SICI being apparent when the test MEP was 20-30% M_max, whereas the least SICI was observed at 0–10% and 40–50%
Using two different approaches (Experimental Series 1 and 2), we found that there was no effect of normalised test MEP amplitude on SICI in resting FDI (Fig. 2.3C, 2.4C). This discrepancy could occur because we had a smaller range of test MEP amplitudes that are more commonly used experimentally (< 30% $M_{\text{max}}$), compared with the larger MEP amplitudes (up to 50% $M_{\text{max}}$) obtained by Lackmy and Marchand-Pauvert (2010). In contrast to the rest state, estimates of SICI during activation of FDI showed an increase in inhibition with an increase in the normalised test MEP amplitude (Fig. 2.3D, 2.4D). This could occur because of a reduced $I_3$ wave contribution in the active state (see above), which is increased in response to the greater activation of motor units when applying the higher TMS intensity required to achieve a larger MEP (Di Lazzaro et al., 1998b). The greatest SICI was observed at 20–30% $M_{\text{max}}$, but was reduced at higher levels of normalised test MEP amplitude (> 30% $M_{\text{max}}$). This effect could be due to the stronger TMS intensity activating D waves that are not influenced by SICI circuits (Di Lazzaro et al., 1998b).

2.5.3. Influence of normalised test MEP amplitude on LICI

In contrast with SICI, LICI uses a suprathreshold conditioning pulse that does produce a descending volley (Di Lazzaro et al., 2002b), suggesting that inhibition of the test MEP could have both cortical and spinal contributions. Although recent work using stimulation of the cervicomedullary junction has supported a spinal contribution to LICI when using a 100 ms ISI (McNeil et al., 2009, 2011), we used an ISI of 150 ms in the present study, which is likely to reduce the contribution from spinal circuits (Inghilleri et al., 1993). Nonetheless, given that SICI and LICI both modulate the amplitude of late I-waves (Di Lazzaro et al., 1998c; Di Lazzaro et al., 2002b), we expected that LICI would also vary with increasing normalised test MEP amplitude in a similar way to SICI (Lackmy & Marchand-Pauvert, 2010). However, using both approaches (Fig. 2.3E, 2.4E), we found that LICI in resting FDI was reduced with increased normalised test MEP amplitude, which is inconsistent with the results obtained for
SICI. This observation, therefore, provides further support to the view that different factors contribute to SICI and LICI, such as the activation of different inhibitory circuits (Sanger et al., 2001), and/or a greater contribution to LICI from spinal circuits (McNeil et al., 2009, 2011).

In contrast to the rest state, LICI measurements recorded during tonic contraction of FDI were not significantly influenced by normalised test MEP amplitude in Experimental Series 1. This finding was surprising, given that there was increased LICI with increased test TMS intensity (Fig. 2.2F), and this was obtained with test MEP amplitudes that produced a high variability between subjects (Fig. 2.2B). Normalising the test MEP amplitude to $M_{max}$ produced a more homogeneous sample of test MEP amplitudes within each bin (Fig. 2.3B), but also reduced the number of samples obtained at higher normalised test MEPs, which may have contributed to the lack of statistical significance with this analysis. In contrast, when each subject made an equal contribution to each bin, there was a significant effect of normalised test MEP amplitude on active LICI (Experimental Series 2; Fig. 2.4F), although the magnitude of LICI in the active state was small. Furthermore, the variability in the LICI response did not seem to be influenced by the variability in the test alone MEPs that were obtained in each bin, suggesting that the normalised test MEP amplitude plays only a small role in the assessment of LICI. This is supported by the weak correlations between test TMS intensity/normalised test MEP amplitude and LICI in active muscle (Fig. 2.5).

2.5.4. Test TMS intensity vs. normalised test MEP amplitude

With current non-invasive TMS techniques, it is not possible to isolate the relative contribution of the descending volley (I-wave contribution) from the effect of an increase in test MEP amplitude on SICI and LICI, because both of these factors change as a result of an increase in test TMS intensity. However, this study was based on the interpretation that our
two different assessments of SICI and LICI allow us to consider the relative importance of these two factors in the modulation of SICI and LICI. For example, estimating SICI and LICI using similar test TMS intensities provides a more homogenous sample of responses relative to the characteristics of the descending volley (Sanger et al., 2001; Hammond & Vallence, 2007; Garry & Thomson, 2009), without consideration of the test MEP amplitude (see between subject MEP variability in Figs. 2.2A, B). In contrast, normalisation of the test MEP to $M_{\text{max}}$ provides a more homogenous sample of responses based on the size of the normalised test MEP between different subjects, without consideration for the characteristics of the test TMS intensity that influences the descending volley. Although an increase in the test MEP still includes a contribution from both sources (descending volley and MEP size), a comparison between the two approaches allows the possibility to establish whether increased normalised test MEP amplitude (and therefore increased motor unit activation) might contribute to the change in SICI and LICI with increasing test TMS intensity. Using this interpretation, we show that increasing TMS intensity influenced SICI and LICI in both resting and active FDI muscle, but this effect was removed only for SICI in resting muscle when data were expressed relative to the normalised test MEP amplitude. Although other factors are likely to contribute, this finding indicates that the reduction in SICI with increasing test TMS intensity in resting FDI muscle is unlikely to be due to an increase in normalised test MEP amplitude.

2.5.5. Physiological Significance

The physiological significance of this study lies in the interpretation that the $M_{\text{max}}$ response represents the activation of the whole motor neuron pool, with a submaximal response corresponding to a consistent percentage of $M_{\text{max}}$, leading to the assumption that a comparable proportion of motor neurons have been activated in each subject (Crone et al., 1999; Calder et al., 2005; Lackmy & Marchand-Pauvert, 2010). Accordingly, an increase in MEP size within
the same subject means that larger motor units have been activated by TMS, which can be assumed to activate a greater proportion of the motor neuron pool. Furthermore, larger motor units would be expected to have a greater influence on the MEP because they have larger innervation ratios, so a conditioning TMS that inhibits a large MEP involving higher threshold motor units will have a greater influence on the test MEP than a conditioning TMS that inhibits a small MEP involving lower threshold motor units. Despite this rationale, the most common approach to the assessment of SICI and LICI is to use a relative test TMS intensity (120% RMT) or an absolute test MEP amplitude (1 mV), which does not standardise the proportion of motor neurons activated between subjects. To reduce the differential effect of motor unit size on the MEP, SICI and LICI should therefore be assessed when the test TMS pulse produces an MEP that represents a similar proportion of \( M_{\text{max}} \) in each subject. When this procedure was performed using two different approaches, we found that SICI in resting FDI muscle was not modulated by increasing normalised test MEP amplitude, suggesting that using a test TMS intensity or absolute test MEP amplitude is a reasonable strategy in this instance. In contrast, we found that LICI at rest and SICI and LICI in active FDI were modulated by increasing normalised test MEP amplitude, suggesting that the proportion of motor neurons activated by the test TMS is an important consideration in the assessment of SICI and LICI under these circumstances.

In conclusion, we have used two different approaches to examine the effect of normalised test MEP amplitude (\( \%M_{\text{max}} \)) on SICI and LICI in rest and active FDI muscle. Although increasing test TMS intensity decreased SICI, there was no effect of normalised test MEP amplitude on SICI in resting FDI muscle. In contrast, SICI in active FDI muscle and LICI at rest were modulated by normalised test MEP amplitude. To reduce the differential effect of motor unit size on the test MEP, these findings suggest that the test MEP should be normalised to the maximum muscle response when assessing LICI at rest and SICI in active
FDI muscle. This would be particularly important when comparing different subject populations that may demonstrate altered $M_{\text{max}}$ characteristics (e.g. healthy elderly subjects, patient populations), or after interventions that may change $M_{\text{max}}$ amplitude (e.g. fatigue, muscle disuse).
CHAPTER III

AGE-RELATED DIFFERENCES IN SHORT- AND LONG-INTERVAL INTRACORTICAL INHIBITION IN A HUMAN HAND MUSCLE

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Contribution: Experimental design, subject recruitment, collection and analysis of data, interpretation of data, wrote manuscript

Signed…

Date: 12/6/2015

John Semmler

Contribution: Experimental design, supervised development of work, data interpretation, critical manuscript evaluation, acted as corresponding author

Signed…

Date: 12/6/2015
3. Age-related differences in short- and long-interval intracortical inhibition in a human hand muscle

3.1. Abstract

Effects of age on the assessment of intracortical inhibition with paired-pulse transcranial magnetic stimulation (TMS) have been variable, which may be due to between-study differences in test TMS intensity and test motor evoked potential (MEP) amplitude. The aim of the current study was to investigate age-related differences in short- (SICI) and long-interval intracortical inhibition (LICI) across a range of test TMS intensities and test MEP amplitudes. In 22 young and 18 older subjects, SICI and LICI were recorded at a range of test TMS intensities (110%–150% of motor threshold) while the first dorsal interosseous (FDI) muscle was at rest, or producing a precision grip of the index finger and thumb. Data were subsequently compared according to the amplitude of the MEP produced by the test alone TMS. When pooled across all test TMS intensities, SICI in resting muscle and LICI in active muscle were similar in young and older adults, whereas SICI in active muscle and LICI in resting muscle were reduced in older adults. Regrouping data based on test MEP amplitude demonstrated similar effects of age for SICI and LICI in resting muscle, whereas more subtle differences between age groups were revealed for SICI and LICI in active muscle. Advancing age influences GABA-mediated intracortical inhibition, but the outcome is dependent on the experimental conditions. Age-related differences in SICI and LICI were influenced by test TMS intensity and test MEP amplitude, suggesting that these are important considerations when assessing intracortical inhibition in older adults, particularly in an active muscle.
3.2. Introduction

The ageing process causes extensive changes to the structure and function of many brain areas, including the primary motor cortex (M1). For example, older adults show decreased thickness of M1 (Salat et al., 2004), degradation of corticospinal tract white matter (Salat et al., 2005), and demonstrate increased activation and reduced lateralisation of cortical activity during motor tasks (Cabeza, 2001, 2002). Paired-pulse transcranial magnetic stimulation (TMS) is an increasingly utilised method to further investigate age-related changes in M1 function. When a subthreshold conditioning stimulus precedes a suprathreshold test stimulus by 1–5 ms, there is a reduction of the test motor evoked potential (MEP) amplitude that is likely to involve GABA_A-receptors (Ziemann et al., 1996a), and is referred to as short-interval intracortical inhibition (SICI; Kujirai et al., 1993). However, when both conditioning and test stimuli are suprathreshold and separated by 100–200 ms, there is a reduction of the test MEP amplitude that involves GABA_B-receptors (Werhahn et al., 1999), and is referred to as long-interval intracortical inhibition (LICI; Valls-Sole et al., 1992). Several studies have shown that these GABAergic intracortical inhibitory circuits may be affected by advancing age (Peinemann et al., 2001; McGinley et al., 2010; Heise et al., 2013), although other studies have shown no difference between young and older adults (Oliviero et al., 2006; Smith et al., 2009; Cirillo et al., 2010), even when using a range of conditioning TMS intensities (Rogasch et al., 2009; Smith et al., 2009). The factors that contribute to these discrepancies between studies are currently unknown.

One important methodological consideration when performing paired-pulse TMS studies is the approach used to obtain the test MEP. In young subjects, the magnitude of inhibition recorded during SICI and LICI depends on test TMS intensity (Garry & Thomson, 2009) and test MEP amplitude (Sanger et al., 2001). Previous studies examining changes with advancing age have therefore matched one of these variables between age groups (Kossev et al., 2002;
Cirillo et al., 2010). Furthermore, recent research suggests that the magnitude of intracortical inhibition in young subjects also depends on the proportion of the test alone MEP relative to the maximum muscle response (\(M_{\text{max}}\); Lackmy and Marchand-Pauvert, 2010). These findings suggest that comparisons of intracortical inhibition between young and older subjects, which typically exhibit different \(M_{\text{max}}\) characteristics (Cirillo et al., 2011), may confound the estimation of SICI and LICI between subject groups. Furthermore, it is not clear whether changes in test TMS intensity or test MEP amplitude (absolute or normalised) have similar effects on the magnitude of SICI and LICI in young and older adults. The aims of the current study were therefore to compare the magnitude of SICI and LICI with increasing test TMS intensity between young and older subjects, and to assess the effects of absolute and normalised test MEP amplitude on age-related comparisons of SICI and LICI.

3.3. Materials and Methods

18 older (mean ± SD; 70.8 ± 5.0 years, 10 females) healthy subjects were recruited to participate in the current study. These data were compared with the data from 22 young (mean ± SD; 22.3 ± 3.1 years) subjects, the results of which have been reported previously (Chapter 2). Standard exclusion criteria were applied (Rossi et al., 2009) and each subject provided written, informed consent prior to participation. All experimentation was approved by the University of Adelaide Human Research Ethics Committee and conducted in accordance with the declaration of Helsinki.

3.3.1. Experimental arrangement

Subjects were seated in a comfortable chair with their right arm and hand relaxed on a support placed next to them. Surface electromyography (EMG) was recorded from the first dorsal interosseous (FDI) muscle of the right hand using two Ag–AgCl surface electrodes in a belly-tendon montage. EMG was amplified (300 X), band-pass filtered (20 Hz–1 kHz) and digitized
at 2 kHz (Cambridge Electronic Design data acquisition system, Cambridge, UK), before being recorded and stored offline for analysis.

Force and acceleration were recorded using a manipulandum that has been described previously (Todd et al., 2010) and is designed specifically for assessing performance during a grip-lift task involving the thumb and index finger (Johansson & Westling, 1984). Force signals were amplified (x1000–10,000) and filtered (100 Hz), while both force and acceleration signals were digitised at 400 Hz (Cambridge Electronic Design data acquisition system, Cambridge, UK) and stored offline for analysis.

### 3.3.2. Experimental Procedures

**Maximal Voluntary Contraction**. The maximal voluntary contraction (MVC) was assessed while subjects produced maximal precision grip force between the index finger and thumb for 3 s. Several contractions were performed, separated by 30 s rest, until the three greatest trials were within a 10% margin. The largest of these was chosen as the subjects’ MVC.

**Transcranial magnetic stimulation**. TMS was applied to left primary motor cortex using a figure-of-eight coil (external wing diameter 9 cms) with two Magstim 200 magnetic stimulators connected through a Bistim unit (Magstim, Dyfed, UK). The coil was held tangentially to the scalp at an angle of 45° to the sagittal plane, with the handle pointed backwards and laterally. The coil was positioned on the scalp over the location producing an optimum response in the relaxed FDI muscle. This location was marked on the scalp for reference and continually checked throughout the experiment. Resting motor threshold (RMT) was defined as the minimum TMS intensity producing a response amplitude ≥ 50 μV in three out of five trials in resting FDI muscle (Carroll et al., 2001). Active motor threshold (AMT) was defined as the minimum TMS intensity producing a response amplitude ≥ 300 μV
in three out of five trials (Rothwell et al., 1999) while FDI was active in performing a precision grip held at 5% MVC. TMS was delivered at 0.2 Hz for all conditions.

Intracortical inhibition. SICI and LICI were assessed while FDI was relaxed (rest state) or active in performing a precision grip at 5% MVC (active state). SICI used an 80% AMT conditioning stimulus and an interstimulus interval (ISI) of 3 ms (Kujirai et al., 1993), while LICI used a 120% RMT conditioning stimulus and a 150 ms ISI (Valls-Sole et al., 1992). Both paradigms used the same test TMS intensities (110%–150% of motor threshold in 10% increments). At rest, these intensities were normalised to RMT, while during activation they were normalised to AMT. The order in which test TMS intensities were applied was pseudo-randomised between subjects. Both paired-pulse paradigms were applied in the same experimental block, allowing normalisation of conditioned responses to a common test alone state. Using this design, each experimental block contained 20 paired-pulse trials (10 SICI and 10 LICI) and 10 test-alone trials, with each subject receiving a total of 300 stimuli (5 test TMS intensities and 2 activity states).

Grip-lift task. Hand function was assessed during a grip-and-lift procedure, during which subjects held the manipulandum between index finger and thumb using a precision grip, lifted it to a height of approximately 10 cm and then set it down again. No practice was allowed and each subject completed 5 lifts for 3 loads of different mass (100 g, 200 g and 300 g).

Maximal compound muscle action potential (\(M_{\text{max}}\)). Electrical stimulation applied at the wrist was used to stimulate the ulnar nerve, generating maximal compound muscle action potentials within FDI. Stimuli were applied using a constant-current stimulator (DS7AH, Digitimer, UK) and bipolar surface electrodes with the cathode positioned distally. Each stimulus was a square wave pulse of 100 µs duration and intensity set at 120% of that required to produce a
maximal response in FDI (i.e. 120% $M_{\text{max}}$). $M_{\text{max}}$ was obtained by averaging the responses to 5 stimuli delivered at the end of the experiment.

### 3.3.3. Data Analysis

For each test TMS intensity, and in both activity states, SICI and LICI were quantified by expressing individual conditioned MEPs as a percentage of the average unconditioned test alone MEP. When expressing inhibition measurements based on test alone MEP amplitude, data were grouped into 1 mV bins for absolute amplitude and 10% $M_{\text{max}}$ bins for normalised amplitude. Although this alternative analysis resulted in an unequal number of responses from each subject in each bin (due to between-subject differences in test MEP and $M_{\text{max}}$ amplitudes), we have previously shown that this sampling procedure produces a similar magnitude of SICI and LICI compared with when each subject contributes a sample to each bin (Chapter 2).

For grip-lift data, the temporal phases of movement (preload, load, transition) were defined according to previously established criteria (Johansson & Westling, 1984) using the first derivatives of the grip force (GF), lift force (LF) and acceleration (Acc) traces. The maximum GF ($GF_{\text{max}}$ – expressed as a percentage of LF at the time of occurrence), LF ($LF_{\text{max}}$) and Acc ($Acc_{\text{max}}$) were recorded from raw data traces. Cross-correlations between the first derivatives of GF and LF were calculated and assessed via the maximum cross-correlation coefficient ($\rho_{\text{max}}$). The time shift of GF (relative to LF) required to achieve $\rho_{\text{max}}$ (lag time) was also assessed.

### 3.3.4. Statistical Analysis

RMT, AMT and $M_{\text{max}}$ amplitude were compared between groups using unpaired student’s $t$ tests. Individual mixed-model analyses were used to compare the effects of test TMS intensity (110%, 120%, 130%, 140% & 150% RMT/AMT), absolute test MEP amplitude (rest, 0–1, 1–
2 and > 2 mV; active, 0–1, 1–2, 2–3 and > 3 mV) and normalised test MEP amplitude (rest, 0–10%, 10–20% and > 20% $M_{\text{max}}$; active, 0–10%, 10–20%, 20–30% and > 30% $M_{\text{max}}$) on SICI and LICI between young and older subjects, in resting and active muscle. Subject was included as a random effect and significant main effects and interactions were further investigated using Bonferroni corrected custom contrasts. Repeated-measures analysis of variance (ANOVA$_{\text{RM}}$) was used to assess the impact of lift trial (1, 2, 3, 4, 5), weight (100g, 200g, 300g) and age (young, older) on grip-lift performance. Significant main effects and interactions were further investigated using unpaired student’s $t$ tests with Bonferroni correction. For all significant between-group interaction effects, the estimated mean difference and corresponding 95% confidence interval (CI) was also calculated as an unstandardised indication of effect size. Linear regression of individual subject data was used to investigate interactions between measures of corticospinal excitability and grip-lift performance indices. Significance was set at $P < 0.05$ for all comparisons and data are shown as mean and 95% CI [lower limit, upper limit], unless otherwise stated.

3.4. Results

As the results of the young cohort have been previously reported (Chapter 2), and the primary interest of the current study concerns age-related effects, only findings involving main effects or interactions of age will be described in detail. No differences were found between groups for RMT (young, 46.4 [43.3, 49.4] % MSO; older, 48.2 [43.2, 53.1] % MSO, $P = 0.5$) or AMT (young, 38.2 [35.2, 41.2] % MSO; older, 38.4 [34.3, 42.5] % MSO, $P = 0.9$), but $M_{\text{max}}$ amplitude was 40% larger in young subjects (19.0 [17.4, 20.6] mV) than in older subjects (13.5 [12.0, 15.0] mV; $P < 0.0001$).
Chapter 3  
Ageing, I/O Properties & ICI

3.4.1. Influence of test TMS intensity on the test alone MEP in young and old subjects

Increasing test TMS intensity resulted in larger absolute test MEP amplitudes in both resting ($P < 0.0001$; Fig. 3.1A) and active ($P < 0.0001$; Fig. 3.1C) muscle. At rest, the amplitude of the absolute test MEPs at each test TMS intensity were unaffected by age ($P = 0.1$) and there was no interaction between age and test TMS intensity ($P = 0.2$). With FDI active, absolute test MEP amplitude was reduced in older subjects ($P = 0.0001$) and there was an interaction between age and test TMS intensity ($P < 0.0001$). Post hoc testing showed that young subjects had larger absolute test MEP amplitudes than older subjects at 130% (estimated mean difference: $0.8 \text{ mV}$, 95% CI $[0.3, 1.2]$, $P = 0.001$), 140% (estimated mean difference: $1.1 \text{ mV}$, 95% CI $[0.7, 1.6]$, $P < 0.0001$) and 150% RMT (estimated mean difference: $1.7 \text{ mV}$, 95% CI $[1.3, 2.2]$, $P < 0.0001$).

After normalising absolute amplitude test MEPs to individual subject $M_{max}$, increasing test TMS intensity produced larger amplitude normalised test alone MEPs in both resting ($P < 0.0001$; Fig. 3.1B) and active ($P < 0.0001$; Fig. 3.1D) muscle. At rest, no effect of age was found ($P = 0.2$), but there was an interaction between age and test TMS intensity ($P < 0.0001$). Between-group post hoc analysis showed larger normalised test MEP amplitudes in older subjects when applying the 150% RMT test intensity (estimated mean difference: $7.3 \%M_{max}$, 95% CI $[1.3, 13.4]$, $P = 0.02$). In active muscle, normalised test MEP amplitude was not different between age-groups ($P = 0.4$) and there was no interaction between age and test TMS intensity ($P = 0.4$).
Figure 3.1 Changes in the amplitude of the test alone MEP with increasing test TMS intensity. Data show the average amplitude of the absolute (mV; A, C) and normalised (% \( M_{\text{max}} \); B, D) test MEP amplitude recorded at each test TMS intensity for young (black circles) and older (white circles) subjects in resting (top panels) and active (bottom panels) FDI muscle. Positively directed error bars show the upper limit of the 95% CI, negatively directed error bars show the lower limit of the 95% CI. *P < 0.05. Abbreviations: mV, millivolts; RMT, resting motor threshold; AMT, active motor threshold; \( M_{\text{max}} \), maximum compound muscle action potential.

3.4.2. Influence of age on SICI and LICI

The main effects of age on SICI and LICI in resting and active muscle pooled across all test TMS intensities are shown in Figure 3.2. In resting muscle, SICI was not different between groups (\( P = 0.3; \) Fig. 3.2A) whereas LICI was significantly reduced in older subjects (\( P = 0.007; \) Fig. 3.2C). With the muscle active, SICI was significantly reduced in older subjects (\( P = 0.02; \) Fig. 3.2B) but LICI was unaffected by age (\( P = 0.3; \) Fig. 3.2D).
3.4.3. Influence of test TMS intensity and test MEP amplitude on age-related changes in SICI

For SICI at rest, increasing test TMS intensity resulted in reduced inhibition ($P < 0.0001$; Fig. 3.3A), but there was no interaction between age and test TMS intensity ($P = 0.5$). In active muscle, greater test TMS intensity produced increased inhibition ($P < 0.0001$; Fig. 3.3D). However, interactions between age and test TMS intensity were not significant ($P = 0.7$).

When SICI data were regrouped according to absolute test MEP amplitude, resting SICI was reduced in response to larger amplitude test alone MEPs ($P = 0.003$; Fig. 3.3B) but this was not different between age groups ($P = 0.2$). However, SICI in active muscle was increased
with larger absolute amplitude test alone MEPs ($P < 0.0001$; Fig. 3.3E) and there was a significant interaction between age and absolute test MEP amplitude ($P < 0.0001$). Age-related comparisons within each bin showed that older subjects had increased SICI for test MEP amplitudes of 0–1 mV (estimated mean difference: 17.9%, 95% CI [4.9, 30.8], $P = 0.007$) and 2–3 mV (estimated mean difference: 10.9%, 95% CI [1.2, 20.6], $P = 0.03$), but reduced SICI for test MEP amplitudes $> 4$ mV (estimated mean difference: 16.4%, 95% CI [11.1, 21.8], $P < 0.0001$).

