# Surround inhibition in the human motor system

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**DECLARATION**

I, Panagiotis Kassavetis confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

Signature Date

1st February 2015

**ABSTRACT**

Human dexterity is unique within the animal kingdom. The human hand, the final product of long evolutionary process is the most fascinating and refined motor systems in nature. This thesis approaches the neural control of finger movements through the scope of surround inhibition, a neural process well described in the sensory system and recently associated with the motor system. Individuation of finger movements was explored by means of electromyography (EMG) and transcranial magnetic stimulation (TMS) during a brief flexion of the index finger. A thorough description of the motor evoked potentials and EMG activity in three intrinsic hand muscles is provided initially (Chapter 4). The role of cerebellum as a modulator of moto-cortical output was explored during the same movement and was found to modulate the motor output in a non- muscle specific manner (Chapter 5). In Chapter 6, brain plasticity, a fundamental neural process was probed by means of peripheral nerve stimulation with electrical and mechanical tools in a successful attempt to modulate the strength of surround inhibition in the motor cortex. Finally, data from patients suffering from dystonia is presented and compared with previously published literature (Chapter 7). Lack of significant differences between the dystonia and healthy groups raised questions about the credibility of the proposal that dystonia is disease model for loss of inhibition in the motor system. The thesis calls for a reappraisal of our approach to the role of SI in the motor system and in particular in the pathophysiology of movement disorders such as dystonia.

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I would like to thank my friends and colleagues who contributed significantly to the work presented in this thesis (in alphabetic order): Daniele Belvisi, Britt Sofie Hoffland, Maja Kojovic, Isabel Pareés, Anna Sadnicka, Tabish A Saifee and Medhi Van Den Bos.

## STATEMENT OF PARTICIPATION IN STUDIES DESCRIBED

The main concepts for the thesis were generated by the principal PhD supervisor Dr Mark J Edwards and further developed to experimental protocols by myself. I completed ethics and R&D application with the assistance and guidance of Dr Mark J Edwards. Subject recruitment, data collection, data analysis and data presentation was conducted exclusively by me in all studies except for the study described in Chapter 6.2 for which Dr Daniele Belvisi contributed significantly and the study in Chapter 5.2 for which I received the valuable assistance of Dr Anna Sadnicka. Dr Mark J Edwards was involved in all stages of all projects described in the thesis. Dr John Rothwell played a key role in critical appraisal of all the studies and he provided essential input especially for the study design, data analysis and interpretation. A number of co-authors contributed significantly in the production of individually published papers originated from the studies described in this thesis.

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| | |
|---|---|
| ADM | Abductor Digiti Minimi |
| AMT | Active Motor Threshold |
| ANOVA | Analysis Of Variance |
| APB | Abductor Pollicis Brevis |
| CBI | Cerebellar Brain Inhibition |
| CD | Cervical Dystonia |
| EMG | Electromyography |
| FCR | Flexor Carpi Radialis |
| FDI | First Dorsal Interosseous |
| FHD | Focal Hand Dystonia |
| GABA | γ-Aminobutyric Acid |
| LICI | Long Interval Intracortical Inhibition |
| M1 | Primary Motor Cortex |
| MD | Musician's Dystonia |
| MEP | Motor Evoked Potential |
| MSO | Maximum Stimulation Output |
| PAS | Paired Associative Stimulation |
| PMd | Dorsal Premotor Cortex |
| PMv | Ventral Premotor Cortex |
| RMT | Resting Motor Threshold |
| SAI | Short Afferent Inhibition |
| SEM | Standard Error of the Mean |
| SI | Surround Inhibition |

| | |
|---|---|
| SICI | Short Interval Intracortical Inhibition |
| SP | Cortical Silent Period |
| tDCS | Transcranial Direct Current Stimulation |
| TMS | Transcranial Magnetic Stimulation |
| WC | Writer’s Cramp |

# Chapter 1. INTRODUCTION

## 1.1. **Motor control of fingers in healthy humans**

Humans' ability to use their hands and fingers is extraordinary and it truly sets them apart from other species in the animal kingdom including other primates. Although the peripheral neuromuscular apparatus of human hand does not significantly differ from other primates (including the opposable thumb) the precision and smoothness of human movements is unique. At the early stages of life when the nervous system is not fully developed the only skilful movement that both humans and other primates are able to do is grasping [1]. The rest of the movements are broken and appear random, resembling movement disorders which manifest when the nervous system suffers from disease. However in adult life when the nervous system is fully developed, human hand movements become more precise and efficient. The ability to generate movements of such quality is called 'dexterity', which although is intuitively easy to understand, it is hard to be described in scientific terms because of its complexity and various dimensions (learning, coordination, problem solving, tool use etc.) which are poorly understood at a neuroscientific level. This thesis is focused on one of the dimensions of dexterity, finger individuation.

The ability to individuate finger movements increases along the phylogenetic scale. In those reptiles and amphibians which have fingers their use for grasping is non-existent

or minimal. In contrast, mammals with fingers, like rats and cats use their forepaws to hold food but cannot use individual fingers and only rarely grasp objects with one forepaw [2, 3]. Primates other than humans can pinch small objects between the tip of the thumb and the side of the index but they do not show the sophisticated individual finger movements observed in humans [2, 4]. From an evolutionary perspective, finger individuation has been greatly facilitated by the bipedal locomotion which freed the hands from carrying body weight and lead its evolution towards more dexterous tasks [5]. The human hand as the final product of this long evolutionary process is undoubtedly one of the most fascinating and refined motor systems in nature.

Individuation of fingers increases the degrees of freedom of hand movements and therefore increases the range of activities that can be performed but it also increases the computational capacity necessary to accurately control them. Given the natural statistics of human finger movements in daily living where only a few dominant patterned movements are used it would be great waste of energy for the system to equally represent and capacitate all possible finger movement combinations. For this reason the existence of constraints in the biomechanical but mainly in the neuronal level facilitates formation of movement patterns and makes finger movement modular rather than completely independent [6, 7]. This effect is also described as muscle enslavement and is more prominent in high levels of neural activity for generation of maximal or submaximal forces [8, 9] . It is well known that muscle enslavement does not represent only biomechanical constrains in the level of tendons and muscles but it is also related to the modular neuronal control of fingers. [10, 11].

Motor cortex plays a significant role in the generation of neuronal signals that control the intrinsic and extrinsic hand muscles and consequently generate hand and finger movements. Although the neuroarchitectonic structure of motor cortex is well described, our understanding of how this architecture is relevant in generating individual finger movement for optimal motor control is limited. In particular, the divergence of neuronal signal from single motocortical neurons to multiple muscles and the convergence of signal from multiple neurons to single muscles, in combination with the presence of horizontal inhibitory and facilitatory interconnections, indicate that a simple topographic organisation/representation of finger movements in the cortical level is not adequate to explain the degree of individuation of finger movements.[12-17].

The concept of suppression of unwanted movements in adjacent fingers by inhibitory horizontal intracortical connections resembles the well described concept of surround or lateral inhibition (SI) in the sensory system. Before discussing how SI can be relevant for the study of motor control of individual fingers a quick overview of SI in the sensory system is necessary.

### 1.2. **Surround (or lateral) inhibition**

Surround inhibition (SI) was firstly described in the retinal cells of Limulus polyphemus, (commonly called the "horseshoe crab") by Keffer Hartline who was

awarded the Nobel Prize in Physiology in 1967 for this work. Since then it has been described in several sensory systems including visual, somatosensory, auditory and olfactory [18-21].

### 1.2.1. In the sensory system

SI has been hypothesised to serve several functions in the pre-processing of visual stimuli. The most prominent proposition was that SI plays the role of a filter for enhancing the edges of the retinal image before being processed by higher areas in the visual system [22, 23]. In addition SI increases efficiency of neuronal encoding of information by removing redundancy from the visual inputs [24]. In other words, SI cancels out a constant bias of the signal (DC offset) in order to maintain the neuronal signal distribution within the dynamic range of the receptive neurons[20, 24]. This was an interesting concept and although it was originally qualitative it triggered the generation of quantitative predictive coding models. These models take into account the intrinsic noise within the nervous system, a limiting factor for the amount of information that can be encoded by a single neuron with a given dynamic range. In addition these models use the input values in a particular spatial region to generate statistical estimates for sensory inputs in adjacent regions by using the natural statistics of environment (e.g. spatial correlations of images) [20]. As an extension to predictive coding, dynamic predictive coding models have been developed to incorporate dynamic adjustments of spatio-temporal receptive fields during changes of visual scenes [25].

These dynamic adjustments are mediated in the neuronal level through plastic changes in the synaptic level of retinal cells[25].

SI has been most extensively studied in the visual system but has also been described in the auditory, somatosensory and olfactory systems. Furthermore the concept of lateral inhibition as a normalisation process has been used for computational modelling of higher functions such as attention[26] and value based decision making [27]

### 1.2.2. In the motor system

More recently an electrophysiological phenomenon has been proposed to reflect the presence of SI in the motor system[28]. Motor SI has been probed with transcranial magnetic stimulation (TMS) applied over the primary motor cortex at the onset of an isolated voluntary finger movement. It was found that at the onset of movement the corticospinal excitability in muscles which are adjacent to the active muscles but irrelevant to the task was reduced when compared to resting baseline excitability. This reduction of corticospinal excitability in the surround muscles was proposed to reflect the presence of active inhibitory processes within the motor system as a neural correlate of SI in the sensory system [29]. However at this point, a direct link of this phenomenon with sensory SI was only hypothetical. Furthermore it was initially unknown if this is truly an inhibitory process or it reflected different processes like withdrawal of facilitation. A number of follow up neurophysiological studies attempted to answer

these questions and link this phenomenon to known intracortical inhibitory networks. The first candidate was short intracortical inhibition (SICI) as a potential inhibitory network driving SI [28, 30]. SICI was tested in the surround muscles at the onset of an isolated finger movement but results were contradictory. It was found that SICI did not follow a similar pattern of muscle-specific modulation [28, 30]. However, other investigators had used a similar paradigm and reported muscle specific modulation of SICI at the onset of a finger movement [31]. The relationship of SICI and SI is still under question which remains to be answered.

Other intracortical networks such as long intracortical inhibition (LICI), short afferent inhibition (SAI), silent period (SP), ventral premotor cortex (PMv) and dorsal premotor cortex (PMd) connectivity to primary motor cortex have been investigated but no definitive relationship of SI to any of those networks has been established [32-36].

In parallel to the studies which attempted to investigate the relationship of SI with other intracortical networks, another series of studies have investigated different aspects of this phenomenon. In particular its temporal profile was examined in relation to the onset of the EMG activity in the active muscle and was found that suppression initiates approximately 100ms before onset of the EMG activity and continues until it fades out around 100ms after the onset (regardless of whether the contraction of the active muscle continues or stops)[28, 30, 37]. The temporal profile of SI depends on the intended magnitude of force of the active muscle and the -100ms,+100ms temporal profile is

present only when the intended force of the active muscle is 10% of the maximum voluntary force (MVF). When the intended force is higher (20% or 40% MVF) or lower (5%MVF) SI was found to have a different temporal profile[38]. It was also found to have bigger effect in the dominant hemisphere compared to the non-dominant probably reflecting the unbalanced motor control of the two hands [39]. Task difficulty was also found to significantly influence the temporal profile of SI with earlier onset of the suppressive effect in a choice reaction paradigm compared to simple reaction time paradigm where the suppressive effect started later and it was overall weaker [37].

The above studies were important in the characterisation of SI as a neuronal process in healthy volunteers. Although there is no experimental evidence to prove a direct link between SI and motor dexterity, it has been hypothesised that SI is essential in shaping motor commands during fine voluntary actions that require dexterity. This hypothesis has been derived from two lines or argument. Firstly, an indirect link between SI and dexterity was assumed as both the current concept of motor SI and the notion of dexterity incorporate the idea of shaping or focussing motocortical output as an essential factor for optimal movement generation. Secondly, patients who suffer from diseases that cause abnormal manual dexterity have been found to have impaired motor SI [40]. The hypothetical link between SI and dexterity provides an opportunity to use TMS to access the neuronal signals that potentially drive dexterity and gain insight to its underlying mechanisms. In particular, the study of SI is focused on the down-regulation of excitability in neuronal pathways that control muscles adjacent to the active muscles but not involved in the executed movement. Break down of motor SI

could potentially cause excessive movement in those muscles leading to impaired dexterity. This thesis focuses on motor SI as a way to understand impaired dexterity in patients with movement disorders, in particular focal hand dystonia.

## 1.3. **Focal hand dystonia**

Dystonia is a neurological condition where involuntary muscle spasms lead to abnormal postures of the affected body part.

### 1.3.1. Clinical features

The clinical presentation of dystonia is variable and there is great heterogeneity in distribution of the symptoms, in the age of onset and the aetiology. Adult onset task-specific focal hand dystonia is a particular type of dystonia that manifests with involuntary spasms of the hand during performance of specific tasks, for example during writing (writer's cramp - WC), or when playing a musical instrument (musician's dystonia - MD). For those affected with WC or MD the symptoms can be very disabling and some affected patients(particularly professional musician's) may have to terminate their careers. The available treatment options for focal hand dystonia are currently very limited [41-44].

### 1.3.2. Pathophysiology

Our current understanding on the pathophysiology of focal hand dystonia has in part been derived from electrophysiological studies exploring the sensorimotor system in affected patients. Loss of inhibition in the central nervous system was early identified as a neural deficit contributing significantly in the expression of dystonic symptoms [45]. Specific neural networks in the sensory [46-50] and motor system [30, 51-55] have been found to be abnormal in dystonia.

In addition to loss of inhibition, the plasticity response to several neurophysiological protocols was also found to be abnormal in dystonia. These studies have suggested that the cellular mechanisms responsible for regulation of plasticity responses to stimuli fail in patients with dystonia [56, 57]. Failure of regulation of plasticity lead to generation of abnormal sensorimotor associations which effectively manifest as abnormal motor control and dystonic spasms [58]. The hypothesis of abnormal plasticity in dystonia has dominated the literature but its reproducibility has recently been questioned [59] .

### 1.3.3. Surround inhibition in focal hand dystonia

Motor surround inhibition (SI) has been explored in focal hand dystonia where it was found to be abnormal [29, 30, 38, 40, 45]. Impaired SI in FHD is indeed an intriguing concept which provides a theoretical framework to explain abnormal overflow of muscle activity into muscles not involved in the desired movement and it can also explain lack of dystonic symptoms at rest. However there is still lack of understanding regarding the actual mechanism how impaired surround inhibition relates to manifestation of dystonic symptoms. This thesis is essentially an attempt to provide evidence on the mechanisms of generation of SI in the motor system and on the link of SI with finger movements in health and focal dystonia.

# Chapter 2. SCOPE OF DISSERTATION AND HYPOTHESES

The scope of this dissertation is to investigate individuation of finger movements in healthy volunteers and patients with focal hand dystonia by means of non-invasive brain stimulation and other electrophysiology methods. We approached this matter in 4 different ways.

## 2.1. **EMG activity during finger movements**

Basic understanding of the recruitment of active and non-active intrinsic hand muscles was the first step to understand how the different parameters of finger movement (cortical excitability, electromyographic (EMG) activity, force) are linked together. It is often proposed that SI is essential for successful generation of isolated finger movements[29] but no direct experimental evidence has been reported.

With this study we attempted to provide essential evidence on the basic electrophysiological characteristics of SI. We investigated the relationship between SI and muscle activation in the hand in a large cohort of normal volunteers at the onset of an isolated finger movement. We hypothesised that EMG activity will be modulated in a muscle specific pattern similar to the pattern that MEPs are modulated at the onset of an individual finger movement. We provide a complete description of the profile of SI

by providing descriptive data of corticospinal excitability and comparisons with EMG activity. Such comparisons are an essential first step in exploring the proposal that SI is a mechanism for the reduction/inhibition of muscle activity in surround muscles.

## 2.2. **The role of cerebellum in motor surround inhibition**

It is currently not known which structures within the central nervous are important for the generation of SI. Some favour a neocortical mechanism following the observation that hemispheric dominance and task difficulty modulate the magnitude of SI [37, 39] .

The cerebellum plays a major role in temporal encoding and coordination of movements and deficiencies in hand control and individual finger movements are seen in patients with cerebellar disease [60]. It also has a net inhibitory effect on the cerebral cortex via the cerebello-dentato-thalamo-cortical pathway [60] . These characteristics make the cerebellum a suitable candidate that may functionally contribute to the generation of SI in the motor system.

We used two different types of cerebellar stimulation to assess its role in the generation of SI. Firstly, single pulse TMS was employed to assess phasic modulation of excitability of the dentato-thalamo- cortical pathway during individual finger movements. We hypothesised that excitability would be modulated in a muscle specific

fashion if the driving force of SI was originated in the cerebellar cortex. Secondly we used tDCS to test how a global reduction of cerebellar output may reflect on modulation of SI. We hypothesised that reduced cerebellar control over the motor cortex would lead to break down of finger coordination and consequently to less strong surround inhibition.

### 2.3. **Brain plasticity and motor surround inhibition**

As discussed above previous studies have provided evidence that patients with focal hand dystonia (FHD) have impaired SI. We attempted to increase the strength of SI in normal volunteers with the hope to use the same paradigms in patients with FHD. We attempted to change SI in two ways, firstly by introducing sensory noise during repetitive finger movements and secondly by inducing plastic changes in the motor cortex with non-invasive brain stimulation.

In the first approach we used muscle vibration to stimulate the muscle spindles of a surround muscle during movement of a different active muscle. We hypothesised that sensory feedback indicating unwanted contraction of surround muscles will induce adaptive changes in the strength of SI. Importantly, in order to achieve causal inference of the spindle stimulation and the movement, we accurately matched the timings of the movement and the vibration.

In the second approach we used the paired associative stimulation (PAS) protocol to enhance corticospinal excitability in a single muscle of interest (active muscle) expecting that this will increase the excitability imbalance between the active and non-active muscles. We hypothesised that this imbalance will be reflected in SI measurements after the PAS protocol.

### 2.4. **Motor surround inhibition in dystonia**

Following initial reports where SI was found to be abnormal in patients suffering from dystonia [28] several studies have compared SI between patients with hand dystonia and healthy controls. However, 10 years later there is still uncertainty about the way that SI relates to the pathophysiology and clinical manifestation of dystonia. In the final approach to SI in this dissertation, we assessed SI in a group of patients with FHD and focal cervical dystonia (CD). We hypothesised that SI is abnormal in patients with FHD and normal in patients with CD. We put this data in perspective with the rest of the published literature. We summarise the current evidence on SI and we go one step further by critically appraising the significance of existing patients' data for designing future studies.

## 2.5. **Summary of hypotheses**

- That EMG activity in the intrinsic hand muscles is modulated in a muscle specific pattern similar to the pattern that MEPs are modulated at the onset of an individual finger movement

- That excitability of the dentato-thalamo- cortical pathway is modulated in a muscle specific fashion at the onset of an individual finger

- That decreased cerebellar inhibitory output will lead to breakdown of finger coordination and consequently to less strong surround inhibition.

- That sensory feedback indicating unwanted contraction in surround muscles will induce adaptive changes in the strength of SI.

- That artificially induced imbalance between the excitability of the active and non-active muscles will be reflected in SI measurements at the onset of a voluntary finger movement.

- That SI is abnormal in patients with FHD and normal in patients with CD.

# Chapter 3. GENERAL METHODS

## 3.1. **Electromyography**

Electromyography is a technique used for assessment of muscle activity by measurement of electric fields generated in the muscles during contraction. Different types of EMG can be used for evaluation of several different aspects of muscle activity. For the purposes of the majority of the experiments described in this dissertation, we used bi-polar surface EMG. Two Ag-AgCl electrodes where placed over the surface of the skin on three intrinsic hand muscles (FDI, APB, ADM) and one ground electrode over the wrist. A belly-tendon montage was used, with one electrode over the centre of the belly of the muscle and the other electrode over the tendon of the muscle. Appropriate preparation of the skin with exfoliating agents and use of high conductance gel ensured impedance of less than 5kΩ between the electrodes and the skin. The electrodes where connected to an amplifier with gain of 1000 and analog to digital converter (ADC) with sampling frequency of 5KHz and band-pass filter of 20-2000Hz . All recordings were stored in a computer and analysed off-line. The same setup was used for assessment of motor evoked potentials (MEPs) after delivery of TMS pulses. The details of the setup for each study are described in the methods sections

## 3.2. **Transcranial magnetic stimulation**

Transcranial magnetic stimulation (TMS) is a non-invasive technique for stimulating the surface of the brain of awake and conscious humans. It has been used for almost 40 years since it was firstly introduced in 1985 by Anthony Barker and his colleagues in Sheffield, UK. A coil is held over the scalp of the subject and a rapidly changing magnetic field induces weak electric currents over the surface of the brain causing depolarisation of cortical neurons[61].

Several different areas of the cortex can be approached and be stimulated with different shaped coils. For the purposes of the studies in this thesis we used single TMS pulses delivered with a figure-of-eight shaped coil (external loop diameter of 9 cm) over the motor cortex and with a double-cone coil (110mm mean diameter) over the right cerebellar hemisphere. A monophasic Magstim 200 stimulator (Magstim Co, Carmarthenshire, Wales, UK) was used for all experiments. The details of the setup for each study are described in the methods sections.

### 3.3. **Transcranial Direct Current Stimulation**

Transcranial direct current stimulation (tDCS) is a neuromodulatory technique that was used in the past for treatment of psychiatric disorders and has become popular within the last decade as a technique to explore mechanisms of brain plasticity through the application of weak polarizing currents to the brain of awake and functioning humans. tDCS has been applied in several cortical areas and recently cerebellar tDCS has been

gaining popularity[62]. tDCS has been demonstrated to modify the excitability of the cerebellar-thalamo-cortical pathway in a polarity specific manner with effects lasting approximately 30minutes.

For the study described in Chapter 5.2 cerebellar was used to modulate the excitability of the cerebellar-thalamo-cortical pathway. 2 mA of constant current were delivered using a tDCS device through 25 $cm^2$ saline-soaked surface sponge electrodes (Eldith-Electro-Diagnostic & Therapeutic Systems GmbH, Germany). One electrode was centred on the right cerebellar cortex, 3 cm lateral to the inion and the other electrode was positioned on the right buccinator muscle. Anodal or cathodal current was delivered over the cerebellum for 15 min. In the sham session, anodal current was applied for 30 s. At the onset and offset of all interventions (anodal, cathodal, and sham) current was changed in a ramp-like manner over 10 s.

### 3.4. **Paired associative stimulation**

Paired associative stimulation (PAS) is a stimulation protocol used to induce plastic changes in the cortical level by repetitively pairing two stimuli (a TMS pulse and a peripheral nerve electrical stimulation pulse). In study 6.2 we used a standardized PAS protocol which consisted of 200 electrical stimuli to the median nerve at the wrist paired with TMS stimuli over the APB hot spot, delivered at the rate 0.25 Hz. Each TMS stimulus was preceded by an electrical stimulus by 21.5 ms. Intensity of electrical

stimulus was 300% of the perceptual threshold; while TMS intensity was adjusted to the intensity that evoked MEPs of 0.5–1 mV in APB muscle. Median nerve electrical stimulation was applied through a bipolar electrode, with the cathode positioned proximally (Digitimer DS 7 stimulator; Digitimer Ltd, Welwyn Garden City, Herts, UK). The electrical pulses were square wave pulses with a pulse width of 200 μs.

### 3.5. **Muscle vibration**

In study 6.1 we used muscle vibration to stimulate the muscle spindles of the ADM muscle and interpolate false feedback signals through Ia afferent nerve fibres. Vibration was applied to the right ADM muscle using an electromagnetic mechanical stimulator (Ling Dynamics System) with a 3 cm diameter circular probe. The probe was positioned orthogonally to, and under slight pressure against, the belly of the right ADM between the EMG electrodes. The frequency of the vibration was 80Hz and the amplitude was 0.2– 0.5 mm [63]. Vibration of the same properties has been found to be effective for stimulation of the muscle spindle primary endings (Ia fibres) [64]. During vibration, EMG activity of both muscles was monitored for voluntary activation or induction of the tonic vibration reflex [65].

# Chapter 4. MEP VARIABILITY AND EMG ACTIVITY DURING FINGER MOVEMENTS

## 4.1. **Muscle activation in the hand during individual finger movements**

*(Published as: Kassavetis P, Sadnicka A, Saifee TA, Belvisi D, van den Bos M, Pareés I, Kojovic M, Rothwell JC, Edwards MJ. Motor 'surround inhibition' is not correlated with activity in surround muscles. Eur J Neurosci. 2014 Aug;40(3):2541-7.)*

As discussed above motor SI has been probed with transcranial magnetic stimulation (TMS) applied over the primary motor cortex at the onset of an isolated voluntary finger movement. It refers to the reduction of corticospinal excitability in muscles which are non-active but adjacent to the active muscles [28, 29]. Although there is evidence of the presence of intracortical inhibitory networks in the primate motor cortex [66] with similarities to sensory surround inhibition, the electrophysiological phenomenon described as motor surround inhibition is only hypothetically mediated through these networks and there is no direct experimental data to prove a link with sensory SI. Furthermore the different cyto-architecture of the primary motor cortex and primary visual cortex (agranular motor cortex, presence of Betz cells in motor cortex, layering of visual cortex, different intracortical connectivity etc.) makes evidence acquired in the visual system not easily transferred directly to the motor system. In addition, it is still uncertain whether motor SI really involves inhibitory neuronal networks [28, 31] or it is an epiphenomenon of reduced excitation rather than active inhibition.

It is often proposed that SI is essential for successful generation of isolated finger movements [29]. This assumption has mainly been derived from electrophysiological studies demonstrating impairment of SI in movement disorders including dystonia, Parkinson's disease and paroxysmal kinesigenic dyskinesias [30, 38, 67-70]. However, these movement disorders are diverse in their phenomenology and, at least in the case of paroxysmal kinesigenic dyskinesia; there is no clinically apparent movement disorder during performance of the motor task during which SI is assessed.