When SICI data were grouped according to normalised test MEP amplitude, measurements in resting muscle were unaffected by changes in amplitude ($P = 0.09$; Fig. 3.3C), but a significant interaction between age and normalised amplitude was found ($P = 0.001$). However, age-related comparisons within individual bins were not significant. In active muscle, a main effect of normalised MEP amplitude was found ($P < 0.0001$) and there was an interaction between age and normalised test MEP amplitude ($P < 0.0001$; Fig. 3.3F). Age-related comparisons within each bin showed that older subjects had increased SICI for normalised MEP amplitudes of 0–10% $M_{max}$ (estimated mean difference: 13.7%, 95% CI [4.0, 23.3], $P = 0.005$), but reduced SICI for normalised MEP of 10–20% (estimated mean difference: 8.8%, 95% CI [1.6, 16.0], $P = 0.02$) and $> 30$% $M_{max}$ (estimated mean difference: 18.6%, 95% CI [13.0, 24.2], $P < 0.0001$).
Figure 3.3 Effects of test TMS intensity (A, D), absolute test MEP amplitude (B, E) and normalised test MEP amplitude (C, F) on SICI in young (black circles) and old (white circles) adults at rest (top panels) and during activation (bottom panels) of FDI. The dotted horizontal line represents no inhibition, with values below 100% representing inhibition of the test MEP. Positively directed error bars show the upper limit of the 95% CI, negatively directed error bars show the lower limit of the 95% CI. *P < 0.05. Abbreviations: MEP, motor evoked potential; RMT, resting motor threshold; AMT, active motor threshold; $M_{\text{max}}$, maximum compound muscle action potential.
3.4.4. Influence of test TMS intensity and test MEP amplitude on age-related changes in LICI

Increasing test TMS intensity reduced the magnitude of LICI in resting muscle ($P < 0.0001$; Fig. 3.4A) and there was a significant interaction between age and test TMS intensity ($P = 0.004$). Age-related comparisons within each test intensity showed that older subjects had less inhibition than young subjects at 110% RMT (estimated mean difference: 30.0%, 95% CI [6.6, 53.4], $P = 0.01$), 120% RMT (estimated mean difference: 28.7%, 95% CI [5.3, 52.2], $P = 0.02$), 130% RMT (estimated mean difference: 39.6%, 95% CI [16.2, 63.1], $P = 0.001$) and 140% RMT (estimated mean difference: 40.4%, 95% CI [17.0, 63.9], $P = 0.01$). In active muscle, increasing test TMS intensity produced increased LICI ($P < 0.0001$; Fig. 3.4D) and a significant interaction between age and test TMS intensity was found ($P < 0.0001$), with age-related comparisons within individual test intensities showing that older subjects had more LICI than young subjects at 110% RMT (estimated mean difference: 46.2%, 95% CI [5.9, 86.5], $P = 0.03$).

When LICI data were regrouped according to absolute test MEP amplitude, measurements in resting muscle were reduced in response to larger absolute amplitudes ($P < 0.0001$) and there was an interaction between age and absolute test MEP amplitude ($P = 0.02$; Fig. 3.4B). Age-related comparisons within each bin showed that older subjects had less inhibition than young subjects for absolute amplitudes that were 0–1 mV (estimated mean difference: 43.0%, 95% CI [17.5, 68.6], $P = 0.001$) and 1–2 mV (estimated mean difference: 34.1%, 95% CI [8.1, 60.1], $P = 0.01$). In active muscle, LICI was increased when assessed using larger absolute test MEP amplitudes ($P < 0.0001$) and there was an interaction between age and absolute test MEP amplitude ($P < 0.0001$; Fig. 3.4E). Post hoc comparisons showed that older subjects had significantly more LICI than young subjects for absolute test MEP amplitudes of 0–1 mV.
(estimated mean difference: 52.4%, 95% CI [10.9, 94.0], \( P = 0.01 \)) and 1–2 mV (estimated mean difference: 41.7%, 95% CI [1.1, 82.4], \( P = 0.04 \)).

When LICI data were regrouped according to normalised test MEP amplitude, measurements in resting muscle were reduced in response to larger normalised test MEPs (\( P < 0.0001 \)) but there was no interaction between factors (\( P = 0.4 \)). Age-related comparisons within individual bins showed that LICI was significantly reduced in older subjects for test MEP amplitudes that were 0–10% (estimated mean difference: 29.8%, 95% CI [4.6, 55.0], \( P = 0.02 \)) and 10–20% \( M_{\text{max}} \) (estimated mean difference: 35.3%, 95% CI [8.6, 61.9], \( P = 0.01 \)). In active muscle, LICI was increased in response to larger normalised test MEP amplitudes (\( P < 0.0001 \)) and there was a significant interaction between age and normalised amplitude (\( P < 0.0001 \); Fig. 3.4F). Between-group comparisons showed a trend towards increased inhibition in older subjects for test MEP amplitudes that were 0–10% \( M_{\text{max}} \) (estimated mean difference: 40.1%, 95% CI [0.3, 80.6], \( P = 0.05 \)).
Figure 3.4 Effects of test TMS intensity (A, D), absolute test MEP amplitude (B, E) and normalised test MEP amplitude (C, F) on LICI in young (black circles) and old (white circles) adults at rest (top panels) and during activation (bottom panels) of FDI. The dotted horizontal line represents no inhibition, with values below 100% representing inhibition of the test MEP. Positively directed error bars show the upper limit of the 95% CI, negatively directed error bars show the lower limit of the 95% CI. *P < 0.05. Abbreviations: MEP, motor evoked potential; RMT, resting motor threshold; AMT, active motor threshold; $M_{\text{max}}$, maximum compound muscle action potential.
3.4.5. Grip-lift performance and linear regression

Grip-lift data were obtained from 10/22 young subjects (mean age ± SD; 22.1 ± 1.2 years) and all 18 older subjects. Age-related comparisons of performance parameters are shown in Table 3.1. Linear regression analysis found significant associations between $M_{max}$ amplitude and the duration of both preload ($r^2 = 0.46, P = 0.0001$) and transition ($r^2 = 0.18, P = 0.03$) phases and significant association were also found between the magnitude of LICI in resting muscle and the duration of the transition phase ($r^2 = 0.04, P = 0.02$). All other comparisons were not significant.
### Table 3.1 Grip-lift performance parameters using 3 different loads in young and old subjects

<table>
<thead>
<tr>
<th></th>
<th>Young</th>
<th>Old</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100 g</td>
<td>200 g</td>
</tr>
<tr>
<td><strong>Finger force / coordination</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$GF_{\text{max}}$ (% LF)</td>
<td>268.7 (194.4, 343.0)</td>
<td>228.1 (184.9, 271.3)</td>
</tr>
<tr>
<td>$LF_{\text{max}}$ (N)</td>
<td>2.6 (2.5, 2.7)</td>
<td>3.8 (3.7, 3.9)</td>
</tr>
<tr>
<td>$Acc_{\text{max}}$ (g)</td>
<td>1.8 (1.4, 2.2)</td>
<td>1.7 (1.3, 2.1)</td>
</tr>
<tr>
<td>$\rho_{\text{max}}$</td>
<td>0.84 (0.81, 0.86)</td>
<td>0.86 (0.85, 0.88)</td>
</tr>
<tr>
<td><strong>Phase Duration (ms)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preload</td>
<td>49 (38, 60)</td>
<td>52 (39, 64)</td>
</tr>
<tr>
<td>Load</td>
<td>145 (111, 179)</td>
<td>168 (124, 211)</td>
</tr>
<tr>
<td>Transition</td>
<td>1107 (937, 1277)</td>
<td>1097 (935, 1258)</td>
</tr>
</tbody>
</table>

Values are shown as mean (95% CI; lower limit, upper limit). *P < 0.05 when compared to the same weight in young subjects. Abbreviations: ms, milliseconds


3.5. Discussion

The current study examined age-related differences in SICI and LICI in resting and active muscle with increasing test TMS intensity. This approach produced a broad range of MEP responses, allowing an investigation of age-related differences in inhibition at different absolute and normalised (relative to $M_{\text{max}}$) test MEP amplitudes. When data were pooled across test TMS intensities, SICI in active muscle and LICI in resting muscle were reduced in older compared with young subjects, but there were no age-related differences in SICI at rest and LICI in active muscle. However, these effects varied depending on the approach used to compare the test response (test TMS intensity/test MEP amplitude) between groups, suggesting that this is an important consideration when assessing age-related differences in SICI and LICI.

3.5.1. Advancing age influences SICI in active but not resting muscle

Within the current study, SICI in resting muscle was not affected by age, suggesting maintenance of resting GABA$_A$-mediated intracortical inhibition in old adults (Ziemann et al., 1996a). This finding supports the results of several previous studies (Oliviero et al., 2006; Rogasch et al., 2009; Smith et al., 2009; Cirillo et al., 2010; Cirillo et al., 2011; Fujiyama et al., 2011; Smith et al., 2011; Fujiyama et al., 2012b) but is in contrast to others (Peinemann et al., 2001; Kossev et al., 2002; McGinley et al., 2010; Marneweck et al., 2011; Heise et al., 2013). Although the reasons for these discrepancies are unclear, they are commonly attributed to methodological differences between studies. For example, previous studies have matched either test TMS intensity or test MEP amplitude between groups, as both factors are thought to influence estimates of SICI in resting muscle (Sanger et al., 2001; Garry & Thomson, 2009). We investigated whether estimates of SICI at rest, assessed using several different approaches to compare the test alone MEP, were differentially effected by age. We found that resting SICI did not differ between age groups when data were matched for test TMS
intensity or test MEP amplitude (absolute or normalized), suggesting that these factors are unlikely to contribute to previous inconsistencies between studies. Furthermore, as conflicting effects of age on SICI have been reported from studies that have used the same conditioning intensity and ISI (Kossev et al., 2002; Smith et al., 2009; Hinder et al., 2011), it seems unlikely that variations in stimulus parameters alone can account for these inconsistencies.

We therefore suggest that factors other than TMS parameters may contribute to inter-study variations in effects of age on resting SICI, such as target muscle, subject characteristics (i.e., health status, physical activity levels, or habitual hand function) and age-related changes in measurement reliability.

In contrast with resting muscle, SICI in active FDI was significantly reduced in older subjects, but the effect varied depending on the approach used to compare the test MEP between groups. When data were pooled over all test TMS intensities, SICI in active muscle was less in older adults. However, regrouping data relative to test MEP amplitude (absolute or normalised) showed that older adults had increased SICI for small amplitude test MEPS (0–1 mV/0–10% $M_{\text{max}}$) but decreased SICI for large amplitude test MEPS (>4 mV/>30% $M_{\text{max}}$).

Only one previous study has examined age-related changes in SICI during muscle activation (although age-effects during movement preparation have been investigated; Fujiyama et al., 2011; Fujiyama et al., 2012b; Heise et al., 2013). Using a 2 mV test MEP and low-intensity (15% MVC) contraction of the flexor carpi radialis muscle (FCR), McGinley and colleagues failed to observe any effect of age on SICI in active muscle (McGinley et al., 2010). The results of the current study support this, as there was no difference in active SICI between groups when matching absolute test MEP amplitude at 1–2 mV (Fig. 3.2E). However, differences were observed when smaller or larger test MEPS were used, suggesting that test MEP amplitude has important implications for the comparison of active SICI between young and older adults. Furthermore, the effects of age on active SICI were variable and dependent
on the specific test MEP amplitude used, suggesting that more than one test MEP amplitude should be used to adequately characterise age-related differences in SICI in active muscle.

3.5.2. Advancing age influences LICI in resting and active muscle

The most striking difference between young and older adults in the present study was a reduction in resting LICI in older adults. This effect was observed irrespective of how the data were grouped, suggesting strong age-related alterations in resting GABA\textsubscript{B} mediated intracortical inhibition (Werhahn et al., 1999). This effect was most pronounced at low-moderate test TMS intensities (< 140% RMT) and test MEP amplitudes (< 2 mV/ < 20% M\textsubscript{max}). In contrast to our findings, the only previous study to examine age-related differences in resting LICI showed an increase in LICI in older adults (McGinley et al., 2010). We suspect that the use of different ISI’s within each study may have contributed to these divergent findings. Our study used an ISI of 150 ms rather than the 100 ms interval used previously (McGinley et al., 2010), because recent research suggests that inhibition observed using the shorter ISI (100 ms) may be influenced by changes in spinal excitability (McNeil et al., 2011). These possible timing-dependent effects of age on LICI may be consistent with recent suggestions that measurements of LICI using a 100 or 150 ms ISI do not represent activation of the same cortical process (Chu et al., 2008; Chu et al., 2009; Vallence et al., 2012). Nonetheless, for low-moderate test TMS intensities and test MEP amplitudes that are commonly used experimentally, our data show a relatively consistent reduction in resting LICI in older adults.

Our assessment of LICI in active FDI muscle did not find any main effect of age. However, separating data based on test TMS intensity or test MEP amplitude revealed increased LICI in older subjects at low test TMS intensities (110% AMT) and absolute test MEP amplitudes (< 2 mV), with no difference between age groups for normalised test MEP amplitude. In the
only other study to examine age-related changes in LICI in an active muscle, no effect of age was found when data in FCR muscle was matched between groups using a 2 mV test MEP amplitude (McGinley et al., 2010). As suggested above, it is possible that the use of different ISI’s may have contributed to these contradictory findings. However, the contraction intensity also varied between studies (15% MVC by the previous study, 5% MVC in the current study) and, as increasing contraction intensity has been shown to have non-linear effects on the magnitude of active LICI (McNeil et al., 2011), age-related variations in this effect may also have contributed to the contrasting results.

Interestingly, when low test TMS intensities and test MEP amplitudes were used to compare LICI between groups, young subjects demonstrated MEP facilitation, whereas older subjects displayed MEP inhibition. A previous study in young subjects assessing LICI in active muscle also reported a tendency for MEP facilitation when using low intensity test stimuli (110% RMT) and ISIs of 150–160 ms (Wassermann et al., 1996). Furthermore, two recent studies have observed a period of cortical disinhibition at long intervals (> 165 ms) after application of suprathreshold TMS (Cash et al., 2010; Caux-Dedeystère et al., 2014), which was suggested to relate to the previously observed MEP facilitation (Cash et al., 2010). Although our differential effect of test TMS intensity on LICI in active muscle may therefore suggest an age-related reduction in this cortical disinhibition, the lack of any strong correlation of LICI with grip-lift performance suggests that the functional implications, at least during this task, are relatively minor.

In the present study, it was not possible to directly compare the magnitude of intracortical inhibition in resting and active states because the test TMS intensities were not the same under both conditions, due to normalisation to either the resting (in the rest state) or the active (in the active state) motor threshold. Despite this caveat, the magnitude of LICI in older adults over a range of test TMS intensities was less than young adults at rest, but the effect was
removed (or even reversed at some TMS intensities) in the active muscle. These results suggest that the activity-related modulation of LICI may be reduced in older adults, which supports recently reported observations for SICI (Fujiyama et al., 2012b; Heise et al., 2013). The activity-related disinhibition of SICI has been suggested to stem from an increased contribution of $I_1$ waves to the MEP (Hanajima et al., 1998; Zoghi et al., 2003), as well as a reduced inhibition of $I_3$ waves from SICI circuits (Zoghi et al., 2003). Given that SICI and LICI both modulate the amplitude of late I waves (Di Lazzaro et al., 1998c; Di Lazzaro et al., 2002b), age-related differences in the activity-dependent modulation of LICI may therefore reflect differences in the ability to modulate these intracortical inhibitory circuits, or age-related differences in the way these inhibitory circuits influence the descending volley during muscle activation. Nonetheless, the mechanisms contributing to the disinhibition of LICI during muscle activation, along with the functional implications of age-related changes in these mechanisms, remain to be explored.

In conclusion, we found age-dependent differences in the magnitude of SICI and LICI, suggesting alterations to GABA$_A$- and GABA$_B$-mediated intracortical inhibition. However, the nature of these effects depended on the activity state of the target muscle, the technique used to compare the test response between groups (test TMS intensity/test MEP amplitude) and the specific characteristics of the test response. Our findings suggest that future studies investigating age-related changes in SICI and LICI during muscle activation should consider the use of multiple test TMS intensities or test MEP amplitudes when quantifying the magnitude of intracortical inhibition.
CHAPTER IV

TASK-RELATED CHANGES IN INTRACORTICAL INHIBITION ASSESSED WITH PAIRED- AND TRIPLE-PULSE TRANSCRANIAL MAGNETIC STIMULATION

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STATEMENT OF AUTHORSHIP

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By signing the Statement of Authorship, each author certifies that their stated contribution to the publication is accurate and that permission is granted for the publication to be included in the candidate’s thesis

**George Opie (Candidate)**

**Contribution:** Experimental design, subject recruitment, collection and analysis of data, interpretation of data, wrote manuscript

Signed…

Date…

**Michael Ridding**

**Contribution:** Experimental design, data interpretation, edited manuscript

Signed…

Date…

**John Semmler**

**Contribution:** Experimental design, supervised development of work, data interpretation, critical manuscript evaluation, acted as corresponding author

Signed…

Date…
4. Task-related changes in intracortical inhibition assessed with paired- and triple-pulse transcranial magnetic stimulation

4.1. Abstract

Recent research has demonstrated a task-related modulation of postsynaptic intracortical inhibition within primary motor cortex for tasks requiring isolated (abduction) or synergistic (precision grip) muscle activation. The current study sought to investigate task-related changes in pre- and postsynaptic intracortical inhibition in motor cortex. In 13 young adults (22.5 ± 3.5 years), paired-pulse transcranial magnetic stimulation (TMS) was used to measure short- (SICI) and long-interval intracortical inhibition (LICI) (i.e. postsynaptic motor cortex inhibition) in first dorsal interosseous muscle, and triple-pulse TMS was used to investigate changes in SICI-LICI interactions (i.e. presynaptic motor cortex inhibition). These measurements were obtained at rest and during muscle activation involving isolated abduction of the index finger or during a precision grip using the index finger and thumb. SICI was reduced during abduction and precision grip compared with rest, with greater reductions during precision grip. The modulation of LICI during muscle activation depended on the interstimulus interval (ISI; 100 and 150 ms), but was not different between abduction and precision grip. For triple-pulse TMS, SICI was reduced in the presence of LICI at both ISIs in resting muscle (reflecting presynaptic motor cortex inhibition), but was only modulated at the 150 ms ISI during index finger abduction. Results suggest that synergistic contractions are accompanied by greater reductions in postsynaptic motor cortex inhibition than isolated contractions, but the contribution of presynaptic mechanisms to this disinhibition is limited. Furthermore, timing-dependent variations in LICI provide additional evidence that measurements using different ISIs may not represent activation of the same cortical process.
Chapter 4  

Motor task & LICI-SICI interactions

4.2. Introduction

Throughout the central nervous system (CNS), inhibitory neurotransmission mediated through the activity of \( \gamma \)-aminobutyric acid (GABA) and its associated receptors represents a fundamental component of brain function. Important examples of this can be seen in the involvement of GABA in oscillatory activity and synaptic plasticity, processes thought to be important in learning and memory (Paulsen & Moser, 1998; Mann & Paulsen, 2007), as well as in facilitating sensory acuity via surround inhibition (Kyriazi et al., 1996; Binns & Salt, 1997; Vučinić et al., 2006). Furthermore, alterations in GABAergic function are thought to be associated with several pathological states, such as epilepsy (Treiman, 2001) and schizophrenia (Benes & Berretta, 2001; Lewis et al., 2005). Within human primary motor cortex, the activity of GABAergic inhibitory circuits can be studied non-invasively using paired-pulse transcranial magnetic stimulation (TMS). When a subthreshold conditioning stimulus is applied at short interstimulus intervals (ISI; 1–5 ms) prior to a suprathreshold test stimulus, the amplitude of the test motor evoked potential (MEP) is reduced (Kujirai et al., 1993). This is referred to as short-interval intracortical inhibition (SICI) and is thought to be due to activation of postsynaptic GABA\(_A\) receptors (Ziemann et al., 1996b). Furthermore, when both conditioning and test stimuli are suprathreshold and separated by long ISIs (100–150 ms), a reduction of the test MEP amplitude is referred to as long-interval intracortical inhibition (LICI; Valls-Sole et al., 1992) and is thought to be due to activation of postsynaptic GABA\(_B\) receptors (Werhahn et al., 1999). The magnitude of SICI and LICI may be altered in some movement disorders such as Parkinson’s disease, Huntington’s disease and dystonia (Berardelli et al., 2008), suggesting that these inhibitory circuits are important for basic motor control.

Voluntary activation of target muscles causes reductions in the magnitude of both SICI (Ridding et al., 1995) and LICI (Hammond & Vallence, 2007), and this change in
postsynaptic inhibition is thought to be functionally relevant (Sohn et al., 2002; Zoghi et al., 2003). In support of this, a recent study has shown that intracortical inhibition varies between tasks requiring different patterns of hand muscle activation, with greater reductions in both SICI and LICI occurring during synergistic as opposed to isolated muscle recruitment (Kouchtir-Devanne et al., 2012). Similar effects have also been reported for the EMG silent period (SP), with greater reductions in SP duration occurring during synergistic tasks (Tinazzi et al., 2003). This increased cortical disinhibition during synergistic tasks may facilitate the functional coactivation of the cortical representations for task-related muscles (Kouchtir-Devanne et al., 2012), resulting in improved task performance.

One factor that may influence these changes in SICI and LICI is a task-dependent modulation of presynaptic motor cortex inhibition, which can be assessed by quantifying the interaction between LICI and SICI (Ni et al., 2011b). This is examined in human motor cortex using a triple-pulse TMS protocol, where the conditioning and test stimuli used to assess SICI are preceded by a conditioning stimulus for LICI (Sanger et al., 2001). In resting muscle, this pattern of stimulation results in a reduced inhibition of the test MEP relative to the inhibition observed during application of SICI in isolation (Sanger et al., 2001). Several lines of evidence suggest that this disinhibition of SICI circuitry occurs by GABA_B receptor (LICI) mediated presynaptic motor cortex inhibition (Werhahn et al., 1999; Sanger et al., 2001; McDonnell et al., 2006; Muller-Dahlhaus et al., 2008; Ni et al., 2011a). This presynaptic interaction of SICI by LICI is impaired in Parkinson’s’ disease patients, which may contribute to their movement deficits (Chu et al., 2009). However, these previous studies have largely examined presynaptic motor cortex inhibition in resting muscles, and it is unknown whether presynaptic motor cortex inhibition is modulated during the performance of different tasks, and whether this is related to task-dependent changes in postsynaptic intracortical inhibition.
Chapter 4  

The main aim of the current study was therefore to investigate task related variations in pre and postsynaptic GABA-mediated intracortical inhibition. This was accomplished by using paired- and triple-pulse TMS to examine SICI, LICI and LICI-SICI interactions in resting first dorsal interosseous muscle (FDI), or when it was active during isolated index finger abduction or precision grip (involving synergistic opposition of the index finger and thumb). Given that presynaptic motor cortex inhibition may be important for fine motor control (Chu et al., 2009), we would expect to see greater alterations in presynaptic and postsynaptic motor cortex inhibition during more demanding precision grip tasks. In addition, as the time course of presynaptic motor cortex inhibition in humans is not clear (Chu et al., 2008; Cash et al., 2010), a secondary aim was to compare any task-related changes in paired- and triple-pulse TMS measurements using two commonly used ISIs (100 ms and 150 ms).

4.3. Methods

13 young (mean ± standard deviation; 22.3 ± 3.8 years) healthy subjects were recruited from the university and wider community to participate in the current study. Exclusion criteria included a history of neurological or psychiatric disease, or current use of psychoactive medication (sedatives, antipsychotics, antidepressants etc.). Hand preference and laterality was assessed using the Edinburgh Handedness Inventory (Oldfield, 1971). Each subject provided written, informed consent prior to participation. All experimentation was approved by the University of Adelaide Human Research Ethics Committee and conducted in accordance with the declaration of Helsinki.

4.3.1. Experimental arrangement

For the duration of the experiment, subjects were seated in a comfortable chair with their right arm abducted approximately 45° at the shoulder. This allowed the forearm and hand to sit comfortably on an arm support placed next to them. Surface electromyography (EMG) was
used to record responses from the first dorsal interosseous (FDI) muscle of the right hand. Two Ag–AgCl electrodes (3.2 cm diameter) were attached to the skin over the muscle in a belly-tendon montage, with a grounding strap around the wrist acting as a reference. EMG was amplified (300 X) and band-pass filtered (20 Hz high pass, 1 kHz low pass) using a CED1902 (Cambridge Electronic Design, Cambridge, UK), and digitized at 2 kHz using a CED1401 interface (Cambridge Electronic Design), before being recorded and stored offline for analysis. To facilitate muscle relaxation when required, real-time EMG signals were displayed under high gain (50 µV/ division) on an oscilloscope placed in front of the subject.

Each subject participated in two experimental sessions held on separate days, each of 2–3 hours duration. Within each session, TMS was applied during complete relaxation of FDI and while FDI was active in producing one of two low intensity (5% of maximum force) contractions, performed in random order. For one of the sessions, subjects were required to produce an isolated abduction of the index finger, whereas in the other session they were required to activate the muscle by performing a precision grip of the index finger and thumb. As prolonged contractions were required to complete the multiple stimulation conditions needed for triple-pulse TMS (see below), assessing each active task on separate days reduced the likelihood of fatiguing the target muscle, which may have confounded measurements of intracortical inhibition (Benwell *et al.*, 2006; Benwell *et al.*, 2007; Vucic *et al.*, 2011). Within each experimental session, all TMS conditions (see below) were applied twice, once with the target muscle at rest, and again with the target muscle active (either abduction or precision grip). Furthermore, paired-pulse TMS was always performed before triple-pulse TMS for all subjects, allowing the experimenter to monitor baseline levels of inhibition before applying triple-pulse TMS. During active state measurements, stimulation began after subjects had reached stable force application.
4.3.2. Experimental Procedures

Maximal Voluntary Contraction At the beginning of each experiment, maximum voluntary contractions (MVC) were assessed for each subject. This was performed for both index finger abduction and during a precision grip using the index finger and thumb. During index finger abduction, the subject’s right hand was positioned with the palm facing downwards and the index finger isolated from the middle, ring and little fingers. When instructed, subjects abducted the lateral surface of the index finger against a force transducer (LC1205-K020; A&D Mercury Pty Ltd, Australia) placed in-line with the distal interphalangeal joint. During precision grip, subjects opposed the index finger and thumb against a purpose built manipulandum that has been described previously (Chapters 2 & 3). The procedure to assess the MVC was identical for both index finger abduction and precision grip: subjects were required to produce maximum force for 3 s in several repetitions, separated by 30 s rest, until the maximal force of three trials were within a 10% margin. The largest force recorded during these trials was chosen as the subject’s MVC. To optimise force production, feedback was displayed on a computer monitor placed at eye level in front of the subject, and verbal encouragement was provided by the experimenter.

Transcranial magnetic stimulation TMS was applied to the left primary motor cortex using a figure-of-eight coil (external wing diameter 9 cms) with three Magstim 200 magnetic stimulators connected via two Bistim units (Magstim, Dyfed, UK). Within this setup, two stimulators were connected via the first Bistim unit, while the third stimulator and the output from the first Bistim unit were connected via the second Bistim unit. The coil was then connected to the output of the second Bistim unit. This allowed application of up to 3 stimuli at very short intervals and different intensities through the same coil, but is associated with a reduction in stimulus strength of approximately 15% (Sanger et al., 2001). During testing, the coil was held tangentially to the scalp at an angle of 45° to the sagittal plane, with the handle
pointed backwards and laterally, producing a current flow in the brain with a posterior to anterior direction. The coil was positioned on the scalp over the location producing an optimum response in the relaxed FDI muscle. This location was marked on the scalp for reference and continually checked throughout the experiment. TMS was delivered at 0.2 Hz for all conditions.

Resting and Active motor thresholds (RMT and AMT, respectively) were obtained in FDI while the TMS coil was placed at the optimal location over primary motor cortex. RMT was defined as the minimum TMS intensity producing a response amplitude $\geq 50 \mu V$ in three out of five trials in resting FDI muscle, and expressed relative to the maximum stimulator output (MSO). Active motor threshold (AMT) was defined as the minimum TMS intensity producing a response amplitude $\geq 300 \mu V$ in three out of five trials while FDI was active at 5% MVC. Force feedback was provided via an oscilloscope placed at eye level in front of the subject, with a target force set on the oscilloscope that was adjusted to 5% of each subject's MVC.

Intracortical inhibition The magnitude of intracortical inhibition was assessed using 4 experimental conditions (Table 4.1, Conditions A–D). SICI was measured with a subthreshold conditioning stimulus set at 80% AMT and an interstimulus interval (ISI) of 2 ms (Condition B; Kujirai et al., 1993) while LICI was assessed using a suprathreshold conditioning stimulus set at 120% RMT and two ISIs of 100 and 150 ms (Conditions C and D; Valls-Sole et al., 1992). For both SICI and LICI, the intensity of the test stimulus was set at the level producing an MEP with peak-to-peak amplitude of 1 mV when given alone (Condition A; Stim$_{1mV}$). Representative data from a single subject for each of these experimental conditions in resting FDI is shown in the top 3 traces of Figure 4.1. Both paired-pulse TMS paradigms were applied in the same experimental block, allowing normalisation of all paired-pulse responses to a common test alone state. As 30 conditioned trials (10 SICI, 20 LICI) and 10 test alone
(control) trials were included within a block, and each block was repeated with the muscle at rest and during activation, a total of 80 trials were used to assess baseline levels of intracortical inhibition.

Table 4.1 TMS protocol

<table>
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<tr>
<th>Condition</th>
<th>CS\textsubscript{150}</th>
<th>CS\textsubscript{100}</th>
<th>CS\textsubscript{2}</th>
<th>Test Stimulus</th>
</tr>
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<tbody>
<tr>
<td>A</td>
<td>Test MEP</td>
<td>—</td>
<td>—</td>
<td>Stim\textsubscript{1mV}</td>
</tr>
<tr>
<td>B</td>
<td>SICI</td>
<td>—</td>
<td>—</td>
<td>Stim\textsubscript{1mV}</td>
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<tr>
<td>C</td>
<td>LICI\textsubscript{100}</td>
<td>—</td>
<td>120% RMT</td>
<td>Stim\textsubscript{1mV}</td>
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<tr>
<td>D</td>
<td>LICI\textsubscript{150}</td>
<td>120% RMT</td>
<td>—</td>
<td>Stim\textsubscript{1mV}</td>
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<td>—</td>
<td>120% RMT</td>
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<tr>
<td>L</td>
<td>LICI\textsubscript{150}-SICI</td>
<td>120% RMT</td>
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</tbody>
</table>

Abbreviations: CS\textsubscript{150}, conditioning stimulus applied 150 ms prior to the test stimulus; CS\textsubscript{100}, conditioning stimulus applied 100 ms prior to the test stimulus; CS\textsubscript{2}, conditioning stimulus applied 2 ms prior to the test stimulus; MEP\textsubscript{1mV}, stimulus intensity producing an MEP with peak-to-peak amplitude of 1 mV; MEP\textsubscript{100}, stimulus intensity producing an MEP with peak-to-peak amplitude of 1 mV when preceded by CS\textsubscript{100}; MEP\textsubscript{150}, stimulus intensity producing an MEP with peak-to-peak amplitude of 1 mV when preceded by CS\textsubscript{150}; RMT, resting motor threshold; AMT active motor threshold.
Figure 4.1 Representative data from a single subject showing the MEP response generated during each of the different experimental conditions. Data are showing responses in resting muscle only. Refer to table 1 for a description of each condition. *Conditioned MEP amplitude as a percentage of the control MEP amplitude.
The effect of LICI on SICI was assessed using triple-pulse TMS (Table 4.1, Conditions G–J). The conditioning stimulus used to activate LICI circuitry was set at 120% RMT and applied at two intervals of 100 ms (CS₁₀₀, Condition I) and 150 ms (CS₁₅₀, Condition J) in separate blocks. Within both blocks, the conditioning stimulus used to activate SICI circuitry was set at 80% AMT and applied using a 2 ms ISI (CS₂). The effect of LICI on SICI (LICI-SICI interaction) was quantified by comparing the amplitude of the test MEP generated by application of all three stimuli (CS₁₀₀/CS₁₅₀, CS₂ and a test stimulus) to the amplitude of the test MEP generated by application of the LICI conditioning stimulus and the test stimulus. The intensity of the test stimulus was adjusted to the level producing an MEP response of 1 mV when preceded by either CS₁₀₀ (Stim₁₀₀, Condition G) or CS₁₅₀ (Stim₁₅₀, Condition H). This intensity was set individually for each ISI. Representative data from a single subject for each of these experimental conditions is shown in the bottom 2 traces of Figure 4.1. As increasing test TMS intensity reduces the magnitude of SICI (Garry & Thomson, 2009; Chapters 2 & 3) the higher intensity Stim₁₀₀ and Stim₁₅₀ could account for changes in SICI observed during triple-pulse TMS. Therefore, additional measurements of SICI using Stim₁₀₀ (SICI_adj₁₀₀) and Stim₁₅₀ (SICI_adj₁₅₀) for the test stimulus were recorded as control states (Conditions E and F, respectively). We will refer to the test MEP generated during SICI_adj₁₀₀ and SICI_adj₁₅₀ as MEP_adj₁₀₀ and MEP_adj₁₅₀, respectively. As 10 conditioned and 10 control trials were applied within four experimental blocks, and each block was repeated with the muscle at rest and during activation, a total of 160 trials were used to assess interactions between LICI and SICI.

4.3.3. Data Analysis

Data analysis was completed manually by visual inspection of offline EMG. Within the rest state, traces showing muscle activity > 20 µV in peak-to-peak amplitude during the 150 ms prior to the MEP were excluded from analysis. MEP amplitudes from each trial were
measured peak-to-peak and expressed in mV. Paired- and triple-pulse measurements of intracortical inhibition were quantified by expressing the amplitude of individual conditioned MEPs as a percentage of the average control MEP amplitude. Furthermore, to quantify the absolute change in SICI in response to adjusted intensity test stimuli (SICI_{adj100} / adj_{150}) and triple pulse stimulation (LICI_{100}SICI / LICI_{150}SICI), baseline SICI measurements were subtracted from adjusted intensity and triple-pulse SICI measurements for each task. For active trials, muscle activation was assessed by quantifying the root mean squared (rms) EMG amplitude (normalised to the maximum rmsEMG amplitude recorded during MVC) in the 100 ms leading up to application of CS_{150}, CS_{100} or CS_{2} (depending on stimulation condition).

4.3.4. Statistical Analysis

RMT and AMT were compared between sessions using paired t-tests. Stim_{1mV}, Stim_{100} and Stim_{150} in resting muscle were also compared between sessions using paired t-tests. Subject to no significant inter-session differences, these data were pooled to investigate the effect of task (rest, abduction & precision) on test stimulus intensity. This was assessed using a two-way repeated-measures analysis of variance (ANOVA_{RM}), with factors of test stimulus condition (Stim_{1mV}, Stim_{100} & Stim_{150}) and task. Main effects and interactions were further investigated using one-way ANOVA’s with Fishers PLSD post-hoc test. Normalised EMG amplitude prior to TMS was assessed for SICI, LICI and LICI-SICI interactions using individual two-way ANOVA_{RM} and Fishers PLSD post-hoc test.

Mixed-model analysis was used to investigate the effects of test MEP condition (MEP_{1mV}, MEP_{adj100}, MEP_{adj150}, MEP_{100} & MEP_{150}) and task on the amplitude of the test alone MEP. Individual mixed-model analyses were used to compare the effects of task on the magnitude of inhibition for SICI and LICI. The effect of LICI on SICI was also assessed using mixed-model analysis, with factors of SICI condition (baseline, SICl_{adj} & LICI-SICI) and task. This
was investigated using separate models for each ISI. For all models, subject was included as a random effect, and significant interactions were further investigated using custom contrasts with Bonferroni correction. The absolute change in SICI relative to baseline within each stimulus condition was compared to ‘0’ (i.e., no change in inhibition) using one-sample t-tests with Bonferroni correction. As a non-standardised indication of effect size, estimated mean differences (EMD) and corresponding 95% confidence intervals (CI) were provided for post hoc comparisons. Significance was set at \( P < 0.05 \) for all comparisons and data are shown as mean [95% CI lower limit, upper limit], unless otherwise stated.

4.4. Results

All subjects completed both experimental sessions in full and without adverse reaction. The subject cohort consisted of 7 females (21.4 [20.2, 22.6] years) and 6 males (23.3 [18.5, 28.1] years) and all subjects were right-hand dominant (average laterality quotient, 0.92 [0.8, 1.0]). No differences were found between sessions for RMT (Session 1: 61.8 [57.0, 66.5]% MSO, Session 2: 60.5 [55.8, 65.1]% MSO, \( P = 0.2 \)) or AMT (Session 1: 47.0 [42.5, 51.5]% MSO, Session 2: 47.6 [42.8, 52.4]% MSO, \( P = 0.6 \)). MVC force was significantly greater during precision grip (51.0 [42.9, 59.2] N) than index finger abduction (29.7 [23.3, 36.0] N, \( P < 0.01 \)), whereas MVC EMG was significantly greater during index finger abduction (0.95 [0.8, 1.1] mV) than precision grip (0.60 [0.4, 0.8], \( P < 0.01 \)).

4.4.1. Test MEP characteristics

The amplitude of the test alone MEP in each test stimulus condition is reported in Table 4.2. Analysis of these data revealed significant main effects of test MEP condition (\( F_{4,1857} = 555.6, P < 0.01 \)) and task (\( F_{2,1109} = 113.8, P < 0.01 \)), as well as a significant interaction between factors (\( F_{4,1848} = 113.3, P < 0.01 \)). As expected, these effects were driven by MEP\(_{\text{adj100}}\) and MEP\(_{\text{adj150}}\) being significantly larger than all other conditions (all \( P \)-values < 0.01).
Furthermore, compared with rest, both MEP\textsubscript{adj100} and MEP\textsubscript{adj150} were significantly larger during index finger abduction and precision grip (all \(P\)-values < 0.01), but there was no difference in amplitude between abduction and precision grip for either variable. No significant differences in the amplitude of the adjusted test MEPs (MEP\textsubscript{1mV}, MEP\textsubscript{100} and MEP\textsubscript{150}) was found between stimulus conditions or tasks (all \(P\)-values > 0.05), suggesting that these were well matched to each other (Table 4.2). Test stimulus intensities for each test stimulus condition are shown in Table 4.2. Significant main effects of test stimulus condition (\(F_{2,49} = 96.7, P < 0.01\)) and task (\(F_{2,49} = 23.1, P < 0.01\)) were found, and there was a significant interaction between factors (\(F_{4,98} = 15.4, P < 0.01\)). Post hoc testing showed, for all conditions, test TMS intensities in resting muscle were larger than during either abduction or precision grip (all \(P\)-values < 0.02), but there was no difference in intensities between abduction and precision grip (all \(P\)-values > 0.3). Furthermore, in resting muscle, Stim\textsubscript{100} and Stim\textsubscript{150} were both larger than Stim\textsubscript{1mV} (all \(P\)-values < 0.02), whereas during both abduction and precision grip, Stim\textsubscript{100} was larger than the other two states (all \(P\)-values < 0.002), but there was no difference between Stim\textsubscript{1mV} and Stim\textsubscript{150} (all \(P\)-values > 0.3).
Table 4.2 Test MEP characteristics

<table>
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<th></th>
<th>Amplitude</th>
<th>TMS Intensity</th>
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<tr>
<td></td>
<td>MEP&lt;sub&gt;1mV&lt;/sub&gt;</td>
<td>MEP&lt;sub&gt;adj100&lt;/sub&gt;</td>
</tr>
<tr>
<td></td>
<td>(Condition A)</td>
<td>(Condition E)</td>
</tr>
<tr>
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<td>2.1 [1.8, 2.3]</td>
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<tr>
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<td>7.2 [6.9, 7.5]*</td>
</tr>
<tr>
<td>Precision</td>
<td>1.1 [0.8, 1.4]</td>
<td>7.3 [6.9, 7.6]*</td>
</tr>
</tbody>
</table>

See text for a definition of MEP amplitude conditions. Values are shown as mean [95% CI; lower limit, upper limit]. *P < 0.05 compared to rest; #P < 0.05 compared to Stim<sub>1mV</sub>; §P < 0.05 compared to Stim<sub>1mV</sub> and Stim<sub>150</sub>
4.4.2. Intracortical inhibition

Representative data from a single subject in resting FDI is shown in Figure 4.1. For this subject, RMT was 69% MSO, AMT was 53% MSO and MEP-Stim$_{1\text{mV}}$ was 80% MSO, while both MEP-Stim$_{100}$ and MEP-Stim$_{150}$ were 87% MSO. Baseline SICI for this subject was 60%, while baseline LICI was 50% and 26% for 100 and 150 ms ISIs, respectively. During triple-pulse TMS, this subject demonstrated reduced SICI in the presence of LICI, with SICI of 114% observed when preceded by LICI at 100 ms, whereas SICI of 95% was observed when preceded by LICI at 150 ms.

*Short-interval intracortical inhibition* The magnitude of baseline SICI for the three task conditions is shown in Figure 4.2. These data show that the magnitude of SICI varied between tasks ($F_{2,158} = 142.9$, $P < 0.01$), with inhibition being significantly reduced (reflected by larger values) during both index finger abduction (EMD: 51.9%, 95%CI [41.1, 62.7], $P < 0.01$) and precision grip (EMD: 76.5%, 95%CI [63.9, 89.2], $P < 0.01$) compared with measurements in resting muscle (Fig. 4.2). Furthermore, measurements during precision grip were also significantly reduced relative to those recorded during index finger abduction (EMD: 24.6%, 95%CI [10.2, 39.1], $P < 0.01$). Normalised pre-stimulus rmsEMG amplitude during SICI measurements was significantly greater during precision grip than index finger abduction ($P < 0.01$; Table 4.3). To address whether this increased muscle activation influenced SICI during precision grip, we reanalysed a subset of 10 subjects that showed similar levels of muscle activation between abduction and precision grip. This subpopulation had average normalised EMG amplitudes of 11.0 [7.5, 14.5]% MVC EMG for abduction and 13.7 [10.6, 16.8]% MVC EMG for precision grip ($P = 0.2$). Reanalysis of the SICI data in this subpopulation showed results similar to the original sample, with SICI of 95 [82.4, 107.6]% during index finger abduction and 131 [113.7, 148.9]% during precision grip ($P < 0.01$).
Figure 4.2 Task-dependent variations in the magnitude of SICI. The dotted line represents no inhibition, with values below 100% showing increased inhibition. Error bars show the upper limit of the 95% CI. *P < 0.05 when compared to measurements at rest; #P < 0.05 when compared to measurements during rest and abduction; *P < 0.05. Abbreviations: MEP, motor evoked potential.

<table>
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<th>Precision</th>
<th>P-value</th>
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</thead>
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<td></td>
<td></td>
</tr>
<tr>
<td>SICI</td>
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<td>16.3 [12.5, 20.1]</td>
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<tr>
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<td>15.2 [11.6, 18.8]</td>
<td>&lt; 0.01</td>
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<tr>
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<td>15.7 [11.9, 19.4]</td>
<td>&lt; 0.01</td>
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<td>21.3 [14.5, 28.2]</td>
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<td>22 [14.3, 29.7]</td>
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<tr>
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<td>23 [12.7, 33.2]</td>
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<td>&lt; 0.01</td>
</tr>
<tr>
<td>LICI-SICI&lt;sub&gt;100&lt;/sub&gt;</td>
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<tr>
<td>Conditioned</td>
<td>10.6 [7.6, 13.7]</td>
<td>21.3 [15.0, 27.7]</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Test MEP</td>
<td>9.3 [6.4, 12.2]</td>
<td>21.1 [14.6, 27.7]</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>LICI-SICI&lt;sub&gt;150&lt;/sub&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conditioned</td>
<td>10.4 [7.6, 13.1]</td>
<td>17.9 [13.2, 22.6]</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Test MEP</td>
<td>8.8 [6.1, 11.4]</td>
<td>18.7 [12.8, 24.6]</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

Values are shown as mean [95% CI: lower limit, upper limit]
Long-interval intracortical inhibition Task- and timing-dependent variations in the magnitude of LICI are shown in Figure 4.3. Main effects of task failed to reach significance ($F_{2,189} = 2.4, P = 0.1$), whereas the magnitude of inhibition varied between ISI’s ($F_{1,240} = 90.7, P < 0.01$), and there was a significant interaction between factors ($F_{2,189} = 67.5, P < 0.01$). For measurements using a 100 ms ISI, LICI was significantly increased during both index finger abduction (EMD 40.7%, 95%CI [30.1, 51.3], $P < 0.01$) and precision grip (EMD: 39.1%, 95%CI [28.4, 49.8], $P < 0.01$) compared with resting muscle. However, comparisons between abduction and precision grip showed that LICI$_{100}$ was not differentially affected by the type of task performed ($P = 0.1$). In contrast, for measurements using a 150 ms ISI, LICI was significantly reduced in response to index finger abduction (EMD: 36.7%, 95%CI [15.6, 56.9], $P < 0.01$) and precision grip (EMD: 58.1%, 95%CI [37.2, 79.0], $P < 0.01$) compared with resting muscle, although no differences in this effect were found between tasks ($P = 0.2$). Timing-related comparisons within each task condition demonstrated that, relative to LICI$_{150}$, LICI$_{100}$ was significantly reduced in resting muscle (EMD: 17.6%, 95%CI [8.3, 26.8], $P < 0.01$), whereas it was significantly increased during both abduction (EMD: 59.4%, 95%CI [42.9, 75.9], $P < 0.01$) and precision grip (EMD: 79.6%, 95%CI [62.9, 96.4], $P < 0.01$). Normalised pre-stimulus rmsEMG amplitude during LICI measurements was significantly affected by both task and ISI, with approximately 6% greater EMG amplitude during precision grip than index finger abduction ($P < 0.01$) and 1% greater EMG amplitude at 100 ms compared to 150 ms ($P < 0.01$). The interaction between these factors failed to reach significance though ($P = 0.1$; Table 4.3).
**Figure 4.3** Task- and timing-dependent variations in the magnitude of LICI. Measurements of LICI using interstimulus intervals of 100 ms (black bars) and 150 ms (white bars) during relaxation of FDI (Rest), isolated index finger abduction (Abduction) or precision grip of the index finger and thumb (Precision). The dotted line represents no inhibition, with values below 100% showing increased inhibition. Error bars show the upper limit of the 95% CI. *P < 0.05 when compared to measurements in resting muscle.*#P < 0.05. Abbreviations: MEP, motor evoked potential.
SICI in the presence of LICI. Figure 4.4A shows the effect of LICI on SICI at an ISI of 100 ms. Analysis of these data showed significant influences of both stimulation state ($F_{2,298} = 16.3, P < 0.01$) and task ($F_{2,317} = 51.0, P < 0.01$), and a significant interaction between these factors ($F_{4,262} = 26.9, P < 0.01$). With the muscle relaxed, the magnitude of inhibition was reduced during $\text{SICI}_{\text{adj}}^{100}$ (i.e. when SICI was reassessed using the increased test TMS intensity required for triple-pulse TMS) relative to baseline (EMD: 21.7%, 95%CI [13.0, 30.5], $P < 0.01$). During triple-pulse TMS, SICI at rest was reduced relative to both baseline (EMD: 63.0%, 95%CI [52.2, 73.8], $P < 0.01$) and $\text{SICI}_{\text{adj}}^{100}$ (EMD: 41.3%, 95%CI [29.9, 52.6], $P < 0.01$). During index finger abduction, the magnitude of inhibition did not vary between stimulation states ($P$-values ranged from 0.06 to 0.9). However, during precision grip, $\text{SICI}_{\text{adj}}^{100}$ demonstrated increased inhibition relative to baseline (EMD: 29.6%, 95%CI [16.8, 42.3], $P < 0.01$), whereas the magnitude of inhibition produced by triple-pulse TMS was not different to either baseline ($P = 0.1$) or $\text{SICI}_{\text{adj}}^{100}$ ($P = 0.4$). Task-related comparisons within each stimulation state showed that $\text{SICI}_{\text{adj}}^{100}$ in resting muscle was significantly increased relative to measurements recorded during both abduction (EMD: 19.8%, 95%CI [11.3, 28.3], $P < 0.01$) and precision grip (EMD: 25.3%, 95%CI [16.3, 34.2], $P < 0.01$), but that no differences were found between abduction and precision tasks ($P = 0.3$). Furthermore, no differences were found between all 3 task conditions during triple-pulse TMS. The absolute change in SICI, relative to baseline, was significant for all tasks during $\text{SICI}_{\text{adj}}^{100}$ (all $P$-values < 0.004), but only in resting muscle during LICI$^{100}$SICI ($P < 0.004$; Fig. 4.4B).
Figure 4.4 Task- and timing-dependent changes in LICI-SICI interactions. Measurements of SICI when preceded by LICI_{100} (A) or LICI_{150} (C) using triple pulse stimulation (LICI100-SICI, Condition I; LICI150-SICI, Condition J) during relaxation of FDI (black bars), isolated index finger abduction (white bars) or precision grip of the index finger and thumb (grey bars). A re-assessment of baseline SICI using the increased intensity test stimulus used during triple pulse stimulation is included as a control state (SICI_{adj100}, Condition E; SICI_{adj150}, Condition F). See Table 4.1 for a description of experimental conditions. The dotted line represents no inhibition, with values below 100% showing increased inhibition. The magnitude of change in SICI from baseline for the 100 ms and 150 ms intervals is also quantified in panels B and D. Positively directed error bars show the upper limit of the 95% CI, negatively directed error bars show the lower limit of the 95% CI. #P < 0.05 when compared to Baseline SICI; †P < 0.004 when compared to Baseline SICI. *P < 0.05. Abbreviations: MEP, motor evoked potential; SICI, short-interval intracortical inhibition.
The effect of LICI on SICI at 150 ms is shown in Figure 4.4C. Significant main effects of stimulation state ($F_{2,286} = 8.5, P < 0.01$) and task ($F_{2,339} = 135.6, P < 0.01$) were found, and there was a significant interaction ($F_{4,277} = 6.5, P < 0.01$). In resting muscle, inhibition recorded during $\text{SICI}_{adj150}$ was significantly reduced when compared to baseline (EMD: 19.5%, 95%CI [10.8, 28.3], $P < 0.01$), while inhibition recorded during triple-pulse TMS was reduced relative to both baseline (EMD: 32.5%, 95%CI [23.7, 41.3], $P < 0.01$) and $\text{SICI}_{adj150}$ (EMD: 13.0%, 95%CI [3.5, 22.4], $P < 0.01$). During index finger abduction, $\text{SICI}_{adj150}$ was not different to baseline (EMD: 6.7%, 95%CI [6.2, 19.5], $P = 0.6$), whereas triple-pulse TMS produced reduced inhibition relative to both baseline (EMD: 28.9%, 95%CI [1.0, 56.8], $P = 0.04$) and $\text{SICI}_{adj150}$ (EMD: 35.5%, 95%CI [7.7, 63.4], $P < 0.01$). However, during precision grip, the magnitude of inhibition did not vary between stimulation states. Task-related comparisons within each stimulation state showed that the magnitude of $\text{SICI}_{adj150}$ was progressively reduced from rest to index finger abduction (EMD: 25.7%, 95%CI [14.5, 36.9], $P < 0.01$) and precision grip (EMD: 27.1%, 95%CI [10.5, 43.8], $P < 0.01$). In contrast, during triple-pulse TMS, inhibition was significantly greater at rest compared with both abduction (EMD: 48.3%, 95%CI [21.0, 75.5], $P < 0.01$) and precision grip (EMD: 45.9%, 95%CI [24.3, 67.4], $P < 0.01$), but differences between abduction and precision grip were not significant. The absolute change in SICI, relative to baseline, was significant in resting muscle during $\text{SICI}_{adj150}$ ($P < 0.004$), but for both rest and index finger abduction during $\text{LICI}_{150}\text{SICI}$ ($P < 0.004$; Fig. 4.4D). When triple-pulse TMS was applied using the 100 ms ISI, normalised pre-stimulus rmsEMG amplitude was approximately 11% greater during precision grip than index finger abduction ($P < 0.01$). However, this difference was reduced to 7% when using the 150 ms ISI ($P = 0.04$; Table 4.3).
4.5. Discussion

The current study used paired- and triple-pulse TMS to investigate task-dependent variations in the modulation of intracortical inhibition. SICI, LICI and the interaction between SICI and LICI were assessed while subjects were at rest, or active in producing either isolated index finger abduction or synergistic precision grip of the index finger and thumb. At least three new findings can be drawn from the novel experimental approach used in this study. First, we found task-related variations in postsynaptic intracortical inhibition, with SICI (but not LICI) being particularly sensitive to the type of task performed (abduction vs. precision grip). Second, presynaptic motor cortex inhibition (assessed through LICI-SICI interactions) was modulated differently between abduction and precision grips, but this was evident for one ISI only (150 ms). Third, there was a divergent effect on LICI for different ISIs when the muscle was active, with LICI increasing at one ISI (100 ms) and decreasing at another (150 ms) for both tasks, compared with rest.