With this study we attempt to provide essential evidence on the basic electrophysiological characteristics of SI. We investigated the relationship between SI and muscle activation in the hand in a large cohort of normal volunteers at the onset of an isolated finger movement. We provide a complete description of the profile of SI by providing descriptive data of corticospinal excitability and comparisons with EMG activity based on the hypothesis that SI is not only responsible for suppression of corticospinal excitability measured with TMS but also for general suppression of motor output reflected in EMG activity of surround muscles . Such comparisons are an essential first step in exploring the proposal that SI is a mechanism for the reduction/inhibition of muscle activity in surround muscles.

### 4.1.1. Methods

## Participants

The data from a total of 31 right-handed healthy adults (mean age 27.4 years, SD=7.2, 16 women) were analysed. The participants had no history of any neurological condition and they were not professional musicians. Written informed consent was obtained from all participants and the study was approved by the local ethics committee and conducted in accordance with the Declaration of Helsinki.

## Motor task

During the experiments, the subjects sat with their right hand resting on a desk. While their hand was lying flat and relaxed on the desk, the tip of their index finger was placed on a small button. They were asked to briefly press the button with a self-paced delay after a 'go' signal (an auditory tone), by flexing their index finger at the metacarpo-phalangeal joint. FDI is a synergist for this movement and previous studies have shown that this movement induces activation of FDI and suppression of corticospinal excitability in ADM [28, 71]. Subjects were asked to perform the movement with 10% of their maximum EMG activity. Duration of the movement was aimed to be approximately 100ms and the subjects were also asked to keep their other fingers relaxed while they were performing the movement. Visual feedback of the EMG activity from all three muscles (FDI, APB and ADM) was displayed on a screen in front of the subjects.

## EMG recordings

EMG activity was recorded from the right FDI, APB and ADM using a pair of Ag–AgCl surface electrodes in a belly-tendon montage. The EMG signal was amplified (1000x) and band-pass filtered (bandwidth 10–1,000 Hz) with a Digitimer D360 amplifier (Digitimer Ltd, UK), digitized at a sampling rate of 5 kHz (CED 1401 laboratory interface; Cambridge Electronic Design, Cambridge, UK) and fed into a laboratory computer for storage and off-line analysis. Data were collected with SIGNAL® software V4.00 (Cambridge Electronic Design, Cambridge, UK).

## Transcranial magnetic stimulation

A figure-of-eight shaped coil (external loop diameter of 9 cm) connected to a monophasic Magstim 200 stimulator (Magstim Co, Carmarthenshire, Wales, UK) delivered transcranial magnetic stimulation (TMS). The intersection of the coil was positioned tangentially on the scalp over the left motor cortex. The handle of the coil was pointing backwards and laterally at a $45^0$ angle to the sagittal plane in order to induce posterior–anterior directed current in the brain and to activate corticospinal neurons trans-synaptically [72, 73]. The “hot spot” was defined as the optimal scalp

position for eliciting motor evoked potentials (MEPs) of maximal amplitude in the contralateral ADM and it was marked with a felt pen in order to ensure consistent coil position during the experiment. The intensity of the stimulation was set to evoke MEPs with average peak-to peak amplitude of approximately 1mV-1.5mV at rest in the right ADM muscle. For the assessment of SI, single TMS pulses were delivered at rest and at the onset of the movement. Each trial started with a self-paced movement after the "go" signal and lasted for 10 seconds when the next "go" signal was presented. A total of 40trials were collected. During each trial one single TMS pulse was delivered. In 20 trials we assessed the MEP amplitude size at the onset of the movement with the TMS being triggered immediately when EMG activity in right FDI above 100 μV was detected. In other 20 trials we assessed the MEP amplitude size at rest by delivering the TMS pulse 5 seconds after the onset of the brief movement while the subjects were resting waiting for the next "go" signal. This time point is considered to be sufficient for measurements at rest since the duration of the movement was aimed to be 100ms, meaning that the pulse was delivered with a delay of approximately 4900 ms after the end of the movement when neither SI or any other post activation inhibitory or facilitatory effect are known to be active and the corticospinal excitability has returned to baseline [28]. The 20 trials for the MEPs at rest and the 20 trials for the MEPs at the onset of the movement were randomised (Fig. 4.1.1). When MEPs were collected at the onset of the movement the muscle twitch due to the TMS pulse did not allow measurement of the exact amount of force that was intended if the twitch had not have happened. Therefore no trials were excluded. The subjects were only getting feedback of the amount of force they applied in the trials that the pulse was delivered 5 seconds

after the movement (rest trials). In this way we could ensure that the subjects were consistently pressing 10% of their maximum force.

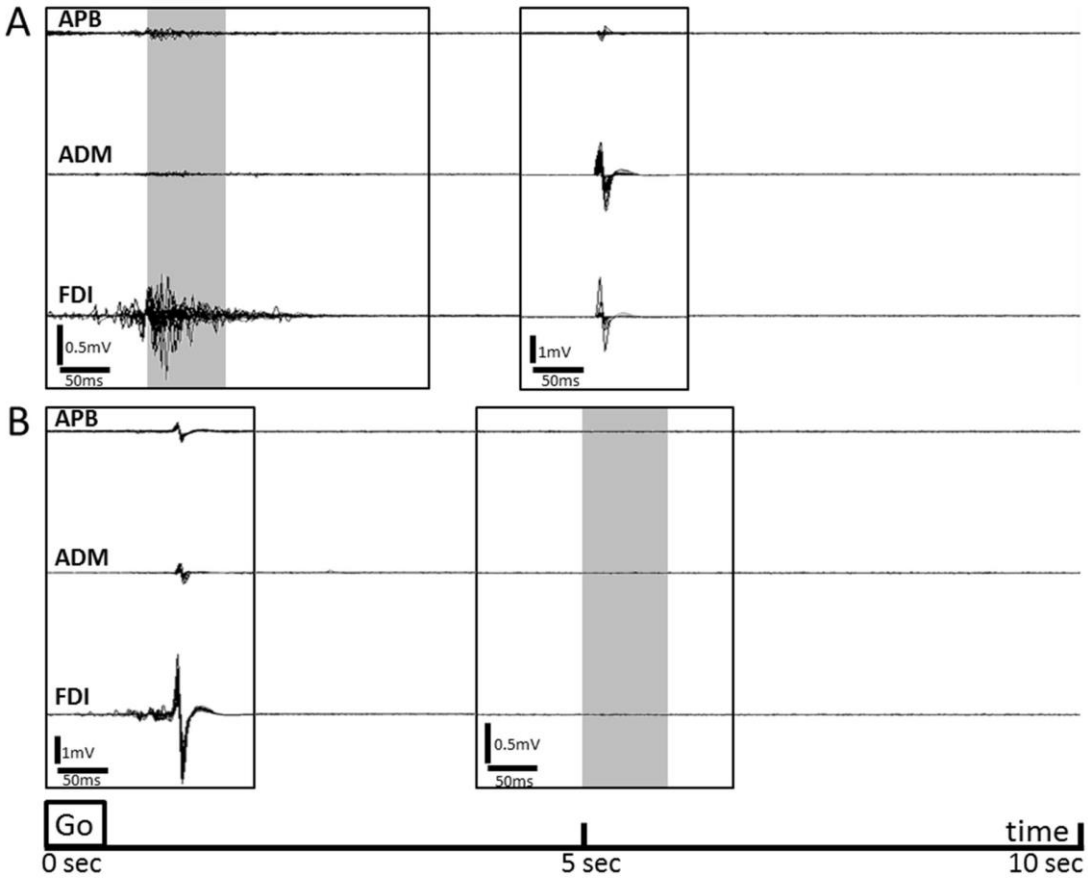


*Fig. 4.1.1. (A) Overdraw EMG traces of FDI muscle in trials where MEP was recorded at rest (TMS pulse 5 s after the onset of the movement). (B) Overdraw EMG traces of FDI muscle in trials where MEP was recorded at the onset of the movement (TMS pulse at 0 ms after the onset of the movement). The epochs where the EMG activity was analysed are represented in grey colour (epochs 3 and 4 as described in the text). Note that the scale is bigger in the boxes in order to visualise the EMG activity better*

## Data analysis

*EMG activity*

The EMG activity in all three muscles was assessed in four different epochs for two purposes. First purpose was to detect minimal background activity/noise before the MEPs, and second purpose was to compare the EMG activity during movement and at rest.

For the first purpose we measured the EMG activity during the period of 20ms preceding the MEP delivered at the onset of the movement (epoch 1) and a period of 200ms preceding the MEP delivered at rest (epoch 2). The end of the epochs was just before the onset of the TMS artefact.

For the second purpose, to assess the EMG activity during FDI contraction, we used the 20 trials where the TMS pulse was delivered at rest (TMS pulse 5 seconds after movement) and we used an epoch of 100ms after onset of FDI contraction (activity above 100μV) (epoch 3, Fig 4.1.1). The epoch duration of 100ms was chosen as this is a time period in which SI has been found to be active [28]. We also assessed the EMG activity at rest (essentially background activity/noise) during an epoch (epoch 4, Fig 4.1.1) which started at 5000ms after the onset of the movement and lasted for 100ms in the 20 trials where the TMS pulse was delivered at the onset of the movement. We chose this epoch to match with the time period when rest MEPs were assessed. The

EMG activity measured during the above epochs was expressed as the root mean square (RMS) amplitude of the raw EMG signal.

*Corticospinal excitability*

Peak-to peak MEP amplitudes from the three muscles were measured off-line and the means were calculated for the 20 MEPs at rest and the 20 MEPs at the onset of the movement. SI was expressed as the ratio of the mean MEP amplitudes at the onset of the movement to the mean MEP amplitudes at rest.

*Common neuronal drive*

In order to explore possible common neuronal drive in different muscles we performed cross correlation analysis for the EMG epochs 3 and 4 described above. Cross correlation has been used in the past as one of the methods to assess muscle cross talk and motor unit synchronization [74, 75]. It essentially calculates the magnitude of the common component between two recorded signals by overlapping one signal over the other and extracting the differential at every possible lag time. If the two recorded signals are totally independent the cross correlation coefficient equals 0, if the signals are identical the cross correlation coefficient equals 1 and if they are identical but of the opposite polarity the cross correlation coefficient equals -1. Possible changes in EMG

amplitudes do not confound the analysis because the relative amplitude of the signals does not affect the cross correlation coefficient. The analysis was performed independently for the recordings at the onset of the movement and for the recording at rest. At rest we were not expecting to observe increased coefficients as there was no muscle activity. However this measurement would be a good estimation of possible common background noise from external sources other than EMG activity (e.g. power line noise). For both rest and movement analysis the cross correlation coefficients were calculated for all 20 trials and for all 3 pairs of muscles (FDI and ADM, FDI and APB, ADM and APB) at 0 ms lag-time. The relatively small distance between the electrodes on the hand justify the use of 0ms lag-time.

**Statistical analysis**

The SPSS Statistics software (version 19.0.0) was used for the statistical analysis. Normality of data distribution was explored with Kolmogorov-Smirnoff test.

In order to explore differences between genders we used parametric (t-test) and non-parametric (Mann-Whitney U test) independent samples comparisons for MEP amplitudes and RMS EMG amplitude in all conditions and all muscles. No significant difference was found therefore the data from both genders were pooled together for the rest statistical tests.

In order to explore the changes in the EMG activity and MEP amplitudes we used two-way repeated measures analysis of variance (rmANOVA). Post hoc paired tests were used for the exploration of significant effects.

Bivariate correlations between the MEP amplitude ratios (onset/rest) were explored in order to investigate simultaneous modulation of corticospinal excitability in the three pairs of muscles. Furthermore the relationship of the RMS amplitude of the EMG activity preceding the MEPs and the actual MEP amplitudes was explored with bivariate correlation.

For the exploration of muscle synchronisation, cross correlation analysis was performed with MATLAB (2007b, The MathWorks). For each muscle pair the mean cross correlation coefficient across the 20 EMG recordings at rest and 20 EMG recordings at the onset of the movement were compared in a two-way rmANOVA design.

If data were not normally distributed Log10 transformation was used and normality was re-assessed. The data used in the ANOVAs were always normally distributed after log10 transformation. All descriptive statistics correspond to untransformed data. The data presented in the figures correspond to the data used for the statistical analyses. Statistical significance was set to $p \leq 0.05$.

### 4.1.2. Results

**EMG activity preceding MEPs**

The background EMG activity preceding the MEPs delivered at rest (200ms epoch) never exceeded 20 μV in any subject and any channel, (FDI: median 5.1μV, IQR=3.1-7.1 μV, ADM: median=3.5 μV, IQR=2.8-5.3 μV, APB: median=4.3 μV, IQR=3.0-6.9 μV). The EMG activity preceding the MEPs at the onset of the movement (20ms epoch) were also not exceeding 20 μV in ADM and APB (ADM: median=5.5 μV, IQR=3.7 - 8.1μV, APB: mean=9.6 μV, SD=4.8 μV) but it was higher than 20 μV in the active FDI muscle (FDI: median 24.5μV, IQR=21.7- 33.3μV) since the trigger threshold was set at 100 μV. rmANOVA with factors MUSCLE (FDI,ADM,APB) and MOVEMENT (REST, ONSET) showed significant effect of MOVEMENT $F(1,30)=270.8$, $p<0.001$, significant effect of MUSCLE $F(2,60)=55.34$, $p<0.001$ and significant interaction MUSCLE*MOVEMENT $F(2,60)=93.1$, $p<0.001$. Paired comparisons of the EMG activity just before the MEPs at rest and the MEPs at the onset of the movement showed significant increase of EMG activity at movement onset in all muscles (FDI: $t(30)=-19.85$, $p<0.001$, ADM: $t(30)=-5.15$, $p<0.001$, APB: $t(30)=-8.01$, $p<0.001$) (Fig. 4.1.2) .

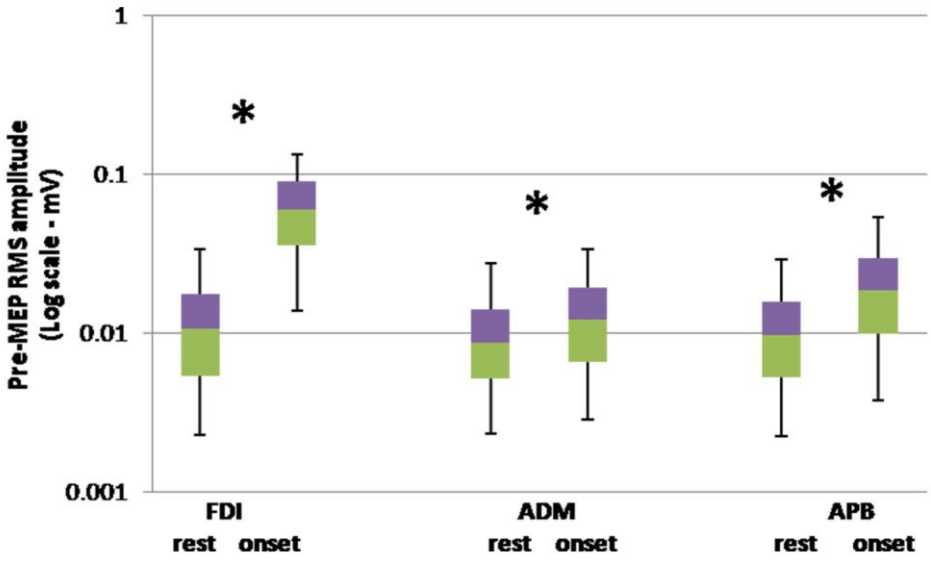


*Fig. 4.1.2. Pre-MEP RMS amplitudes. Box plots present median, first and third quartiles, and extremes (minimum and maximum values). * indicate statistical significant differences.*

**EMG activity during the movement**

rmANOVA with factors MUSCLE (FDI,ADM,APB) and MOVEMENT (REST, ONSET) showed significant effect of MOVEMENT $F(1,30)=303.2$, $p<0.001$, significant effect of MUSCLE $F(2,60)=113.3$, $p<0.001$ and significant interaction MUSCLE*MOVEMENT $F(2,60)=192.0$, $p<0.001$. Post-hoc pair-wise comparisons

showed that the RMS amplitude of EMG activity was significantly increased in the FDI muscle during activation (Rest: median=6.1µV, IQR= 3.8 – 8.7µV, Onset: median=77.9 µV, IQR=53.3- 106.4µV) t(30)=-22.1, p<0.001. Similarly, the EMG activity in ADM muscle increased during FDI contraction (Rest: median=5.1µV, IQR= 3.1-9.0µV, Onset: median=9.3 µV, IQR=5.6-13.5µV) t(30)=-8.1, p<0.001. Also in APB the RMS amplitude was significantly smaller at rest (median=5.3 µV, IQR= 3.2-9.4µV) than during FDI movement (mean=19.5 µV, SD= 11.4µV), t(30)=-9.9, p<0.001 (Fig. 4.1.3).

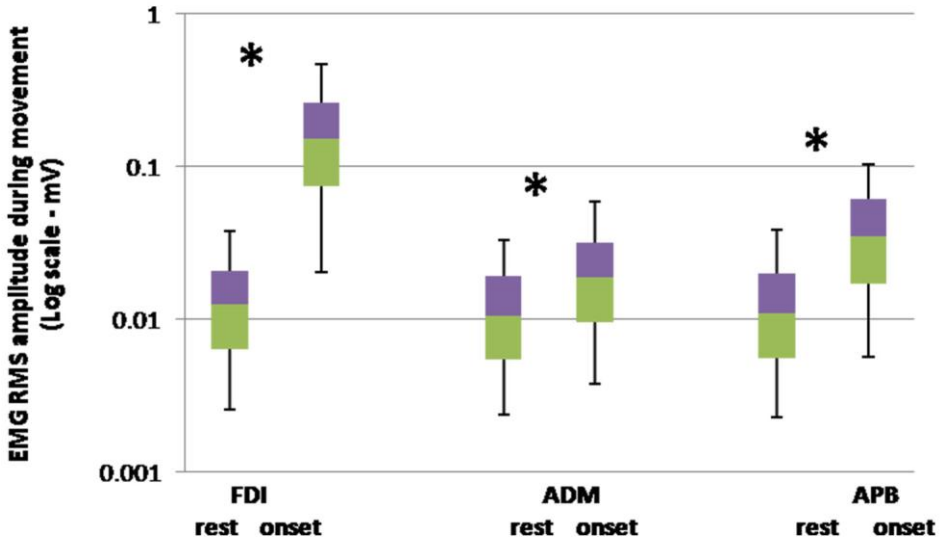


*Fig. 4.1.3. EMG RMS amplitude during movement and at rest. Box plots present median, first and third quartile and extremes (minimum and maximum values). * indicate statistical significant differences.*

**Corticospinal excitability**

Mean absolute stimulation intensity used was 58.42±12.42 % of maximum stimulator output.

rmANOVA with factors MUSCLE (FDI,ADM,APB) and MOVEMENT (REST, ONSET) showed significant effect of MOVEMENT $F(1,30)=14.53$, $p=0.001$, significant effect of MUSCLE $F(2,60)=110.9$, $p<0.001$ and significant interaction MUSCLE*MOVEMENT $F(2,60)=45.42$, $p<0.001$.Corticospinal excitability in the active muscle (FDI) was significantly increased at the onset of the movement (mean=6.50mV, SD=1.43) when compared to rest (mean=2.80mV, SD=1.72) $t(30)=-9.45$, $p<0.001$. In the ADM muscle, there was a significant decrease of the MEP amplitude at the movement onset (median=0.67 mV, IQR=0.43-1.28) in comparison to the MEPs at rest (median=1.11 mV, IQR=0.86-1.75), $t(30)=5.3$, $p<0.001$, confirming the presence of SI. In total, 25 out of the 31 subjects (81% of the cohort) showed decreased corticospinal excitability in ADM at the onset of the movement (ratio of mean MEP onset/mean MEP rest <1). In the APB muscle, the presence of SI could not be confirmed as the difference between the MEP amplitudes at rest (median=0.93 mV, IQR=0.43-2.44) and at the onset of the movement (median=1.09 mV, IQR=0.75-1.83) was not significant, $t(30)=-1.65$, $p=0.11$. 48% of our subjects (15 out of 31 – chance level) showed ratio of mean MEP onset/mean MEP rest <1 (Fig. 4.1.4).

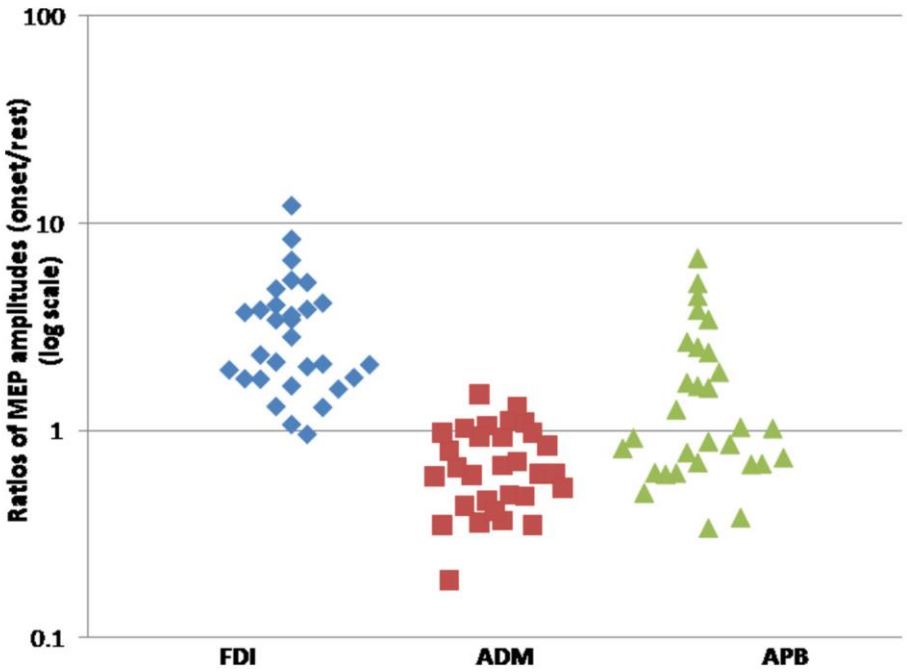


*Fig. 4.1.4. Corticospinal excitability ratios (onset/rest) in three muscles in 31 individual healthy volunteers. The subjects in each group were arbitrarily spread along the x-axis in order to facilitate visualisation.*

The ratio of the MEP amplitudes (onset/rest) in FDI correlated significantly and positively with the ratio in APB $R^2$=0.45, p=0.011 but the correlation was not significant between FDI and ADM muscle, $R^2$= -0.36, p=0.047. Finally the ratios between ADM and APB did not correlate significantly $R^2$=-0.18, p=0.35 (level of significance after Bonferroni correction for multiple comparisons <0.017)

Interestingly RMS amplitude of EMG activity preceding MEPs at the onset of the movement did not correlate with the MEP amplitudes in any of the muscles FDI: $R^2$=0.11, p=0.56, ADM: $R^2$=0.09, p=0.64, APB: $R^2$=0.39, p=0.031 (level of significance after Bonferroni correction for multiple comparisons <0.017).

**Muscle common drive**

In order to explore motor unit synchronisation we performed cross-correlation analysis and we statistically compared the coefficients. rmANOVA with MOVEMENT (rest and onset) and MUSCLE_PAIR (ADM-APB, ADM-FDI and APB-FDI) showed significant effect of MOVEMENT F (1,30)=39.32, p<0.001, significant effect of MUSCLE_PAIR F(2,60)=90.55, p<0.001 and significant interaction MOVEMENT x MUSCLE_PAIR F(2,60)=99.29, p<0.001. Post hoc comparisons showed that for the muscle pair FDI-ADM the mean cross correlation coefficients at rest (mean=0.06, SD=0.17) and at the onset of the FDI contraction (mean=0.10, SD=0.20) were not significantly different t(30)=0.80, p=0.42. However the comparison for the pair FDI-APB revealed significantly increased cross correlation coefficients during movement (mean=-0.59, SD=0.15) in comparison to rest (mean=-0.03, SD=0.16), t(30)=-17.7, p<0.001 and significant difference was also found for the pair ADM and APB (onset: mean=0.20, SD=0.18, rest: mean=0.04, SD=0.14) t(30)=4.63, p<0.001 (Fig. 4.1.5).

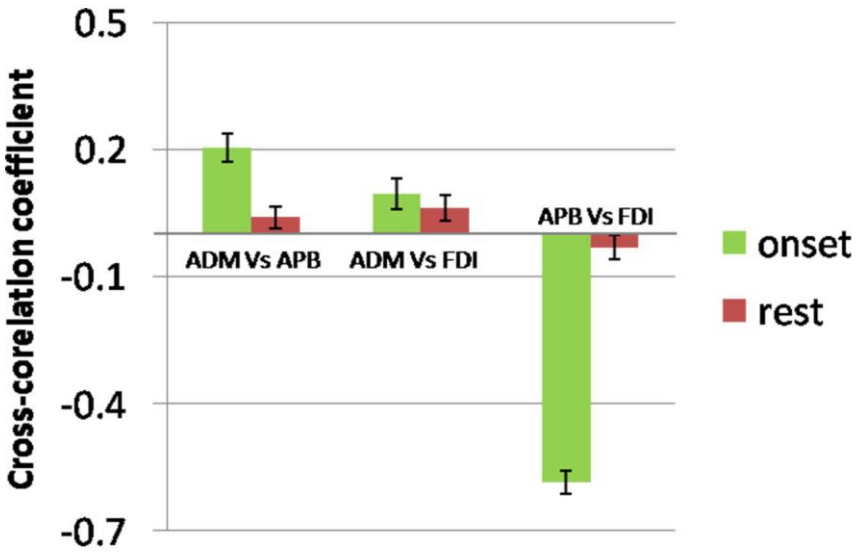


***Fig. 4.1.5. Cross correlation coefficients for three muscle pairs during FDI contraction and at rest. Error bars indicate SEM.***

### 4.1.3. Discussion

We have characterised SI in a large cohort of healthy participants and explored its relationship with EMG activity in the active and surround muscles. As reported by others [28], we found evidence of SI in the ADM muscle at the onset of FDI contraction. We could not confirm the presence of SI in the APB muscle at the same time. Importantly the analysis of the EMG signals showed increased EMG activity in all three muscles at the onset of FDI contraction despite the fact that corticospinal excitability measured with TMS was reduced in the ADM muscle. There was no correlation between the SI and EMG activity in surround muscles.