4.5.1. Task-related variations in postsynaptic intracortical inhibition (SICI and LICI)

During voluntary contraction, a reduction in GABAergic tone within primary motor cortex is thought to facilitate the activation of cortical areas innervating task-related muscles, subsequently allowing the generation of descending commands for movement (Matsumura et al., 1991; Matsumura et al., 1992). In humans, this reduction in inhibitory tone has been demonstrated for different GABA receptor subtypes by activity-dependent changes in SICI (Ridding et al., 1995), LICI, and the SP (Hammond & Vallence, 2007). In support of these observations, the current study observed reductions in both SICI and LICI in active muscle, although this effect for LICI depended on the ISI (see below).

Activity-dependent changes in GABAergic inhibition have also been suggested to differ between tasks requiring different muscle activation patterns, which may help to facilitate the
coactivation of cortical representations of muscles involved in the task. For example, reductions in the magnitude of SICI have been observed in control of a target muscle when a synergistic muscle is concurrently activated (Devanne et al., 2002; Kouchtir-Devanne et al., 2012). In support of this, we observed a progressively greater reduction in SICI from rest, to isolated index finger abduction, to synergistic precision grip. However, the extent of disinhibition in an active muscle observed by us was greater than reported previously (Kouchtir-Devanne et al., 2012), with SICI almost completely absent during index finger abduction, and facilitation of the test MEP during precision grip. Contraction intensities and TMS characteristics were similar between studies, so it is unlikely that they would have contributed to these differences. One possible explanation for these variations between studies could relate to differences in task performance, with the current study using a constant contraction force between tasks (i.e., 5% MVC), and the previous study using constant EMG (Kouchtir-Devanne et al., 2012). As a result, normalised pre-stimulus EMG was significantly greater (~ 6%) during precision grip than during index finger abduction in the present study. However, subsequent analysis of these data in a subgroup of subjects with similar EMG between tasks showed similar SICI modulation compared with the original subject cohort, suggesting that variations in muscle activation do not confound our findings. Nonetheless, our findings support previous studies suggesting that a progressively greater reduction in SICI occurs in tasks requiring the fine coordination of multiple task-related muscles.

In addition to SICI, Kouchtir-Devanne et al. (2012) also found that LICI was modulated by the task performed, with paired-pulse TMS measurements during precision grip showing a facilitation of the test MEP (Kouchtir-Devanne et al., 2012). Although we found that LICI was altered by muscle activation, this was not different between index finger abduction and precision grip, demonstrating a lack of task specificity. These findings suggest that LICI is insensitive to the demands of the task under the conditions of the present study. Given that the
experimental conditions (contraction and TMS intensities) were similar between this and the previous study by Kouchtir-Devanne et al. (2012), we can only speculate as to why the two studies have produced divergent findings. One possible reason is related to differences in the requirements of the precision grip task. For example, Kouchtir-Devanne et al. (2012) performed a precision grip using an unsupported cylinder held vertically between the thumb and index finger, and it is possible that this task required a higher level of functional coupling between the two digits compared with the fixed manipulandum used in the present study.

4.5.2. Task-related variations in presynaptic motor cortex inhibition (LICI-SICI interactions)

All previous investigations have assessed task-related changes in postsynaptic SICI and LICI, whereas the current study examined the presynaptic interaction between SICI and LICI during different tasks. This interaction is seen as a reduction in the magnitude of SICI when assessed in the presence of LICI (Sanger et al., 2001), and is thought to represent activation of presynaptic GABA\textsubscript{B}-receptors on the terminal of SICI neurons by LICI collaterals (Werhahn et al., 1999; Sanger et al., 2001; McDonnell et al., 2006; Muller-Dahlhaus et al., 2008). Although the functional relevance of this presynaptic inhibition is unclear, deficits have been observed in subjects with Parkinson’s disease (Chu et al., 2009) and a role in sensorimotor organisation has been suggested (Rosenkranz & Rothwell, 2003, 2004; Rosenkranz et al., 2008; Rosenkranz et al., 2009). In resting muscle, we observed the expected reduction in SICI in the presence of LICI at ISIs of 100 (LICI\textsubscript{100}SICI) and 150 ms (LICI\textsubscript{150}SICI), compared with SICI in isolation (Baseline SICI and SICI\textsubscript{adj100}/SICI\textsubscript{adj150}; Figure 4.4), although the effect was more pronounced at 100 ms. Two previous assessments of ISI-dependent changes in the LICI-SICI interaction in resting muscle have reported reduced SICI only at 100 ms (Chu et al., 2008), and at both 100 ms and 150 ms (Cash et al., 2010). These conflicting findings introduced uncertainty as to the duration of presynaptic motor cortex inhibition in humans. In
support of Cash et al. (2010), our observed disinhibition of SICI by LICI at both intervals suggests that presynaptic inhibition is in effect for at least 150 ms after the activation of LICI circuitry in humans.

In active muscle, the interpretation of the response to triple-pulse TMS is complicated by measurements of baseline SICI being markedly disinhibited, making further reductions in SICI difficult to observe. Nonetheless, during index finger abduction, SICI was significantly reduced (relative to baseline SICI and SICI_{adj150}) when preceded by LICI at an ISI of 150 ms. In contrast, SICI was not influenced (relative to baseline SICI and SICI_{adj100}/SICI_{adj150}) by LICI during both tasks at the 100 ms ISI, or during the precision grip task at the 150 ms ISI. These findings suggest that, for measurements assessed at 100 ms, or during precision grip at 150 ms, presynaptic mechanisms are unlikely to contribute to task-dependent changes in postsynaptic motor cortex inhibition observed during baseline SICI measurements (Sanger et al., 2001; Ni et al., 2011b). However, our findings also suggest that the disinhibition of baseline SICI observed during index finger abduction is associated with an increase in the activity of GABA_B-mediated presynaptic motor cortex inhibition at a latency of 150 ms (Sanger et al., 2001; Muller-Dahlhaus et al., 2008; Ni et al., 2011b; Chin et al., 2012).

As mentioned above, no study has specifically assessed the effect of muscle activation on presynaptic motor cortex inhibition. However, one study has investigated changes in SICI during the SP. Using an index finger abduction task at 20% MVC, Ni et al. (2007) measured SICI at three time points within the SP, two of which (110 ms and 140 ms) were comparable to the intervals investigated by the current study. Relative to measurements in resting muscle, this previous study found significant reductions in SICI at both intervals (Ni et al., 2007). The timing-dependent nature of our effects of muscle activation on LICI-SICI interactions therefore only partially supports these findings. Despite this, comparisons between the two studies are limited due to methodological differences, including differences in contraction.
intensity (5% MVC by us, 20% MVC by Ni and colleagues), variations in the intensity of the conditioning stimulus (to activate SICI circuitry; 80% AMT by us, 95% AMT by Ni and colleagues), and the use of a different ISI to assess SICI (2 ms by us, 2.5 ms by Ni and colleagues).

4.5.3. Timing-dependent variations in intracortical inhibition

Within the current study, one of the most notable findings was that task-dependent changes in LICI differed between ISIs. In these data, the transition from resting to active muscle produced an increase in inhibition assessed using the 100 ms ISI, but a decrease in inhibition assessed using the 150 ms ISI. These findings are inconsistent with previous work for the 100 ms ISI (Hammond & Vallence, 2007; McNeil et al., 2011), but are novel observations for the 150 ms ISI. In addition to these timing-dependent effects on LICI, effects of task on LICI-SICI interactions also varied between ISIs (during index finger abduction, inhibition was significantly reduced during LICI-SICI\textsubscript{150} but not LICI-SICI\textsubscript{100}) and Stim\textsubscript{100} was significantly greater than Stim\textsubscript{150}. As the conditioning TMS used to activate LICI circuitry within the current study (i.e., 120% RMT) could be expected to elicit a SP of approximately 150 ms (Chin et al., 2012), resolution of the SP and the onset of post-SP disinhibitory events (Chin et al., 2012) could be suggested to explain these timing-dependent effects. However, we found no evidence of EMG activity prior to the test stimulus for LICI\textsubscript{150}, so it is reasonable to suggest that the test stimulus for both LICI and LICI-SICI measurements was applied during the SP, suggesting this mechanism is unlikely to explain our findings.

Alternatively, our timing-dependent effects of task on LICI and LICI-SICI interactions may provide further evidence for suggestions that LICI at different ISIs may have contributions from non-identical cortical circuits. Although the ISIs that contribute to LICI (i.e., greater than 50 ms; Inghilleri et al., 1993; Brasil-Neto et al., 1995) are often assumed to represent
activation of the same cortical processes, a growing body of evidence suggests that this may not be an appropriate assumption. For example, LICI inhibits SICI at 100 ms but not 150 ms in resting muscle (Chu et al., 2008; but see Cash et al. 2010, and the current study for opposing findings), ischemic nerve block increases LICI at 150 ms but not 80 ms (Vallence et al., 2012), LICI at 100 ms is apparent when using low intensity conditioning stimuli (100 – 105% RMT) whereas LICI at 150 ms is not (Vallence et al., 2014) and LICI at 100 ms is increased by continuous theta burst stimulation applied to the cerebellum, whereas LICI at 150 ms is unaffected (Koch et al., 2008). If measurements at different ISIs do represent activation of different cortical circuits, our findings could also reflect independent sensitivities of these circuits to voluntary contraction, possibly suggesting unique roles in motor control. This possibility remains to be explored.

In conclusion, our results demonstrate strong task-related variations in postsynaptic inhibition (SICI), but limited task-related variations in presynaptic inhibition (LICI-SICI interaction). While SICI was progressively reduced from rest, to index finger abduction, to precision grip, LICI was only sensitive to muscle activation, and not the way in which the muscle was activated. Furthermore, a task-related modulation of presynaptic motor cortex inhibition was only observed during index finger abduction using a 150 ms ISI, suggesting a limited involvement of presynaptic mechanisms in the task-dependent disinhibition of motor cortex. Finally, timing-dependent variations in the effect of muscle activation on LICI and LICI-SICI interactions may further support previous suggestions that non-identical processes contribute to LICI at different ISIs. As most previous studies have been performed in resting muscle, these findings provide new insight into the functional role of these inhibitory circuits, and how they interact, during the performance of different motor tasks.
CHAPTER V

AGE-RELATED DIFFERENCES IN PRE- AND POST-SYNAPTIC MOTOR CORTEX INHIBITION ARE TASK DEPENDENT

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By signing the Statement of Authorship, each author certifies that their stated contribution to the publication is accurate and that permission is granted for the publication to be included in the candidate’s thesis

George Opie (Candidate)

Contribution: Experimental design, subject recruitment, collection and analysis of data, interpretation of data, wrote manuscript

Signed…

Date…

Michael Ridding

Contribution: Experimental design, data interpretation, edited manuscript

Signed…

Date…

John Semmler

Contribution: Experimental design, supervised development of work, data interpretation, critical manuscript evaluation, acted as corresponding author

Signed…

Date…
5. Age-related differences in pre- and post-synaptic motor cortex inhibition are task dependent

5.1. Abstract

Previous research has shown age-related differences in short- (SICI) and long-interval intracortical inhibition (LICI) in both resting and active hand muscles, suggesting that healthy ageing influences post-synaptic motor cortex inhibition. However, it is not known how the ageing process affects the pre-synaptic interaction between LICI and SICI, and how these pre- and post-synaptic intracortical inhibitory circuits are modulated by the performance of different motor tasks in older adults. The objective of this study was therefore to examine age-related differences in pre- and post-synaptic motor cortex inhibition at rest, and during index finger abduction and precision grip. In 13 young (22.3 ± 3.8 years) and 15 old (73.7 ± 4.0 years) adults, paired-pulse transcranial magnetic stimulation (TMS) was used to measure SICI (2 ms interstimulus interval; ISI) and LICI (100 and 150 ms ISI), whereas triple-pulse TMS was used to investigate SICI when primed by LICI. We found no age-related difference in SICI at rest or during index finger abduction, but significantly greater SICI in older subjects during precision grip. Older adults showed reduced LICI in resting muscle (at an ISI of 150 ms), with no age-related differences in LICI during either task. When SICI was primed by LICI, disinhibition of motor cortex was reduced in older adults at rest (100 ms ISI) and during index finger abduction (150 ms ISI), but not during precision grip. Our results support age-related differences in pre- and post-synaptic motor cortex inhibition, which may contribute to impaired hand function during task performance in older adults.
5.2. Introduction

Motor deficits are a major aspect of the ageing process that can significantly impede the performance of essential activities of daily living. Subsequently, age-related deficits in motor function may lead to reduced independence, decreased quality of life and institutionalisation (Scherder et al., 2008). Despite this, our understanding of how the ageing process affects function within core components of the motor system, such as the motor areas of the brain, is somewhat rudimentary. Nonetheless, within the primary motor cortex (M1), age-related changes in inhibitory neurotransmission mediated by γ aminobutyric acid (GABA) have been increasingly investigated as a factor potentially contributing to age-related motor deficits (for review, see; Levin et al., 2014). This line of investigation stems from the established importance of intracortical inhibition in motor control (Ridding et al., 1995; Reynolds & Ashby, 1999; Zoghi et al., 2003; Buccolieri et al., 2004) and has been facilitated by the use of non-invasive transcranial magnetic stimulation (TMS).

In humans, TMS allows an assessment of distinct GABAergic processes by applying pairs of magnetic stimuli to M1 (paired-pulse TMS), or an assessment of interactions between GABAergic processes by applying 3 magnetic stimuli to M1 (triple-pulse TMS). During paired-pulse TMS, application of a subthreshold conditioning stimulus at short-intervals (1–5 ms) prior to a suprathreshold test stimulus produces inhibition of the test motor evoked potential (MEP) via activation of post-synaptic GABA_A receptors (Ziemann et al., 1996b). This process is known as short-interval intracortical inhibition (SICI; Kujirai et al., 1993). However, when both stimuli are suprathreshold and separated by a long inter-stimulus interval (100–150 ms; ISI), inhibition of the test MEP is thought to involve post-synaptic GABA_B receptors (Werhahn et al., 1999) and is known as long-interval intracortical inhibition (LICI; Valls-Sole et al., 1992). During triple-pulse TMS, the interaction between LICI and SICI is assessed by preceding the conditioning and test stimuli for SICI by the conditioning stimulus for LICI (Sanger et al., 2001). This protocol results in reduced inhibition of the test MEP and
is thought to involve activation of pre-synaptic GABA\(_B\) receptors (Werhahn et al., 1999; Sanger et al., 2001; McDonnell et al., 2006; Ni et al., 2007; Muller-Dahlhaus et al., 2008).

In young subjects, activity-dependent changes in both SICI (Ridding et al., 1995; Reynolds & Ashby, 1999; Buccolieri et al., 2004) and LICI (Hammond & Vallence, 2007; McNeil et al., 2011) are well established. Furthermore, the nature of this modulation is thought to be task-dependent (Devanne et al., 2002; Kouchtir-Devanne et al., 2012). In contrast, task-related changes in inhibition in older adults have been limited to measurements made during tonic contractions (McGinley et al., 2010; Chapter 3) or in the period prior to contraction (Fujiyama et al., 2011; Fujiyama et al., 2012b; Heise et al., 2013). However, some evidence suggests that the task-dependency of inhibitory tone in M1 is modified by age (Sale & Semmler, 2005). Interestingly, task-dependent changes in SICI and LICI have been suggested to be mediated by pre-synaptic mechanisms (Kouchtir-Devanne et al., 2012), suggesting that effects of age on the task-dependent modulation of inhibition may be influenced by changes in pre-synaptic motor cortex inhibition.

Pre-synaptic inhibition in M1 has not been previously compared between young and old adults. However, Chu and colleagues (Chu et al., 2008) investigated LICI-SICI interactions in a group of older adults (age 54-68 years) using two ISIs of 100 ms and 150 ms. While this study observed the expected reduction in inhibition using the 100 ms ISI, no change in inhibition was seen when the 150 ms interval was used (Chu et al., 2008). However, subsequent investigations assessing the duration of pre-synaptic inhibition in young subjects have shown that effects can last > 200 ms (Cash et al., 2010). Comparing the findings of these studies suggests that LICI-SICI interactions are reduced by the ageing process, but only at longer ISIs (i.e., 150 ms). This observation may reflect a timing-dependent reduction in presynaptic inhibition in M1.
The aim of the current study was therefore to compare the magnitude of SICI, LICI and LICI-SICI interactions between young and old subjects during relaxation, index finger abduction and precision grip (between the index finger and thumb) – tasks that have previously produced specific changes in intracortical inhibition in young subjects (Kouchtir-Devanne et al., 2012; Chapter 4). Also, as effects of age on pre-synaptic motor cortex inhibition were expected to be timing-dependent, LICI-SICI interactions were assessed using two ISIs of 100 ms and 150 ms. Based on previous studies (Chu et al., 2008; Cash et al., 2010), we expected that old subjects would show reduced pre-synaptic M1 inhibition at 150 ms. Furthermore, as the activity-dependent modulation of inhibitory tone is reduced in older adults (Heise et al., 2013), we expected that task-dependent changes in this modulation would also be influenced by advancing age.

5.3. Materials and methods

15 old (73.7 ± 4.0 years) healthy subjects were recruited to participate in the current study via advertisements placed in local media. These data were compared to those from 13 young (mean ± standard deviation; 22.3 ± 3.8) healthy subjects, the results of which have been presented previously (Chapter 4). Exclusion criteria included a history of stroke, history of neurological or psychiatric disease, or current use of psychoactive medication (sedatives, antipsychotics, antidepressants etc.). Hand preference and laterality was assessed using the Edinburgh Handedness Inventory (Oldfield, 1971). Each subject provided written, informed consent prior to participation. All experimentation was approved by the University of Adelaide Human Research Ethics Committee and conducted in accordance with the declaration of Helsinki.

5.3.1. Experimental arrangement

For the duration of each experimental session, subjects were seated in a comfortable chair with their right arm abducted approximately 45° at the shoulder. This allowed the forearm and
hand to sit comfortably on an arm support placed next to them. Surface electromyography (EMG) was used to record responses from the first dorsal interosseous (FDI) muscle of the right hand. Two Ag–AgCl electrodes (1.6 cm diameter) were attached to the skin over the muscle in a belly-tendon montage, with a strap around the wrist grounding the electrodes. 

EMG was amplified (300 X) and band-pass filtered (20 Hz high pass, 1 kHz low pass) using a CED1902 (Cambridge Electronic Design, Cambridge, UK), and digitized at 2 kHz using a CED1401 interface (Cambridge Electronic Design), before being recorded and stored offline for analysis. To facilitate muscle relaxation when required, real-time EMG signals were displayed under high gain (50 µV/ division) on an oscilloscope placed in front of the subject.

Each subject participated in two experimental sessions held on separate days, each of 2–3 hours duration. Within each session, TMS was applied during complete relaxation of FDI and while FDI was active in producing one of two low intensity (5% of maximum force) contractions, performed in random order. For one of the sessions, subjects were required to produce an isolated abduction of the index finger, whereas in the other session they were required to perform a precision grip of the index finger and thumb. As prolonged contractions were required to complete the multiple stimulation conditions needed for triple-pulse TMS (see below), assessing each active task on separate days reduced the likelihood of fatiguing the target muscle, which may have confounded measurements of intracortical inhibition (Benwell et al., 2006; Benwell et al., 2007; Vucic et al., 2011). Within each experimental session, all TMS conditions (see below) were applied twice, once with the target muscle at rest, and again with the target muscle active (either abduction or precision grip). Furthermore, paired-pulse TMS was always performed before triple-pulse TMS for all subjects, allowing the experimenter to monitor baseline levels of inhibition before applying triple-pulse TMS. During active state measurements, stimulation began after subjects had reached stable force application.
5.3.2. Experimental Procedures

Maximal Voluntary Contraction At the beginning of each experiment, maximum voluntary contractions (MVC) were assessed for each subject. This was performed for both index finger abduction and precision grip using the index finger and thumb. During index finger abduction, the subject’s right hand was positioned with the palm facing downwards and the index finger isolated from the middle, ring and little fingers. When instructed, subjects abduced the lateral surface of the index finger against a force transducer (LC1205-K020; A&D Mercury Pty Ltd, Australia) placed in-line with the distal interphalangeal joint. During precision grip, subjects opposed the index finger and thumb against a purpose built manipulandum that has been described previously (Chapters 2, 3 & 4). The procedure to assess the MVC was identical for both index finger abduction and precision grip: subjects were required to produce maximum force for 3 s in several repetitions, separated by 30 s rest, until the maximal force of three trials were within a 10% margin. The largest force recorded during these trials was chosen as the subject’s MVC. To optimise force production, feedback was displayed on a computer monitor placed at eye level in front of the subject, and verbal encouragement was provided by the experimenter.

Transcranial magnetic stimulation TMS was applied to the left primary motor cortex using a figure-of-eight coil (external wing diameter 9 cms) with three Magstim 200 magnetic stimulators connected via two Bistim units (Magstim, Dyfed, UK). Within this setup, two stimulators were connected via the first Bistim unit, while the third stimulator and the output from the first Bistim unit were connected via the second Bistim unit. The coil was then connected to the output of the second Bistim unit. This allowed application of up to 3 stimuli at very short intervals through the same coil, but is associated with a reduction in stimulus strength of approximately 15% (Sanger et al., 2001). During testing, the coil was held tangentially to the scalp at an angle of 45° to the sagittal plane, with the handle pointed
backwards and laterally, producing a current flow in the brain with a posterior to anterior
direction. The coil was positioned on the scalp over the location producing an optimum
response in the relaxed FDI muscle. This location was marked on the scalp for reference and
continually checked throughout the experiment. TMS was delivered at 0.2 Hz for all
conditions.

Resting and Active motor thresholds (RMT and AMT, respectively) were obtained in FDI
while the TMS coil was placed at the optimal location over primary motor cortex. RMT was
defined as the minimum TMS intensity producing a response amplitude ≥ 50 µV in at least
three out of five trials in resting FDI muscle, and expressed relative to the maximum
stimulator output (MSO). Active motor threshold (AMT) was defined as the minimum TMS
intensity producing a response amplitude ≥ 300 µV in at least three out of five trials while
FDI was active at 5% MVC. Force feedback was provided via an oscilloscope placed at eye
level in front of the subject, with a target force set on the oscilloscope that was adjusted to 5%
of each subject’s MVC.