At least for the FDI-ADM muscle pair, the increase in ADM activity was unlikely to have been due to EMG crosstalk between the surface recordings from each muscle. During FDI contraction, the cross-correlation between EMG activity in the ADM and FDI was comparable to the rest condition, suggesting that EMG activity in ADM is unlikely to have common origins with the signal driving FDI. In contrast, the cross-correlation between FDI and APB became stronger and had a negative sign, which is likely due to cross talk between the two muscles or strengthening of the reciprocal inhibitory drive between the two muscles. Interestingly, the coupling between APB and ADM appeared to be stronger during FDI contraction. ADM and APB are not directly involved in the task yet both are in the surroundings of the agonist muscle. We speculate that the EMG synchronisation between them reflects a common (subcortical, see below) drive quite separate from the (possibly cortical) drive to the agonist.

Sohn & Hallett (2004) had previously noted that spinal cord F-waves were enhanced at the same time as MEPs were reduced and concluded that spinal excitability was enhanced. However, F-waves are now thought to be an unreliable indicator of the excitability of spinal motoneurones [76] and results on the modulation of EMG activity have been contradictory so far [28, 30] . Our data therefore show that reduced corticospinal excitability does not necessarily lead to reduced EMG activity, and conversely that increased EMG activity does not always lead to larger MEPs. Another condition when similar dissociation may be present is startle. Previous studies have shown that startling acoustic stimuli can produce EMG activity and non-startling acoustic stimuli can suppress motor cortex excitability [77-79]. However the latter has

been tested only in the absence of any startle-evoked EMG and it is unclear whether MEPs evoked during a startle EMG burst would be larger or smaller than at rest.

Given the previous experiments of Di Lazzaro et. al. [72] who studied the effect of voluntary activity on descending corticospinal activity evoked by TMS, by recording from cervical epidural electrodes at the same time as measuring MEPs, our results seem surprising. In the above study [72] it was found that a low to moderate level of volitional contraction produced only a small increase in the descending activity and the authors concluded that much of the increase in MEPs was caused by increasing excitability of spinal motoneurones and interneurons. Thus, reduction of MEPs in the present experiment suggests that the effect of SI on excitability of cortical projections to "surround" muscles is quite strong.

Perhaps initiation of a focal voluntary movement results in a relatively generalised motor excitation which under the presence of spatially specific inhibitory networks (including SI) is 'shaped' to form a motor command that carries spatial and temporal parameters of the desired movement. The basal ganglia may play a significant role in this respect as has been hypothesised previously [80]. In the surround muscles, these mechanisms fail to completely suppress the general tendency towards excitation during movement, leading to 'leakage' of neuronal activity and consequently increase in the recorded EMG activity. Given that corticospinal excitability at this time is suppressed,

the increase in EMG must be produced by other pathways, such as the uncrossed corticospinal tract, the rubrospinal tract, or the reticulospinal tract.

The role of volition in the generation of movement is another factor which may be important for the interpretation of our results. A simple direct comparison of the pattern of the intended voluntary movement and the spatial pattern of the MEP and EMG modulation leaves no doubt that volition is better reflected in the topographic specificity of MEP modulation and not the EMG modulation. Several studies on the modulation of MEPs during performance or imagery of voluntary actions have shown strong correlations of the modulation of MEPs and intentions of action [16]. However, in the case of surround muscles the increase in EMG activity is non voluntary and the MEPs are modulated in the opposite direction. In more broad terms volition has greater effect on modulating excitability at the motor cortical level (reflected in MEPs) but not in other structures which may cause the involuntary activation of EMG in the surround muscles, at a subcortical or spinal level. Similarly to volition, attention may have played a significant role in muscle specific modulation of MEPs and different levels of attentional balance between the active and non-active muscles may explain across-subjects variability of MEP suppression.

Another concept that may play a significant role in the generation of SI and its dissociation for EMG activity are the distinctive brain oscillatory patterns in the cortical representation area of the active and surround fingers. There is evidence that pre-TMS

motor-cortical oscillations play a significant role in regulation of corticospinal excitability [81, 82] and that cortical activity in Beta (15-30Hz) and Piper (30-60Hz) bands in the motor cortex drives EMG activity in a somatotopic manner [83]. The muscle specific regulation of corticospinal excitability may be related to changes in different brain rhythms power through synchronisation and desynchronisation (for example inhibitory alpha activity 'flip-flop' mechanism in active vs surround muscles [84]) which could explain reduction of MEP amplitude and generation of motor SI. Although speculative, this concept is interesting and worth further investigation in the exploration of potential mechanisms for generation of SI.

Regarding the general profile of SI in the cohort of our subjects it seems that SI is not present in all subjects and that there is a considerable between -subject variability which should be taken into account when designing future studies on SI. In contrast the increase in the EMG RMS amplitude was relatively consistent across almost all of our subjects. We could not replicate previous results on the presence of SI in APB muscle during FDI contraction ([37, 38, 54], and this may reflect differences in the experimental set up, i.e. relative placement of the hand to the experimental apparatus.

The present study is limited by lack of assessment of the corticospinal excitability (MEP amplitudes) during the whole period when SI is known to be active (100ms after the onset of the movement). However, the temporal pattern of the modulation of corticospinal excitability at the onset of a voluntary movement has been replicated

multiple times [28, 71] and it is not completely relevant to the points raised by this study. In addition, in the present set-up there is no objective measurement of volition or attention. Therefore we cannot infer with certainty the significance of the roles of attention or volition in modification of MEPs. However, in all the experiments we used the same set up and the same wording to instruct the subjects to make an isolated movement of the index finger without movement of the other fingers. Therefore we believe that attentional focus and movement planning (intention) was comparable in our subjects although it was not objectively measured. Another limitation of this study is that the cortical hotspot of ADM was also used for MEP assessment in the APB and FDI muscles. Given the fact that we consistently recorded MEP of high amplitudes in both APB and FDI muscle (see results) we are certain that at least part of the cortical representation of these muscles was stimulated and therefore any systematic modulation of corticospinal excitability was captured. Furthermore, in this way we could assess modulation of corticospinal excitability in the same trials controlling for variability of task performance between trials. Finally we acknowledge the limitation of single pulse TMS paradigms to infer cortically mediated effects.

Where do these data take us in better understanding motor SI? We believe that they question a simplistic view of SI as a phenomenon reflected by a reduction in muscle activity in surround muscles. There may well be a role for SI in finessing performance of fine motor behaviour, but as yet this is not proven. Two electrophysiological studies in healthy humans have assessed the relationship of SI and plasticity of the nervous system [85, 86]. SI was assessed before and after introduction of distorted sensory

feedback or repetitive simultaneous movements of two fingers. In both studies after the training session SI was found to be altered, but there was no measurement made in either study of motor performance or individuation of finger movement. Although these studies demonstrate that SI can be modified, neither provides evidence on whether increased SI is “good” or “bad” for individual finger movement execution, or whether it is beneficial for some movements in some circumstances but not in others.

Interestingly, professional musicians who are capable of great skill in the performance of isolated finger movements have reduced SI [87]. This is a counterintuitive result which has been used to explain why a small proportion of professional musicians develop dystonia [87]. However, it could also be argued that reduced SI could be advantageous when fast sequences of isolated movements have to be executed or that enlargement of cortical finger representations associated with motor skill acquisition in musicians reduces SI independent of any effect on motor performance [88], or indeed that SI is not related to motor performance at all. Another possibility is that SI reflects the natural statistics of fingers movements and the presence of muscle synergies whose patterns are influenced by everyday life and significantly differ amongst individuals ([85]. The relationship between SI and behaviour remains a key unanswered question in understanding the role of SI in hand motor performance.

# Chapter 5. THE ROLE OF CEREBELLUM IN MOTOR SURROUND INHIBITION

## 5.1. **Surround inhibition modulation by phasic cerebellar output**

*(Published as: Kassavetis P, Hoffland BS, Saifee TA, Bhatia KP, van de Warrenburg BP, Rothwell JC, Edwards MJ. Cerebellar brain inhibition is decreased in active and surround muscles at the onset of voluntary movement. Exp Brain Res. 2011 Mar;209(3):437-42. )*

Cerebellar brain inhibition (CBI) is an inhibitory circuit which is thought to be mediated through the dentato-thalamo-cortical pathway [89, 90]. Using transcranial magnetic stimulation (TMS), the size of the motor evoked potential elicited by a TMS pulse over the hand motor area is significantly reduced by a TMS pulse, delivered over the contralateral cerebellar hemisphere, 5-7ms earlier. CBI occurs at rest but has been found to be reduced in hand muscles during tonic activation of proximal arm muscles [91].

It is not known how CBI may be modulated in active and surround muscles during movement preparation and at movement onset when SI is most prominent. Here, we aimed to probe the relationship between SI and CBI. We hypothesized that, if such a relationship existed, CBI during movement initiation would be differentially modified in an active and surround muscle, being reduced in the contracted muscle and increased in the surrounding muscles.

### 5.1.1. Methods

**Participants**

16 healthy volunteers (mean age 29 ± 9 years; range 22-52 years; 9 men and 7 women) participated in the study after giving their written informed consent. All of them, except for one, were right-handed and none of them had any history of neurological disease. The study was approved by local ethics committee and conducted in accordance with regulations laid down in the Declaration of Helsinki.

**Electromyographic recordings**

Electromyographic (EMG) activity was recorded from right first dorsal interosseus (FDI) and abductor digiti minimi (ADM) muscles using a pair of Ag-AgCl surface electrodes in a belly-tendon montage. Ground electrode was placed above the styloid process of the right ulna. The EMG signal was amplified (1000x) and band-pass filtered (bandwidth 20Hz to 2000Hz) with a Digitimer D360 amplifier (Digitimer Ltd, UK), digitized at a sampling rate of 5KHz (CED 1401 laboratory interface; Cambridge Electronic Design, Cambridge, UK) and fed into a laboratory computer for storage and off-line analysis. Data was analysed using SIGNAL software V4.00 (Cambridge Electronic Design, Cambridge, UK).

**Motor task**

During the experiments the subjects were sitting in a comfortable chair with their right hand resting on a desk. While their hand was lying flat and relaxed on the desk, the tip of their index finger was placed on a small button. They were asked to briefly press the button after a 'go' signal (an auditory tone) with a self-paced delay, by flexing their index finger in the metacarpo-phalangeal joint. FDI is a synergist rather than a primary muscle for this movement but previous studies have shown that this movement induces activation of FDI and suppression of ADM through SI [28]. At the beginning of the experiment we measured the individual maximum EMG activity which could be produced in FDI by briefly pressing the button. Then we asked the subjects to perform the same brief movement with 10% of their maximum EMG activity. They were also asked to keep their ADM muscle totally relaxed while they were doing the task. Visual feedback of the EMG activity from both muscles (FDI and ADM) was displayed on a screen in front of the subjects. Training sessions before the start of the experiments were needed for a consistent performance of the desired movement to be attained by the subjects with EMG activity in ADM not to exceed 100μV. We examined SI and CBI at rest and at the onset of the movement.

**Transcranial magnetic stimulation**

A figure-of-eight shaped coil (external loop diameter of 9cm) connected to a monophasic Magstim 200 stimulator (Magstim Co, Carmarthenshire, Wales and UK)

delivered TMS over the left motor cortex. The intersection of the coil was positioned tangentially on the scalp over the left motor cortex at the optimal site for eliciting motor evoked potentials (MEP) of maximal amplitude in the right ADM. The handle of the coil was pointing backwards and laterally at a 45º angle to the saggital plane in order to induce trans-synaptically a posterior-anterior directed current in the brain to activate the corticospinal tract [72, 73]. The hot spot was marked with a felt pen in order to ensure consistent coil position during the experiment. For the assessment of SI single TMS pulses were delivered at rest and at the onset of the movement. TMS at movement onset was achieved using the peri-triggering function of SIGNAL software which was set to trigger TMS immediately when EMG activity in right FDI above 100 μV was detected. The intensity of the stimulation was set to evoke MEPs with average peak-to-peak amplitude of approximately 0.5mV–1mV at rest in ADM and FDI, which was found from previous studies to be ideal for CBI assessment [89, 91-93].

The cerebellar conditioning stimulus (CS) was delivered over the right cerebellar hemisphere with a double-cone coil (110mm mean diameter). This type of coil has been found in previous studies to be the most efficient for cerebellar stimulation in CBI paradigms [89, 94]. The exact position of the coil was 3cm lateral to the inion on the line connecting the inion and the external auditory meatus [89, 90, 93]. The current of the coil was directed downwards in order to induce an upwards current in the cerebellar cortex [89, 92, 93]. In line with previous studies on CBI, cerebellar stimulation intensity was set at 5% below the pyramidal tract active motor threshold (AMT) [93, 95], in order to minimise confounding effects due to brainstem or nerve root stimulation [89, 96]. The AMT for pyramidal tract was measured with the coil positioned on the inion

while subjects maintained background EMG activity of 10% of their maximum force in FDI [93]. Five trials of each intensity were averaged and the minimum intensity which induced MEP responses of 50μV or more above the background activity was considered to be the pyramidal tract AMT. Threshold was determined to the nearest 5% of the stimulator output [91, 93]. The Interstimulus interval (ISI) between the CS and the test stimulus (TS) of motor cortex was set at 5ms. This ISI was found by Saito et al. to be the optimal for CBI and its effect is attributed to cerebellar cortex stimulation rather than stimulation of other peripheral structures (e.g. muscle, nerve, plexus) [90, 91, 93, 94]. For the assessment of CBI at the onset of the movement we used the peri-triggering function of SIGNAL software set to elicit the CS immediately after the detection of EMG activity above 100μV in FDI followed 5ms later by the TS.

**Experimental design**

There were four blocks of experimentation: assessment of MEP size at rest (single pulses), assessment of MEP size at movement onset (single pulses), CBI at rest (paired pulses), CBI at movement onset (paired pulses). For each of the blocks 15 stimulation trials were recorded. In the blocks assessing MEP size or CBI at movement onset we also included 15 trials with no stimulation mixed with the 15 stimulation trials in a randomised fashion. This ensured that subjects continued to perform the movement during these blocks, and were not aware of when a stimulation trial might occur. The order of the blocks was also randomised between participants.

**Statistical analysis**

Peak-to-peak MEP amplitude for each trial was measured off-line and the average amplitude in 15 trials was calculated for each session. CBI was expressed as the ratio of conditioned MEPs to unconditioned MEPs. SI was expressed as the ratio of MEP amplitudes during peri-triggered trials to MEP amplitudes in control trials. The effects of SI and CBI were evaluated through repeated measures analysis of variance (ANOVA). Wherever significant interactions were observed, we did post hoc tests with Bonferroni corrections to further analyse the results. Statistical significance was set to $p<0.05$. Unless otherwise stated all results are expressed as mean values ± 1 standard deviation (SD).

### 5.1.2. Results

None of the subjects reported side effects from the experiments. 16 participants completed the study. Seven further participants (5 men and 2 women), recruited for the study, were unable to complete the experiments because either they found cerebellar stimulation too uncomfortable or after a practice session of 30 minutes they could not constantly maintain their right ADM quiet enough (background EMG activity less than 100μV) while they were performing the task.

**Surround Inhibition**

Two-way repeated measures ANOVA revealed significant difference of MEP amplitudes in ADM and FDI at rest and on the onset of the movement. We found significant main effects of MUSCLE (levels: ADM and FDI) ($F(1,15)=78.20$, $p<0.01$), and CONDITION (levels: Rest and Onset of the movement) ($F(1,15)=88.66$, $p<0.01$) and their interaction MUSCLE × CONDITION ($F(1,15)=134.55$, $p<0.01$). Post hoc pairwise comparisons demonstrated significant mean difference for the factor MUSCLE [$p<0.001$, mean difference=3.80 (95%CI= 2.88 – 4.72)] and for the factor CONDITION [$p<0.001$, mean difference=2.11 (95%CI= 1.64 – 2.59)] (Fig. 5.1.1, 5.1.2). The significant suppression of ADM MEP size confirms the existence of surround inhibition in our participants.

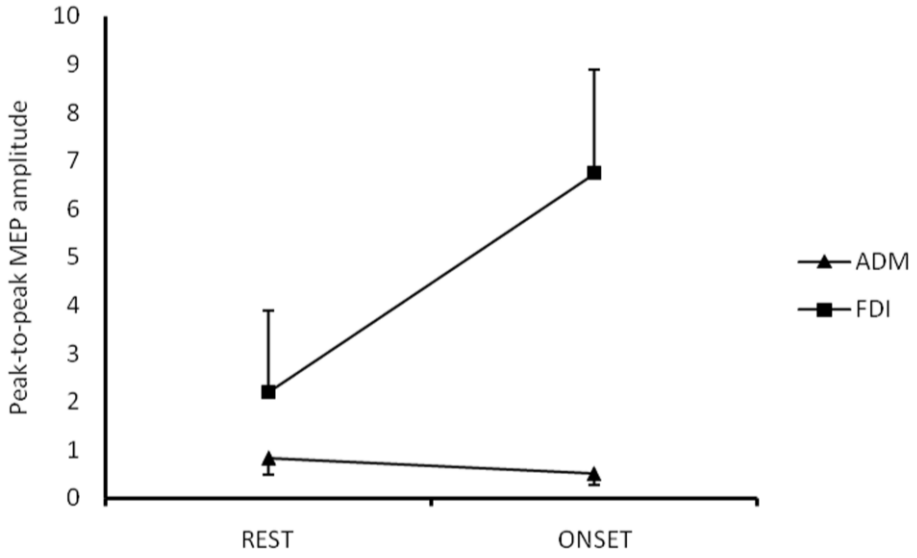


*Fig. 5.1.1 Surround inhibition. FDI is highly facilitated ($p<0.01$) at the onset of the movement. Non-active ADM is suppressed due to SI ($p<0.01$). Error bars indicate SD*

**Cerebellar Brain Inhibition**

We expressed CBI as the ratio of MEP amplitudes of conditioned responses to MEP amplitudes of unconditioned responses. An increase in this ratio therefore indicates a reduction of CBI. Repeated measures ANOVA revealed significant effects of the factor CONDITION (levels: Rest and Onset of the movement) ($F(1,15)=6.48$, $p=0.02$) and no significant effect of the factor MUSCLE ($F(1,15)=0.22$, $p=0.65$) or their interaction MUSCLE x CONDITION ($F(1,15)=0.08$, $p=0.78$) (Fig. 5.1.2, 5.1.3). Post hoc pairwise comparisons showed significant mean difference of the factor CONDITION=0.27(95%CI= 0.04 – 0.50) due to a reduction in CBI at the onset of the movement compared to CBI at rest in both muscles.

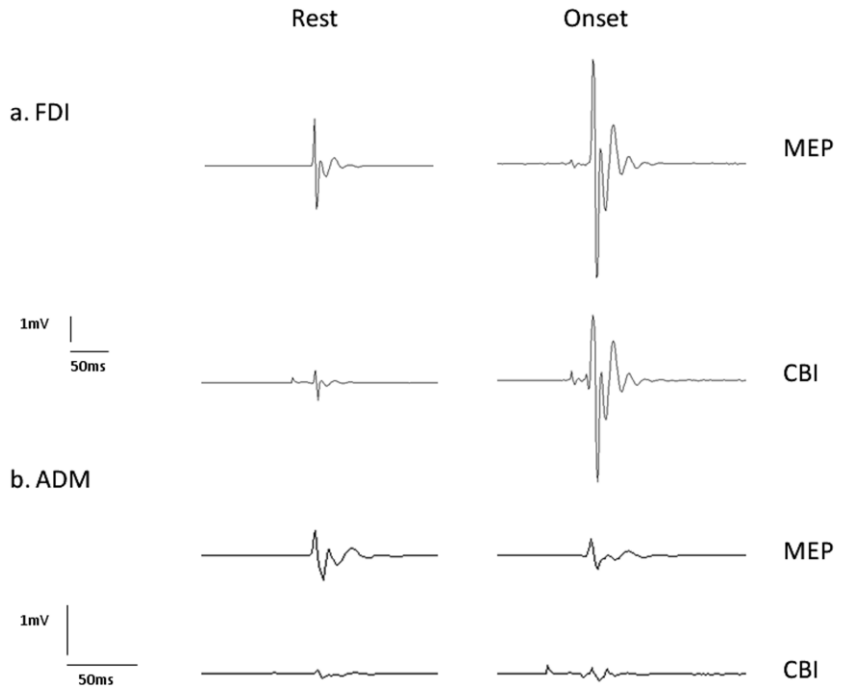

*Fig. 5.1.2 Example trace of raw data from one subject showing an increase in FDI MEP and b decrease in ADM MEP at the onset of movement with a corresponding decrease in CBI in both muscles. Note that the scales for traces recorded from FDI and ADM are different for the sake of clarity of the figure.*

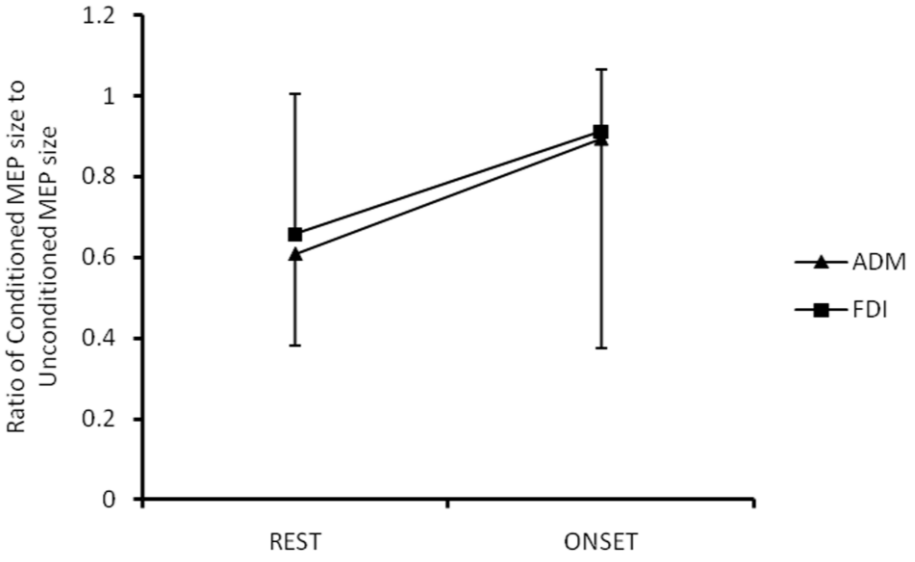


*Fig. 5.1.3 Significant decrease of CBI was found in both muscles (P = 0.02). CBI reduction is not significantly different in the two muscles (P = 0.65). Error bars indicate SD*

**MEP matching**

MEP sizes in FDI and ADM changed significantly at movement onset, due to muscle activation (in FDI) and SI (in ADM). In order to determine if the change in MEP size itself might be responsible for any changes in level of CBI [89] at the onset of movement we performed further recordings of CBI at the onset of the movement in 6 subjects with adjusted TS intensity. Firstly, we increased the intensity of the motor cortex stimulation to a level at which the MEP responses in ADM elicited by the TS alone at the onset of the movement were of the same amplitude as the MEP responses we recorded at rest. Then, we used this new intensity to record CBI at the onset of the movement. We did the same for FDI but this time we decreased the TS intensity in order to achieve MEPs at the onset of the movement of the same amplitude as the ones we recorded when the muscle was relaxed (Mean TS intensity for the main experiment was 52% of the maximum output of the stimulator – range from 36% to 70%, Mean TS intensity for ADM matching experiment was 55% of the maximum output of the stimulator – range from 39% to 75%, Mean TS intensity for FDI matching experiment was 34% of the maximum output of the stimulator – range from 23% to 45%). Paired samples t-test showed that there was no significant difference between the MEP size at rest and the matched MEP size at the onset of the movement for both ADM ($t(5)=1.27$, $p=0.27$) and FDI ($t(5)=0.34$, $p=0.75$). Repeated measures ANOVA revealed no significant effect of the factors GROUP (levels: CBI at movement onset, CBI at movement onset with matched MEPs) ($F(1,5)=3.14$, $p=0.14$) or MUSCLE (levels: ADM, FDI) ($F(1,5)=0.11$, $p=0.75$) or their interaction GROUP x MUSCLE

(F(1,5)=3.10, p=0.14) (Fig. 5.1.4). This indicates that the reduction in CBI observed in ADM and FDI at the onset of movement cannot simply be explained by the change in MEP size occurring at this time in ADM and FDI.

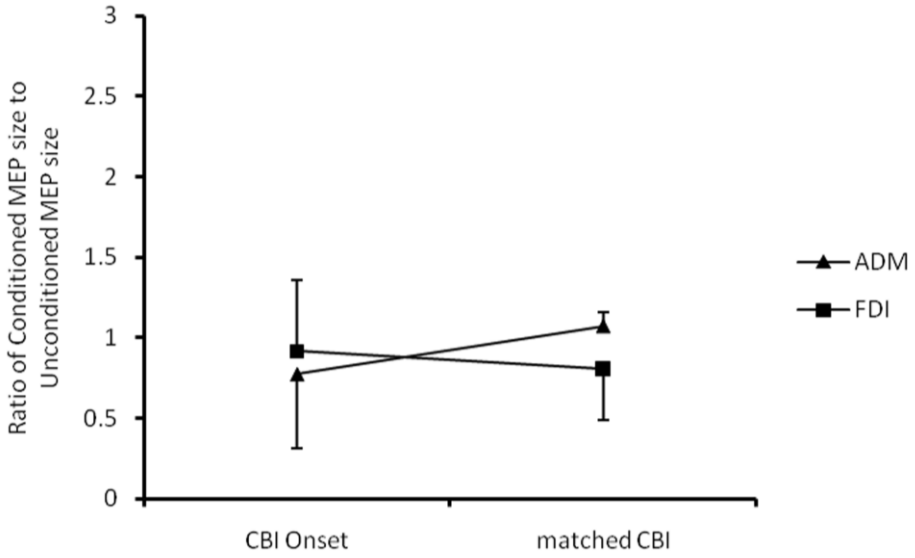


*Fig. 5.1.4 MEP matching on the onset of the movement. There is no significant difference between CBI at movement onset and CBI with TS size adjustment. Increased TS intensity was used for matched CBI in ADM and decreased TS intensity for matched CBI in FDI. Error bars indicate SD.*

### 5.1.3. Discussion

With this study we demonstrated that CBI is reduced in both active and surround muscles at the onset of movement. While our initial hypothesis that there may be muscle specific modulation of CBI at onset of movement in parallel with SI was not confirmed, the data do provide novel evidence of a change in cerebellar inhibitory drive to the motor cortex at onset of movement.

Our data extend the findings of one previous study that has explored the effect of muscle activity on CBI. Pinto and Chen (2001) [91] compared CBI in FDI at rest and when FDI was relaxed, but subjects also maintained their ipsilateral or contralateral arm outstretched. Activation of ipsilateral proximal arm muscles led to a significant reduction of CBI in FDI. However, this study only examined the effect of tonic muscle contraction in a distant muscle, and any possible effects of prolonged shoulder extension on the MEP size in the otherwise relaxed FDI were not controlled for [91].

In both active FDI and the surround muscle ADM we identified the same amount of reduction of CBI at movement onset, the time at which the effects of SI are most prominent [28, 37]. Identical CBI reduction in both active and surrounding muscles makes it unlikely that this specific cerebellar inhibitory mechanism is responsible for driving inhibition of surround muscles. What might, therefore, be the contribution of this reduction in cerebellar inhibitory drive to movement preparation and execution?

There is evidence to show that cerebellum is involved in movement initiation processes. Changes in the blood flow in the ipsilateral cerebellar hemisphere are associated with changes in reaction time of voluntary movement [97]. In addition, patients with

cerebellar dysfunction have increased reaction time [98] and moreover ischemic lesions in the cerebellum lead in decreased premovement corticospinal excitability [99]. These findings imply that the cerebellum may have a role in movement initiation, and therefore it is possible that modification of CBI could contribute to the implementation of this function. Furthermore, according to the model proposed by Houk and Wise (1995) for planning and controlling movement, the triggering process for a movement may be different from the programming process. In this regard the cortical-cerebellar loop is hypothesised to be involved in triggering the initiation of the action command [100]. Within this model, our finding of a non-muscle specific CBI reduction at the onset of the movement fits with a triggering role for the cerebellum through withdrawal of motor cortex inhibition. In contrast, SI may be more important for the programming process through muscle-specific regulation of corticospinal excitability. It would be of interest to further explore the time course of modulation of CBI in the preparation and execution phases of movement.

Although the role of afferent cerebellar input in voluntary movement initiation and execution is not well understood, it is known that CBI still exists even when cerebellar input pathways are damaged [94]. Lack of CBI dependence on input from the periphery implies that it is highly unlikely for CBI to have a corrective role, but it does not exclude the possibility that it has a role in preparedness for possible future corrections. Reduction of inhibition in both active and surrounding muscles at the onset of the movement might be responsible for bringing the motor system into a state where future corrections can be efficiently performed even if they implicate surrounding muscles, for example to allow for rapid adjustments to improve movement stability.

During the MEP recordings, TMS stimulation was given immediately on the onset of the movement (0ms delay), when EMG activity exceeded the peri-triggering threshold. For CBI recordings at the onset of the movement the CS was given at the onset of the movement (0ms delay), and the TS 5ms later (5ms delay). Although this introduces a small time difference in the two recordings, previous studies examining SI have found that the inhibitory effect on the surround muscle only begins to disappear 100ms after the onset of the movement [28]. Therefore, a delay of 5ms in the timing of the TS delivery is highly unlikely to have had any significant effect on the results. We included one left handed subject, and are aware that surround inhibition has been reported to be asymmetric [39], being less marked on the non-dominant side. However, the results of this subject with regard to SI (MEP amplitude in ADM at rest/MEP amplitude in ADM on the onset=0.59) and change in CBI at movement onset (MEP amplitude elicited by conditioned stimulation/MEP amplitude elicited by unconditioned stimulation in ADM at rest=0.88, on the onset=1.16, in FDI at rest=0.87, on the onset=0.97) were of a similar direction and magnitude to the group means.

In conclusion, we found that CBI is modulated at the onset of a brief movement in the active FDI muscle and the surrounding ADM muscle. This does not provide evidence of a functional link between CBI and SI. Instead, we found significant non-topographically specific reduction in the excitability of cerebello-thalamo-cortical inhibitory connections at movement initiation which implies a potential role for the cerebellum in triggering the onset of voluntary movement.

## 5.2. **Surround inhibition modulation by tonic cerebellar output**

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As mentioned in the introduction it is still not known which structures within the central nervous are important for the generation of SI. Some favour a neocortical mechanism following the observation that hemispheric dominance and task difficulty modulate the magnitude of SI[29, 39] . However electrophysiological studies examining the dependency of SI on dorsal and ventral premotor and motor cortex interactions to date have failed to support this notion[35, 36].

The cerebellum plays a major role in temporal encoding and coordination of movements and deficiencies in hand control and individual finger movements are seen in patients with cerebellar disease[60]. It also has a net inhibitory effect on the cerebral cortex via the cerebello-dentato-thalamo-cortical pathway[60]. These characteristics make the cerebellum a suitable candidate to functionally contribute to the generation of SI.

In Chapter 5.1 we examined cerebellar brain inhibition (CBI) during individual finger movements and we demonstrated a nonspecific decrease in cerebellar inhibition to active and surround muscles at the onset of movement but no functional link between SI

and CBI [101]. However, CBI relies on a powerful (and painful) phasic non-topographically specific magnetic stimulation of the cerebellum that may not reveal subtle changes in paradigms such as SI. As an extension of the previous study on the role of cerebellum on SI, in this study we utilised cerebellar transcranial direct current stimulation (tDCS), which has emerged as an important technique by which to enhance (anodal) or decrease (cathodal) cerebellar excitability. This effect has been confirmed neurophysiologically (measuring CBI) and behaviourally (measuring rates of adaptation to sensory perturbations, a cerebellar-dependent learning task); anodal tDCS increases CBI and leads to faster rates of adaptation and cathodal tDCS decreases CBI [62, 102]. In addition, tDCS can be used to assess the cerebellar contribution to neurophysiological paradigms; recently, the cerebellum was shown to be a critical structure for the generation of motor cortex plasticity responses to paired associative stimulation (PAS) with an interstimulus interval of 25 ms[103]

Our hypothesis was that stimulatory anodal tDCS would enhance SI and cathodal tDCS would impair SI. Investigating techniques that may have the potential to modulate SI is important for patients with disorders such as focal hand dystonia and Parkinson's disease in which impaired SI is seen [40, 104]. The multiple session design of this study gave us additionally the opportunity to assess intrasubject and intersubject variability of SI.

### 5.2.1. Methods

**Subjects**

Twelve right-handed healthy subjects (mean age, 25 years; range, 19–35 years; 9 male) with no history of neurological or psychiatric disease participated in the study. Handedness was determined by the Edinburgh Handedness Inventory. Written informed consent was obtained from all participants, and the study was approved by the local ethics committee and conducted in accordance with the Declaration of Helsinki 2008.

**Recording**

Disposable surface silver-silver chloride electromyographic (EMG) electrodes were placed on right first dorsal interosseus (FDI) and abductor digiti minimi (ADM) muscles using a belly-tendon montage. The signal from the EMG electrodes was amplified (gain, 1000), bandpass filtered (20–2000 Hz) (Digitimer D360 amplifier) and digitized at a sampling rate of 5 kHz and stored in a laboratory computer for off-line analysis by CED 1401 hardware and Signal software (Cambridge Electronic Design Ltd).

**Transcranial magnetic stimulation**

Monophasic transcranial magnetic stimulation (TMS) pulses were delivered from a Magstim $200^2$ stimulator. A figure-of-eight coil (external loop diameter of 9 cm) was held tangentially on the scalp at an angle of 45° to the midsagittal plane with the handle pointing laterally and posteriorly to deliver the pulses. Corticospinal tract excitability was measured as the peak-to-peak amplitude of the motor evoked potential (MEP) generated by single pulse TMS. TMS was applied to the motor "hot-spot" of the right ADM muscle that was defined as the point where a magnetic stimulus of slightly suprathreshold intensity consistently elicited a MEP in ADM of the highest amplitude. This position was marked on a tight fitting neoprene cap in order to ensure consistent coil position during the experiment.

**Cerebellar transcranial direct current stimulation**

tDCS was applied to the cerebellum as described previously[62]. It was delivered with an intensity of 2mA, using a DC stimulator through 25 cm2 saline-soaked surface sponge electrodes (Eldith-Electro-Diagnostic & Therapeutic Systems GmbH, Germany). One electrode was centred on the right cerebellar cortex, 3-cm lateral

to the inion and the other electrode was positioned on the right buccinator muscle [102]. Anodal or cathodal tDCS was delivered over the cerebellum for 15 minutes.
In the sham session, anodal tDCS was applied for 30 seconds in order that a true sham condition was simulated (some subjects experience tingling at site of electrodes when stimulation is initiated). At the onset and offset of all interventions (anodal, cathodal and sham), current was changed in a ramp-like manner over 10 seconds. Subjects were supervised during tDCS and listened to a radio documentary. They were asked to keep all movement, specifically finger movements, to a comfortable minimum.

**Motor task**

Subjects were seated in a chair with their right hand resting in a relaxed position on a desk. They were asked to briefly depress a small button with the index finger after a 'go' signal (an auditory tone of 50 ms) with a self-paced delay. FDI is a synergist rather than a primary muscle for this movement and previous studies have shown that this movement induces activation of FDI and suppression of the MEPs elicited in the ADM muscle[28]. Subjects were first asked to press with maximal force, and amplitude of mean EMG activity in FDI was noted. Subjects were then trained to perform the movement to the amplitude of 10% maximal EMG activity while visual feedback of the muscle activity was projected on a screen in front of them. Duration of the movement was approximately 100 ms. We favoured a short movement duration to facilitate production of a clean onset and offset of EMG activity as SI has been found to be active

only during the initiation of the movement and not later during tonic muscle contraction[28]. Subjects were also asked to keep the surround muscle ADM relaxed while they were performing the movement. Training was continued until subjects achieved consistent performance of the desired movement and EMG signal in ADM muscle was not in excess of 100 μV.

**Experimental design**

Each subject took part in a cross over study, which consisted of each of the three types of stimulation (sham, cathodal or anodal) in randomised order. Each session was separated by a week. Resting motor threshold (RMT) was measured and was defined as the lowest intensity [expressed as a percentage of maximum stimulation output (MSO)]that evoked a response of about 50 μV in the relaxed ADM in at least five of ten trials[105]. The intensity of the stimulation was then set to evoke ADM MEPs with average peak-to-peak amplitude of approximately 1 mV at rest for the remainder of the experiment.

For the assessment of SI, five states of self-triggered TMS were applied in a random order at variable intervals between EMG onset and TMS trigger (0, 50, 100, 200 ms and 5 seconds). This allowed us to assess the magnitude of SI at time 0ms and also assess if tDCS induced changes in the timing profile of inhibition/SI at later time intervals. The TMS pulse was triggered when EMG signal of right FDI rose above 100 μV. Twenty

trials of 5 seconds (rest) and 15 trials of the other four intervals (0, 50, 100 and 200 ms) were collected. Five seconds after the onset of movement is considered to be sufficient for measurements at rest as no post-activation inhibitory or facilitatory effect are known to be active at this time[28].

**Data analysis and statistics**

For each subject peak-to-peak MEP amplitude for each trial was measured off-line and the mean MEP at rest and at each time interval was calculated. For each interval, mean MEP amplitude was then divided by mean rest MEP amplitude for the respective muscle (labelled in graphs as percentage of resting MEP). If the ratio is less than 1, there is evidence for SI. When it is greater than or equal to 1, there is no SI. Unless otherwise stated, all results are expressed as mean ± standard error of the mean (SEM). We used SPSS software (version 19) for statistical analysis (SPSS Ltd., IBM, Armonk, NY, USA). Kolmogorov–Smirnov test was used to explore the normality of the data distribution, and Levene's test was used to explore the homogeneity of variance. Log10 transformation was performed when data were not normally distributed. Repeated measures analysis of variance (rmANOVA) was used to confirm the presence of SI in ADM and to assess the effects of tDCS on the magnitude of SI before and after stimulation. Bonferroni's correction for multiple comparisons was used for post hoc t tests. To quantify intrasubject and intersubject variability, the coefficient of variation

(COV) was expressed as a percentage. The COV is the ratio of the standard deviation to the mean.

### 5.2.2. Results

All subjects completed the three sessions without any adverse events, and each experimental session lasted 2 hours.

**Baseline measures**

The mean stimulus intensity for RMT of ADM across the 3 sessions for all patients was 41% (±2.3%). The stimulus intensity required for a 1mV MEP in ADM ranged from 38% to 80% across patients with a mean value of 57% (± 3.4%). The mean stimulus intensity required for a 1mV MEP in ADM was on average 137% of the RMT.

**SI present in ADM**

Fig. 5.2.1 demonstrates the profile of MEP sizes in the FDI and ADM muscles for each of the intervals tested. MEPs are expressed as % resting MEP and the group mean is derived from the individual mean of the 3 baseline measurements of SI taken at each

session. Log10 transformation was performed and the data satisfied the assumptions for parametric tests after the transformation. One-way rmANOVA revealed a significant effect of INTERVAL in the ADM muscle $F(3) = 22.84$, $p < 0.001$ and FDI muscle $F(3) = 15.84$, $p < 0.001$ (Fig. 5.2.1).

Post hoc paired sample t-tests of raw MEP data at rest (5 s) and during movement (0 ms, 50 ms, 100 ms and 200 ms) revealed that SI was present at time interval 0 ms, thus MEPs in ADM were significantly inhibited at time interval 0 ms $t(11) = 4.93$, $p < 0.001$. There was no significant inhibition at the other time intervals and it can be seen from Fig. 5.2.1 that the MEP size gradually increases. Only one subject had a mean ADM MEP amplitude at the onset of the movement (interval 0 ms), which was not less than the resting MEP (mean SI = 1.12 ± 0.04 across three baseline sessions). The MEP was still suppressed in this subject (as there is an increase in spinal excitability at 0 ms [2]), but it is not by definition inhibited. In FDI, there was significant enhancement of MEP amplitudes at all of the time intervals (0, 50, 100 and 200 ms) compared with rest (0 ms $t(11) = -8.77$, $p < 0.001$; 50 ms $t(11) = -5.46$, $p < 0.001$; 100 ms $t(11) = -4.27$, $p = 0.001$; 200 ms $t(11) = -3.45$, $p = 0.005$).

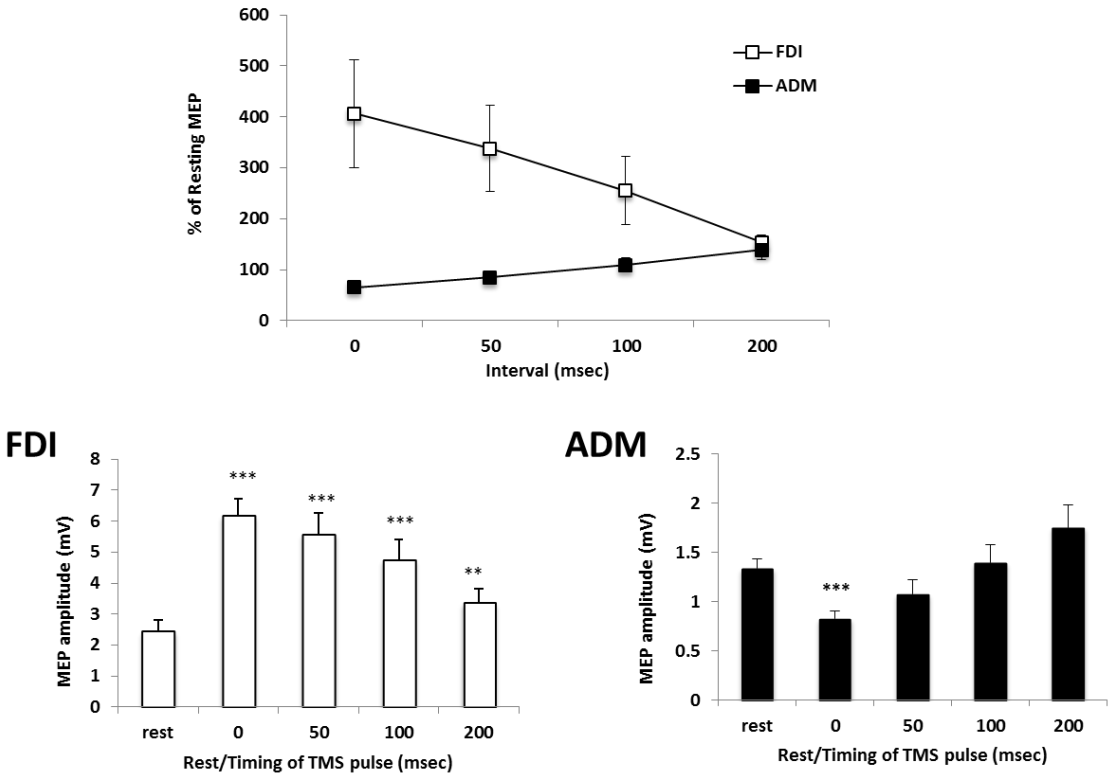


*Fig. 5.2.1. Profile of SI. This figure demonstrates the group mean of the individual means across the three baseline sessions. In the upper panel, the normalised data are shown for both muscles. Raw MEP data are given for individual muscles below. The surround muscle ADM is significantly inhibited at time interval 0 ms. Note the reduction of variability in the ADM muscle MEPs (as indicated by the error bars demonstrating the standard error). The active muscle FDI is facilitated at the onset of movement and the later time intervals tested (∗∗$p \leq 0.01$; ∗∗∗$p \leq 0.001$).*

**Effect of tDCS on SI**

To explore the effect of tDCS on SI, we looked at the magnitude of SI at 0 ms in the muscle ADM at each of the time points measured (baseline, T0, T20) (Fig. 5.2.2A). rmANOVA with factors TIME (baseline, T0, T20) and tDCS (sham, anodal, cathodal) revealed no significant effect of TIME [$F(2,10) = 1.09$, $p = 0.35$], tDCS [$F(2,10) = 1.03$, $p = 0.38$] or their interaction [$F(4,8) = 1.05$, $p = 0.39$]. There was also no significant effect of tDCS on MEP profile at any of the other intervals tested (50, 100 or 200 ms) (Fig. 5.2.2B–D). On the basis of these results, we conclude that the cerebellum does not seem to have a role in the generation of SI.

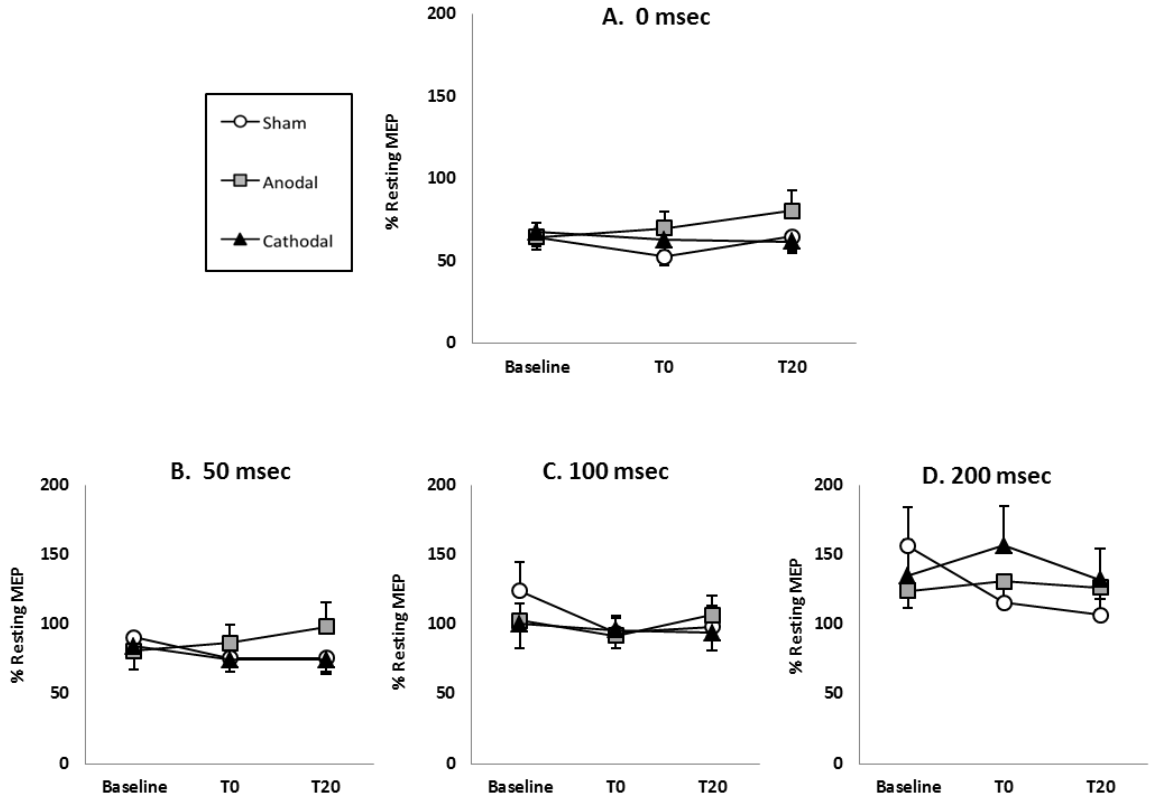


*Fig. 5.5.2. Effect of sham, anodal and cathodal tDCS on the magnitude of SI in ADM. There was no significant modulation of the magnitude of SI by tDCS.*

**Intra-subject and inter-subject variability of SI**

In order to quantify variability of SI we examined SI seen in ADM at the onset of index finger movement (interval 0 ms) as measure of 'maximal' SI (see Table 5.2.1). Intrasubject variation of SI (range of SI responses exhibited by a single subject) as assessed by COV had a mean value of 27% (range from 14% to 48%). Inter-subject variability (different subjects) had a mean value of 44% (range from 40% to 46%).

| Subject | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | Inter-subject COV (each session) | Mean (n= 12) Inter-subject COV |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **SI (session 1)** | 0.29 | 0.39 | 0.47 | 0.56 | 0.62 | 0.42 | 0.84 | 0.94 | 0.41 | 1.02 | 0.80 | 0.94 | **40%** | **44%** |
| **SI (session 2)** | 0.46 | 0.42 | 0.72 | 0.37 | 0.44 | 0.66 | 0.65 | 0.51 | 1.17 | 0.56 | 0.52 | 1.26 | **45%** | |
| **SI (session 3)** | 0.35 | 0.31 | 0.26 | 0.53 | 0.46 | 0.56 | 0.78 | 0.82 | 0.81 | 0.89 | 1.20 | 1.14 | **46%** | |
| **Mean SI for each subject** | **0.37** | **0.37** | **0.48** | **0.49** | **0.51** | **0.55** | **0.76** | **0.76** | **0.80** | **0.82** | **0.84** | **1.12** | | |
| **Intra-subject COV (individual values)** | **24%** | **15%** | **48%** | **22%** | **19%** | **22%** | **13%** | **29%** | **47%** | **28%** | **41%** | **14%** | | |
| **Mean (n=12) Intra-subject COV** | **27%** | | | | | | | | | | | | | |

*Table 5.2.1. Intrasubject and intersubject variability of SI exhibited in ADM muscle at the onset of movement (interval 0 ms). Values are shown for each session before any stimulation. Each measure of SI is given as a ratio of mean resting MEP for ADM (normalised values). Intrasubject and intersubject variability are expressed using the coefficient of variation (COV).*

### 5.2.3. Discussion

Motor surround inhibition was clearly demonstrated across subjects; at 0 ms there was consistent and statistically significant inhibition of MEPs in ADM. The study design allowed three measures of SI on different sessions in the same subjects and SI was confirmed to be stable within subjects. Given the intrinsic variability of MEPs this marks out the measurement of SI a robust and reproducible TMS paradigm. This is in contrast to some other commonly used electrophysiological paradigms. For example, a common measure of motor cortex plasticity is paired associative stimulation (PAS) in which repetitive pairing of median nerve stimulation and TMS pulses to the motor cortex lead to facilitation of MEPs in APB [106]. However, if individual PAS responses are displayed, it is seen that some subjects have facilitatory responses while others have inhibitory responses to PAS. Furthermore, if PAS is tested in the same subjects at another session, the direction of the MEP response may change, subjects can switch between facilitators and inhibitors and vice versa [107]. This is not seen with SI when tested across the three sessions and quantified by the COV (Table 5.2.1). This reemphasises the importance of the deficiency of SI seen in diseases of motor control such as focal hand dystonia and Parkinson's disease[29] . Attempting to modulate the strength of SI, as in this study, remains an important potentially therapeutic goal in neurophysiological studies of SI.

SI is defined as the functional inhibition of surround muscles seen during the movement initiation phase (and just before and during the first phase of EMG onset [29]. The mechanisms of how and where it is generated are less well characterised. At the spinal level there is a non-spatially selective facilitation at these time points (shown by F-wave and H-reflex studies) and thus SI is thought to reflect a supraspinal control mechanism. We find no evidence that modulating the cerebellum in isolation can change the magnitude of SI. This adds to previous work examining CBI, which did not find a functional link between SI and CBI [101]. In addition, no association between activity in premotor cortex (both ventral and dorsal) and SI has been demonstrated[35, 36]. It may be that SI is a fundamental inhibitory mechanism within the nervous system and subtle alteration of the activity of one of the nodes within the SI network does not allow a meaningful change in SI to be observed. Alternatively the genesis of SI may reside within other areas such as the basal ganglia nuclei. It should be possible in the future to explore this hypothesis by measuring SI in patients with Parkinson's disease or dystonia before and after deep brain stimulation.