Intracortical inhibition The magnitude of post-synaptic intracortical inhibition was assessed
using 4 experimental conditions (Table 5.1, Conditions A–D). SICI was measured with a
subthreshold conditioning stimulus set at 80% AMT and an interstimulus interval (ISI) of 2
ms (Condition B; Kujirai et al., 1993) while LICI was assessed using a suprathreshold
conditioning stimulus set at 120% RMT and two ISIs of 100 and 150 ms (Conditions C and
D; Valls-Sole et al., 1992). For both SICI and LICI, the intensity of the test stimulus was set
at the level producing an MEP with peak-to-peak amplitude of 1 mV (Stim1mV, Condition A).
Both paired-pulse TMS paradigms were applied in the same experimental block, allowing
normalisation of all paired-pulse responses to a common test alone state. As 30 conditioned
trials (10 SICI, 10 LICI_{100}, 10 LICI_{150}) and 10 control trials were included within a block, and
each block was repeated with the muscle at rest and during activation, a total of 80 trials were used to assess baseline levels of intracortical inhibition.

### Table 5.1 TMS protocol

<table>
<thead>
<tr>
<th>Condition</th>
<th>CS\textsubscript{150}</th>
<th>CS\textsubscript{100}</th>
<th>CS\textsubscript{2}</th>
<th>Test Stimulus</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Test MEP</td>
<td>—</td>
<td>—</td>
<td>Stim\textsubscript{1mV}</td>
</tr>
<tr>
<td>B</td>
<td>SICI</td>
<td>—</td>
<td>80% AMT</td>
<td>Stim\textsubscript{1mV}</td>
</tr>
<tr>
<td>C</td>
<td>LICI\textsubscript{100}</td>
<td>—</td>
<td>120% RMT</td>
<td>Stim\textsubscript{1mV}</td>
</tr>
<tr>
<td>D</td>
<td>LICI\textsubscript{150}</td>
<td>120% RMT</td>
<td>—</td>
<td>Stim\textsubscript{1mV}</td>
</tr>
<tr>
<td>E</td>
<td>MEP\textsubscript{adj100}</td>
<td>—</td>
<td>—</td>
<td>Stim\textsubscript{100}</td>
</tr>
<tr>
<td>F</td>
<td>MEP\textsubscript{adj150}</td>
<td>—</td>
<td>—</td>
<td>Stim\textsubscript{150}</td>
</tr>
<tr>
<td>G</td>
<td>SICI\textsubscript{adj100}</td>
<td>—</td>
<td>80% AMT</td>
<td>Stim\textsubscript{100}</td>
</tr>
<tr>
<td>H</td>
<td>SICI\textsubscript{adj150}</td>
<td>—</td>
<td>80% AMT</td>
<td>Stim\textsubscript{150}</td>
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<tr>
<td>I</td>
<td>Test MEP\textsubscript{100}</td>
<td>—</td>
<td>120% RMT</td>
<td>Stim\textsubscript{100}</td>
</tr>
<tr>
<td>J</td>
<td>Test MEP\textsubscript{150}</td>
<td>120% RMT</td>
<td>—</td>
<td>Stim\textsubscript{150}</td>
</tr>
<tr>
<td>K</td>
<td>LICI-SICI\textsubscript{100}</td>
<td>—</td>
<td>120% RMT</td>
<td>Stim\textsubscript{100}</td>
</tr>
<tr>
<td>L</td>
<td>LICI-SICI\textsubscript{150}</td>
<td>120% RMT</td>
<td>80% AMT</td>
<td>Stim\textsubscript{150}</td>
</tr>
</tbody>
</table>

Abbreviations: CS\textsubscript{150}, conditioning stimulus applied 150 ms prior to the test stimulus; CS\textsubscript{100}, conditioning stimulus applied 100 ms prior to the test stimulus; CS\textsubscript{2}, conditioning stimulus applied 2 ms prior to the test stimulus; MEP\textsubscript{1mV}, stimulus intensity producing an MEP with peak-to-peak amplitude of 1 mV; MEP\textsubscript{100}, stimulus intensity producing an MEP with peak-to-peak amplitude of 1 mV when preceded by CS\textsubscript{100}; MEP\textsubscript{150}, stimulus intensity producing an MEP with peak-to-peak amplitude of 1 mV when preceded by CS\textsubscript{150}; RMT, resting motor threshold; AMT, active motor threshold.

The effect of LICI on SICI was assessed using triple-pulse TMS (Table 5.1, Conditions E–L).

The conditioning stimulus used to activate LICI circuitry was set at 120% RMT and applied at two intervals of 100 ms (CS\textsubscript{100}, Condition K) and 150 ms (CS\textsubscript{150}, Condition L) in separate blocks. Within both blocks, the conditioning stimulus used to activate SICI circuitry was set at 80% AMT and applied using a 2 ms ISI (CS\textsubscript{2}). The effect of LICI on SICI (LICI-SICI interaction) was quantified by comparing the amplitude of the test MEP generated by application of all three stimuli (CS\textsubscript{100}/CS\textsubscript{150}, CS\textsubscript{2} and a test stimulus) to the amplitude of the test MEP generated by application of the LICI conditioning stimulus and the test stimulus.
The intensity of the test stimulus was adjusted to the level producing an MEP response of 1 mV when preceded by either CS\textsubscript{100} (Stim\textsubscript{100}, Condition I) or CS\textsubscript{150} (Stim\textsubscript{150}, Condition J) and this intensity was set individually for each ISI. As increasing test TMS intensity reduces the magnitude of SICI (Garry & Thomson, 2009; Chapter 2 & 3) the higher intensity Stim\textsubscript{100} and Stim\textsubscript{150} could account for changes in SICI observed during triple-pulse TMS. Therefore, as a control state, additional measurements of SICI were recorded using the higher intensity Stim\textsubscript{100} (SICI\textsubscript{adj100}) and Stim\textsubscript{150} (SICI\textsubscript{adj150}) as the test stimulus intensity (Conditions G and H, respectively). We will refer to the amplitude of the test MEP generated during SICI\textsubscript{adj100} and SICI\textsubscript{adj150} as MEP\textsubscript{adj100} (Condition E) and MEP\textsubscript{adj150} (Condition F), respectively. As 10 conditioned and 10 control trials were applied within four experimental blocks, and each block was repeated with the muscle at rest and during activation, a total of 160 trials were used to assess interactions between LICI and SICI.

5.3.3. Data Analysis

Data analysis was completed manually by visual inspection of offline EMG. Within the rest state, traces showing muscle activity > 20 µV in peak-to-peak amplitude during the 150 ms prior to the first conditioning stimulus (for conditioned trials) or the test stimulus (for unconditioned trials) were excluded from analysis. MEP amplitudes from each trial were measured peak-to-peak and expressed in mV. Paired- and triple-pulse measurements of intracortical inhibition were quantified by expressing the amplitude of individual conditioned MEPs as a percentage of the average control MEP amplitude. For measurements of adjusted SICI and LICI-SICI interactions, the absolute change in inhibition from baseline was assessed by subtracting the amplitude of individual conditioned MEPs from the average conditioned MEP amplitude for the same condition. This value will be referred to as SICI\textsubscript{diff}. For active trials, muscle activation was assessed by quantifying the root mean squared (rms) EMG
amplitude (normalised to the maximum rmsEMG amplitude recorded during MVC) in the 100 ms leading up to application of CS$_{150}$, CS$_{100}$ or CS$_2$ (depending on stimulation condition).

5.3.4. Statistical Analysis

RMT and the stimulus intensities used for MEP$_{1mV}$, MEP$_{100}$ and MEP$_{150}$ in resting muscle were compared between sessions using paired $t$-tests. Subject to no significant inter-session differences, these data were pooled across sessions. AMT was also compared between sessions using a paired $t$-test. Pooled RMT and handedness scores (laterality quotient) were compared between age groups using unpaired $t$-tests, while pooled AMT was compared between age groups and sessions using a two-way repeated measures analysis of variance (ANOVA$_{RM}$). A three-way ANOVA$_{RM}$ was used to investigate effects of age (young, old), test stimulus condition (Stim$_{1mV}$, Stim$_{100}$ & Stim$_{150}$) and task (rest, abduction, precision) on test stimulus intensity. Individual two-way ANOVA’s were used to assess the effects of age and task on normalised EMG amplitude prior to TMS for LICI at each ISI, SICI in each stimulus condition (i.e., baseline, SICI$_{adj100}$, SICI$_{adj150}$, LICI-SICI$_{100}$, LICI-SICI$_{150}$) and the corresponding test alone MEPs. All main effects and interactions were further investigated using one-way ANOVA’s with Fishers PLSD post hoc test. Mixed-model analysis was used to investigate the effects of test MEP condition (MEP$_{1mV}$, MEP$_{adj100}$, MEP$_{adj150}$, MEP$_{100}$ & MEP$_{150}$), task and age on the amplitude of the test alone MEP. Effects of task on SICI, LICI and LICI-SICI interactions were compared between young and old adults using mixed model analyses. For LICI, individual models were used for each ISI while, for LICI-SICI interactions, individual models were used for both task and ISI. For all models, subject was included as a random effect, and significant main effects and interactions were further investigated using custom contrasts with Bonferroni correction. For assessments of adjusted SICI and LICI-SICI interactions, the absolute change in SICI relative to baseline within each stimulus condition was compared to ‘0’ (i.e., no change in inhibition) using one-sample $t$-tests.
with Bonferroni correction. The change in SICI was also compared between groups and stimulation states using linear mixed model analysis, with separate models used for each ISI and activity state. Significant main effects and interactions were also investigated using custom contrasts with Bonferroni correction. Linear regression of individual subject data was used to further investigate age-related changes in the interactions between each measurement. As measurements in resting muscle were repeated in each session, values were averaged across sessions within each subject (subject to no significant difference between sessions, assessed using paired *t*-test’s), with the resulting value used for regression analysis. Significance was set at $P \leq 0.05$ for all comparisons and data are shown as mean ± standard error of the mean (SEM), unless otherwise stated.

### 5.4. Results

As the results of the young cohort have been previously reported (Chapter 4), and the primary interest of the current study concerns age-related effects, only findings involving main effects or interactions of age will be described in detail. All subjects completed the experiment in full and without adverse reaction. The young cohort consisted of 6 males (23.3 ± 5.4) and 7 females (21.4 ± 1.4), whereas the old cohort consisted of 8 males (75.0 ± 3.8 years) and 7 females (72.1 ± 3.8 years). Handedness was not different between age groups (average laterality quotient: young, 0.92 ± 0.04; old, 0.87 ± 0.06; $P = 0.5$). As RMT did not vary between sessions ($P = 0.5$), values were pooled and compared between age groups, with no significant difference found (young, 61.1 ± 1.5% MSO; old, 62.0 ± 2.2% MSO; $P = 0.7$). For AMT, values were not different between sessions ($P = 0.4$) or age groups ($P = 0.6$)(abduction - young: 47.0 ± 2.1% MSO, old: 48.7 ± 2.6% MSO; precision - young: 47.6 ± 2.2% MSO, old: 49.6 ± 2.8% MSO). Normalised prestimulus EMG for each stimulus condition and task is compared between young and old adults in Table 5.2.
### Table 5.2 Normalised prestimulus EMG (% MVC EMG)

<table>
<thead>
<tr>
<th>Condition</th>
<th>Young</th>
<th></th>
<th>Old</th>
<th></th>
<th>Main effects (P-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Abduction</td>
<td>Precision</td>
<td>Abduction</td>
<td>Precision</td>
<td>Age</td>
</tr>
<tr>
<td><strong>Baseline ICI</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SICI</td>
<td>9.6 ± 1.4</td>
<td>16.3 ± 1.7</td>
<td>20.7 ± 1.9</td>
<td>21.6 ± 3.2</td>
<td>0.0007</td>
</tr>
<tr>
<td>LICI&lt;sub&gt;100&lt;/sub&gt;</td>
<td>9.4 ± 1.4</td>
<td>16.0 ± 1.7</td>
<td>20.9 ± 2.1</td>
<td>20.3 ± 2.7</td>
<td>0.0005</td>
</tr>
<tr>
<td>LICI&lt;sub&gt;150&lt;/sub&gt;</td>
<td>9.1 ± 1.3</td>
<td>15.2 ± 1.7</td>
<td>21.5 ± 2.1</td>
<td>21.3 ± 2.9</td>
<td>0.0001</td>
</tr>
<tr>
<td>Test MEP</td>
<td>7.7 ± 0.9</td>
<td>15.7 ± 1.7</td>
<td>21.0 ± 2.1</td>
<td>20.9 ± 2.9</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td><strong>SICI&lt;sub&gt;adj100&lt;/sub&gt;</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conditioned</td>
<td>10.9 ± 1.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>21.3 ± 3.1</td>
<td>24.5 ± 2.9</td>
<td>21.9 ± 2.3</td>
<td>0.008</td>
</tr>
<tr>
<td>Test MEP</td>
<td>9.3 ± 1.2&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>22.0 ± 3.5</td>
<td>23.7 ± 2.5</td>
<td>22.8 ± 2.4</td>
<td>0.004</td>
</tr>
<tr>
<td><strong>SICI&lt;sub&gt;adj150&lt;/sub&gt;</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conditioned</td>
<td>10.7 ± 1.3</td>
<td>23.0 ± 4.7</td>
<td>21.2 ± 1.7</td>
<td>25.9 ± 3.4</td>
<td>0.03</td>
</tr>
<tr>
<td>Test MEP</td>
<td>9.2 ± 1.4</td>
<td>21.8 ± 4.1</td>
<td>22.0 ± 1.8</td>
<td>26.1 ± 3.6</td>
<td>0.006</td>
</tr>
<tr>
<td><strong>LICI-SICI&lt;sub&gt;100&lt;/sub&gt;</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conditioned</td>
<td>10.6 ± 1.4</td>
<td>21.3 ± 2.9</td>
<td>23.5 ± 2.4</td>
<td>25.1 ± 2.9</td>
<td>0.002</td>
</tr>
<tr>
<td>Test MEP</td>
<td>9.3 ± 1.3</td>
<td>21.1 ± 3.0</td>
<td>22.7 ± 2.0</td>
<td>25.7 ± 2.9</td>
<td>0.0005</td>
</tr>
<tr>
<td><strong>LICI-SICI&lt;sub&gt;150&lt;/sub&gt;</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conditioned</td>
<td>10.4 ± 1.3</td>
<td>17.9 ± 2.2</td>
<td>20.4 ± 2.2</td>
<td>23.5 ± 2.6</td>
<td>0.02</td>
</tr>
<tr>
<td>Test MEP</td>
<td>8.8 ± 1.2</td>
<td>18.7 ± 2.7</td>
<td>21.3 ± 2.2</td>
<td>23.5 ± 2.9</td>
<td>0.0006</td>
</tr>
</tbody>
</table>

<sup>a</sup>P < 0.05 compared to the same task in old subjects; <sup>b</sup>P < 0.05 compared to precision grip

#### 5.4.1. Test MEP characteristics

The amplitude of the test alone MEP in each test MEP condition and task is compared between young and old subjects in Table 5.3. For this comparison, all main effects and interactions were significant (all P-values < 0.0001). Age-related differences were found only for MEP<sub>adj100</sub> and MEP<sub>adj150</sub>, (i.e., the test MEPs recorded using the increased test stimulus intensity applied during triple-pulse TMS) and these varied between activity states (see Table 5.3). Furthermore, no differences in MEP<sub>1mV</sub>, MEP<sub>100</sub> or MEP<sub>150</sub> were found between age-groups in any task (P-values ranging from 0.1 – 0.9). These comparisons demonstrate that, for those test MEP conditions which aimed to produce a response of 1 mV (i.e., MEP<sub>1mV</sub>, MEP<sub>100</sub> and MEP<sub>150</sub>), the amplitude was well matched between conditions and groups. However, for those conditions which did not adjust MEP amplitude, but instead adjusted the intensity of the
test stimulus in order to produce a descending volley comparable to that which could be expected during triple-pulse TMS (i.e., MEP<sub>adj100</sub> and MEP<sub>adj150</sub>; see Discussion for explanation of methodology), the test MEP amplitude was significantly increased. The intensity of the test stimulus for each stimulus condition is shown in Table 5.4. All main effects and interactions of age failed to reach significance for these data (all \(P\)-values > 0.3).

### Table 5.3 Test MEP amplitude

<table>
<thead>
<tr>
<th></th>
<th>Young</th>
<th></th>
<th>Old</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MEP&lt;sub&gt;1mV&lt;/sub&gt;</td>
<td>MEP&lt;sub&gt;adj100&lt;/sub&gt;</td>
<td>MEP&lt;sub&gt;adj150&lt;/sub&gt;</td>
<td>MEP&lt;sub&gt;100&lt;/sub&gt;</td>
</tr>
<tr>
<td>Rest</td>
<td>1.1 ± 0.1</td>
<td>2.0 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.4 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.1 ± 0.1</td>
</tr>
<tr>
<td>Abduction</td>
<td>1.2 ± 0.2</td>
<td>7.2 ± 0.2&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>3.2 ± 0.2&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>1.2 ± 0.2</td>
</tr>
<tr>
<td>Precision</td>
<td>1.1 ± 0.2</td>
<td>7.3 ± 0.1&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>3.1 ± 0.1&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>1.1 ± 0.2</td>
</tr>
</tbody>
</table>

See text for a definition of MEP amplitude conditions. \(^aP < 0.05\) compared to the same MEP condition in old subjects; \(^bP < 0.05\) compared to rest.

### Table 5.4 Test TMS intensity

<table>
<thead>
<tr>
<th></th>
<th>Young</th>
<th></th>
<th>Old</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stim&lt;sub&gt;1mV&lt;/sub&gt;</td>
<td>Stim&lt;sub&gt;100&lt;/sub&gt;</td>
<td>Stim&lt;sub&gt;150&lt;/sub&gt;</td>
<td></td>
</tr>
<tr>
<td>Rest</td>
<td>65.3 ± 1.8</td>
<td>72.5 ± 2.2</td>
<td>73.5 ± 2.3</td>
<td>67.1 ± 2.9</td>
</tr>
<tr>
<td>Abduction</td>
<td>45.5 ± 2.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>64.2 ± 2.6</td>
<td>50.0 ± 3.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>46.9 ± 2.4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Precision</td>
<td>44.7 ± 2.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>60.4 ± 3.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>47.4 ± 3.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>48.7 ± 3.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

See text for a definition of MEP amplitude conditions. Differences between age groups were not significant. \(^aP < 0.05\) compared to rest.
5.4.2. Short-interval intracortical inhibition

Measurements of SICI in young and old subjects during each task are shown in Figure 5.1. The magnitude of inhibition was affected by age \( (P = 0.007) \) and there was a significant interaction between age and task \( (P = 0.001) \). Age-related comparisons within each task showed no difference in SICI during rest \( (P = 0.2) \) and index finger abduction \( (P = 0.2) \), but significantly greater inhibition in old subjects during precision grip \( (P < 0.0001) \).

![Figure 5.1](image)

**Figure 5.1** Age-dependent changes in the effect of task on SICI. Measurements of SICI were compared between young (black bars) and old (white bars) subjects during rest, index finger abduction and precision grip of the index finger and thumb. The dotted horizontal line represents no inhibition, with values below 100% showing an increase in inhibition. \# \( P < 0.05 \) when compared to values in resting muscle; † \( P < 0.05 \) when compared to values in resting muscle and during index finger abduction. * \( P < 0.05 \) between young and old adults.

5.4.3. Long-interval intracortical inhibition

Effects of age and task on LICI are shown in Figure 5.2. LICI_{100} was not significantly affected by age \( (P = 0.9) \) and there was no interaction between age and task \( (P = 0.1; \text{Fig. 5.2A}) \). For LICI_{150}, inhibition was again unaffected by age \( (P = 0.4) \), but the interaction between age and task reached significance \( (P = 0.02; \text{Fig. 5.2B}) \). Age-related comparisons in each task showed that older subjects had significantly reduced LICI_{150} in resting muscle \( (P = 0.01) \), but no
difference in LICI between groups during index finger abduction ($P = 0.1$) or precision grip ($P = 0.4$).

Figure 5.2 Task-related changes in LICI compared between young and old adults. The magnitude of LICI was compared between young (black bars) and old (white bars) subjects during rest, index finger abduction and precision grip tasks using interstimulus intervals of 100 ms (A) and 150 ms (B). The dotted horizontal line represents no inhibition, with values below 100% showing an increase in inhibition. *$P < 0.05$ when compared to values in resting muscle; †$P < 0.05$ when compared to values in resting muscle and during index finger abduction. *$P < 0.05$ between young and old adults.
5.4.4. \textit{SICI in the presence of LICI}

The effect of LICI on SICI in resting muscle is compared between young and old subjects in Figure 5.3. When using the 100 ms ISI, the magnitude of inhibition was not affected by age ($P = 0.1$), but there was an interaction between age and stimulation state ($P = 0.02$; Fig. 5.3A). Age-related comparisons showed that $\text{SICI}_{\text{adj100}}$ did not differ between groups ($P = 0.08$), whereas older subjects had a significantly smaller reduction in SICI during LICI-$\text{SICI}_{100}$ than young subjects ($P = 0.05$). Within each stimulation state, there was a significant change in SICI from baseline ($\text{SICI}_{\text{diff}}$) for both $\text{SICI}_{\text{adj100}}$ ($P < 0.0001$) and LICI-$\text{SICI}_{100}$ ($P < 0.0001$) in young subjects, but only LICI-$\text{SICI}_{100}$ ($P < 0.0001$) in old subjects (Fig. 5.3B). Furthermore, comparing $\text{SICI}_{\text{diff}}$ between groups showed significant effects of age ($P < 0.0001$) and stimulation state ($P < 0.0001$) but no interaction between factors ($P = 0.9$). When SICI in the presence of LICI was assessed using the 150 ms ISI, inhibition was not affected by age ($P = 0.5$) and there was no interaction between age and stimulation state ($P = 0.5$; Fig. 5.3C). For $\text{SICI}_{\text{diff}}$ at 150 ms, values were significant for all stimulation states in both groups (all $P$-values $< 0.001$; Fig. 5.3D). However, although $\text{SICI}_{\text{diff}}$ showed a significant effect of stimulation state ($P < 0.0001$), there was no effect of age ($P = 0.2$) and no interaction between factors ($P = 0.5$).
Figure 5.3 Effects of age on the interaction between SICI and LICI in resting muscle. Triple-pulse TMS measures were compared between young (black bars) and old (white bars) adults at two interstimulus intervals of 100 ms (A) and 150 ms (C) during complete relaxation of FDI. For all panels, SICI_{adj|100/150} refers to conditions G/H, and LICI-SICI_{100/150} refers to conditions K/L. The magnitude of change in SICI from baseline (i.e., SICI_{diff}) for the 100 ms and 150 ms intervals is also quantified in panels B and D. The dotted horizontal line represents no inhibition, with values below 100% showing an increase in inhibition. #P < 0.05 when compared to baseline SICI and adjusted SICI; †magnitude of change from baseline is significant (P < 0.01); *P < 0.05 between young and old adults.
LICI-SICI interactions during index finger abduction are shown in Figure 5.4. For LICI-SICI_{100}, effects of age failed to reach a conventional significance level ($P = 0.06$).

Furthermore, there was no interaction between stimulation state and age ($P = 0.1$; Fig. 5.4A). For both age groups, SICI_{diff} was significant for SICI_{adj100} (young, $P < 0.0001$; old, $P = 0.002$) but failed to reach significance for LICI-SICI_{100} (young, $P = 0.05$; old, $P = 0.3$; Fig. 5.4B). SICI_{diff} failed to show effects of stimulation state ($P = 0.1$) or age ($P = 0.3$) and there was no interaction between factors ($P = 0.1$). For measurements using the 150 ms ISI, inhibition was significantly greater in older subjects ($P = 0.04$), but the interaction between factors was not significant ($P = 0.2$; Fig. 5.4C). SICI_{diff} was not significant for either group during SICI_{adj150} (young, $P = 0.5$; old, $P = 0.5$), or for old subjects during LICI-SICI_{150} ($P = 0.2$), but did reach significance for young subjects during LICI-SICI_{150} ($P = 0.003$; Fig. 5.4D). SICI_{diff} at 150 ms failed to show an effect of age ($P = 0.08$), whereas an effect of stimulation state ($P = 0.002$) and an interaction between factors ($P = 0.04$) was found. Age-related comparisons within each stimulation state showed no differences between age groups for SICI_{adj150} ($P = 0.7$) whereas SICI_{diff} was significantly greater in young subjects during LICI-SICI_{150} ($P = 0.04$).
Figure 5.4 Effects of age on the interaction between SICI and LICI during index finger abduction. Triple-pulse TMS measures were compared between young (black bars) and old (white bars) adults at two interstimulus intervals of 100 ms (A) and 150 ms (C) during isolated abduction of the index finger. For all panels, SICI_{adj100/150} refers to conditions G/H, and LICI-SICI_{100/150} refers to conditions K/L. The magnitude of change in SICI from baseline (i.e., SICI_{adj}) for the 100 ms and 150 ms intervals is also quantified in panels B and D. The dotted horizontal line represents no inhibition, with values below 100% showing an increase in inhibition. \#P < 0.05 when compared to baseline SICI and adjusted SICI; † magnitude of change from baseline is significant (P < 0.01); *P < 0.05 between young and old adults.
LICI-SICI interactions during precision grip are shown in Figure 5.5. For measurements using the 100 ms ISI, a significant effect of age ($P = 0.009$) and interaction between age and stimulation state was found ($P = 0.0002$; Fig. 5.5A). However, age-related comparisons showed no differences between groups for $\text{SICI}_{100}$ ($P = 0.1$) and LICI-SICI$_{100}$ ($P = 0.1$). For $\text{SICI}_{100}$, $\text{SICI}_{\text{diff}}$ was significant for young ($P < 0.0001$) but not old ($P = 0.9$) subjects, whereas $\text{SICI}_{\text{diff}}$ was not significant for either group during LICI-SICI$_{100}$ (young, $P = 0.2$; old, $P = 0.6$; Fig. 5.5B). $\text{SICI}_{\text{diff}}$ at 100 ms showed a significant effect of age ($P = 0.001$), but no effect of stimulation state ($P = 0.9$) or interaction between factors ($P = 0.07$). For measurements using the 150 ms ISI, inhibition was significantly reduced in younger subjects ($P < 0.0001$) but there was no interaction between factors ($P = 0.5$; Fig. 5.5C). $\text{SICI}_{\text{diff}}$ was significant during $\text{SICI}_{150}$ for old ($P < 0.0001$) but not young subjects ($P = 0.6$). Furthermore, $\text{SICI}_{\text{diff}}$ failed to reach significance for either group during LICI-SICI$_{150}$ (young, $P = 0.1$; old, $P = 0.3$; Fig. 5.5D). $\text{SICI}_{\text{diff}}$ showed a significant stimulation state effect ($P = 0.002$) but no effect of age ($P = 0.2$) or interaction between factors ($P = 0.5$).
Figure 5.5 Effects of age on the interaction between SICI and LICI during precision grip. Triple-pulse TMS measures were compared between young (black bars) and old (white bars) adults at two interstimulus intervals of 100 ms (A) and 150 ms (C) during precision grip between the index finger and thumb. For all panels, SICI$_{adj}$100/150 refers to conditions G/H, and LICI-SICI$_{100/150}$ refers to conditions K/L. The magnitude of change in SICI from baseline (i.e., SICI$_{adj}$) for the 100 ms and 150 ms intervals is also quantified in panels B and D. The dotted horizontal line represents no inhibition, with values below 100% showing an increase in inhibition. # $P < 0.05$ when compared to baseline SICI and adjusted SICI; † magnitude of change from baseline is significant ($P < 0.01$); *$P < 0.05$ between young and old adults.
5.4.5. Linear regression

Linear regression of individual subject data was used to investigate if task- and age-related changes in the activity of one inhibitory circuit was related to altered activity in either of the other inhibitory circuits. Subsequently, all inhibitory measurements (i.e., SICI, LICI and LICI-SICI interactions) in each task (rest, abduction, precision grip) were regressed against each other. In young but not old subjects, significant interactions were found between measurements of LICI\textsubscript{150} at rest and during index finger abduction (young: $r = 0.73$, $F_{1,12} = 12.24$, $P = 0.005$; old: $r = 0.39$, $F_{1,14} = 2.38$, $P = 0.15$; Fig. 5.6A, 5.6C), between LICI\textsubscript{150} at rest and during precision grip (young: $r = 0.58$, $F_{1,12} = 5.57$, $P = 0.04$; old: $r = 0.31$, $F_{1,14} = 1.34$, $P = 0.3$; Fig. 5.6B, 5.6E) and between LICI-SICI\textsubscript{100} and LICI-SICI\textsubscript{150} at rest (young: $r = 0.60$, $F_{1,12} = 6.11$, $P = 0.03$; old: $r = 0.38$, $F_{1,14} = 2.22$, $P = 0.16$). For old but not young subjects, significant interactions were found between measurements of LICI\textsubscript{100} at rest and during precision grip (young: $r = -0.16$, $F_{1,12} = 0.29$, $P = 0.6$; old: $r = 0.76$, $F_{1,14} = 17.65$, $P = 0.001$), and between LICI\textsubscript{100} and LICI\textsubscript{150} at rest (young: $r = 0.52$, $F_{1,12} = 4.05$, $P = 0.07$; old: $r = 0.84$, $F_{1,14} = 32.04$, $P < 0.0001$). In both groups, interactions between LICI\textsubscript{150} during abduction and precision grip were significant (young: $r = 0.72$, $F_{1,12} = 11.55$, $P = 0.006$; old: $r = 0.56$, $F_{1,14} = 6.06$, $P = 0.03$; Fig. 5.6C, 5.6F). All other regressions failed to reach significance.
Figure 5.6 Interactions between LICI_{150} during different tasks in young and old subjects. In young (black circles) but not old (white circles) subjects, LICI_{150} in resting muscle was significantly related to LICI_{150} during both index finger abduction (A/D) and precision grip (B/E). For both groups, significant interactions were found between LICI_{150} during index finger abduction and precision grip (C/F).
5.5. Discussion

The current study assessed age-related differences in pre- and post-synaptic M1 inhibition during relaxation, index finger abduction and precision grip. This was achieved by using paired- and triple-pulse TMS to assess SICI, LICI and the interaction between LICI and SICI in young and old adults. At least 3 new findings were obtained from this novel experimental approach. First, we found age-related differences in SICI during the precision grip task, but not at rest or during index finger abduction. Second, age-related differences in LICI were only evident in resting muscle (at an ISI of 150 ms), with no age-related differences during task performance. Third, we found age-related differences in LICI-SICI interactions at rest (100 ms ISI) and during index finger abduction (150 ms ISI), but not during precision grip. Taken together, these findings suggest that there are subtle differences in pre- and post-synaptic M1 inhibition with advancing age, which may contribute to deficits in motor performance during some tasks in older adults.

5.5.1. Effects of age on short-interval intracortical inhibition

Although age-related changes in SICI have been extensively investigated, the majority of studies have focussed on measurements in resting muscle, and have produced conflicting findings. Within the current study, SICI in resting muscle was not different between young and old adults, suggesting that resting post-synaptic GABA\textsubscript{A} mediated inhibitory tone in motor cortex is maintained with age (Ziemann \textit{et al.}, 1996b). This supports previous investigations from within our lab (Rogasch \textit{et al.}, 2009; Cirillo \textit{et al.}, 2010; Cirillo \textit{et al.}, 2011; Chapter 3), and from elsewhere (Wassermann, 2002; Oliviero \textit{et al.}, 2006; Smith \textit{et al.}, 2009), but is in contrast to reports of reduced (Peinemann \textit{et al.}, 2001; Marneweck \textit{et al.}, 2011; Heise \textit{et al.}, 2013) or increased (Kossev \textit{et al.}, 2002; McGinley \textit{et al.}, 2010) SICI with age. The reasons for these inconsistencies are currently unclear, but likely relate to variations in subject characteristics and methodological approach (Chapter 3).
In contrast to measurements in resting muscle, only two previous studies have investigated age-related changes in SICI during muscle activation, reporting no effect of age (McGinley et al., 2010) or reduced SICI in old subjects (Chapter 3). These inconsistent findings have been previously suggested to stem from variations in test MEP characteristics (Chapter 3). In the current study, we expanded this investigation by including different motor tasks, as the disinhibition of SICI during muscle activation has been shown to also be task-dependent in young subjects (Devanne et al., 2002; Kouchtir-Devanne et al., 2012). For example, greater reductions in SICI were observed during a synergistic precision grip between the index finger and thumb than during an isolated index finger abduction (Kouchtir-Devanne et al., 2012). This greater disinhibition of motor cortex during synergistic contractions has been suggested to contribute to the functional coactivation of cortical areas innervating task-related muscles (Kouchtir-Devanne et al., 2012).

Consistent with these previous findings, the current study observed greater reductions in SICI during precision grip in young subjects. In contrast, although inhibition was reduced in both tasks relative to rest in old subjects, the magnitude of this reduction did not differ between abduction and precision grip states, suggesting a lack of task-dependency with advancing age. Small (8%) differences in pre-stimulus EMG that we observed between age groups is unlikely to contribute to this effect, as the increased EMG in older adults was consistent for both tasks, but a difference in SICI was only observed for precision grip. Therefore, despite maintaining the ability to reduce post-synaptic GABA\textsubscript{A}-mediated inhibition within motor cortex during tonic muscle activation, old adults demonstrate a reduced modulation of inhibitory tone for tasks requiring more complex activation of primary motor cortex. This may contribute to age-related impairments in motor performance during this task, as has been shown previously (Cole, 1991; Cole et al., 1999). Alternatively, a recent study by Fujiyama and colleagues (Fujiyama et al., 2012b) showed that greater SICI during the foreperiod of a warned reaction
task was associated with faster reaction times in old but not young subjects. This could suggest that the increased inhibition observed during precision grip is a compensatory mechanism to maintain performance. This remains to be explored by future research.

5.5.2. Effects of age on long-interval intracortical inhibition

In resting muscle, we found that LICI\(_{100}\) was unaffected by age, whereas LICI\(_{150}\) was significantly reduced in older adults. These observations suggest that the strength of postsynaptic GABA\(_B\) mediated inhibitory tone in motor cortex (Werhahn \textit{et al.}, 1999) at rest may be reduced in older adults in a timing-dependent manner. Age-related changes in the magnitude of LICI have only been investigated by two previous studies. The first reported increased inhibition with age (McGinley \textit{et al.}, 2010), whereas the second, from our group, reported reduced inhibition with age (Chapter 3). As McGinley and colleagues used a 100 ms ISI, whereas our previous study used a 150 ms ISI, the current results contradict previous findings for the shorter interval, but support previous findings for the longer interval.

For LICI\(_{100}\) and LICI\(_{150}\) in active muscle, we found that the magnitude of inhibition was not significantly different between age groups for both tasks, supporting a previous study in active muscle at the 100 ms ISI (McGinley \textit{et al.}, 2010). However, we also found that the activity-dependent modulation of LICI\(_{150}\) was different in older adults. Specifically, young subjects showed a progressive reduction in LICI\(_{150}\) from rest, to index finger abduction, to precision grip (Fig. 5.2B), and these changes in inhibition were all significantly related to each other (Fig. 5.6A–C). However, in old subjects, there was no modulation of LICI\(_{150}\) from rest to index finger abduction, and no significant relationship between LICI\(_{150}\) in resting and active muscle (irrespective of task; Fig. 5.6D, 5.6E). These observations suggest that the ageing process changes the way in which LICI\(_{150}\) is modulated during the transition from resting to active muscle, but that once the muscle is active, old adults maintain the ability to
Chapter 5  

Age, Task & LICI-SICI interactions

modulate LICI<sub>150</sub> according to task demands. However, as the functional role of LICI is still not clear, the ramifications of this altered inhibitory modulation require further investigation.

### 5.5.3. Effects of age and task on the interaction between LICI and SICI

All previous investigations of age-related changes in intracortical inhibition have focussed on the conventional paired-pulse TMS measurements of SICI and LICI. The current study is the first to compare the difference in the interaction between these paradigms in young and old adults. The LICI-SICI interaction is seen as reduced SICI when assessed in the presence of LICI (Sanger et al., 2001), and several lines of evidence suggest that this is due to the activation of pre-synaptic GABA<sub>B</sub> receptors on the terminal of SICI neurons by collateral branches of LICI neurons (Werhahn et al., 1999; Sanger et al., 2001; McDonnell et al., 2006; Muller-Dahlhaus et al., 2008; Ni et al., 2011a), providing a measure of pre-synaptic motor cortex inhibition. LICI-SICI interactions may be altered in active muscle (Ni et al., 2007; Chapter 4) and are reduced in individuals with Parkinson’s disease (Chu et al., 2009), suggesting a role for pre-synaptic motor cortex inhibition in motor function.

In the current study, LICI-SICI interactions were investigated using two intervals (between LICI’s conditioning stimulus and the test stimulus) of 100 ms and 150 ms, as inconsistent findings from previous studies suggested that pre-synaptic inhibition in older adults may be timing-dependent (Chu et al., 2008; Cash et al., 2010). In resting muscle, although both groups showed reduced SICI in the presence of LICI at each ISI, the magnitude of this effect was reduced in old subjects at 100 ms, but not different between groups at 150 ms. Although we expected to see a timing-dependent effect of age on the LICI-SICI interaction, the findings of Chu and colleagues suggested that it would occur at 150 ms, not 100 ms. The reasons for this discrepancy are currently unclear, but seem unlikely to stem from the minor methodological differences between studies. Despite this, these findings suggest that the
ageing process may cause a timing specific reduction in pre-synaptic GABA$_B$-mediated intracortical inhibition within motor cortex. Interestingly, results of our linear regression analysis further supports a timing-dependent dissociation of pre-synaptic inhibition in old adults, with young but not old subjects showing a significant interaction between resting LICI-SICI$_{100}$ and LICI-SICI$_{150}$.

In active muscle, LICI-SICI$_{100}$ measurements during both abduction and precision grip failed to show any effects of age. Furthermore, while a consistent age effect was observed across stimulus conditions for LICI-SICI$_{150}$ during precision grip, it seems likely that this effect was driven by the age-related increase in baseline SICI observed during precision grip (Fig. 5.1). Despite this, for LICI-SICI$_{150}$ during index finger abduction, the absolute change in inhibition from baseline (i.e., SICI$_{diff}$; Fig. 5.4D) was significant for young but not old subjects, and the magnitude of SICI$_{diff}$ was significantly greater in young subjects. These observations show an age-related reduction in the interaction between SICI and LICI during index finger abduction at 150 ms, reflecting reduced presynaptic motor cortex inhibition in older adults under these conditions.

One possible reason for these observed changes in LICI-SICI interactions could be due to age-related differences in SP duration (Sale & Semmler, 2005; Oliviero et al., 2006; McGinley et al., 2010). However, the majority of studies reporting age-related changes in the SP suggest a reduced duration with age (Sale & Semmler, 2005; Oliviero et al., 2006), whereas our observed changes in LICI-SICI interactions in older adults could only be explained by an increased SP duration with advancing age. It therefore seems unlikely that these changes in LICI-SICI interactions were confounded by SP duration, but instead reflect an age-related reduction in the activation of pre-synaptic GABA$_B$ receptors when performing isolated index finger abductions. As pre-synaptic GABA$_B$ receptors limit the release of GABA from inhibitory neurons (for review, see; Benarroch, 2012), this reduced presynaptic
inhibition may reflect a less specific cortical activation that impairs task performance. For example, both TMS and neuroimaging studies have shown that old adults demonstrate more non-specific patterns of cortical activation during performance of a motor task (Carp et al., 2011; Bernard & Seidler, 2012), and that this increased activation may be detrimental to task performance (Bernard & Seidler, 2012). Therefore, these age-related changes in inhibitory tone may represent a compensatory mechanism to regain more specific patterns of cortical activation.

In line with previous studies (Sanger et al., 2001; Chu et al., 2008; Muller-Dahlhaus et al., 2008), the current study was able to match the test MEPs under all conditions by adjusting the test TMS intensity. This process assumes that a similar test MEP reflects a comparable descending volley involving a similar contribution of early (I$_1$) and late (I$_3$) I-waves, even when preceded by the activation of inhibitory circuits. This interpretation has been supported by experimental evidence from epidural recordings recorded during relaxation of target muscles (Ni et al., 2011a), although opposing results may be found in some subjects (Weise et al., 2013). Nonetheless, the contribution of early and late I waves to the MEP are known to be altered when the target muscle is active (Di Lazzaro et al., 1998b), which may complicate the interpretation of the interactions between inhibitory circuits in active muscle. Furthermore, it is not known whether the composition of the descending volley is different in older adults, although recent evidence comparing MEP latencies between antero-posterior (preferential I$_3$ wave activation) and posterior-anterior (preferential I$_1$ wave activation) TMS showed similar recruitment of I waves in older adults (Sale et al., 2015). Further research is therefore needed to confirm whether the descending volley (that produces similar MEP amplitudes) is comparable in young and old adults, particularly when the muscle is active.

In conclusion, the current study has demonstrated complex effects of age on the task-dependent modulation of intracortical inhibition. In resting muscle, SICI was unaffected by
age, whereas LICI and LICI-SICI interactions showed timing-dependent reductions in old adults. During muscle activation, older adults showed a reduced modulation of both SICI and LICI, resulting in reduced SICI during precision grip in older adults, and no age-related difference in LICI. When SICI was primed by LICI, disinhibition of motor cortex was reduced in older adults at rest (100 ms ISI) and during index finger abduction (150 ms ISI), but not during precision grip. These findings suggest that there are age-related differences in pre- and post-synaptic motor cortex inhibition that are dependent on the task performed, which may occur due to a reduced ability to modulate inhibitory circuits in the ageing motor cortex.
CHAPTER VI

INTRACORTICAL INHIBITION IS MODULATED DURING SLOW SHORTENING AND LENGTHENING CONTRACTIONS IN YOUNG AND OLD ADULTS

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INTRACORTICAL INHIBITION IS MODULATED DURING SLOW SHORTENING AND LENGTHENING CONTRACTIONS IN YOUNG AND OLD ADULTS

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By signing the Statement of Authorship, each author certifies that their stated contribution to the publication is accurate and that permission is granted for the publication to be included in the candidate’s thesis.

George Opie (Candidate)

Contribution: Experimental design, subject recruitment, collection and analysis of data, interpretation of data, wrote manuscript

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Date 12/6/2015

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Contribution: Experimental design, supervised development of work, data interpretation, critical manuscript evaluation

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Chapter 6

Ageing, ICI & Movement

6. Intracortical inhibition is modulated during slow shortening and lengthening contractions in young and old adults

6.1. Abstract

The modulation of intracortical inhibition is thought to be impaired in older adults, which may contribute to their reduced fine motor control, particularly during muscle lengthening. The purpose of this study was to quantify the magnitude of intracortical inhibition and movement performance during postural, shortening and lengthening contractions of a hand muscle in young and old adults. In 18 young (23.2 ± 4.2) and 16 old (70.6 ± 6.5) subjects, paired-pulse transcranial magnetic stimulation (TMS) was used to assess short- (SICI) and long-interval intracortical inhibition (LICI) during a movement task involving the first dorsal interosseous muscle. The task required a load (50 g) to be slowly lifted and lowered using the index finger while single- or paired-pulse TMS was delivered during the shortening or lengthening contraction. Relative to postural contractions, SICI during shortening contractions was reduced by 35% in young subjects ($P < 0.0001$) and 47% in old subjects ($P < 0.0001$), whereas SICI during lengthening contractions was reduced by 15% in young subjects ($P = 0.0004$) and 37% in old subjects ($P < 0.0001$). Furthermore, differences between groups were significant for both phases (both $P$-values < 0.01). For LICI, inhibition was unaffected by contraction type in young subjects, whereas old subjects showed a 20% reduction in LICI during lengthening contractions ($P < 0.0001$). Furthermore, LICI in old subjects was significantly less than young subjects in each phase (both $P$-values < 0.05). These findings suggest that movement is associated with a modulation of GABAergic inhibition, and that this modulation is modified by the ageing process.
6.2. Introduction

A growing body of evidence suggests that the neural control of lengthening contractions represents a unique component of movement. This includes observations that voluntary activation, electromyography (EMG), force generation and spinal motoneuron excitability are all different during lengthening contractions (Enoka, 1996; Duchateau & Enoka, 2008). Furthermore, more recent evidence from studies using a range of neuroimaging techniques have supplied compelling support for lengthening contractions being associated with distinct patterns of cortical activity (Abbruzzese et al., 1994; Fang et al., 2001; Sekiguchi et al., 2001; Sekiguchi et al., 2003; Fang et al., 2004; Gruber et al., 2009; Ducaly et al., 2011; Kwon & Park, 2011; Ducaly et al., 2014). In addition to demonstrating unique neural control mechanisms, lengthening contractions are also known to be associated with reduced motor performance (Christou & Carlton, 2002b; Christou et al., 2003; Neto et al., 2012).

Interestingly, the magnitude of this deficit is thought to be increased by advancing age, with greater impairments in performance observed in old adults during lengthening movements (Burnett et al., 2000; Graves et al., 2000; Laidlaw et al., 2000; Christou & Carlton, 2002a; Christou et al., 2003), which may contribute to the increased incidence of falls in the elderly (Carville et al., 2007).

Although age-related differences in neuromuscular function are well established (Enoka et al., 2003) our current understanding of the CNS mechanisms contributing to this movement deficit in old adults is limited. One factor that may contribute to this impaired motor performance is changes in inhibitory neurotransmission within primary motor cortex (M1) mediated by GABA. In young subjects, the modulation of local GABAergic inhibition has been associated with motor function by studies using transcranial magnetic stimulation (TMS; Ridding et al., 1995; Reynolds & Ashby, 1999; Stinear & Byblow, 2003; Zoghi et al., 2003; Buccolieri et al., 2004). Furthermore, variations in GABAergic inhibition during shortening
and lengthening muscle contractions have also been proposed in young subjects (Sekiguchi et al., 2007; Duclay et al., 2011; Howatson et al., 2011; Duclay et al., 2014), suggesting that these circuits may contribute to the accurate performance of anisometric contractions. However, these previous studies have relied on the assessment of GABAergic inhibition by measuring the silent period (SP) in the EMG following TMS during shortening and lengthening contractions (Sekiguchi et al., 2007; Duclay et al., 2011, 2014), which is difficult to interpret and is highly sensitive to changes in spinal excitability (for review, see; Rossini et al., 2015). As previous studies with paired-pulse TMS in old adults have shown that a reduced ability to modulate local inhibition prior to contraction is associated with impaired motor performance (Heise et al., 2013), it is therefore possible that age-related changes in the modulation of local GABAergic inhibition during movements (particularly lengthening contractions) may contribute to the movement performance deficits commonly observed in the elderly.

The main aim of the current study was therefore to investigate variations in GABAergic inhibition within contralateral M1 during shortening and lengthening contractions in young and old adults. We examined GABAergic inhibition using paired-pulse TMS to assess short-(SICI) and long-interval intracortical inhibition (LICI), which provide more consistent information on GABAergic processes within primary motor cortex compared with the EMG silent period (Ziemann et al., 2014). Although technically challenging, we set out to match the test motor evoked potential (MEP) amplitudes during each of the contraction phases, so that the cortical circuits activated by TMS were equivalent during each task (Sanger et al., 2001). As previous studies suggest that lengthening contractions are associated with disinhibition of contralateral M1 (Sekiguchi et al., 2007; Duclay et al., 2011, 2014), we expected that lengthening movements would also be associated with a reduction in SICI and LICI. Furthermore, as the activity-dependent modulation of inhibitory tone is thought to be reduced
in old adults (Fujiyama et al., 2012b; Heise et al., 2013; Chapter 5), and this has been related to impaired motor performance in the elderly (Heise et al., 2013), we expected that old individuals would demonstrate less modulation of cortical inhibition during movement, and that this would be associated with age-related motor deficits.

6.3. Methods

6.3.1. Ethical approval

All experimentation was approved by the University of Adelaide Human Research Ethics Committee and conducted in accordance with the declaration of Helsinki. Each subject provided written, informed consent prior to participation.

6.3.2. Subjects

18 young (mean ± SD: 23.3 ± 4.2 years; 9 females) and 16 old (70.6 ± 6.5 years, 9 females) healthy subjects were recruited from the university and wider community to participate in the current study. Exclusion criteria included a history of stroke, history of neurological or psychiatric disease, or current use of psychoactive medication (sedatives, antipsychotics, antidepressants etc.). Hand preference and laterality was assessed using the Edinburgh Handedness Inventory (Oldfield, 1971).

6.3.3. Experimental arrangement

For the duration of the experiment, subjects were seated in a chair with their right arm abducted approximately 45° at the shoulder. The right hand and forearm was placed pronated on a purpose built manipulandum that was located on a table in front of the subject. The index finger was extended over a cavity within the manipulandum, while the third, fourth and fifth fingers were flexed around the edge of the cavity at the level of the metacarpophalangeal (MCP) joint. The thumb was extended against a padded support on the manipulandum and the
forearm was strapped to an adjustable rest. A strap was also placed across the hand to minimise movement. This position allowed abduction-adduction of the index finger that was isolated to activation of the first dorsal interosseous (FDI) muscle. A circular plastic cast placed around the distal end of the index finger was attached to a 50 g load via a length of low compliance line. The line ran over a pulley attached to the edge of the manipulandum, suspending the load in mid-air.

Surface EMG was used to record responses from the FDI muscle of the right hand. Two Ag–AgCl electrodes (3.2 cm diameter) were attached to the skin over the muscle in a belly-tendon montage, with a strap around the wrist grounding the electrodes. Acceleration of the index finger in the abduction-adduction plane was measured using a uniaxial accelerometer (V94-41, Coulbourn Instruments, Whitehall, PA) that was placed on the medial surface of the plastic cast attached to the index finger. Position of the index finger was assessed via a potentiometer that’s rotational axis was aligned with the MCP joint and securely attached along the length of the index finger. EMG was amplified (300 X) and band-pass filtered (20 Hz high pass, 1 kHz low pass) using a CED1902 (Cambridge Electronic Design, Cambridge, UK). EMG, position and acceleration signals were digitized at 2 kHz using a CED1401 interface (Cambridge Electronic Design), before being recorded and stored offline for analysis. To facilitate muscle relaxation when required, real-time EMG signals were displayed under high gain (50 µV/ division) on an oscilloscope placed in front of the subject.