At the synaptic level a GABA-ergic mechanism for SI has been proposed largely based on animal work[29]. In humans, proving the link between GABAergic circuits and SI is less certain. No functional link has been shown between SI and short intracortical inhibition (SICI) and cortical silent period, which are indirect markers for $GABA_A$ and $GABA_B$ receptor function respectively[28, 33]. Other inhibitory projections to M1 are reduced at the onset of movement and do not consistently demonstrate the action

specific modulation of muscle excitability unique to SI (LICI, SAI, LAI, IHI, CBI)[29, 101].

There is increasing evidence that SI is an adaptive phenomena. It has previously been shown that SI is more pronounced in the dominant hemisphere, is stronger with low force levels, and starts earlier with increasing task difficulty [37-39]. More recently it has been demonstrated that the magnitude of SI is increased by carefully timed vibration training[86] (*this study is presented in Chapter 6*). Conversely, 30 minutes of finger exercises with synchronised movements of the index and little finger in contrast to little finger movements alone, reduces the magnitude of SI, perhaps blurring individuation of digits as measured by SI or implicating a role for fatigue on SI modulation[85].

The failure of tDCS to modulate SI was surprising. We believe tDCS to be an excellent tool to explore the functional network that contributes to SI; indeed in the visual cortex anodal tDCS has recently been found to change surround suppression, a comparable paradigm to SI in the visual system[108]. It is an interesting question whether the degree of adaptation of SI may be increased or decreased by stimulation techniques; one might expect tDCS to modify the adaptation seen with vibration training.

Further characterisation of SI remains a challenging field. It is worth restating that the first study of SI found comparable amounts of inhibition in ADM when the paradigm is

triggered by mouth or leg movement (risorius: 77%; tibialis anterior: 68%)[28] . This finding has never been replicated but suggests a less spatially specific mechanism for SI than is currently discussed, particularly when SI is mentioned in the context of models of focal hand dystonia. Additionally the current literature freely moves between using the term surround inhibition as a cellular mechanism in the senses, neurophysiological mechanism in motor (SI) and sensory systems (SSEPs[46]), as a mechanism for selecting motor programmes[109] and as an explanation for psychophysical phenomena[110]. To move away from a purely descriptive term that represents the capability of organisms to attach saliency to inputs or produce specific commands, we must examine the similarities and differences between surround inhibition at each hierarchical level and modality to understand its mechanisms further.

A limitation of our study is that subtle differences in experimental conditions across the three sessions may have led to incorrect acceptance of the null hypothesis that the cerebellum does not functionally contribute in the generation of SI (both subject dependent, e.g. level of attention to task and experimental, e.g. differences in placement position of TMS coil). We considered increasing the number of subjects but as no trend was seen in our 12 subjects we consider the acceptance of the null hypothesis to be correct.

We find SI to be a robust electrophysiological phenomenon with minimal intrasubject variability over the three sessions in this study. Quantification of intrasubject variability

in this study will allow future therapeutic studies that attempt to modulate SI to be adequately powered. We do not find evidence to suggest that the cerebellum contributes to the neuroanatomical network necessary for the generation of SI. We have reviewed the current literature on SI and identify important future challenges in the field that need further investigation so that the physiology of SI and its deficit in certain diseases is more clearly understood.

# Chapter 6. BRAIN PLASTICITY AND MOTOR SURROUND INHIBITION

## 6.1. **Adaptation of motor surround inhibition**

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Sensory error signals are hypothesised to be the instructive signals for several forms of learning including motor learning. The occurrence of sensory error (for example mismatch between proprioceptive afferent signal and expected signal) leads to feed-forward adaptation of the motor command [111], a process that involves the cerebellum [102, 112]. Motor adaptation has been extensively studied using a variety of motor learning paradigms where sensory error is introduced via manipulating visual or proprioceptive feedback [113-117]. Sensory error appears to require certain characteristics in order to be judged as relevant to the movement and drive adaptation [118-121]. For example, if a sensory input is widely separated from an event in space and time the nervous system infers that it is not related to the event and processes it separately[121].

Fine control of hand function requires a balance between the active and the non-active adjacent muscles: unwanted activation of surround muscles may interfere with accurate task performance. The phenomenon of lateral or “surround” inhibition (SI) has been

proposed to play an important role in this regard. As discussed above surround inhibition has been recognised as an important property of the sensory (e.g. visual) system for some time, but it is only more recently that it has been described in the motor system, specifically in relation to individuation of finger movements [28]. Active inhibition of the surround muscles during a movement is proposed to enhance motor performance and lack of SI in patients with dystonia is proposed as one mechanism behind their abnormal movement control [30]. The physiological mechanism behind the generation of SI remains unclear but its presence in the motor system as a neurophysiological phenomenon is widely accepted. In the experimental paradigm traditionally used to examine SI, subjects are asked to press a button by flexing their index finger while maintaining the surround muscles silent. When subjects initially try the task it is not always immediately possible to keep the surround muscles at rest and a brief practice session is usually required to attain consistent motor performance [30, 37]. It is well known that it is hard for humans [122] and for primates [123] to perform individual finger movements without invoking motion of adjacent fingers. Presumably, during these first trials unintended contractions are noted and the motor command for future trials is updated in a feed forward manner through an adaptation process as described above. If SI adapts in this manner, it should be possible to use electrophysiological techniques to record these adaptive changes.

Here we investigated the proposal that SI is adaptable and that its adaptation involves a process of error-based learning that depends on sensory feedback. Specifically, we hypothesised that sensory feedback indicating unwanted contraction of surround

muscles will induce adaptive changes in the strength of SI. We interpolated false feedback signals from muscle spindles by applying vibration to a surround muscle during movement. Importantly, in order to achieve causal inference of the spindle stimulation and the movement, we accurately matched the timings of the movement and the stimulation. To control for a general effect of vibration on SI, in a separate session we performed surround muscle vibration with a short delay (100ms) after movement onset. We expected that as with adaptation in other paradigms, a learning effect would persist when the error signal was withdrawn, and would slowly return to baseline, and that this effect would only be seen with prior exposure to vibration closely timed to movement onset.

### 6.1.1. Methods

**Participants**

A total of 30 right-handed healthy adults with no history of neurological disorder took part in two experiments. Written informed consent was obtained from all participants and the study was approved by the local ethics committee and conducted in accordance with the Declaration of Helsinki.

**EMG recordings**

Electromyographic (EMG) activity was recorded from right first dorsal interosseus (FDI) and right abductor digiti minimi (ADM) muscles using a pair of Ag–AgCl surface electrodes in a belly-tendon montage. The EMG signal was amplified (1000x) and band-pass filtered (bandwidth 20–2,000 Hz) with a Digitimer D360 amplifier (Digitimer Ltd, UK), digitized at a sampling rate of 5 kHz (CED 1401 laboratory interface; Cambridge Electronic Design, Cambridge, UK) and fed into a laboratory computer for storage and off-line analysis. Data were analysed using SIGNAL software V4.00 (Cambridge Electronic Design, Cambridge, UK).

**Motor task**

During the experiments, the subjects sat in a comfortable chair with their right hand resting on a desk. While their hand was lying flat and relaxed on the desk, the tip of their index finger was placed on a small button. They were asked to briefly depress the button after a ‘go’ signal (an auditory tone) with a self-paced delay, by flexing their index finger at the metacarpo-phalangeal joint. FDI is a synergist rather than a primary muscle for this movement but previous studies have shown that this movement induces activation of FDI and suppression of ADM through SI [28]. Subjects were asked to perform the movement with 10% of their maximum EMG activity and we provided training, including visual feedback, to allow them to achieve this target force intensity. Duration of the movement was aimed to be approximately 100ms and the subjects were

also asked to keep their ADM muscle relaxed while they were performing the movement. Visual feedback of the EMG activity from both muscles (FDI and ADM) was displayed on a screen in front of the subjects during a brief training session before the start of the experiments. The training was needed for a consistent performance of the desired movement to be attained by the subjects with EMG activity in ADM not in excess of 100 μV. During this session verbal instructions were given to subjects.

**Vibration**

Vibration was applied to the right ADM muscle using an electromagnetic mechanical stimulator (Ling Dynamics System) with a 3 cm diameter circular probe. The probe was positioned orthogonally to, and under slight pressure against, the belly of the right ADM between the EMG electrodes. The frequency of the vibration was 80Hz and the amplitude was 0.2– 0.5 mm [63]. Vibration of the same properties has been found to be effective for stimulation of the muscle spindle primary endings (Ia fibres) [64, 124]. Duration of stimulation was 100ms, similar to the duration that SI has been found to be active after the onset of a brief contraction of a hand muscle [28]. Spatial attentional focus has been found to significantly influence the effects induced by vibration on the intrinsic hand muscles [125, 126]. Therefore, in order to ensure similar attentional levels between sessions, subjects were asked to look at their vibrated hand, to focus their attention on the vibrated muscle and to count and report the number of cycles of vibration that they received. Vibration at movement onset was achieved using the peri-

triggering function of SIGNAL software which was set to trigger the stimulator immediately when EMG activity above 100 μV was detected in right FDI. During vibration, EMG activity of both muscles was monitored for voluntary activation or induction of the tonic vibration reflex [65].

**Transcranial magnetic stimulation**

A figure-of-eight shaped coil (external loop diameter of 9 cm) connected to a monophasic Magstim 200 stimulator (Magstim Co, Carmarthenshire, Wales, UK) delivered transcranial magnetic stimulation (TMS) over the left motor cortex. The intersection of the coil was positioned tangentially on the scalp over the left motor cortex. The handle of the coil was pointing backwards and laterally at a $45^0$ angle to the saggital plane in order to induce transsynaptically a posterior–anterior directed current in the brain to activate the corticospinal tract [72, 73]. The "hot spot" was defined as the optimal scalp position for eliciting motor evoked potentials (MEPs) of maximal amplitude in the contralateral ADM and it was marked with a felt pen in order to ensure consistent coil position during the experiment. The intensity of the stimulation was set to evoke MEPs with average peak-to peak amplitude of approximately 1mV at rest in the right ADM. For the assessment of SI, single TMS pulses were delivered at rest and at the onset of the movement. 20 trials were collected at rest and 20 separate trials at the onset of the movement (40 trials in total) in a randomised way by using the peri-triggering function of SIGNAL software. Peak-to-peak MEP amplitude for each trial

was measured off-line. For assessment of MEP size at the onset of the movement TMS was set to be triggered immediately when EMG activity in right FDI above 100 μV was detected. For the assessment of MEP size at rest, TMS pulses were delivered 5 seconds after the onset of the brief movement. This time point is considered to be sufficient for measurements at rest since the duration of the movement was aimed to be 100ms, meaning that the pulse was delivered with a delay of approximately 4900 ms after the end of the movement when neither SI or any other post activation inhibitory or facilitatory effect are known to be active and corticospinal excitability has returned to baseline [28]. The time interval between each self-paced movement and the next 'go' signal was 10s, so the shortest time interval between two consecutive TMS pulses was at least 5s plus the self-paced delay.

**Experimental design**

*Experiment 1: Timed vibration*

Vibration was applied to the belly of ADM for 100ms starting immediately at the onset of FDI contraction ($VIB_{onset}$). Subjects were asked to repeat the motor task described above 100 times at a self-paced delay after a tone, which followed the previous trial by 3 seconds. SI was assessed before and immediately after the "training" session and no TMS pulses were delivered during the vibration session. MEP amplitudes at the onset of the movement were compared to the resting condition as an assessment of SI for each

trial (ratio of the MEP amplitudes at the onset of the movement for each TMS trial to the mean MEP amplitude at rest for the examined block).

*Experiment 2: Delayed vibration*

In order to investigate the importance of synchronising vibration with the movement and any possible general effect of vibration itself on SI we repeated the same experiment but instead applied the vibration 100ms after the onset of the movement ($VIB_{100}$). Similarly to experiment 1, SI was assessed before and immediately after the vibration session which again consisted of 100 repetitions of the movement with vibration without any TMS pulses.

**Statistical analysis**

We used the SPSS Statistics software (version 19.0.0) for statistical analysis. Linear regression analysis explored possible changes of SI within the block of recordings. SI was expressed as a ratio of peak-to-peak MEP amplitude at the onset of the movement to peak-to-peak MEP amplitude at rest. Root mean square amplitude of the EMG activity in ADM during the first 100ms of FDI contraction was measured in order to explore the impact of increased SI on motor control. Kolmogorov-Smirnoff test was used to explore the normality of the data distribution and Levene's test was used to

explore the homogeneity of variance. SI data were log transformed in order to normalise the distribution of the data. Repeated measures analysis of variance (rmANOVA) was used to compare data acquired from the two groups of subjects before and after vibration. Follow up ANOVA was used to further explore significant interactions and we did post hoc t-test with Bonferonni corrections to explain the results. Statistical significance was set to $p \leq 0.05$. Unless otherwise stated all results are expressed as mean values ±1 standard error of the mean (SEM).

### 6.1.2. Results

A total of 16 subjects completed $VIB_{onset}$ session and none reported any side effects. Descriptive statistics revealed two outliers (SI above 2SD) who were excluded from the rest of the statistical analysis. In these two subjects MEPs in ADM at the onset of FDI movement were strongly facilitated rather than inhibited (274% and 204% increase respectively); therefore, SI was not present. After the vibration training, their MEPs in ADM were inhibited at the onset of the movement (64% and 33% respectively) supporting presence of SI. The results in these two subjects were in fact in line with the rest of the group but they were excluded because they significantly skewed the baseline group data.

In the remaining 14 subjects, the effects of the vibration on SI were explored in detail. Possible changes in SI in time across the block of recordings after vibration was investigated with linear regression analysis for the individual trials before and after vibration. The trial number of each block (1, 2, 3...20) was used as the predictor variable and the MEP amplitude ratios averaged across subjects as the outcome variable (Fig. 6.1.1). As we had expected, there was no trend in the amount of SI in the blocks prior to vibration ($R^2$=0.01, F(1,19)=1.96, p=0.18). However, there was a significant effect of trial number after vibration ($R^2$=0.24, F(1,19)=5.53, p=0.03). This effect suggests that SI decreased over time after vibration. In view of this, further statistical analysis was performed after division of the post-vibration trials into two bins (10 first trials and 10 last trials).

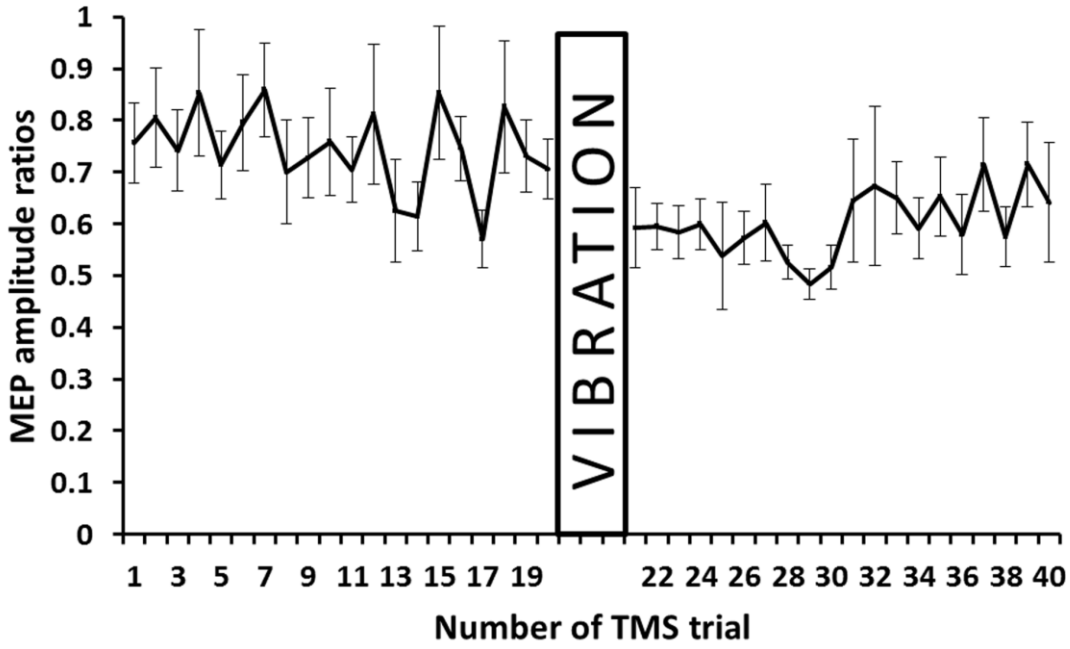


*Fig. 6.1.1 Mean MEP amplitude ratios in TMS trials averaged across subjects in the VIBonset group. MEP ratios were calculated for each trial in each subject by dividing the individual MEP amplitudes at the onset of the movement by the mean of 20 MEP amplitudes at rest for each of the two blocks (before and after vibration). Error bars represent SEM*

In experiment 2, SI was assessed in 14 subjects before and immediately after the delayed vibration training session. No side effects were reported after the experiment. Linear regression showed that there was no effect of trial number either before ($R^2$=0.08, F(1,19)=1.59, p=0.22) or after vibration ($R^2$=0.03, F(1, 19)=0.61, p=0.45) (Fig 6.1.2). However, in order to maintain consistency in statistical methods we again divided the blocks of recordings into two halves (10 first trials and 10 last trials) as for the first experiment.

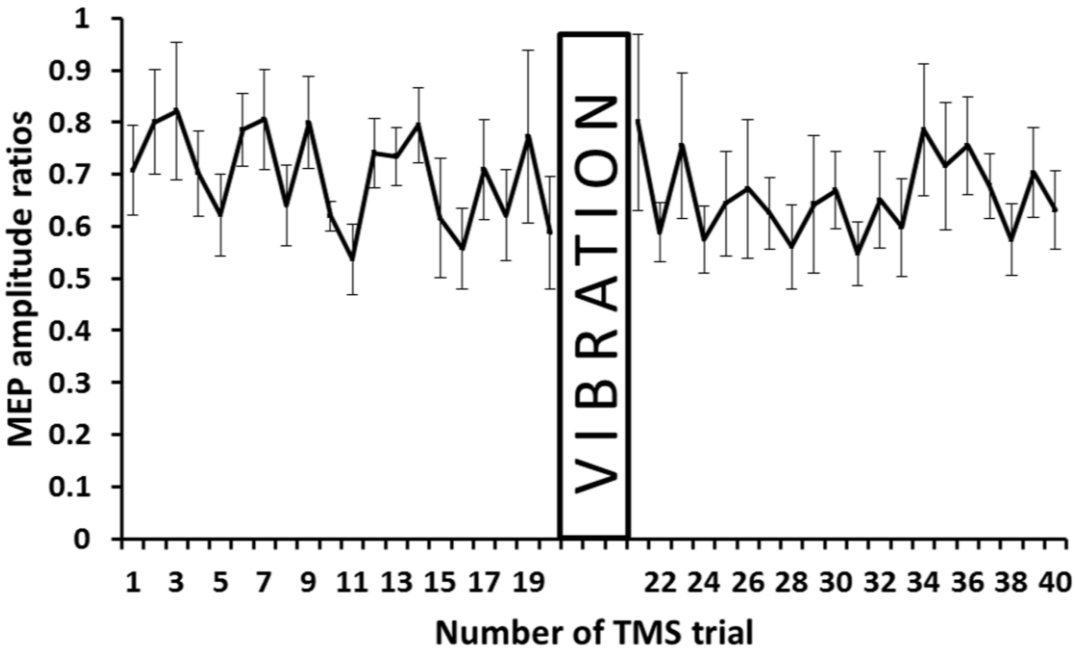



*Fig. 6.1.2 Mean MEP amplitude ratios in TMS trials averaged across subjects in the VIB100 group. MEP ratios were calculated for each trial in each subject by dividing the individual MEP amplitudes at the onset of the movement by the mean of 20 MEP amplitudes at rest for each of the two blocks (before and after vibration). Error bars represent SEM*

In order to explore the effect of vibration on SI in experiments 1 and 2 we compared the data from both groups (Fig. 6.1.3) Using a mixed design ANOVA with TIME (before vibration, 10 first trials after vibration and 10 last trials after vibration) as within subjects factor and GROUP ($VIB_{onset}$ group, $VIB_{100}$ group) as between subject factor.

This revealed a borderline significant effect of TIME F(2,52)=3.04, p=0.06 and a significant TIMExGROUP interaction (F(2,52)=4.43, p=0.02).

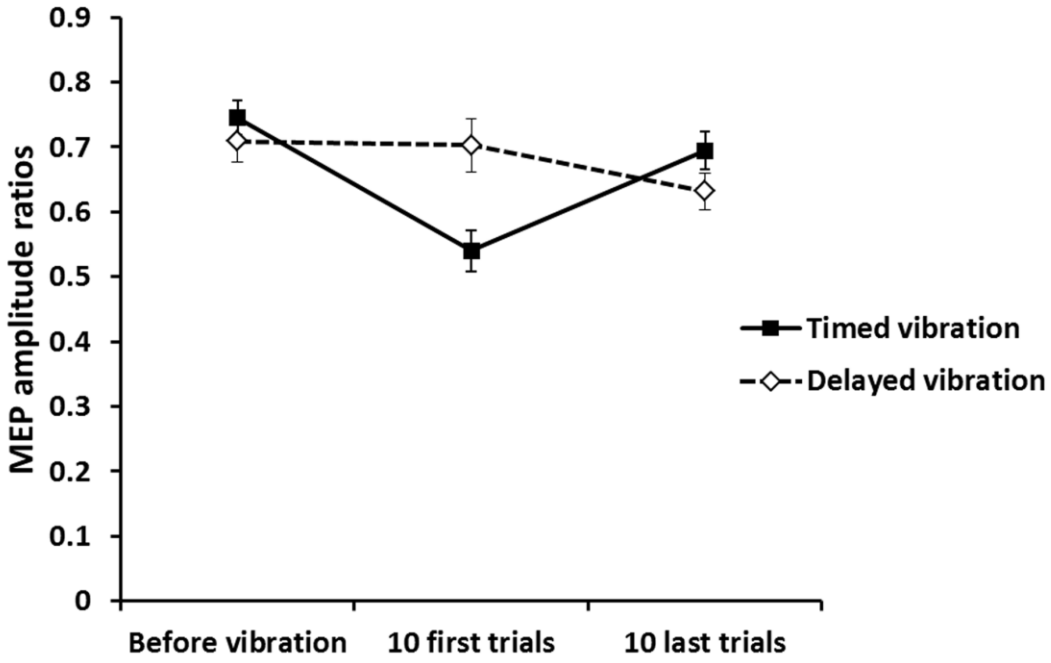



*Fig. 6.1.3 Mean ratio of MEP amplitudes in ADM at the onset of the movement to MEP amplitudes at rest before vibration, in the 10 first trials after vibration and 10 last trials after vibration. Error bars represent SEM*

In order to explore the significant interaction further we performed post hoc rmANOVA for each group using within subjects factor TIME (before vibration, 10 first trials after vibration, 10 last trials after vibration). In the $VIB_{100}$ group there was no effect of TIME

(F(2,26)=0.75, p=0.48) suggesting that delayed vibration had no effect on SI. In contrast, there was a significant effect of TIME for the $VIB_{onset}$ group (F(2,26)=7.38, p<0.01). Further pairwise comparisons showed that SI before vibration training timed to the onset of the movement was significantly less than SI in the 10 first trials after vibration t(13)=5.90, p<0.01, and not significantly different from the 10 last trials after vibration t(13)=0.84, p=0.42. SI in the 10 first trials was also stronger when compared to the last 10 trials t(13)=-2.31, p=0.04.

We also tested whether the change in SI had any effect on the performance of the task by measuring the root mean square amplitude EMG in the ADM muscle during the first 100ms of the FDI contraction. An rmANOVA with TIME as the main factor (before vibration, 10 first trials after vibration, 10 last trials after vibration) found no significant change in the amount of ADM EMG after vibration (F(2,26)=0.68, p=0.52).

In order to control for possible effects of vibration on MEP peak-to-peak amplitudes at rest we performed a rmANOVA on rest MEP data which showed no significant effect of TIME (before vibration, 10 first trials after vibration, 10 last trials after vibration) in the $VIB_{onset}$ group F(2,26)=0.05, p=0.96 or in the $VIB_{100}$ group F(2,26)=0.08, p=0.46.

### 6.1.3. Discussion

The present study demonstrates that sensory input plays an important role in the regulation of SI. Timed sensory feedback from the surround muscle was found to be crucial in adapting SI for future movements. Repeated application of a short period of vibration to ADM that was timed at the onset of FDI contraction increased SI for a short period after withdrawal of vibration. If vibration training was conducted with vibration delayed until 100ms after movement onset, there was no effect on SI.