### 6.3.4. Experimental Procedures

**Maximal compound muscle action potential (M\text{max})** Electrical stimulation applied at the wrist was used to stimulate the ulnar nerve, generating maximal compound muscle action potentials within FDI. Stimuli were applied using a constant-current stimulator (DS7AH, Digitimer, UK) and bipolar surface electrodes with the cathode positioned distally. Each stimulus was a square wave pulse of 100 µs duration and intensity set at 120% of that required to produce a
maximal response in FDI (i.e. 120% $M_{\text{max}}$). $M_{\text{max}}$ was obtained by averaging the responses to 5 stimuli delivered at the beginning of the experiment.

**Maximal Voluntary Contraction** Index finger abduction force during maximum voluntary contraction (MVC) was assessed for each subject. MVCs were conducted with the hand positioned on the manipulandum as described above and with 0° of index finger abduction. When instructed, subjects abducted the lateral surface of the index finger against a force transducer (LC1205-K020; A&D Mercury Pty Ltd, Australia) placed in-line with the distal phalanx. Subjects were required to produce maximum force for 3 s in several repetitions, separated by 30 s rest, until the maximal force of three trials were within a 10% margin. The largest force recorded during these trials was chosen as the subject’s MVC. To optimise force production, feedback was displayed on a computer monitor placed at eye level in front of the subject, and verbal encouragement was provided by the experimenter.

**Postural, shortening and lengthening contractions** Subjects performed two types of low-intensity contractions against a 50 g inertial load; postural contractions, during which the index finger was held abducted at a constant position of 10° from the index fingers neutral position, and anisometric contractions, during which the subject performed abduction-adduction movements of the index finger over a 20° range of motion. For both contraction types, a display screen showing two cursors was placed at eye level in front of the subject. One cursor represented the position of the index finger, while the second represented a target position. Subjects performed the required movement by matching the position cursor to the target cursor. During postural contractions, the target cursor was static, representing the required abduction angle, whereas during anisometric contractions, the target cursor formed a triangular template representing a constant velocity contraction of 4 degs/s. The assessment of intracortical inhibition during postural contractions required subjects to maintain index finger abduction for approximately 4 minutes, while the assessment of intracortical inhibition during
movement required the completion of 72 shortening and 72 lengthening contractions (see below). At the beginning of both postural and anisometric contractions, subjects were instructed to match the position of the target cursor as accurately as possible at all times.

During the contractions, encouragement to perform the task accurately was also provided by the experimenter.

**Transcranial magnetic stimulation** TMS was applied to the left primary motor cortex using a figure-of-eight coil (external wing diameter 9 cms) with two Magstim 200² magnetic stimulators connected via a Bistim unit (Magstim, Dyfed, UK). The coil was held tangentially to the scalp at an angle of 45° to the sagittal plane, with the handle pointed backwards and laterally, producing an anteriorly directed current flow in the brain. The coil was positioned on the scalp over the location producing an optimum response in the relaxed FDI muscle. This location was marked on the scalp for reference and continually checked throughout the experiment. During resting and postural measurements, TMS was delivered at 0.2 Hz. However, during anisometric measurements, TMS was delivered at between 0.08 and 0.14 Hz, depending upon which phase the previous stimulus had been applied.

Resting and Active motor thresholds (RMT and AMT, respectively) were obtained in FDI while the TMS coil was placed at the optimal location over primary motor cortex. RMT was defined as the minimum TMS intensity producing a response amplitude ≥ 50 μV in three out of five trials in resting FDI muscle, and expressed relative to the maximum stimulator output (MSO). Active motor threshold (AMT) was defined as the minimum TMS intensity producing a response amplitude ≥ 200 μV in three out of five trials while subjects performed the postural contraction at 10° of index finger abduction described above. In addition, AMT was also assessed separately for shortening and lengthening contractions. Due to time constraints, this assessment utilised a modified version of the anisometric contraction described above; subjects abducted and adducted the index finger over a 10° range of motion,
from 5° to 15° of index finger abduction, with stimuli applied at 10°. This ensured that stimuli were applied at a comparable muscle length to that used during all other assessments, but considerably reduced the time requirement.

Intracortical inhibition Contraction phase-dependent changes in intracortical inhibition were assessed by examining measurements of SICI and LICI recorded during postural, shortening and lengthening contractions of FDI. To avoid any age-related differences in baseline inhibition, the intensity of the conditioning stimulus for SICI and LICI was adjusted during the postural contraction to produce an ~ 50% reduction (range 25 to 75% reduction) in the amplitude of a 2 mV test MEP (when assessed in isolation). Furthermore, this intensity was determined separately for postural, shortening and lengthening contractions, as levels of cortical and spinal activity are known to vary between contraction types. Measurements of SICI used a 2 ms interstimulus interval (ISI), whereas measurements of LICI used a 150 ms ISI. For the assessment of baseline inhibition during a postural contraction, both paradigms were applied in the same block, allowing normalisation to a common test alone MEP. As 24 conditioned trials (12 SICI, 12 LICI) and 12 unconditioned trials were included in a block, 36 trials were applied to assess baseline inhibition. For the assessment of contraction type-dependent changes in inhibition, TMS was applied at the midpoint of each contraction phase (i.e., 10° of abduction) to match the joint angle used during postural contractions.

Furthermore, while a single movement trial consisted of both shortening and lengthening movements, TMS was only applied on one contraction phase (shortening or lengthening) for each trial. As 24 conditioned (12 SICI, 12 LICI) and 12 unconditioned trials were applied in each phase, a total of 72 trials were used to assess contraction type-dependent changes in intracortical inhibition. However, as a single trial lasted 12 s, the experimental block was broken into 6 blocks of 12 trials, with a 30 s break between blocks, to avoid fatigue and loss of attention.
To improve comparability with previous studies, EMG SP duration was also assessed from a subset of subjects that demonstrated a reliable SP. These measurements were assessed in each contraction type during application of the test alone MEP. EMG was first rectified then SP duration was assessed from the time of TMS to the point at which EMG crossed the pre-stimulus mean (using a 200 ms pre-stimulus period). This was calculated using a modified cumulative sum (CUSUM) method (Brinkworth & Turker, 2003).

6.3.5. Data Analysis

Data analysis was completed manually by visual inspection of offline EMG. MEP amplitudes from each trial were measured peak-to-peak and expressed in mV. Paired-pulse measurements of intracortical inhibition were quantified by expressing the amplitude of individual conditioned MEPs as a percentage of the average unconditioned MEP amplitude within each condition. For all contraction types and in both subject groups, the level of muscle activity in each condition was assessed by quantifying the mean rectified EMG amplitude in the 100 ms leading up to application of the conditioning stimulus for SICI and LICI trials, or the test stimulus for test alone MEP trials. These values were normalised to the mean rectified EMG amplitude recorded during MVC. Motor output during different contraction types was assessed using acceleration SD and the absolute error between position and target cursors. During postural contractions, SD and absolute error were averaged over the 800 ms prior to application of TMS, whereas during anisometric contractions, SD and absolute error were averaged over the middle 3 s of each contraction phase. As the muscle twitch associated with TMS would confound the assessment of performance, only phases in which TMS was not applied were used for analysis of movement performance.
6.3.6. Statistical analysis

Unpaired students t-tests were used to compare Age, Handedness, RMT, $M_{\text{max}}$ amplitude and $M_{\text{max}}$ intensity between young and old subjects. The effects of contraction type (hold, shortening, lengthening) and age (young, old) on AMT, test TMS intensity, conditioning TMS intensity and prestimulus EMG were investigated using individual 2-way repeated measures analysis of variance (ANOVA$_{\text{RM}}$). All main effects and interactions were further investigated using one-way ANOVA’s with Fishers PLSD post hoc test. Individual linear mixed models with repeated measures were used to compare the fixed effects of contraction type and age on indices of performance (acceleration SD, absolute position error). Individual linear mixed models with repeated measures were also used to compare the fixed effects of contraction type and age on SICI, LICI, SP duration and the amplitude of the test alone MEP within SICI and LICI blocks. For all models, subject was included as a random effect and all significant main effects and interactions were further investigated using custom contrasts with Bonferroni correction. Linear regression of individual subject data was used to investigate associations between measurements of inhibition and indices of motor performance derived from postural and anisometric contractions. Significance was set at $P < 0.05$ and all data are presented as mean ± SEM, unless otherwise stated.

6.4. Results

All subjects completed the experiment in full and without adverse reaction. However, it was not possible to produce the required level of baseline SICI or LICI during postural contractions (i.e., ~ 50% inhibition of test MEP amplitude) in some subjects. Subsequently, not all subjects contribute data to the analysis of both measurements. 14 subjects from each age group are included in the analysis of SICI, whereas 15 subjects from each group are included in the analysis of LICI. A total of 11 young and 13 old subjects contributed data to both measurements. Baseline characteristics for all subjects are shown in Table 1. The results
of the Edinburgh Handedness Inventory showed that the study cohort was, on average, right hand dominant, and that this was not effected by age \( (P = 0.1) \). RMT was also unaffected by age \( (P = 0.6) \), whereas \( M_{\text{max}} \) amplitude was significantly greater in young subjects \( (P = 0.001) \). AMT differed between contraction types \( (P < 0.0001) \), with post hoc analysis showing that AMT during lengthening contractions was reduced relative to postural contractions \( (P < 0.0001) \), whereas AMT during shortening contractions was reduced relative to both postural \( (P < 0.0001) \) and lengthening contractions \( (P = 0.03) \). However, there was no difference between age groups \( (P = 0.8) \) and no interaction between factors \( (P = 0.1) \). Pre-stimulus EMG also differed between contraction types \( (P = 0.01) \), with post hoc testing showing increased muscle activity during shortening contractions relative to both postural \( (P < 0.0001) \) and lengthening \( (P < 0.0001) \) contractions, and increased muscle activity during lengthening contractions relative to postural contractions \( (P = 0.003) \). Furthermore, old subjects showed greater pre-stimulus EMG than young subjects \( (P = 0.01) \), but there was no interaction between factors \( (P = 0.7) \).

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<tr>
<th>Table 6.1 Subject Characteristics</th>
<th>Young</th>
<th>Old</th>
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<tr>
<td>Age (years)</td>
<td>23.3 ± 1.0</td>
<td>70.6 ± 1.6 (^a)</td>
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<tr>
<td>Handedness (L.Q)</td>
<td>0.93 ± 0.02</td>
<td>0.84 ± 0.06</td>
</tr>
<tr>
<td>MVC force (N)</td>
<td>31.7 ± 2.7</td>
<td>28.2 ± 2.4</td>
</tr>
<tr>
<td>Mmax amplitude (mV)</td>
<td>16.4 ± 1.1</td>
<td>11.6 ± 0.6 (^a)</td>
</tr>
<tr>
<td>RMT (%MSO)</td>
<td>43.5 ± 1.5</td>
<td>44.8 ± 2.2</td>
</tr>
<tr>
<td>MVC EMG (mV)</td>
<td>0.38 ± 0.03</td>
<td>0.28 ± 0.02 (^a)</td>
</tr>
<tr>
<td>AMT (%MSO)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-Postural</td>
<td>37.7 ± 1.2</td>
<td>37.8 ± 2.1</td>
</tr>
<tr>
<td>-Shortening</td>
<td>35.8 ± 1.2 (^b)</td>
<td>34.5 ± 1.7 (^b)</td>
</tr>
<tr>
<td>-Lengthening</td>
<td>36.3 ± 1.1 (^c)</td>
<td>35.7 ± 1.9 (^c)</td>
</tr>
<tr>
<td>Pre-stimulus EMG (% MVC EMG)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-Postural</td>
<td>6.6 ± 0.9</td>
<td>11.7 ± 1.5</td>
</tr>
<tr>
<td>-Shortening</td>
<td>15.1 ± 1.5 (^b)</td>
<td>22.3 ± 3.5 (^b)</td>
</tr>
<tr>
<td>-Lengthening</td>
<td>9.7 ± 1.1 (^c)</td>
<td>16.5 ± 2.4 (^c)</td>
</tr>
</tbody>
</table>

Data shown as mean ± SEM. \(^a\)P < 0.05 compared to young; \(^b\)P < 0.05 compared to postural; \(^c\)P < 0.05 compared to postural and shortening.
6.4.1. Intracortical inhibition

Representative data from a single old subject (75 years) showing variations in inhibition during each contraction type is shown in Figure 6.1A and 6.1B. For this subject, AMT was 33% MSO, 31% MSO and 32% MSO during postural, shortening and lengthening contractions, respectively, while test TMS intensity was 40% MSO, 37% MSO and 36% MSO during postural, shortening and lengthening contractions, respectively. This subject showed SICI of 66% during postural contractions, which was reduced to 85% during shortening contractions and 68% during lengthening contractions (Figure 6.1A). For LICI, inhibition of 52% was obtained during postural contractions, and this was reduced to 63% and 87% during shortening and lengthening contractions, respectively (Figure 6.1B).
Figure 6.1 Representative data showing variations in SICI (A) and LICI (B) during postural, shortening and lengthening contractions. An example of a single trial for the postural and anisometric tasks, demonstrating the target position, position of the index finger (C) and associated index finger acceleration (D), are also shown.
Short interval intracortical inhibition For all subjects, the amplitude of the test alone MEP used to assess SICI during each contraction type is shown for both young and old subjects in figure 6.2A. The test alone MEP was similar for all contraction types ($P = 0.05$) and was comparable between age groups ($P = 0.9$). Furthermore, there was no interaction between factors ($P = 0.1$). These data suggest that the amplitude of the test alone MEP used for the assessment of SICI was well matched between contraction types and age groups. The TMS intensity used to produce the test alone MEP differed between contraction types ($P < 0.0001$), with post hoc analysis showing that the intensity required during postural contractions (young, 48.7 ± 1.8% MSO; old, 47.6 ± 2.5% MSO) was greater than during either lengthening (young, 43.1 ± 1.6% MSO; old, 42.6 ± 2.2% MSO) or shortening (young, 45.0 ± 1.6% MSO; old, 44.3 ± 2.4% MSO) contractions (P-values < 0.0001), and that the intensity during shortening contractions was greater than during lengthening contractions ($P = 0.001$). However, no effect of age ($P = 0.8$) or interaction between factors ($P = 0.8$) was found.

Variations in SICI during each contraction type are compared between young and old subjects in figure 6.2B. The magnitude of SICI differed between contraction types ($P < 0.0001$) and was reduced in old subjects ($P < 0.02$). Furthermore, a significant interaction between factors was also found ($P = 0.0003$). In young subjects, post hoc analysis showed that SICI during shortening contractions was reduced relative to both postural and lengthening contractions, whereas SICI during lengthening contractions was reduced relative to postural contractions (all $P$-values < 0.001). In old subjects, SICI during both shortening and lengthening contractions was reduced relative to postural contractions ($P < 0.0001$). Age-related comparisons within each contraction type showed that SICI was not different between age groups during postural contractions ($P = 0.5$), but was reduced in old subjects during both shortening ($P = 0.04$) and lengthening ($P < 0.0008$) contractions.
Figure 6.2 Effects of contraction type on SICI compared between young and old adults. The amplitude of the test alone MEP used to assess SICI (A) and the magnitude of SICI (B) are compared between young (black bars) and old (white bars) subjects during postural (left columns), shortening (middle columns) and lengthening (right columns) contractions. The dotted horizontal line represents no inhibition, with values below 100% showing inhibition of the test MEP. ‡P < 0.05 when compared to postural contractions; †P < 0.05 when compared to postural and shortening contractions; *P < 0.05 between age groups.
**Long interval intracortical inhibition** For all subjects, the amplitude of the test alone MEP used to assess LICI in each contraction type is shown in Figure 6.3A. The test MEP did not differ between contraction types ($P = 0.1$) and this was consistent between age groups ($P = 0.5$). Furthermore, there was no interaction between factors ($P = 0.5$). These findings demonstrate that the amplitude of the test alone MEP for the assessment of LICI was well matched between contraction types and age groups. The TMS intensity used to generate the test alone MEP varied between contraction types ($P < 0.0001$), with post hoc testing showing that the intensity of the test stimulus during the postural contraction (young, $47.4 \pm 2.0\%$ MSO; old, $46.8 \pm 2.2\%$ MSO) was greater than during either shortening (young, $41.9 \pm 1.8\%$ MSO; old, $42.7 \pm 2.0\%$ MSO) or lengthening (young, $44.1 \pm 1.7\%$ MSO; old, $44.3 \pm 2.2\%$ MSO) contractions ($P$-values $< 0.0001$), and greater during the lengthening than shortening contraction ($P = 0.002$). However, stimulus intensities were not different between age groups ($P = 0.9$) and there was no interaction between factors ($P = 0.5$).

The magnitude of LICI in each contraction phase is compared between groups in figure 6.3B. LICI differed between contraction types ($P = 0.003$) and was reduced in old subjects ($P = 0.03$). Furthermore, an interaction between factors was also found ($P = 0.002$). For young subjects, post hoc analysis showed that the magnitude of LICI was unaffected by contraction type (all $P$-values $> 0.1$). For old subjects, LICI during lengthening contractions was significantly reduced relative to both postural ($P < 0.0001$) and shortening contractions ($P = 0.02$). Age-related comparisons within each contraction type showed that LICI was not different between groups during postural contractions ($P = 0.5$), but significantly reduced in old subjects during both shortening ($P = 0.02$) and lengthening ($P = 0.005$) contractions.
Figure 6.3 Effects of contraction type on LICI compared between young and old adults. The amplitude of the test alone MEP used to assess LICI (A) and the magnitude of LICI (B) are compared between young (black bars) and old (white bars) subjects during postural (left columns), shortening (middle columns) and lengthening (right columns) contractions. †P < 0.05 when compared to postural and shortening contractions.
**EMG Silent Period** The duration of the SP in each contraction type is compared between a subset of young and old subjects that demonstrated a reliable SP (13 young and 14 old) in Figure 6.4. Subject data was considered reliable if CUSUM analysis returned an accurate SP duration (assessed via visual comparison with raw data) for at least 6 out of 12 trials in all contraction types. Furthermore, the amplitude of the test MEP, which is known to effect SP duration (Orth & Rothwell, 2004), was not different between age groups or contraction types (see above). For all subjects, SP duration was not different between age groups ($P = 0.5$), but differed between contraction types ($P < 0.0001$) and there was an interaction between factors ($P < 0.0001$). In young subjects, post hoc analysis showed that SP duration during lengthening contractions was reduced relative to postural contractions ($P = 0.0001$), whereas SP duration during shortening contractions was reduced relative to both postural ($P < 0.0001$) and lengthening contractions ($P < 0.0001$). In old subjects, the SP during shortening contractions was reduced relative to both postural ($P < 0.0001$) and lengthening ($P = 0.0008$) contractions. Age-related comparisons within each contraction type showed that the SP duration was less in young compared with old subjects during shortening contractions ($P = 0.02$).

![Figure 6.4](image_url)

**Figure 6.4** Effects of age and contraction type on the duration of the EMG SP. Data show the mean SP duration for a subset of 13 young (black bars) and 14 old adults (white bars) during postural (left columns), shortening (middle columns) and lengthening (right columns) contractions. *$P < 0.05$ when compared to postural contractions; †$P < 0.05$ when compared to postural and shortening contractions; ‡$P < 0.001$ when compared to postural and lengthening contractions; *$P < 0.05$ between age groups.
6.4.2. Motor output during isometric movements

Absolute movement error differed between contraction types \((P < 0.0001)\) and was increased in old adults \((P < 0.0001)\). There was also an interaction between these factors \((P < 0.0001; \ \text{Figure 6.5A})\). For both groups, post hoc testing showed that movement error during shortening contractions was greater than during postural contractions \((P < 0.0001)\), and greater during lengthening than either shortening (young, \(P < 0.0001\); old, \(P = 0.001\)) or postural contractions \((P < 0.0001\) for both groups). Age-related comparisons within each contraction type showed that movement error was significantly increased in older adults during shortening \((P < 0.0001)\) and lengthening \((P < 0.0001)\) contractions, but unaffected by age during postural contractions \((P = 0.3)\).

The SD of Acceleration during movement also differed between contraction types \((P < 0.0001)\) and was increased in old adults \((P = 0.002)\). An interaction between factors was also found \((P < 0.0001; \ \text{Figure 6.5B})\). Post hoc testing showed that, in both groups, the SD of acceleration during shortening contractions was greater than postural contractions \((P < 0.0001)\), and greater during lengthening contractions than during either shortening (young, \(P = 0.002\); old, \(P = 0.01\)) or postural contractions \((P < 0.0001)\). Age-related comparisons within each contraction type showed that acceleration SD was significantly increased in old adults during all contraction types (all \(P\)-values < 0.03).

6.4.3. Linear regression

Linear regression of individual subject data was used to investigate associations between intracortical inhibition and movement performance during different contraction types in young and old subjects. However, no significant correlations between motor performance and either the magnitude or modulation of intracortical inhibition was found in either group.
Figure 6.5 Motor performance during different contraction types in young and old adults. Performance during postural, shortening and lengthening contractions was investigated by comparing the absolute error between finger and target positions (A) and the SD of acceleration during movement (B) between young (black bars) and old (white bars) subjects. *P < 0.05 when compared to postural contractions; †P < 0.05 when compared to postural and shortening contractions; *P < 0.05 between age groups.
6.5. Discussion

The current study investigated age-related changes in intracortical inhibition with paired-pulse TMS during postural, shortening and lengthening contractions of the index finger. At least 4 new findings related to the cortical control of movement in young and old adults were obtained from this experimental approach. First, performance of shortening and lengthening contractions is accompanied by a reduction of SICI in both young and old adults. Second, performance of lengthening contractions is accompanied by a reduction of LICI in old but not young subjects. Third, older adults demonstrate a greater modulation of SICI and LICI during both shortening and lengthening contractions. Fourth, these changes in inhibition appear to be unrelated to age-related differences in motor performance during movement.

A pivotal aspect of the current study was that we were able to match the amplitude of the test MEP between young and old adults in each contraction type. This was necessary because the magnitude of inhibition recorded during paired-pulse TMS paradigms varies depending on the amplitude of the test MEP (Sanger et al., 2001), and this effect is thought to stem from the activation of different neuronal populations with individual sensitivities to inhibitory circuits (Sanger et al., 2001). Matching test MEPs suggests that the neuronal populations activated by the test stimulus were consistent across age groups and movements. Furthermore, levels of baseline inhibition were also adjusted to approximately 50% in both groups. This effectively removed any age-related differences in baseline inhibition during tonic muscle activation, ensuring similar activation of inhibitory circuits between groups. Therefore, we argue that this methodological approach has allowed us to reliably compare changes in intracortical inhibition during postural, shortening and lengthening contractions between young and old adults.
6.5.1. SICI is modulated by movement in young and old adults

Changes in the magnitude of SICI during isometric muscle activation have been well documented (Ridding et al., 1995; Reynolds & Ashby, 1999; Stinear & Byblow, 2003; Zoghi et al., 2003; Buccolieri et al., 2004; Zoghi & Nordstrom, 2007), with these changes reflecting modulation of GABA_A inhibition that is cortical in origin due to a subthreshold conditioning stimulus (Ziemann et al., 1996b; Di Lazzaro et al., 1998c). However, the current study is the first to investigate if SICI within contralateral cortex is also modulated during movement when the target muscle is changing length. In young subjects, we found reductions in SICI during movement that differed between contraction types, with greater disinhibition observed during shortening contractions. These observations suggest that although both shortening and lengthening contractions are associated with a reduction of GABA_A-mediated inhibitory tone within M1, a greater disinhibition of this circuit is apparent during shortening contractions. While movement-related changes in SICI have not been previously investigated in contralateral cortex, a recent study by Howatson et al. (2011) assessed changes in SICI within ipsilateral cortex during unilateral shortening and lengthening contractions of a wrist flexor muscle. While a contraction phase-dependent reduction in inhibition was also observed by this study, the greatest reductions in SICI occurred during muscle lengthening (Howatson et al., 2011). The findings of the current study therefore suggest that inhibitory modulation during movement differs between the active and inactive cortical hemispheres. However, as Howatson and colleagues used much greater levels of muscle activation (90% MVC as opposed to ~1.5% MVC in the current study), comparisons with the current study are limited.

Previous studies using EEG in young subjects have reported that movement related cortical potentials are greater during lengthening than shortening contractions (Fang et al., 2001, 2004). This would suggest that reductions in inhibitory tone may be greatest during muscle lengthening, making our findings for SICI counterintuitive. However, a more recent study
using functional magnetic resonance imaging (fMRI) has localised this increased cortical activity during lengthening contractions to higher order motor areas, such as the pre-supplementary motor area and anterior cingulate cortex (Kwon & Park, 2011). The same study reported that activity within the primary motor cortex (the area of focus in the current study) was actually increased during shortening contractions (Kwon & Park, 2011). Considering this finding, a greater disinhibition of SICI during shortening contractions may not be surprising.