We hypothesised that vibratory stimulation of muscle spindles in ADM that was precisely timed to the onset of focal movement in FDI would generate afferent signals indicating that unwanted movement had occurred. Muscle spindles are the principal muscle receptor contributing to sense of limb position and sense of limb movement [127]. In particular, the primary endings (Ia fibers) contribute to kinaesthesia and their stimulation with low amplitude vibration of 80 Hz can increase their firing rates [64, 124, 128-130] . Similar increases in the firing rates of Ia fibers are caused in a physiological manner by passive stretch of the muscle or by voluntary active isometric contraction [124, 131]. Importantly, the firing rate of muscle spindles is not directly correlated to muscle length but rather to a length-tension association. This is why increase in Ia firing rates can be interpreted by the CNS either as passive stretch or as active contraction depending on the circumstance. An increase in the firing rate of Ia fibers caused by vibration, without any change in the actual tension or length of the muscle, generates the illusion of passive muscle stretch [130]. However, vibration likely generates an activation pattern more complicated than a simple passive movement, as prior studies have shown that activation of Ia afferent with vibration can induce plastic

changes in the CNS [125] an effect that is unlikely to be present with simple passive movements [132]. With regards to the experiments presented in this study, vibration of ADM muscle would have been expected to produce an increase in the firing rates of Ia fibers. Similar pattern of afferent signals could theoretically be generated by either active ADM contraction or by passive ADM stretch, as explained above. We suggest that in either case, vibration generated error signals that were interpreted as being due to unintended contraction of surround muscles (ADM or its antagonists). This error signal drove an increase SI in subsequent movements to reduce excitability in the periphery and counteract the unintended afferent input. Similarly, to other adaptation studies we were able to record the aftereffect of this adaptive process after withdrawal of the vibratory input. SI was enhanced for a short period after the vibration session and returned back to baseline towards the end of the recordings. When the afferent signal from the surround muscle was delayed after the movement, there was no effect on SI, confirming the crucial nature of the timing of the afferent input. We suggest that this occurs because only sensory errors that are timely relevant to the motor command induce motor adaptation [121] whereas sensory inputs that arise in other contexts are treated differently. In formal terms, the CNS is thought to infer the causes of sensory input in a probabilistic context according to previous experience [120]. Thus, if muscle spindles are activated by alpha-gamma coactivation during volitional movement the information carried by Ia afferents to CNS is related to the movement and is valuable for motor adaptation. However, if muscle spindles are activated by external passive stretch of the muscle or external vibration this information is not valuable for motor adaptation since the probability of it to being related to important parameters of task performance is low. We propose that timing of afferent input is crucial in distinguishing

relevant and irrelevant sensory error signals. Muscle spindle input that happens simultaneously with a movement is more likely to have a causal relationship with the movement than when it occurs with a temporal discrepancy from the movement. Therefore when stimulation of the muscle spindles of a surround muscle is accurately timed with a movement it can produce a sensory representation of a co-contraction. This is interpreted as a motor error which drives adaptation.

This was confirmed in our second experiment where we delayed ADM vibration by 100ms so that it started after the end of the contraction of FDI when SI is known to be inactive [28]. The results revealed that delayed stimulation of muscle spindles does not induce any adaptive changes to SI. These findings confirm that timing is essential for the distinction of sensory feedback relevant for movement control from sensory feedback caused by external sources. Effectively, SI seems to be intimately related to suppressing "overflow" of activity at onset of movement.

It is significant to note that the importance of timing of inputs is only partially solved by mechanisms that use motor commands to predict expected sensory inputs. Arrangements such as the follow-up length servo of Merton [133] or cerebellar internal models that predict the sensory consequences of motor actions [112], are designed to detect unexpected inputs and treat them as error signals either to adjust the on-going motor command (follow-up servo) or future commands in the same context (internal models). Nevertheless the timing of the sensory inputs is still important since only those

inputs which arrive within a timed window of expected reafference are relevant motor error signals. In the present experiments, unexpected inputs arriving simultaneously with the motor command were used to adapt SI whereas those occurring 100ms were not.

The design of the study did not allow MEP recordings during the training session because of the confounding effects of vibration on the MEP sizes. It is known that MEPs in the vibrated muscle are facilitated and in the surround muscles are inhibited [134]. Therefore, assessment of any changes during the vibration session could not be directly compared to baseline measurements and we were not able to explore adaptation of SI during vibration. In addition, we did not directly assess electrophysiological changes at the level of the spinal cord. However this seems unlikely as there was no effect of vibration in the delayed vibration group and previous work has supported the supraspinal origins of SI [29]. ADM EMG activity was not found to be significantly different before and after training, but we did not assess other potential functional consequences of increased SI (for example reduced ADM activity with increasingly strong contractions of FDI). Therefore, the observed changes in SI may reflect adaptive processes in the motor system but they cannot be directly linked with motor performance at this time. Another potential limitation of the study is the exclusion of two subjects who did not show presence of SI at baseline, as described in the results section. Inclusion of their data would significantly skew the baseline group data introducing variability and decreasing the power of the statistical methods used. Interestingly, independent analysis of the data from these two subjects showed that the

effects of vibration on the SI ratios were in the same direction compared to the rest of the group (reduction of SI ratio after vibration). This is reassuring in that their exclusion for the group analysis would be unlikely to introduce bias on the effects of vibration on SI. In addition, the fact that these two subjects showed facilitation instead of inhibition is in line with prior studies [28, 30, 135] and with the study presented in Chapter 4.1. In a group of normal subjects almost 20 % of the subjects show facilitation of ADM MEPs at the onset of FDI movement instead of inhibition. This is again reassuring that although these subjects were outliers for the baseline SI measurement, their data still lies within a physiological spectrum.

There is some superficial resemblance between protocols of paired associative stimulation (PAS) [106] which are commonly used to induce LTP-like changes in the motor system and our vibration training. However, in our training session there was only one stimulus (vibration) which was paired with self-paced movements. The presence of a single type of external stimulation, the low number of repetitions compared with PAS protocols and the fact that there was no change in the resting MEP amplitudes do not support the possibility of a common mechanism behind the effects of vibration training and the effects of PAS protocols.

In summary with this study we provide evidence that SI can adapt according to sensory feedback and that these adaptive changes are retained for a short period after the end of training. This adaptive property of SI leaves open the possibility of modulation of SI for

possible therapeutic effect in neurological conditions where abnormal SI is thought to play a role in clinical symptoms (e.g. hand dystonia).

## 6.2. **Motor cortex plasticity and motor surround inhibition**

*(Published as: Belvisi D, Kassavetis P, Bologna M, Edwards MJ, Berardelli A, Rothwell JC. Associative plasticity in surround inhibition circuits in human motor cortex. Eur J Neurosci. 2014 Dec;40(12):3704-10.)*

As discussed above SI is a concept originally developed to explain enhancement of spatial contrast boundaries in the visual system [136, 137]. A pattern of short range excitatory connections with longer range inhibitory connections produces a system whereby excitation in a focal area suppresses activity in the surround. This type of organisation can readily be conceived within topographically organised structures such as visual and somatosensory systems [110, 138] but also in the primary motor cortex (M1). Thus, Sohn & Hallett [28] showed that index finger abduction was associated with suppression of corticospinal excitability to the nearby abductor digiti minimi (ADM) muscle even though spinal excitability indexed by the *F* wave was increased. Although the phenomenon may resemble sensory SI, it seems likely that the mechanism is rather different. The reason is that at the level of cortical hand area there are numerous overlapping representations of multiple muscles [139-141]. The distributed output zones to each muscle are likely to have equal numbers of excitatory and inhibitory connections between them [139], allowing, for example, ADM and abductor pollicis brevis (APB) to work together when grasping a wide object in the hand, whereas functioning independently when typing [1]. Motor surround inhibition (SI) could be reproduced by changing patterns of intracortical excitability between appropriate areas when it is required. This could rely on mechanisms internal to M1 or

it could depend on patterned input to motor cortex from other structures such as the basal ganglia [29].

Therefore, it is clear that SI is not hard-wired, but is adaptable. This explains why participants have to practice to move the index finger without activating the ADM [28]. It is also consistent with the variability of SI between individuals[142] and the ability of volunteers to enhance SI with specific feedback training [86] (Chapter 6.1).

We reasoned that if we could bias connectivity at the level of M1 we would be able to modulate the strength of SI when participants subsequently perform a single finger movement task. To do this, we used paired associative stimulation (PAS). Electrical stimuli to the median nerve were paired repeatedly with transcranial magnetic stimulation (TMS) pulses to the hand area of motor cortex. This increased the excitability of corticospinal output to the median nerve-innervated APB muscle without significant spread of activation to the ADM muscle for 30 min[106]. Our subjects performed individuated thumb abduction movements and we measured SI in ADM. Given that median PAS increased the effectiveness of SI onto ADM, we suggest that SI is not an anatomically hard wired phenomenon but it is an actively controlled circuit that can be modified by prior experience.

### 6.2.1. Methods

**Participants**

Fifteen healthy right-handed subjects (eight females; mean age 28± 7 years) participated in the study after giving their written informed consent. Participants had no history of any neuropsychiatric disorders, neurosurgery, or metal or electronic implants and were not on drugs active at CNS level at the time of the experiments. All experimental procedures were approved by the local institutional review board and conducted in accordance with the Declaration of Helsinki and according to international safety guidelines[105] .

**Experimental paradigm**

In order to explore the effects of PAS on SI we measured SI before and after median PAS protocol under which the electrical stimulus and the TMS pulse were paired, with a constant interstimulus interval of 21.5 ms (PAS21.5; [143]). In order to explore the timing specificity of PAS, participants also underwent a control experiment in which SI was also measured before and after a sham-PAS protocol where the electrical stimulation and the TMS pulse were applied with a delay of 100 ms (PAS100;[106, 144, 145]). In order to verify the topographic specificity of the PAS21.5 effect we conducted a further control experiment in 10 subjects, using a median PAS21.5 and ulnar-innervated active first dorsal interosseous (FDI) and surrounding (ADM) muscles for SI. To test whether the PAS21.5 effect on SI was limited to the APB muscle, we also investigated the effect of median PAS21.5 on SI using a different median innervated-active muscle (e.g. flexor carpi radialis; FCR) and ADM as the surrounding muscle in

10 subjects. In order to investigate the PAS21.5 effect on spinal excitability we performed a control experiment in eight subjects, recording ulnar and median *F* waves before and after PAS21.5. For the subjects who participated in all experiments, the sessions were randomised and performed at least 1 week apart.

**Electromyographic recordings**

Electromyographic (EMG) activity was recorded from right APB and ADM muscles using a pair of Ag–AgCl surface electrodes in a belly-tendon montage. The ground electrode was placed above the styloid process of the right ulna. The EMG signal was amplified (1000x) and band-pass filtered (bandwidth 20 Hz–2 kHz) with a Digitimer D360 amplifier (Digitimer Ltd, UK), digitised at a sampling rate of 5 kHz (CED 1401 laboratory interface; Cambridge Electronic Design, Cambridge, UK) and fed into a laboratory computer for storage and off-line analysis. Data were analysed using SIGNAL software V4.00 (Cambridge Electronic Design).

**Motor task**

During the experiments, the subjects were sitting in a comfortable chair with their right hand resting on a desk. The tip of their thumb was placed on a small button. For the assessment of SI, they were asked to perform a motor task. The task involved a brief

press of the button after a ‘go’ signal (an auditory tone) with a self-paced delay, by abducting their right thumb. At the beginning of the experiment, we measured the individual maximum EMG activity which could be produced in APB by briefly pressing the button. Then we asked the subjects to perform brief movements with 10% of their maximum EMG activity while keeping their ADM muscle relaxed. Visual feedback of the EMG activity from both muscles (APB and ADM) was displayed on a screen in front of the subjects. Each subject attended a brief training session before the start of the experiment in order to achieve a consistent performance of the desired movement with EMG activity in ADM not to exceed 100 μV.

During the control experiment performed to test the focality of the PAS21.5 effect on SI, participants were asked to perform a brisk activation of the FDI muscle (active muscle), e.g. index finger flexion, keeping ADM, the surround muscle, at rest.

**TMS**

A monophasic Magstim 200 stimulator (Magstim Co, Whitland, Dyfeld, UK) was used to deliver single TMS pulses. A figure-of-eight coil (external wing 9 cm in diameter) was placed tangentially over the left M1 in the optimal position (hot spot) for eliciting motor-evoked potentials (MEPs) in the right APB and right ADM. The hot spots were marked on the scalp with a soft-tipped pen. For the assessment of SI, single TMS pulses were delivered at rest and at the onset of the movement. The details of the paradigm are

described in a previous study [86, 101]. The intensity of the stimulation was adjusted to induce MEPs of approximately 1 mV in the resting ADM muscle. For the recordings of the MEPs at the onset of the movement the TMS was triggered when EMG activity in right APB (active muscle) > 100 μV was detected. We recorded 20 MEPs at rest and 20 MEPs at the onset of the movement. SI was assessed before the PAS protocol and three times after it, at 0–10 min (T1), 10–20 min (T2) and 20–30 min (T3). For both PAS protocols (PAS21.5 and PAS100), the intensity of the TMS was set to evoke MEPs of 0.5–1 mV in APB while the intensity of the median nerve stimulus (0.2 ms duration) was set at three times perceptual threshold. Two hundred pairs of stimuli were given at a rate of 0.25 Hz. The stimulus intensity to evoke MEPs of approximately 1 mV peak-to-peak amplitude in the APB muscle was used. Twenty rest MEPs were recorded from the median-innervated APB and the ulnar-innervated ADM muscles before and after PAS in a similar timeframe as the MEPs for SI.

**F wave**

We recorded F waves from APB and ADM evoked by supramaximal electrical stimulation at the wrist of the median and ulnar nerves respectively before and after PAS21.5. Twenty APB and ADM F wave were recorded before and 5, 15 and 30 min after PAS21.5. Compound muscle action potentials of APB and ADM were also recorded at 20-ms intervals.

**Data analysis and statistics**

Peak-to-peak motor-evoked potential (MEP) amplitude for each trial was measured off-line and the average amplitude in 20 trials was calculated for each session. SI was expressed as the ratio of MEP amplitudes during peri-triggered trials to MEP amplitudes in control trials [SI (%) = (MEPcond/MEPtest) x 100]. To test SI at baseline we used a two-way repeated measures ANOVA with main factors Muscle (ADM vs. APB) and Condition (rest vs. movement). To compare the effect of PAS21.5 and PAS100 on SI we used a two-way repeated-measures ANOVA with Condition (PAS21.5 vs. PAS100) and Time (T0 vs. T1, T2, T3) as main factors. To test the effect of PAS21.5 or PAS100 on SI we used a follow-up one-way repeated-measures ANOVA with Time (T0 vs. T1, T2, T3) as main factor. To compare the effect of PAS21.5 and PAS100 on APB MEP size we used a two-way repeated-measures ANOVA with Condition (PAS21.5 vs. PAS100) and Time (T0 vs. T1, T2, T3) as main factors. To test the effect of either PAS21.5 or PAS100 on MEPs amplitude recorded from APB and ADM muscles we used a two-way repeated-measures ANOVA with Muscle (APB vs. ADM) and Time (T0 vs. T1, T2, T3) as main factors. To verify whether the PAS21.5 effect on SI was limited to the median innervated system, e.g. to measure PAS21.5 focality, we used a two-way repeated-measures ANOVA with SI (SI from APB to ADM vs. SI from FDI to ADM) and Time (T0 vs. T1, T2, T3) and as main factors. A two-way repeatedmeasures ANOVA with Time (T0 vs. T1, T2, T3,) and Muscle (APB vs. ADM) as main factors was used to verify that PAS21.5 was effective in this control experiment. To verify whether the PAS21.5 effect on SI was limited to the APB muscle,

we checked the presence of SI in ADM during an FCR movement, using a two-way repeated-measures ANOVA with main factors Muscle (ADM vs. FCR) and Condition (rest vs. movement). Subsequently we used a one-way ANOVA with Time (T0 vs. T1,T2,T3) as the main factor.

EMG activity during voluntary movement was calculated in each muscle by assessing the root mean square (RMS) value related to the 100 ms after the onset of the APB voluntary movement. To assess the effect of PAS21.5 on EMG burst, expressed as RMS amplitude, during the voluntary movement we used a two-way repeated-measures ANOVA with Muscle (APB vs. ADM) and Time (T0 vs. T1, T2, T3) as main factors. To analyse the effect of PAS21.5 on each muscle EMG, a one-way repeated-measures ANOVA was run separately for each muscle with main factor Time (T0 vs. T1, T2, T3). Tukey's honest significant difference test was used for all post hoc analyses.

In order to verify the PAS21.5 effect on APB and ADM F wave amplitudes we used a one-way repeated-measures ANOVA with Time as the main factor. Pearson's test was used to test the possible correlation between PAS21.5-induced changes in APB MEP amplitudes and SI. P-values $< 0.05$ were considered to indicate statistical significance. All values are expressed as mean ± SE.

### 6.2.2. Results

**Baseline SI**

SI from APB to ADM was present at baseline. MEPs evoked in the ADM muscle were smaller at the onset of APB contraction than they were at rest; conversely, MEPs in APB were greatly facilitated. This was confirmed using a two-way repeated-measures ANOVA with main factors Muscle and Condition. This revealed significant main effects of Condition ($F_{1,28} = 85.52$; $P < 0.001$) and Muscle ($F_{1,28} = 80.17$; $p < 0.001$) and a significant Condition X Muscle interaction ($F_{1,28} = 106.52$; $p < 0.001$). The latter was due to the fact that contraction of APB increased MEPs in APB but reduced them in ADM (paired t-tests – APB, $p < 0.001$; ADM, $p < 0.003$; Fig. 6.2.1–6.2.6).

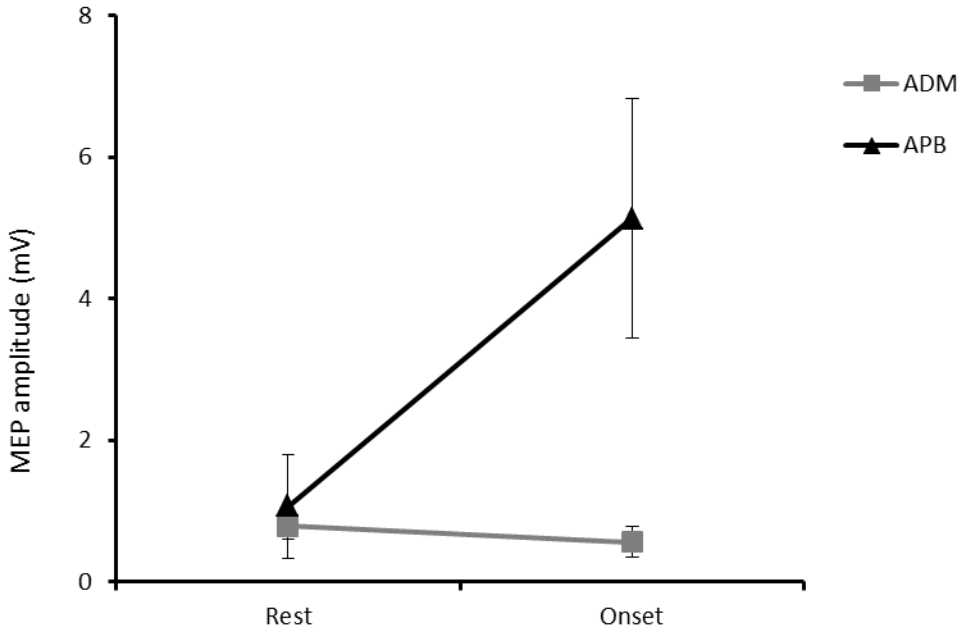



*Fig. 6.2.1 Motor surround inhibition (SI). The onset of the movement induced a strong facilitation in the active muscle abductor pollicis brevis (APB) and a significant suppression in the surround muscle abductor digiti minimi (ADM). SI is expressed as the ratio of motor-evoked potential (MEP) amplitudes during peritriggered trials to MEP amplitudes in control trials [SI (%) = (MEPcond/MEPtest) × 100]. Vertical bars denote SD.*

**Comparison of PAS21.5 and PAS100 on SI**

PAS21.5 increased the amount of SI compared with baseline whereas there was no effect after PAS100. The mean data are shown in Fig. 6.2.2, in which SI is expressed as the percentage change in amplitude of MEPs evoked in ADM during contraction of APB versus rest. Values < 100% represent inhibition. A two-factor ANOVA on this data showed no significant effects of the main factors Condition ($F_{1,28} = 3.60$; $p = 0.09$) or Time ($F_{3,84} = 2.36$; $p = 0.08$) but a significant Condition X Time interaction ($F_{3,48} = 7.94$; $p = 0.001$). Post hoc analysis showed that although the baseline (T0) levels of SI were similar on the two occasions ($p = 0.94$), there was more SI following PAS21.5 than PAS100 at T1 ($p = 0.03$), T2 ($p < 0.03$) and T3 ($p < 0.05$).

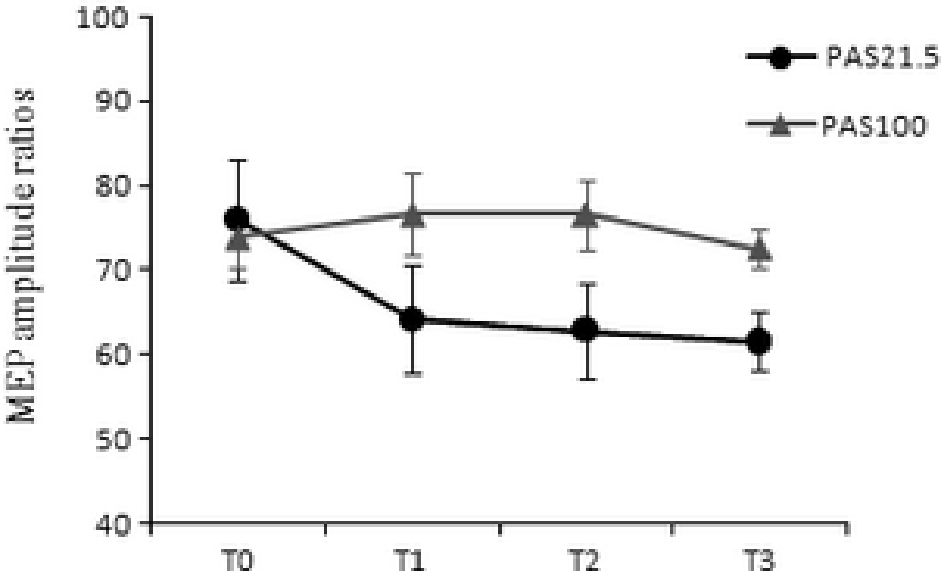



*Fig 6.2.2 Modulation of motor surround inhibition (SI) by PAS21.5 and PAS100. PAS21.5 increased the amount of SI compared with baseline whereas there was no*

*effect after PAS100. The PAS21.5 SI enhancement was significant at 5–10 (T1), 15–20 (T2) and 20–30 (T3) minutes after PAS21.5 Vertical bars denote SD.*

Subsequent follow-up one-way ANOVAs confirmed a significant main effect of Time ($F_{3,42} = 4.53$; $p = 0.007$) after PAS21.5, with post hoc comparisons indicating stronger SI at T1 ($p = 0.02$), T2 ($p < 0.01$) and T3 ($p = 0.03$). There was no significant effect of Time ($F_{3,42} = 5.55$; $p > 0.05$) following PAS100.

**Comparison of PAS21.5 and PAS100 on resting MEP**

We measured the amplitude of MEPs evoked at rest before and after PAS21.5 and PAS100. As expected, PAS21.5 increased MEPs in APB while PAS100 had no effect. A two-way repeated-measures ANOVA revealed a significant Condition X Time interaction ($F_{3,84} = 8.62$; $p < 0.01$) which was due to the fact MEPs only increased after PAS21.5. Post hoc tests showed that despite having similar APB MEP amplitudes at baseline (T0; $p > 0.05$), MEPs following the two forms of PAS differed at T1 ($p < 0.05$), T2 ($p < 0.03$) and T3 ($p < 0.05$; Fig. 6.2.3).

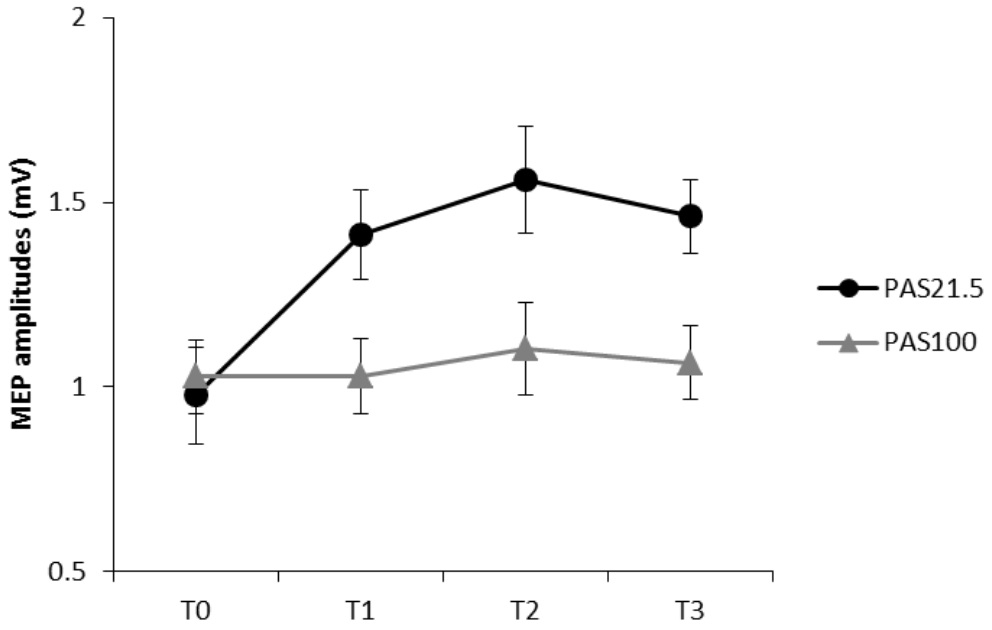



*Fig. 6.2.3 Modulation of resting motor-evoked potentials (MEPs) by PAS21.5 and PAS100 PAS21.5 increased MEPs in abductor pollicis brevis (APB) while PAS100 had no effect. The PAS21.5 APB MEPs enhancement was significant at 5–10 (T1), 15–20 (T2) and 20–30 (T3) minutes after PAS21.5 Vertical bars denote SD.*

In a follow-up analysis we tested whether the effect of PAS21.5 was greater on APB (the homonymous muscle given that PAS employed median nerve stimulation) than on ADM (heteronymous muscle). Figure 6.2.4 shows that although PAS21.5 increased MEPs in APB there was no effect on MEPs in ADM. A two-factor ANOVA showed significant main effects of Muscle ($F_{1,28} = 9.65$; $p < 0.01$) and Time ($F_{3,84} = 36.75$; $p < 0.01$) and a significant Muscle X Time interaction ($F_{3,84} = 6.98$; $p = 0.01$). Post hoc analysis showed that MEPs recorded from ADM muscle were similar before and after

PAS ($p > 0.05$ for all comparisons), but MEPs recorded from APB muscle were facilitated at T1 ($p = 0.01$), T2 ($p < 0.01$) and T3 ($p < 0.01$; Fig. 6.2.4).