In old subjects, a reduction in SICI was also observed during movement. However, in contrast to the young group, the magnitude of this modulation was not different between contraction phases. This lack of phase-specificity could suggest that old adults demonstrate a reduced ability (or need) to modulate GABA\(_A\)-mediated inhibition during movement. We have previously observed age-related reductions in the ability to modulate GABA\(_A\)-mediated inhibition during different motor tasks (Chapter 5), suggesting that old adults demonstrate a loss of specificity in the control of inhibitory neurotransmission. Furthermore, a reduced modulation of SICI in old adults has also been associated with age-related motor performance deficits (Heise et al., 2013). The absence of contraction phase-dependent changes in SICI in old adults may represent an extension of this reduced ability to modulate intracortical inhibitory tone. As previous research has shown that fractionated changes in SICI are an important component of fine motor function (Zoghi et al., 2003), it seems possible that this altered inhibitory modulation could contribute to motor performance deficits associated with ageing (see below).

### 6.5.2. LICI is modulated during lengthening contractions in old but not young subjects

Although this is the first study to investigate movement-related changes in the magnitude of LICI, several previous studies have assessed changes in the duration of the EMG SP, an
alternative assessment of GABA_B-mediated inhibition (Ziemann et al., 1996a), during anisometric contractions in young subjects (Sekiguchi et al., 2007; Duclay et al., 2011, 2014). While two of these reported reduced EMG SP duration during lengthening contractions (Duclay et al., 2011, 2014), the third reported reduced SP duration during shortening contractions (Sekiguchi et al., 2007). In support of Sekiguchi et al. (2007), the greatest reduction in SP duration within the current study was seen during shortening contractions, although this effect was reduced in old adults. As Duclay and colleagues targeted muscles of the lower leg, whereas Sekiguchi and colleagues and the current study targeted an intrinsic hand muscle, it seems possible that differences in target muscle contributed to these inconsistencies (Chen et al., 1998).

Both LICI and the EMG SP are thought to have contributions from GABA_B mediated inhibitory neurotransmission. However, this has been more clearly defined for LICI (Werhahn et al., 1999; Pierantozzi et al., 2004; McDonnell et al., 2006), with some evidence suggesting that the SP may reflect composite activity of both GABA_A and GABA_B receptors (Kimiskidis et al., 2006), as well as have contributions from brain areas ‘upstream’ from M1 (Tergau et al., 1999). The current studies use of LICI therefore provides a more precise assessment of changes in GABA_B mediated inhibition within M1 during shortening and lengthening contractions. In contrast to SP duration, our findings demonstrate that LICI in young subjects was unaffected by contraction type, suggesting that GABA_B mediated inhibition is not modulated within M1 during movement in healthy young subjects. This inconsistency between LICI and SP measurements is not unprecedented (McDonnell et al., 2006; Benwell et al., 2007), and demonstrates the complex relationship between these paradigms. However, as it has been previously suggested that the SP may reflect the duration of GABA_B-mediated inhibitory potentials within corticospinal neurons, whereas LICI may be an indication of the strength of these inhibitory potentials (McDonnell et al., 2006), an alternative interpretation
of our findings could be that while the strength of GABA_B-mediated inhibition (LICI findings) is not modulated by contraction type (at least for an ISI of 150 ms), its temporal characteristics (SP findings) may be. The functional implications of such an effect are not clear, but could relate to alterations in cortical oscillatory activity, with which GABA_B-mediated potentials are thought to be involved (Benarroch, 2012).

Relative to postural contractions, LICI in old adults was not different during shortening contractions, but was significantly reduced during lengthening contractions, suggesting that lengthening movements are associated with a disinhibition of intracortical GABA_B-mediated circuitry in old adults. Although movement-related variations in LICI have not been previously assessed in old subjects, a study by Fujiyama et al. (2012a) investigated age-related changes in SP duration during tasks requiring coordinated movements of the hand and foot. Although this study did not compare phase-dependent variations in inhibition, an age-related reduction in SP duration was observed during the most difficult movement task, with the worst performing subjects demonstrating the shortest SP durations (Fujiyama et al., 2012a). As lengthening contractions represent a more difficult task than shortening contractions (Yao et al., 2014), the greater reduction in LICI observed in old adults during lengthening movements may therefore relate to task complexity.

6.5.3. Old adults demonstrate greater cortical disinhibition during anisometric contractions

As expected, the modulation of inhibitory circuits differed between young and old subjects. However, while we expected a reduced modulation of inhibition with age, we found significantly greater reductions in SICI and LICI in old adults during both contraction types. As previous work has reported that old adults have a reduced ability to modulate inhibitory neurotransmission prior to contraction (Heise et al., 2013), as well as in response to different
motor tasks (Chapter 5), this finding is surprising. One factor that may have contributed to this effect is differences in pre-stimulus EMG between groups. For old adults, muscle activation was increased by ~6-7% MVC EMG across contraction types. However, as this difference in EMG was consistent between postural, shortening and lengthening contractions, whereas inhibition was only reduced in old adults during anisometric contractions, it seems unlikely that variations in EMG could be responsible for our observed age effects. Despite this, this increased modulation may suggest that age-related alterations in inhibitory tone during muscle activation depend on the nature of the contraction, with old adults adopting smaller changes in inhibition to perform isometric contractions (Heise et al., 2013; Chapter 5), but greater changes in inhibition to perform anisometric contractions.

6.5.4. Movement-related changes in inhibition appear unrelated to motor performance

Within the current study, absolute error and acceleration SD were both increased in old adults during performance of the anisometric task, suggesting that old subjects performed the task with reduced accuracy and steadiness. Furthermore, these movement deficits were significantly enhanced during lengthening contractions, supporting previous findings for the index finger (Burnett et al., 2000; Laidlaw et al., 2000), and showing that our subject cohort demonstrated the expected effects of age on fine motor control during movement. Despite this, linear regression analysis of individual subject data failed to demonstrate any significant associations between the indices of performance used in the present study (movement accuracy and steadiness) and measures of SICI, LICI and the EMG SP. Not only does this suggest that inhibitory modulation does not contribute to the accuracy and steadiness of anisometric contractions, but also that age-related changes in inhibitory modulation during movement are unlikely to account for age-related deficits in these specific features of movement. However, as the importance of intracortical inhibition in motor function has been well supported by previous literature (see above), it seems unlikely that the inhibitory
modulation we observed in both young and old adults does not have functional correlates. Rather, an important consideration for future research should be whether variations in inhibition during different contraction types relate to other aspects of movement that were unassessed in the current study, such as the temporal characteristics of contraction (Christou & Enoka, 2011).

In conclusion, GABA_A but not GABA_B mediated inhibition is differentially modulated during shortening and lengthening contractions in young subjects, supporting and extending previous suggestions that anisometric contraction phases involve separate neural control strategies. In contrast, this phase dependent modulation of GABA_A mediated inhibition was different in older adults, demonstrating reduced phase specificity, and an additional modulation of GABA_B mediated inhibition was also observed. However, we were unable to demonstrate causal interactions between age-related changes in the movement-related modulation of inhibitory tone and age-related deficits in fine motor output. The functional implications of these phase-dependent modulations of intracortical inhibition, in both young and old subjects, therefore require further investigation.
Chapter 7

General Discussion

An extensive, ever-growing body of literature demonstrates that inhibitory neurotransmission represents an important component of effective function within the CNS, and that changes in inhibition can result in significant functional deficit. Although the ability to investigate inhibitory processes in animal models has been available for some time, only in the last few decades has this been possible in awake and intact humans. Using TMS, there has been a rapid expansion in our understanding of inhibitory function, especially within the motor areas of the brain. Furthermore, this technique has allowed us to recognise situations in which changes to inhibitory processes, both pathological (for review, see; Berardelli et al., 2008) and non-pathological (Ridding et al., 1995; Reynolds & Ashby, 1999; Zoghi et al., 2003; Buccolieri et al., 2004; Zoghi & Nordstrom, 2007), may contribute to variations in CNS output. The ageing CNS is a good example of this, with many previous studies having investigated age-related changes in intracortical inhibition as a factor potentially contributing to the motor deficits commonly observed in old adults. However, despite relatively narrow lines of investigation, this literature has suffered from inconsistent findings, making it difficult to resolve the consequences of ageing on inhibitory function and subsequently, motor control. Within this thesis, I have attempted to further characterise age-related changes in inhibitory function by assessing intracortical inhibition in young and old adults under a number of different conditions. Specifically, age-related changes in the influence of corticospinal input/output properties, muscle activation, motor task, inhibitory interactions and movement were assessed. The influence of these factors on inhibitory neurotransmission in old adults is either not well understood, or has not been previously investigated. As it is possible to induce changes in inhibitory tone using NIBS (Di Lazzaro et al., 2002a; Huang et al., 2005; Nitsche et al., 2005), an understanding of these factors may provide a novel area of investigation for the development of therapies to manage the often debilitating motor deficits associated with the ageing process.
7.1. Corticospinal input/output properties and ageing

Both central and peripheral factors contribute to the non-linear output characteristics of the corticospinal system (Devanne et al., 1997), and these non-linearities affect the magnitude of inhibition recorded using paired-pulse TMS (Lackmy & Marchand-Pauvert, 2010). These observations suggest that comparing inhibition measurements between groups with different recruitment profiles, such as young and old adults (Pitcher et al., 2003), could produce misleading measurements of intracortical inhibition. In Chapters 2 and 3, I investigated the possibility that these age-related changes in corticospinal recruitment have contributed to previous inconsistencies in the effects of age on intracortical inhibition. This was achieved by assessing SICI and LICI in young and old adults using a number of test TMS intensities (producing test MEP amplitudes covering the range commonly used experimentally), and regrouping inhibition measurements according to both absolute (Chapter 3) and normalised (Chapters 2 and 3) test MEP amplitude.

The results of this study suggested that in both age groups, SICI in resting muscle and LICI in active muscle were unaffected by $M_{\text{max}}$ normalisation, whereas SICI in active muscle and LICI in resting muscle varied depending on test MEP amplitude (absolute and normalised). However, the nature of these effects differed between age groups for measurements in active but not resting muscle. Despite this, the majority of previous studies investigating age-related changes in inhibition have targeted resting muscle. While these results therefore provide good preliminary evidence that variations in motoneuron recruitment are unlikely to explain the inconsistencies within previously reported effects of age on inhibition, further investigation is required to confirm this suggestion. In particular, an assessment of age-related changes in the recruitment of individual components of the corticospinal descending volley is required. As the inhibitory interneurons responsible for the generation of SICI and LICI specifically target the late I-waves (Di Lazzaro et al., 1998c; Hanajima et al., 1998; Di Lazzaro et al., 2002b),
alterations to the recruitment order, or response characteristics of individual waves, could significantly confound comparisons of inhibition between groups. The use of single motor unit recordings to analyse the individual components of the descending volley would provide an effective means of pursuing this important area of investigation (Hanajima et al., 1998). The results of such a study would also provide valuable information for research investigating age-related changes in neuroplasticity, as the techniques used to induce neuroplastic change also target specific components of the descending volley (Di Lazzaro et al., 2010).

Although the findings in Chapters 2 and 3 suggest a limited confounding influence of motoneuron recruitment on the existing literature, it seems that this factor should be considered during the design of future experiments incorporating muscle activation. Ideally, the influence of spinal recruitment on TMS measures could be negated by the use of more direct outcome measures, such as those provided by combined TMS-EEG protocols. However, while a growing body of evidence suggests this technique can demonstrate GABAergic inhibitory processes resembling those recorded during conventional paired-pulse TMS (particularly for LICI), our understanding of the EEG potentials induced by TMS is still limited (Rogasch & Fitzgerald, 2013). Despite this, as age-related changes in the response to TMS have not been previously investigated using TMS-EEG, this would be an interesting topic for future research.

7.2. Inhibitory interactions, motor task and ageing

In addition to the influence of differences in corticospinal recruitment on comparisons of inhibition, one of the most notable effects observed in Chapter 3 was an age-related modulation of LICI, suggesting changes in post-synaptic $\text{GABA}_B$-mediated inhibitory tone. The nature of this inhibitory modulation differed between activity states, with measurements in resting muscle demonstrating a large and consistent reduction in inhibition across test intensities, whereas an increase in inhibition was observed at lower test intensities in active
Chapter 7

General Discussion

muscle. As contradictory effects of age on LICI in both resting and active muscle have been previously reported (McGinley et al., 2010), this result was surprising. However, in conjunction with age-related reductions in SP duration (Sale & Semmler, 2005; Oliviero et al., 2006), these findings suggested that GABA_B mediated inhibition is particularly sensitive to the ageing process. Subsequently, Chapters 4 and 5 further assessed the effects of age on GABA_B-mediated inhibition by investigating if measurements thought to reflect the effects of pre-synaptic GABA_B receptors are altered in old adults. This was accomplished by comparing the interaction between LICI and SICI in young and old subjects (Sanger et al., 2001).

The findings of Chapter 5 again showed that LICI was reduced in old adults, corroborating our previous findings (Chapter 3). Furthermore, the strength of the interaction between LICI and SICI was also reduced in old adults, demonstrating that the age-related modulation of GABA_B-mediated inhibition includes the activity of both pre- and post-synaptic receptors. Although an effect of age on pre-synaptic inhibition has been indirectly suggested by a previous study (Chu et al., 2008), our findings are the first to demonstrate this effect. However, for both LICI and LICI-SICI interactions, effects of age differed between ISI’s, with post-synaptic inhibition reduced at 150 ms, but pre-synaptic inhibition reduced at 100 ms. The reasons for this discrepancy are currently unclear and will require further investigation. Despite this, these effects of age may suggest that the 100 ms ISI provides a more sensitive assessment of changes in pre-synaptic inhibition, whereas the 150 ms ISI is more sensitive to changes in post-synaptic inhibition. Interestingly, this corresponds with the temporal characteristics of pre- and post-synaptic GABA_B receptors, with intracellular recordings having shown that, after receptor activation, peak activity levels occur at 100 ms (Davies et al., 1990) and 150 ms (Deisz, 1999), respectively. Although this requires further investigation, it may be relevant to future studies attempting to characterise changes in GABA_B-mediated neurotransmission using TMS.
In addition to measurements in resting muscle, the experiments described in Chapters 4 and 5 also assessed SICI, LICI and LICI-SICI interactions during a simple (isolated index finger abduction) and more complex (precision grip) motor task. The motivation for these studies stemmed from observations that task-related changes in inhibition are known to be altered with age (Sale & Semmler, 2005) and, based on interactions between inhibitory circuits observed by previous work (Rosenkranz & Rothwell, 2003, 2004), we hypothesised that the reductions in LICI observed in Chapter 3 may contribute to this altered inhibitory modulation through pre-synaptic mechanisms. In keeping with previous findings (Sale & Semmler, 2005), the modulation of SICI was reduced in old adults during the more complex task. However, in contrast, changes in the strength of both LICI and the interaction between LICI and SICI were smallest during the less complex task (although these effects were observed at different ISI’s). Perhaps unsurprisingly, these findings portray complex effects of age on the task-dependent modulation of inhibition. Despite this, the one consistency amongst these observations was that effects of age on inhibitory modulation always resulted in a loss of modulatory capacity.

These findings provide good support for previous observations of reduced inhibitory modulation in old adults prior to contraction (Heise et al., 2013; Heise et al., 2014) and during the performance of different motor tasks (Sale & Semmler, 2005). However, despite some evidence suggesting that this reduced inhibitory modulation correlates with performance outcomes (Heise et al., 2013), our understanding of how these changes relate to altered motor behaviour in the elderly is limited. One way in which we could improve our understanding of this relationship is by manipulating inhibitory tone in young and old adults, and assessing the effects on motor function. This was recently done by Heise et al. (2014), who assessed how changing SICI using anodal transcranial direct current stimulation (anodal tDCS) affected motor performance in young and old adults. While this study reported changes in both inhibition and performance in old adults, these were not correlated (Heise et al., 2014),
suggesting the involvement of additional factors. Despite this, this area of investigation warrants further research. Future work should consider modulating inhibitory tone using interventions that are more specific to M1, such as theta burst stimulation or paired associative stimulation. These may induce changes in inhibition that are more relevant to motor function, potentially providing a better relationship with performance measures. Furthermore, as the specific aspects of motor function that are subject to changes in intracortical inhibition are not well understood, other performance indices should be correlated with changes in inhibitory tone. In addition, an investigation of the influence of other inhibitory mediators such as GABA_B is also required.

As suggested above, the results of Chapter 5 demonstrated that effects of age and task on LICI and LICI-SICI interactions were influenced by the ISI. These observations support several previous studies suggesting that measurements of LICI using different ISI’s may have contributions from non-identical circuits (Chu et al., 2008; Koch et al., 2008; Vallence et al., 2012; Vallence et al., 2014). However, an alternative explanation for these effects is that the ageing process may cause changes to the temporal characteristics of inhibitory neurotransmission. For example, in old subjects, the time required for neuronal activity generated by the conditioning stimulus to modify the excitability of the corticospinal neurons activated by the test stimulus may be different to that required by young subjects. Irrespective of the specific mechanism, the findings of Chapter 5 suggest that investigations of age-related changes in LICI and LICI-SICI interactions should include multiple ISI’s. In addition, although previous work in resting muscle has suggested that age-related changes in SICI are not influenced by the ISI used to assess inhibition (Peinemann et al., 2001; Degardin et al., 2011), it is not known if age-related changes in SICI during muscle activation, or during the performance of different motor tasks, are similarly unaffected by the ISI. Further investigation into how the ageing process affects the temporal characteristics of inhibitory
neurotransmission should therefore be considered, especially relating to the task-dependent effects of age.

One limitation of Chapters 3 and 5 is that inhibitory function was only assessed using a single conditioning stimulus intensity. Subsequently, we were unable to assess if differences in the recruitment of intracortical inhibition contributed to our observed effects of age. Previous work suggests that the ageing process does not change the recruitment of SICI (Smith et al., 2009). However, age-related changes in the recruitment of LICI, as well as in the response of LICI-SICI interactions to variations in conditioning stimulus intensity, have not been investigated. Therefore, an important extension of Chapter 5 will be to compare age-related changes in the recruitment of both pre- and post-synaptic GABA<sub>B</sub>-mediated inhibition by investigating the response of LICI and LICI-SICI interactions to a range of conditioning stimulus intensities. However, even if such an investigation were to reveal altered recruitment profiles, changes in the gain of inhibitory recruitment still represent an important modification of inhibitory neurotransmission that may contribute to age-related functional deficiencies (Rosenkranz et al., 2007).

7.3. Movement and ageing

Although the findings reported in Chapters 3 and 5 demonstrated that GABAergic processes in active muscle are affected by age, these results are limited to isometric muscle activation. As fine motor control is known to be reduced in old adults during movement (Burnett et al., 2000; Graves et al., 2000; Laidlaw et al., 2000; Christou & Carlton, 2002a; Kornatz et al., 2005), investigating if age-related changes in inhibitory tone extended to anisometric contractions seemed a logical extension to the previous studies. Chapter 6 therefore assessed SICI and LICI during shortening and lengthening contractions of the index finger. The results of this study suggested that while young adults showed contraction-dependent (i.e., shortening vs lengthening) disinhibition of SICI, LICI was unaffected. However, old adults showed a
greater contraction-dependent reduction in SICI, as well as reduced LICI during lengthening contractions. For the first time, these observations demonstrate altered GABAergic tone in old adults during movement. Unfortunately, as inhibition failed to correlate with performance measures in both age-groups, more investigation is required. One factor that may have contributed to this lack of correlation is the way in which performance was assessed. Although the performance indices used within Chapter 6 are known to be sensitive to age-related movement deficits (Burnett *et al.*, 2000; Kornatz *et al.*, 2005), the degree to which they reflect inhibitory input is not known. Furthermore, the time frames over which changes in inhibition result in altered motor output during movement are also not known (i.e., at which point should performance be correlated with measurements of inhibition). Therefore, before we can understand the functional implications of this altered movement-related modulation of inhibitory tone in old adults, we need to develop a greater understanding of how intracortical inhibition contributes to motor performance during movement.

In addition to age-related changes in the contraction-dependent modulation of intracortical inhibition, Chapter 6 also found that the disinhibition of both SICI and LICI was greater in old adults irrespective of movement type. In contrast to previous studies (Heise *et al.*, 2013; Heise *et al.*, 2014), this observation suggests an increased modulation of inhibitory tone in old adults. It seems possible that this effect could have both positive and negative functional implications. While a greater disinhibition of motor cortex could allow greater activation of task-related muscles, it could also allow a more non-specific (less graduated) pattern of muscle activation, subsequently reducing the ability for fine motor adjustments. However, the ramifications of this increased cortical disinhibition require further investigation as they also failed to correlate with indices of motor performance.

Deficits in motor performance during movement, especially during lengthening contractions, not only represent a significant functional impediment in the elderly, but also a source of
potential injury (Carville et al., 2007). While the findings of Chapter 6 failed to demonstrate a causal role of intracortical inhibitory circuits in this impediment, this requires significantly more investigation (see above). However, an assessment of the potential role of other cortical circuits thought to be involved with motor control (i.e., intracortical excitatory and interhemispheric inhibitory circuits) seems like an obvious extension to the findings of Chapter 6. Furthermore, as imaging studies have suggested that old adults show contraction-dependent variations in the activity of higher order motor areas (Yao et al., 2014), it would be interesting to assess the contribution of non-primary motor areas to these age-related movement deficits. Several techniques could be used to accomplish such aims, including combined TMS-EEG (Rogasch & Fitzgerald, 2013), or an analysis of corticomuscular coherence (Mima & Hallett, 1999; Liv Hansen & Bo Nielsen, 2004).

During movement, muscles spindles play an important role in providing the nervous system with information about muscle length (Houk & Rymer, 1981). During the lengthening phases of movement, tension within these intrafusal fibres is increased, resulting in increased activity within Ia afferent fibres (Hulliger et al., 1985) and the generation of greater afferent input to motor cortex, subsequently signalling an increase in muscle length. As previous studies have observed that afferent signals can modulate inhibitory tone (Ridding & Rothwell, 1999; Rosenkranz & Rothwell, 2003; Ridding et al., 2005), it could be suggested that variations in afferent activity between contraction phases contributed to the findings of Chapter 6. For SICI in young subjects, this mechanism may have contributed to some of the cortical disinhibition observed during lengthening contractions. However, as the greatest reduction in inhibition was observed during muscle shortening (when the activity of muscle spindles is reduced) it cannot explain the task dependent changes in SICI. Furthermore, as there is evidence for a reduced afferent modulation of intracortical inhibition in old adults (Smith et al., 2011), it seems unlikely that this mechanism can explain any of our findings in the old group.
However, as Chapter 6 did not assess afferent activity, an influence of this factor on the observed changes in inhibitory tone cannot be excluded. Future studies should therefore investigate a potential role of afferent signals in the movement-related modulation of intracortical inhibition.

7.4. Concluding remarks

In this thesis I have provided evidence of age-related changes in several aspects of inhibitory neurotransmission, including reductions in pre- and post-synaptic GABA$_B$-mediated inhibition, changes in the task-dependent modulation of both GABA$_A$- and GABA$_B$-mediated inhibition and variations in inhibitory tone during different phases of movement. Although this has contributed many new findings to the area, the behavioural implications of these alterations to inhibitory function remain unclear. Understanding the impact of these changes on motor function in the elderly therefore needs to be a primary aim within this field of research. Such an understanding could facilitate the development of interventions designed to modulate inhibitory tone, potentially leading to useful clinical outcomes in subject populations known to demonstrate altered inhibitory function, such as Parkinson’s disease (Bareš et al., 2003). Non-invasive brain stimulation techniques provide a unique avenue through which this could be achieved, potentially representing a means of treatment having almost no tangible side-effects. As the average age within the general population continues to increase, the need for such interventions will only increase; as to will the relevance of these interventions to every individual.
8. Appendices

8.1. Appendix I: Publications arising from thesis


8.2. Appendix II: Presentations and abstracts arising from thesis

Title: The estimation of intracortical inhibition is affected by spinal motor neuron activation 2012
5th ACNR Frontier Technologies in Nervous System Function and Repair Workshop, Adelaide, Australia

Title: Modulation of SICI and LICI with increasing motor neuron activation in young and old adults 2013
1st Australasian Brain Stimulation Conference, Melbourne, Australia

Title: Age-related changes in intracortical inhibition within primary motor cortex 2013
Physiology Seminar Series, School of Medical Sciences, University of Adelaide, Adelaide, Australia

Title: Task-related changes in pre- and post-synaptic intracortical inhibition 2014
34th Australasian Neuroscience Meeting, Adelaide, Australia

Title: Age-related changes in intracortical inhibition in resting and active muscle assessed with paired- and triple-pulse transcranial magnetic stimulation 2014
44th Society for Neuroscience Meeting, Washington D.C, America

Title: Age-related changes in pre- & post-synaptic intracortical inhibition in a human hand muscle 2014
34th Australasian Neuroscience Society, Sensorimotor Satellite Meeting, Adelaide, Australia

Title: Inhibitory mechanisms contributing to altered motor function in old adults 2014
Berenson-Allen Centre for Non-invasive Brain Stimulation, Harvard Medical School, Boston, America
Title: Modulation of intracortical inhibition during shortening and lengthening contractions of a hand muscle in young and old adults

1st International Brain Stimulation Meeting, Singapore, Malaysia
Chapter 9

9. Bibliography


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MEGA-PRESS with macromolecule suppression study. *Mol Psychiatry*, DOI: 10.1038/mp.2015.34.