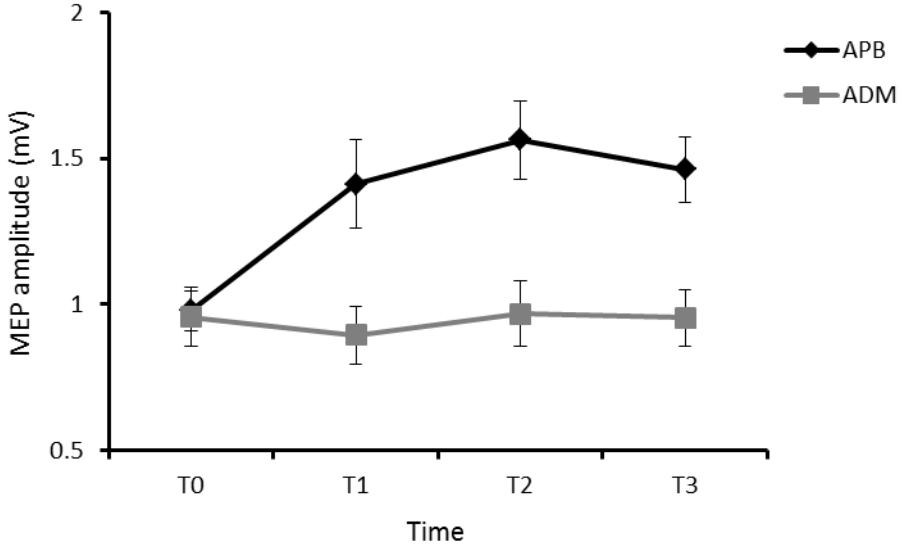



*Fig. 6.2.4Modulation of abductor pollicis brevis (APB) and ADM motor-evoked potentials (MEPs) by PAS21.5. PAS21.5 induced a significant APB (target muscle) MEP facilitation at 5–10 (T1), 15–20 (T2) and 20–30 (T3) minutes after PAS21.5. 5 (T1). Abductor digiti minimi (ADM; non target muscle) remained unchanged after PAS21.5*

A similar analysis on the effects of PAS100 was negative – PAS100 had no effect on MEPs in either APB or ADM. There were no main effects of Muscle ($F_{1,28} = 8.06$; $p = 0.05$) or Time ($F_{3,84} = 2.11$; $p > 0.05$) and no Muscle X Time interaction ($F_{8,84} = 0.94$; $p > 0.05$) (Table 6.2.1).

| | T0 | T1 | T2 | T3 |
|---|---|---|---|---|
| **PAS21.5** | | | | |
| **APB active muscle** | | | | |
| **APB RMT (%)** | 42 | 41 | 42 | 42 |
| **ADM RMT (%)** | 44 | 43 | 44 | 44 |
| **APB RMS (µV)** | 0.136 ± 0.022 | 0.132 ± 0.021 | 0.135 ± 0.019 | 0.134 ± 0.021 |
| **ADM RMS (µV)** | 0.018 ± 0.002 | 0.016 ± 0.002 | 0.016 ± 0.002 | 0.019 ± 0.001 |
| **PAS21.5** | | | | |
| **FDI active muscle** | | | | |
| **APB RMT (%)** | 43 | 43 | 42 | 43 |
| **APB MEP (mV)** | 1.01 ± 0.19 | 1.37 ± 0.15 | 1.47 ± 0.19 | 1.41 ± 0.17 |
| **ADM RMT (%)** | 45 | 44 | 44 | 45 |
| **ADM MEP (mV)** | 1.09 ± 0.11 | 1.07 ± 0.11 | 1 ± 0.12 | 0.99 ± 0.10 |
| **PAS100** | | | | |
| **APB active muscle** | | | | |
| **APB RMT (%)** | 42 | 42 | 41 | 42 |
| **ADM RMT (%)** | 44 | 44 | 44 | 43 |

*Table 6.2.1. Physiological data. (ADM, abductor digiti minimi muscle; APB, abductor pollicis brevis muscle; MEP, motor-evoked potential; RMT, resting motor threshold; RMS, root mean square; T0, baseline; T1, 5 min after PAS21.5/100; T2, 15 min after PAS21.5/100; T3, 30 min after PAS21.5/100).*

**Effect of median PAS21.5 on ADM SI during FDI versus APB movement**

SI from FDI to ADM was present at the baseline. This was confirmed using a two-way repeated-measures ANOVA with main factors Muscle and Condition. This revealed significant main effects of Condition ($F_{1,18} = 796.25$; $p < 0.001$) and Muscle ($F_{1,18} = 111.12$; $p < 0.001$) and a significant Condition X Muscle interaction ($F_{1,18} = 121.54$; $p < 0.001$).

Median PAS21.5 did not modify the amount of SI from FDI to ADM even though it increased SI from APB to ADM. This was demonstrated using a two-way ANOVA, which showed a significant SI X Time interaction ($F_{3,54} = 3.65$; $p < 0.05$), and a significant main effect of Time ($F_{3,54} = 4.36$; $p < 0.01$) but not of SI ($F_{1,18} = 1.95$; $p = 0.17$). Post hoc analysis showed that although the baseline (T0) levels of SI were similar ($p = 0.94$), there was more SI from APB to ADM following PAS21.5 than SI from FDI to ADM at T2 ($p < 0.05$) and T3 ($p < 0.03$), but not at T1 ($p = 0.05$; Fig. 6.2.5).

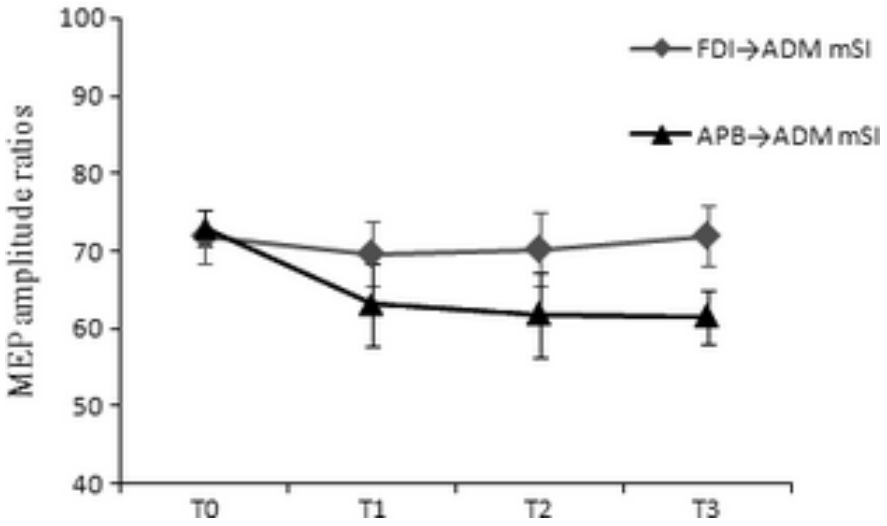



*Fig. 6.2.5 Motor surround inhibition (SI) modulation by PAS21.5 was topographically specific. PAS21.5 changed SI from abductor pollicis brevis (APB) to abductor digiti minimi (ADM) but did not change SI from FDI to ADM. Vertical bars denote SD.*

In the 10 healthy subjects taking part in this control experiment, PAS21.5 was still able to increase MEP size in APB but not in ADM. A two-factor ANOVA showed significant main effects of Muscle ($F_{1,16} = 4.65$; $p < 0.03$) and Time ($F_{3,48} = 3.62$; $p < 0.03$) and a significant Muscle X Time interaction ($F_{3,48} = 5.16$; $p = 0.03$). Post hoc analysis showed that MEPs recorded from ADM muscle were similar before and after PAS ($p > 0.05$ for all comparisons), but MEPs recorded from APB muscle were facilitated at T1 ($p < 0.05$), T2 ($p < 0.03$) and T3 ($p < 0.03$).

**Effect of median PAS21.5 on ADM SI during FCR movement**

SI from FCR to ADM was not present at the baseline This was demonstrated using a one-way repeated-measures ANOVA that showed no significant effect of the factors Muscle ($p > 0.05$) or Condition ($p > 0.05$), and no significant Condition X Muscle ($p > 0.05$) interaction. PAS21.5 did not modify this result ($p > 0.05$) as tested by a one-way ANOVA with non-significant factor Time ($p = 0.1$).

**Effect of PAS21.5 on voluntary EMG activity in APB and ADM**

Finally we asked whether the changes in SI following PAS21.5 might be associated with changes in the movements that participants made during assessment of SI. RMS EMG activity in APB and ADM was measured over the 100 ms following onset of APB contraction in each participant taking part in experiment 1 before and after PAS21.5. A two-way repeated-measures ANOVA showed a significant effect of Muscle ($F_{1,28} = 32.13$; $p < 0.0001$) but not of Time ($F_{3,84} = 1.92$; $p = 0.13$) and no Muscle X Time interaction ($F_{3,84} = 1.79$; $p = 0.15$) Thus, there was no effect of PAS21.5 on the voluntary activity of the two muscles under study (Table 6.2.1).

**Effect of PAS21.5 on APB and ADM F waves**

Median PAS21.5 did not modify APB and ADM F wave amplitudes. This was demonstrated using a one-way repeated-measures ANOVA that showed no significant effect of the factor Time ($p > 0.5$).

**Correlations**

Pearson's test did not show any correlation between PAS21.5-induced changes in either APB MEPs or ADM MEPs amplitudes and SI.

### 6.2.3. Discussion

Previous work has demonstrated SI in the ADM muscle during activation of the FDI or even a mouth or a leg muscle[28]. The present study is the first time SI has been demonstrated from the contracting APB. Our main finding, however, was that a median nerve PAS protocol, which enhances the excitability of corticospinal output to the APB muscle but not the ADM, increased the effectiveness of SI onto ADM for at least 30 min yet had no effect on the SI from FDI onto ADM. Importantly, PAS21.5 had no effect on the amplitude of the EMG activity in APB, thus excluding the possibility that changes in SI were related to changes in contraction force[38]. Confirming previous studies[106], we found that PAS did not modify *F* waves, indicating that there were no major changes in excitability of spinal motoneurones. We suggest that the increase in SI

following PAS21.5 depends on its ability to induce long-term changes in excitability of the APB representation in M1.

As discussed earlier, SI between intrinsic hand muscles needs to be flexible and one potential mechanism could involve the intermingled mosaic of motor output zones to the hand which are interconnected by short- and long-range excitatory and inhibitory connections in M1 [146, 147]. Depending on the required task, the weight of connections could shift from inhibition to excitation or vice versa, allowing ADM and APB muscles to work either reciprocally or synergistically[1, 139]. The fact that we observed a PAS21.5-induced increase in SI from APB to ADM and not from FDI to ADM would then be compatible with the idea that median nerve PAS21.5 shifts the balance of connectivity between APB and ADM representations towards inhibition. Thus when APB is activated, the incoming command as well as the recurrent collateral feedback from pyramidal neurones would be more likely to suppress excitability of output zones to ADM. In line with this hypothesis, Kang *et al*. [85] demonstrated that SI was reduced in the ADM muscle after 30 min of synchronised ADM and FDI contraction while it was unchanged after single ADM activation. Therefore, SI seems to be less effective when hand muscles need to work together and to be enhanced when a focal contraction of a single muscle is made, as in our case.

Previous studies have shown that PAS is able to induce differential effects on the inhibitory mechanisms operating at the M1 level, at least as measured in the output to the target muscle. Facilitatory PAS (including PAS21.5 and PAS25) has no effect on GABA-A inhibition measured with short intracortical inhibition (SICI)[106, 148, 149] ,

while it is able to modify GABA-B inhibition, increasing the cortical silent period (SP; [106, 150, 151] and reducing long intracortical inhibition (LICI) [148]. It also increases the effectiveness of long afferent inhibition (LAI) when the interstimulus interval is 240 ms while it reduces LAI when the interstimulus interval is 150 ms [148]. Further studies showed that there is no direct evidence to conclude that SI employs the same mechanisms that mediate SICI, LICI and LAI [29] . Most data suggests that SICI is not modulated during SI (e.g. Sohn & Hallett, 2004a [28]; but see Stinear & Byblow,2003 [31]). Both LAI180 and LICI are widely reduced in hand muscles before contraction of any one of them[29]. If SI depended on the same interneurones we would expect it to be less effective than at rest.

This leaves open the possibility that the PAS-induced increase in SP might interact with SI. Investigating the size and topography of the cortical areas from which an MEP and SP could be evoked in APB, Wilson *et al*. (1993) [152] that SP area was larger, encompassing and surrounding the MEP area. They raised the possibility that inhibitory processes might act to limit or contain excitatory output. If the GABA-B interneurones involved in the SP contribute to SI, enhancement of the SP by PAS21.5 would tend to facilitate SI. In line with this, patients with focal hand dystonia (FHD), in whom SI has been reported to be reduced, have an abnormally short SP [153-158] and, unlike healthy individuals, PAS does not increase the duration of SP in patients with FHD [57]. This is consistent with the idea that in dystonia an alteration in excitability of SP interneurones causes a deficit in SI and results in a loss of topographic specificity during PAS-induced aftereffects.

Set against this idea that SP interneurones are involved in SI generation is a recent finding by Poston *et al*. (2012) [33]showing that the SP duration in a surround muscle decreased during phasic finger flexion. However, it is not possible to study the more relevant SP in the active muscle because SI requires a phasic movement[29] while SP is evoked during tonic contraction[159, 160].

Another possible explanation of the effect of PAS21.5 is to suggest that SI is mainly the result of the pattern of excitatory and inhibitory input from other motor areas to M1. For example, it may be that a focal motor command to activate APB tends, because of the interleaved representation of the hand muscles in M1, to spread to activate output to ADM. This might normally be suppressed by a concurrent decrease in ADM excitability, perhaps resulting from reduced basal ganglia input [29, 80]. As confirmed by the present results, PAS21.5 produces a focal increase in corticospinal excitability to APB. One consequence of this is that after PAS21.5 a smaller input command might be required to evoke the same level of output to APB. This would mean there was a reduced tendency to overflow into ADM which would then appear to be more highly suppressed by SI from basal ganglia. Although we did not find any correlation between the enhancement of MEP size in APB and SI, this would not necessarily be expected if the amount of excitatory spread to ADM varied from one person to another. The hypothesis that basal ganglia are involved in SI mechanisms again fits well with the abnormalities observed in FHD, in which there is less effective SI[28] and the response to PAS21.5 is less topographically specific than normal[45, 57, 143, 161, 162]. In patients with FHD, PAS induced an abnormal or absent modulation of inhibitory circuits, including SP[57] , LICI and LAI [163], indicating that maladaptive plasticity

also involves inhibitory mechanisms. Even though we did not investigate PAS effects on SI in dystonia in the present study, it is plausible to hypothesise that the reduced SI could contribute to the abnormal plasticity of inhibitory circuits in dystonia.

A limitation of the present study is that investigating the effect of median PAS21.5 on SI from FCR to ADM, we did not find a significant reduction in ADM MEPs (e.g. SI) during the onset of wrist flexor movement, and PAS21.5 did not modify this result. Our explanation is that SI depends on individual finger movements and it is greater when both active and surround muscles are intrinsic to the hand. According to this hypothesis, here we found SI in ADM during APB contraction (previously FDI was usually chosen as the active muscle). A previous study demonstrated that SI was absent in ADM and APB during risorius contraction and it was present in ADM but not in APB during tibialis anterior activation[28] Our group size is probably not large enough to reveal a small phenomenon like SI in ADM during distant muscle contraction. Further studies could be useful to investigate this topic.

Finally it is interesting to note that the effect of median nerve PAS21.5 was specific to SI from APB to ADM whereas SI from FDI to ADM was unaffected. This would be the natural consequence of the focal increase in APB excitability that we confirmed after PAS21.5. However, it is also known that PAS21.5 produces relatively focal increase in the SP, which is greater in APB than ADM[57]); this would fit with the initial suggestion that PAS21.5 affects the distribution of excitability in inhibitory connections between muscle representations of M1.

In conclusion, our data show that preconditioning with a median nerve PAS21.5 protocol increases the amount of SI from APB to ADM. The observation that SI is susceptible to modulation could lead to new therapeutic and rehabilitation approaches in patients with FHD.

# Chapter 7. MOTOR SURROUND INHIBITION IN DYSTONIA

## 7.1. **Motor surround inhibition in primary focal dystonia**

*(Submitted as: Kassavetis P, Sadnicka A, Saifee TA, Pareés I, Kojovic M, Bhatia KP, Rothwell JC, Edwards MJ. Reappraising the role of motor surround inhibition in dystonia. Brain Stimulation – Under review at the time of thesis submission.)*

A clear prediction/assumption of the above studies and all previously published studies on SI is that SI should have a behavioural correlate and specifically that more SI should be associated with less activation of adjacent muscles during single finger movement. In line with this hypothesis, SI has been found to be stronger in the dominant hemisphere which indeed indicates plausible relationship of SI with motor performance. However it has been recently shown that SI does not correlate with EMG activity in adjacent muscles (see chapter 4) [135] and robust data to directly connect SI with performance is still lacking. The argument for the behavioural relevance of SI has instead largely been based on the observation that SI is decreased or absent in patients with focal hand dystonia, a condition characterised by loss of selectivity in activation of individual muscles and overflow of contraction to the muscles not engaged in the movement.

Following initial reports where SI was found to be abnormal in patients suffering from dystonia [40] several studies have compared SI in patients with hand dystonia and healthy controls. However, 10 years later there is still uncertainty on how SI relates to

the pathophysiology and clinical manifestation of dystonia. Instead, the literature is generally limited to reporting between-group differences in SI, while discounting inconsistencies between group data and individual patient data. Finally, the relatively limited statistical description of the neurophysiological profiles of the studied groups has not assisted in qualitative and qualitative analysis of the observed abnormality in SI.

With this study, we attempted to characterise SI in three groups of participants: in healthy volunteers, patients with focal hand dystonia and patients with focal cervical dystonia. We present new data and we critically review published literature. We summarise the current evidence on SI and we go one step further by critically appraising the significance of existing patients' data. For this reason, we did not focus on the mean differences between groups, but we also investigated other dimensions of the data such as the within-group variability of corticospinal excitability and variability of data within individual subjects. Such approach is relevant for critically examining the proposal that focal hand dystonia is a good disease model for the hypothesised consequences of deficits in SI. In addition we used our data as pilot in combination with previously published data to perform power calculations for future studies on the mean SI differences between normal and dystonic groups.

### 7.1.1. Methods

**Participants**

A total of 31 right-handed healthy adults (age 27.4 years, SD=7.2, 16 women), 11 patients with cervical dystonia ( age 54.1 years, SD=10.6, 4 women) and 12 patients with task-specific focal hand dystonia ( age 53.25 years, SD=12.9, 4 women) were recruited. The patients with dystonia were recruited in the movement disorders specialty clinics at the National Hospital for Neurology and Neurosurgery. None of the hand dystonia patients were receiving treatment. The CD patients were all chronically receiving botulinum toxin injects but the most recent were more than three months before the experiment. Written informed consent was obtained from all participants and the study was approved by the local ethics committee. The focal hand dystonia patients were rated with the ADDS scale and the focal cervical dystonia patents with the TWSTRS scale. Demographic and clinical data is presented in Tables 7.1.1 and 7.1.2.

| Patient# | Gender | Age | Disease duration (y) | Last BT injection (months) | TWSTRS |
|---|---|---|---|---|---|
| **1** | M | 43 | 8 | 4 | 28 |
| **2** | M | 55 | 18 | 3 | 30 |
| **3** | F | 72 | 25 | 4 | 26 |
| **4** | F | 54 | 14 | 6 | 18 |
| **5** | M | 46 | 16 | 4 | 15.5 |
| **6** | M | 46 | 16 | 4 | 22.25 |
| **7** | M | 49 | 6 | 3 | 32.25 |
| **8** | F | 70 | 18 | 4 | 26 |
| **9** | M | 41 | 20 | 3 | 22.25 |
| **10** | M | 55 | 40 | 4 | 25 |
| **11** | F | 64 | 14 | 3 | 28.5 |

*Table 7.1.1: Demographic and clinical data of the CD patients.*

| Patient# | Gender | Age | Type of dystonia | Presentation | Duration of disease (y) | ADDS |
|---|---|---|---|---|---|---|
| **1** | M | 86 | MD-clarinet | ring, middle and little finger flexion | 26 | 77 |
| **2** | F | 49 | WC | index and thumb flexion | 10 | 81 |
| **3** | M | 48 | MD-guitar | thumb flexion | 20 | 77 |
| **4** | M | 50 | WC | index and thumb flexion | 11 | 69 |
| **5** | F | 60 | WC | index and thumb flexion | 7 | 77 |
| **6** | M | 56 | MD-guitar | index finger flexion | 8 | 73 |
| **7** | M | 51 | MD-Clarinet | little and ring finger flexion | 5 | 81 |
| **8** | F | 38 | WC | index finger flexion | 17 | 69 |
| **9** | F | 51 | MD-guitar | middle and ring finger flexion | 3 | 73 |
| **10** | M | 51 | MD-saxophone | small finger flexion | 13 | 73 |
| **11** | M | 33 | MD-guitar | ring and little finger flexion | 3 | 81 |
| **12** | M | 66 | WC | index and thumb flexion | 8 | 77 |

*Table 7.1.2: Demographics and clinical data of the FHD patients.*

**Motor task**

The details of the procedure have been described elsewhere [86]. The subjects were asked to briefly depress the button with a self-paced delay after a 'go' signal (an auditory tone), by flexing their index finger at the metacarpo-phalangeal joint. FDI is a synergist for this movement and previous studies have shown that this movement induces an increase in motor evoked potentials (MEPs) in FDI and reduction of MEPs in ADM [28, 71, 86]. EMG activity was recorded in both ADM and FDI muscles. Subjects were asked to perform the movement with 10% of their maximum EMG activity and duration of the movement was aimed to be approximately 100ms.

**Transcranial magnetic stimulation**

A figure-of-eight shaped coil (external loop diameter of 9 cm) connected to a monophasic Magstim 200 stimulator (Magstim Co, UK) delivered transcranial magnetic stimulation (TMS). The intersection of the coil was positioned tangentially on the scalp over the left motor cortex at a $45^0$ angle to the sagittal plane in order to induce trans-synaptically a posterior–anterior directed current in the brain to activate the corticospinal tract [72, 73]. The "hot spot" was defined as the optimal scalp position for eliciting motor evoked potentials (MEPs) of maximal amplitude in the contralateral ADM. The intensity of the stimulation was set to evoke MEPs with average peak-to peak amplitude of approximately 1mV-1.5mV at rest in the ADM muscle. For the assessment of SI, single TMS pulses were delivered at rest and at the onset of the movement. Each trial started with a self-paced movement after the "go" signal and lasted for 10 seconds when the next "go" signal was presented. A total of 40 trials were collected and during each of them a single TMS pulse was delivered. In 20 out of the 40 trials we assessed the MEP amplitude size at the onset of the movement with the TMS being triggered by a closed loop circuit immediately when EMG activity in right FDI above 100 μV was detected. In the rest 20 trials we assessed the MEP amplitude size at rest by delivering the TMS pulse 5 seconds after the onset of the brief movement while the subjects were resting. The 'rest' trials and 'onset' trials were randomised.

**Data analysis**

Peak to peak MEP amplitudes were measured offline. Corticospinal excitability in the three groups at rest and at the onset was assessed with rmANOVA with appropriate post hoc tests. Bivariate correlations between the clinical scales scores and the SI ratios (ADM MEP at onset/ADM MEP at rest) were assessed with Pearson's test and Spearman's Rho test for parametric and non-parametric data respectively.

In order to ensure similar performance of the task between groups, RMS amplitude of EMG activity was assessed during 100ms after the onset of the FDI contraction, in the trials when the MEPs were delivered at rest, so the EMG epoch was not contaminated with MEP or TMS artefact. RmANOVA was used to explore between groups differences.

Finally we explored the individual MEPs variability in our groups of patients with rmANOVA of the coefficient of variation (CV) at rest and at the onset of the movement.

In order to compare our results with previously published studies on SI we reviewed the relevant literature. We searched PubMed with the terms (transcranial magnetic stimulation AND surround inhibition) for studies published until February 2014.

The inclusion criteria for the studies were: 1. Studies that used a similar paradigm/set up (peri-trigerred TMS pulse) 2. Studies that used 10% MVC as the target force for FDI.; 3. Studies that reported the ratio of the MEPs at the onset of the movement to the MEPs at rest either in the manuscript or in figures (data from figures were extracted after digitisation (Plot Digitiser V. 2.6.4.)). 4. Studies in healthy participants or patients with FHD.

In order to explore the variability of TMS measurements in groups of patients with FHD not only in relevance to SI but as a general neurophysiological characteristic we performed another review of studies that have reported SEMs or SDs of MEPs recorded at rest in groups of healthy volunteers and in groups of patients with FHD. We searched PubMed with the search terms (Dystonia AND transcranial magnetic stimulation). We included all studies published until February 2014.

The inclusion criteria were: 1. Studies that reported in the text (not in figures) the absolute peak-to-peak MEP amplitudes and either the SEM or SD in both healthy controls and in patients with FHD 2. Studies that reported the above variables in at least one hand muscle 3. Studies that reported data collected in a single laboratory. We excluded studies that 1. Were multi-center 2. Dystonia groups were heterogeneous including other types of dystonia besides FHD.

### 7.1.2. Results

**Corticospinal excitability**

Mixed design rmANOVA of the MEP amplitudes in ADM and FDI muscles with within subjects factors MOVEMENT (rest, onset) and MUSCLE (ADM, FDI) and between subjects factor Group (CD, FHD, Controls) revealed significant effect of factor MOVEMENT $F(1,51)=46.61$, $p<0.001$, significant effect of MUSCLE $F(1,51)=338.68$, $p<0.001$, significant interaction MUSCLE x MOVEMENT $F(1,51)=123.39$, $p<0.001$. The effect of GROUP ($F(2,51)=1.24$, $p=0.30$) and other main effects and interactions were non-significant.

Post hoc comparisons for ADM muscle was performed to explore surround inhibition. Mixed design rmANOVA of the MEP amplitudes in ADM muscle with within subjects factors MOVEMENT (rest, onset) and between subjects factor Group (CD, FHD, Controls) revealed significant effect of factor MOVEMENT $F(1,51)=24.95$, $p<0.001$due to the significant decrease in MEPs at the onset of the movement. The effect of GROUP and the interaction GROUPxMOVEMENT were not significant ($F(2,51)=1.79$, $p=0.18$ and $F(2,51)=1.47$, $p=0.24$ respectively) (Fig. 7.1.1) thus difference of SI between the groups could not be confirmed.

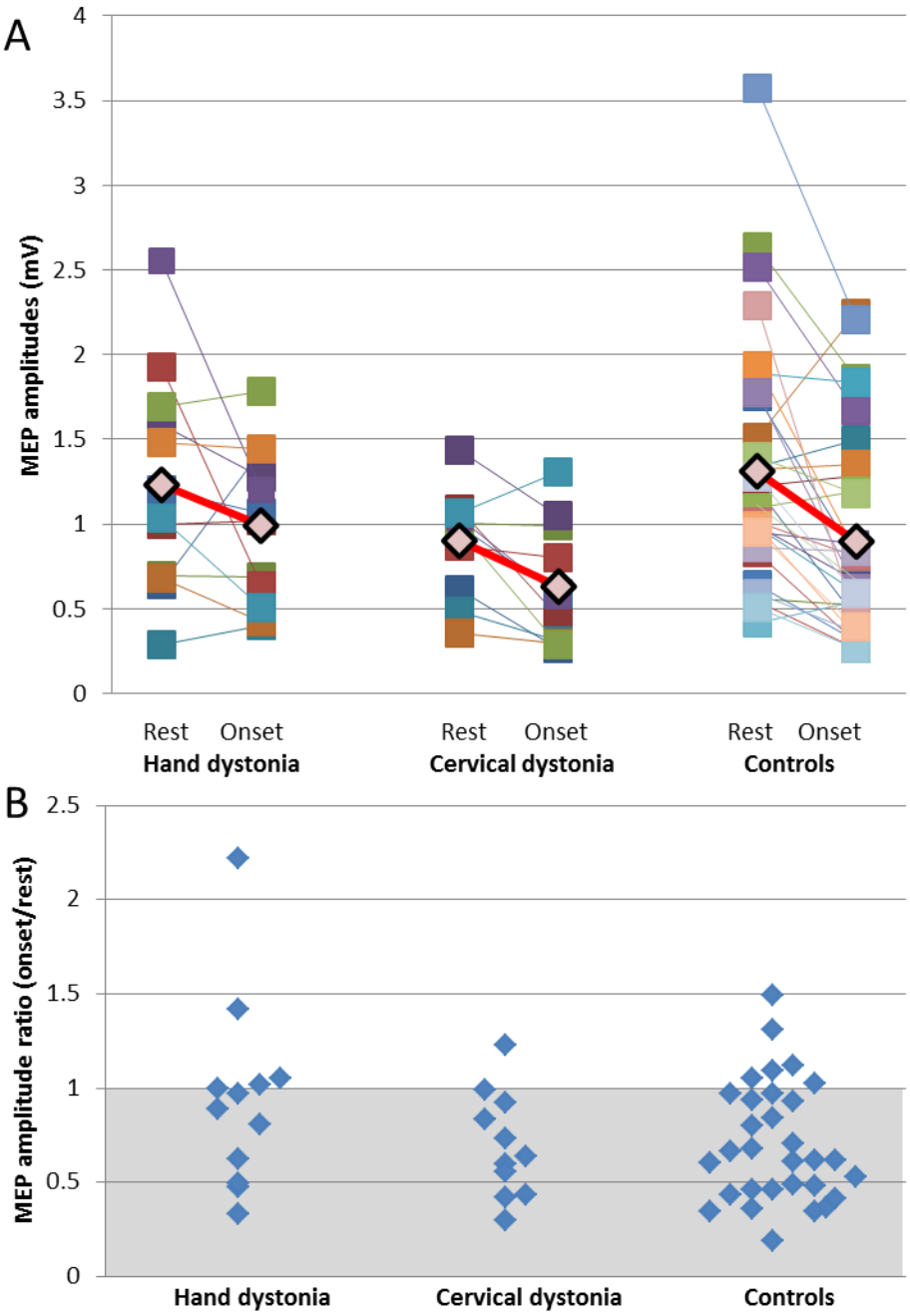



Fig. 7.1.1 A: MEPs at rest and onset of movement in the three groups. Diamonds represent the means. B: SI ratios in the three groups (individual subjects are plotted). Subjects are spread on the x-axis arbitrarily in order to minimize overlapping of subjects and to enhance visualisation. The grey area represents ratios below 1 (MEP at onset<MEP at rest)

No significant correlation was found between the ADDS scores and the SI ratios in the FHD group ($p=0.26$) or the TWSTRS scores and SI ratios in the CD group ($p=0.91$).

Differences in the RMS amplitude of EMG during FDI contraction was assessed with rmANOVA with between group factor MUSCLE (2 levels: FDI and ADM) and between subjects factor Group (CD, FHD, Controls). We found significant effect of MUSCLE ($F(1,53)=773.69$, $p<0.001$) due to increased activation in the active FDI muscle in comparison to the surround ADM muscle. No significant effect of GROUP ($F(2,53)=0.300$, $p=0.74$) or interaction MUSCLExGROUP ($F(2,53)=0.137$, $p=0.87$) was found. Therefore no significance difference in task execution between the groups was detected.

The presence of SI in CD patients is perhaps not surprising since the hands of those patients are not affected by dystonia. However the similarity of the SI profile between the FHD and the control group was unexpected, given the fact that SI in has previously been reported to be decreased or absent in FHD patients. In order to explore if our results are indeed different to previously published data, we performed a review of all previous studies which reported SI in FHD patients and healthy participants.

**Review of studies on SI in healthy and FHD patients.**

36 articles were identified but only 14 fulfilled the inclusion criteria (see methods for details). 4 of the included studies reported both a healthy control group and an FHD group (Table 7.1.3). For the meta-analysis we also included our data and therefore we used 15 groups of healthy volunteers and 5 groups of patients with FHD making a total of 214 healthy volunteers and 64 FHD patients.

| **Healthy** | **Mean SI (%)** | **SEM** | **SD** | **N** |
|---|---|---|---|---|
| *Beck et al. 2009 Exp 2* | 65.8 | 6.3 | 28.2 | 20 |
| *Sohn et al. 2004* | 75.9 | 11.8 | 31.1 | 7 |
| *Houdayer et al. 2012* | 88.9 | 6.5 | 27.4 | 18 |
| *Veugen et al. 2013* | 87.2 | 4.8 | 15 | 10 |
| *Present study* | 70.6 | 5.7 | 31.6 | 31 |
| *Sohn et al. 2004* | 69 | 4.9 | 17 | 12 |
| *Beck et al. 2010* | 84 | 5.2 | 17.2 | 11 |
| *Shin et al. 2009* | 67.2 | 5.1 | 16.2 | 10 |
| *Shin et al. 2010* | 91.8 | 8 | 25.5 | 10 |
| *Shin et al. 2007* | 84.5 | 16.4 | 46.5 | 8 |
| *Beck et al. 2009 Exp 1* | 76.9 | 4.4 | 19.2 | 19 |
| *Kang et al. 2012* | 82.5 | 5.6 | 21.7 | 15 |
| *Sadnicka et al. 2013* | 64.1 | 7.3 | 25.4 | 12 |
| *Kassavetis et al. 2012* | 74.5 | 6.7 | 26.6 | 16 |
| *Shin et al. 2012* | 85.2 | 6.3 | 24.4 | 15 |
| **Dystonia** | | | | |
| *Beck et al. 2009 Exp 2* | 105.9 | 8.7 | 34.8 | 16 |
| *Sohn et al. 2004* | 177.8 | 40.2 | 106.3 | 7 |
| *Houdayer et al. 2012* | 115.7 | 26.8 | 113.6 | 18 |
| *Veugen et al. 2013* | 101 | 8.5 | 32.7 | 15 |
| *Present study* | 94.1 | 14.5 | 50.4 | 12 |

*Table 7.1.3: Studies included in the review of SI in healthy and FHD patients.*

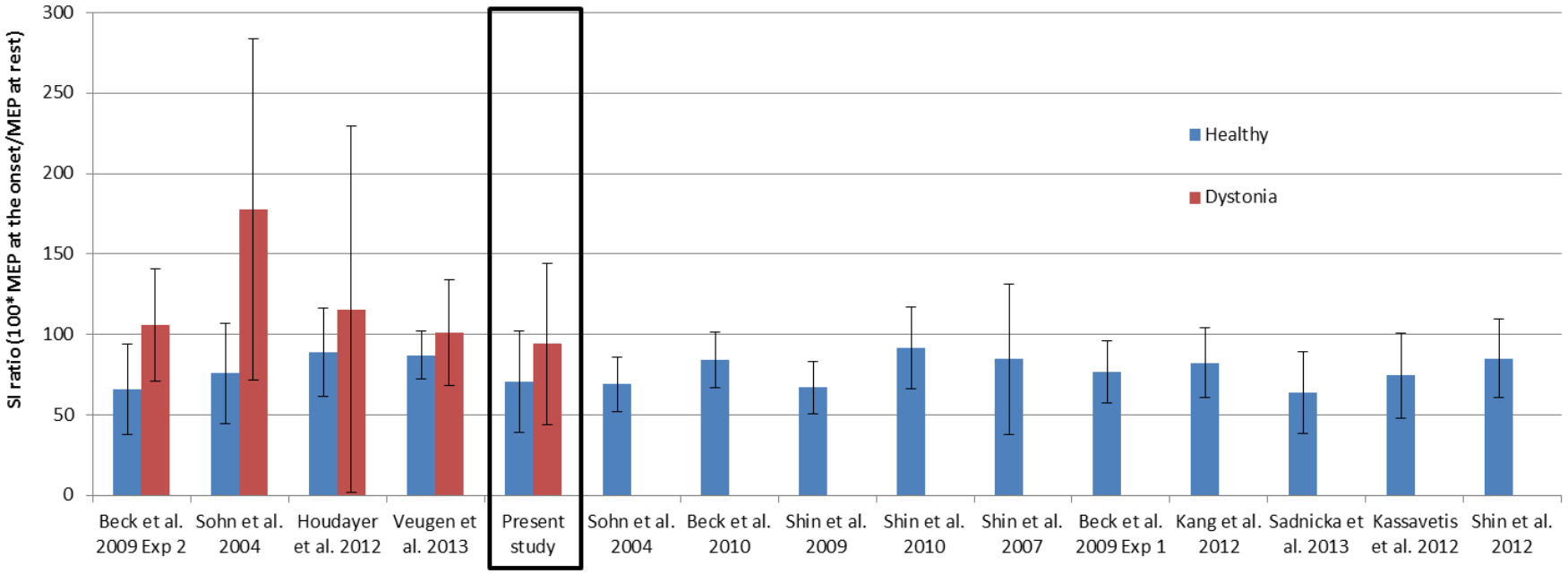



*Fig. 7.1.2: SI ratios in published studies. Error bars indicate SD of the SI ratios as reported in the published papers. Within the black rectangular is the data presented in this paper.*

Fig. 7.1.2 shows that our data fits within the range of SI generally found by others. But is there really a significant difference between FHD and healthy groups? In order to answer this question we calculated the effect sizes in the 4 published studies that have compared SI in FHD and healthy participants and in our study (Table 7.1.4). Table 7.1.4 shows that the effect sizes vary significantly between studies and that our study is indeed within the previously published range. Power calculations with the mean effect size of the 5 studies (d=0.80), alpha error probability of 0.05 and power of 0.80 (beta error =0.20) showed that a total number of 52 subjects (26 subjects in each group) is needed to investigate differences of SI between FHD and healthy participants. This is considerably higher than the sample size in all previous studies.

| | Effect size | |
|---|---|---|
| **Study** | **Cohen's d** | **r** |
| Beck et al. 2009 Exp 2 | 1.26662107 | 0.535038 |
| Sohn et al. 2004 | 1.30058769 | 0.545162 |
| Houdayer et al. 2012 | 0.32452731 | 0.160169 |
| Veugen et al. 2013 | 0.54005418 | 0.26069 |
| Present study | 0.55823775 | 0.268843 |

*Table 7.1.4: Effect sizes of differences of SI as reported in the literature*

Heterogeneity amongst the above studies was investigated with Cohran's Q and $I^2$ statistics [164] which showed non statistical significant low heterogeneity (Table 7.1.5). Forest plot shows the standardized mean difference for all the studies and the overall effect under the fixed and random effects model (Fig. 7.1.3). Different weights are assigned to the different studies for calculating the summary or pooled effect. The weighing is related with the inverse of the standard error (and therefore indirectly to the sample size) reported in the studies (Fig. 7.1.3). Funnel plot shows no obvious publication bias although its utility is limited as the number of studies is small (classically >10 studies are plotted in Funnel plots) (Fig. 7.1.4).

| | |
|---|---|
| **Q** | 4.9527 |
| **df** | 4 |
| **Significance level** | p = 0.2922 |
| **$I^2$** | 19.24% |
| **95% CI for $I^2$** | 0.00 to 84.19 |

*Table 7.1.5: Cohran's Q and $I^2$ statistics show low heterogeneity.*

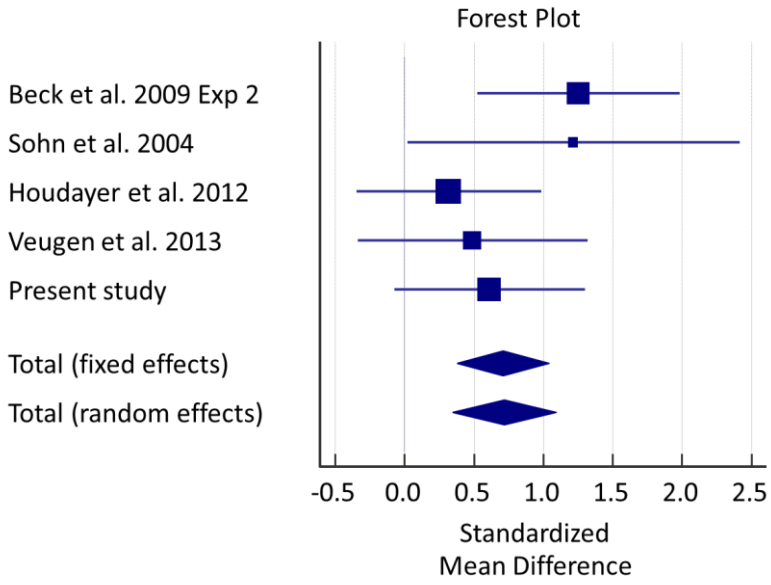


*Fig. 7.1.3: Forest plot of the different studies, with 95% CI, and the overall effect (under the fixed and random effects model) with 95% CI. The marker size varies in size according to the weights assigned to the different studies. In addition, the pooled effects are represented by a diamond which location represents the estimated effect size and its width reflects the precision of the estimate.*

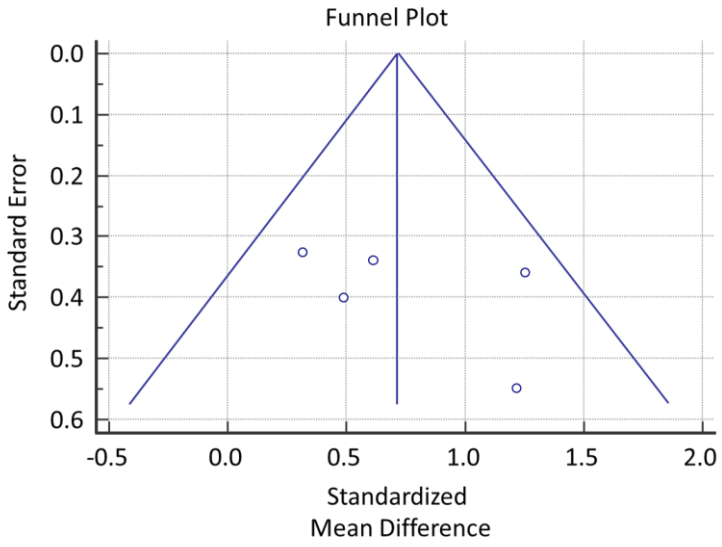



*Fig. 7.1.4: Funnel plot with standard error on the vertical axis [165]. The vertical line represents the summary estimated derived using fixed-effect meta-analysis. The two diagonal lines represent (pseudo) 95% confidence limits (effect ± 1.96 SE) around the summary effect for each standard error on the vertical axis.*

In order to explore variability of SI in FHD and healthy controls, we explored the variability of SI in the existing literature by comparing within group variability in healthy participants and FHD patients with independent sample comparisons of both the SEMs and the SDs. Both SEMs and SDs were found to be significantly different (SEM: t(18)=-3.93, p=0.001, SD: t(18)=-4.16, p=0.001) confirming that the FHD groups are more variable in regards to SI ratios (mean SEM=19.73, mean SD=67.56) in contrast to groups of healthy controls (mean SEM=7.0, mean SD=24.87) (Fig. 7.1.5).

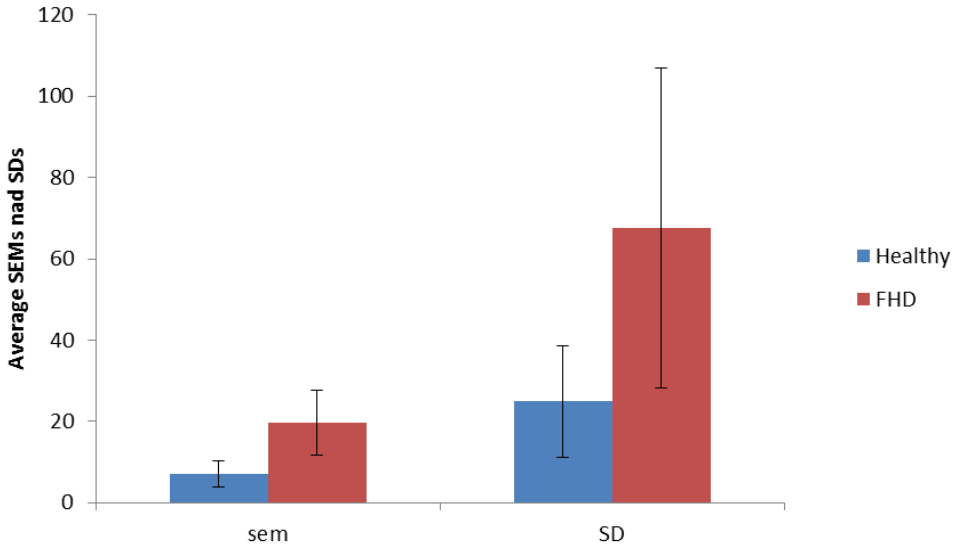



*Fig. 7.1.5: Average SEMs and SDs reported in the literature in groups of healthy volunteers (15 studies) and patients with FHD (5 studies).*

As a secondary analysis we performed multiple regression with dependent variable the SI ratio and independent variables the sample size and the mean age of participants in

the 15 groups of healthy volunteers. The analysis showed that neither age nor the number of subjects influenced SI significantly ($p=0.48$ for age and $p=0.41$ for N).

This is the first time that increased variability of SI ratios in FHD comparing to healthy participants is reported, although this seems to be widely present in the literature. An important question is whether difference in variability of SI ratios reflects differences in MEPs at rest in MEPs during movement? Due to limited published data on the MEPs during movement (only four published studies which mostly report ratios rather raw MEPs) it is hard to draw firm conclusions. However, there is ample data published on rest MEPs in FHD patients. Thus, we performed a second review of the published studies which report measures of variance of MEPs at rest in groups of patients with FHD and healthy controls.

**Review of studies on MEPs at rest in FHD patients.**

222 articles were found and reviewed. 16 of them fulfilled all the inclusion criteria and were included in further analysis. In 13 of the 16 articles more than one recording session was reported, either in more than one hand muscles or in different experiment or different groups. Thus a total of 38 recording groups of healthy volunteers (387 total recordings) and 38 recording groups of FHD patients (370 total recordings) were

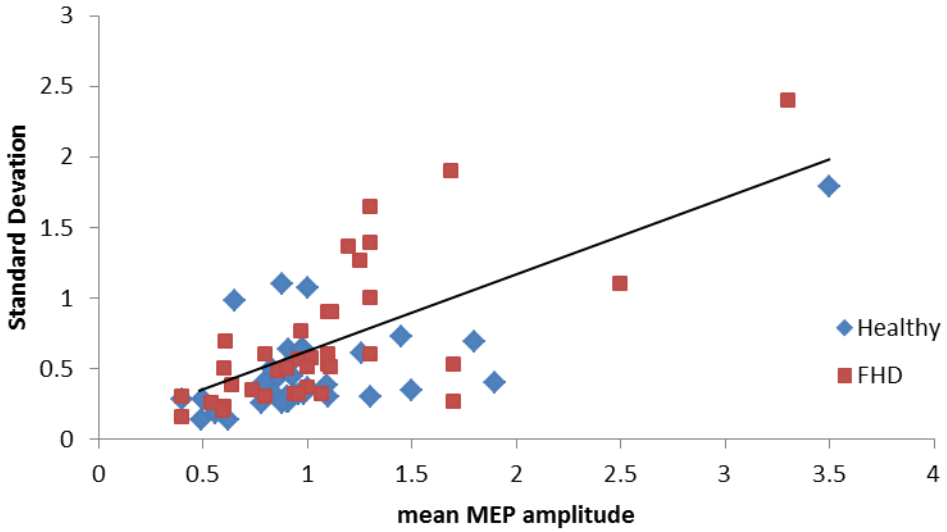
included in the review and statistically analysed. In 24 out of 38 recordings the between subjects SD of the MEP amplitudes were reported. In the remaining 14 recordings the SEMs were reported and SD was calculated.

In the above studies, the MEP sizes were matched between healthy and dystonic groups therefore between-studies heterogeneity was not expected to be high. This assumption was confirmed with heterogeneity measurements which showed very low heterogeneity among studies (Cohran's Q=24.11, df=37, p=0.95 and $I^2$<0.001%, 95% CI 0.00%-3.35%). This meta-analytic approach is informative for between studies differences but not for between group differences of variability. For this reason, a different approach was employed. Multiple regression analysis was used to explore the factors that significantly influenced variability of MEPs. The dependent variable was the SD. Independent variables were the number of subjects in each session (N), the mean age of the subjects, the mean MEP amplitude and the group (healthy controls vs FHD patients). All independent variables were forced into the model and the level of significance was assessed for all of them.

In the generated regression model two independent variables were found to be significant, the mean MEP amplitude (p<0.001) and the Group (p=0.01) (Table 7.1.6 and Fig. 7.1.6). The variables N and age were not found to significantly contribute to the regression model (Table 7.1.6).

| | B | SE B | β | p |
|---|---|---|---|---|
| (Constant) | -0.33 | 0.30 | | 0.27 |
| N | 0.02 | 0.02 | 0.15 | 0.11 |
| Age | 0.00 | 0.01 | 0.02 | 0.82 |
| MEPampl | 0.48 | 0.08 | 0.58 | 0.00 |
| Group | 0.19 | 0.07 | 0.22 | 0.01 |
| Dependent Variable: SD | | | | |

*Table 7.1.6: Regression model parameters*



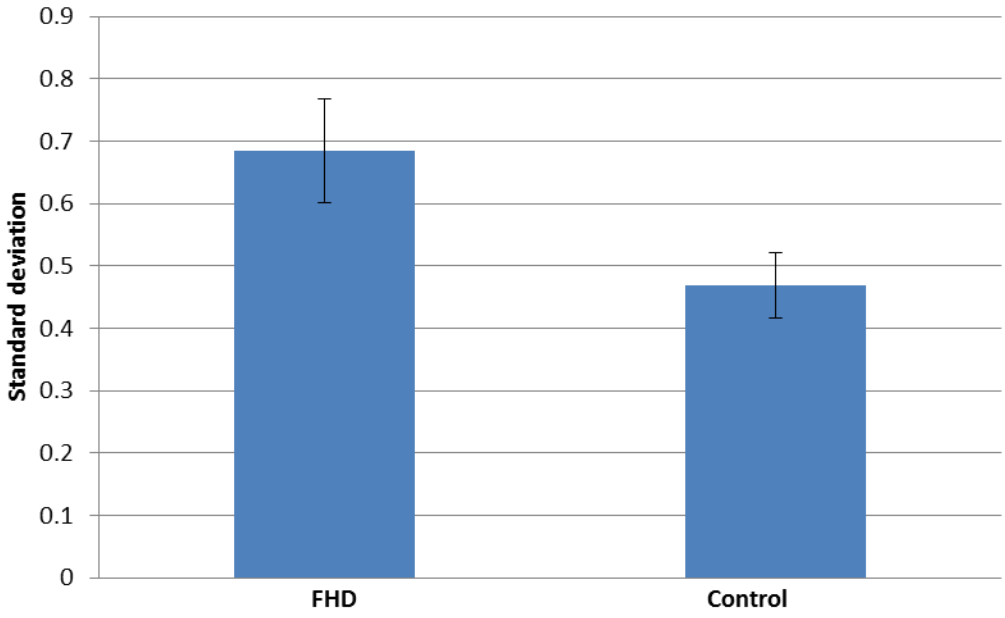



*Fig. 7.1.6: The two significant variables in the regression model which can predict a significant amount of the variability of the dependent variable SD.*

In order to confirm that there was no spurious correlation within the model we explored the relationship between the significant independent variables (mean MEP amplitude and group). Group is a categorical variables therefore independent samples comparisons were performed and showed that the mean MEP amplitudes in healthy volunteers (mean=1.02±0.52mV) and patients with FHD (mean=1.09±0.55mV) did not differ significantly z=-1.32, p=0.19 (Fig. 7.1.7).

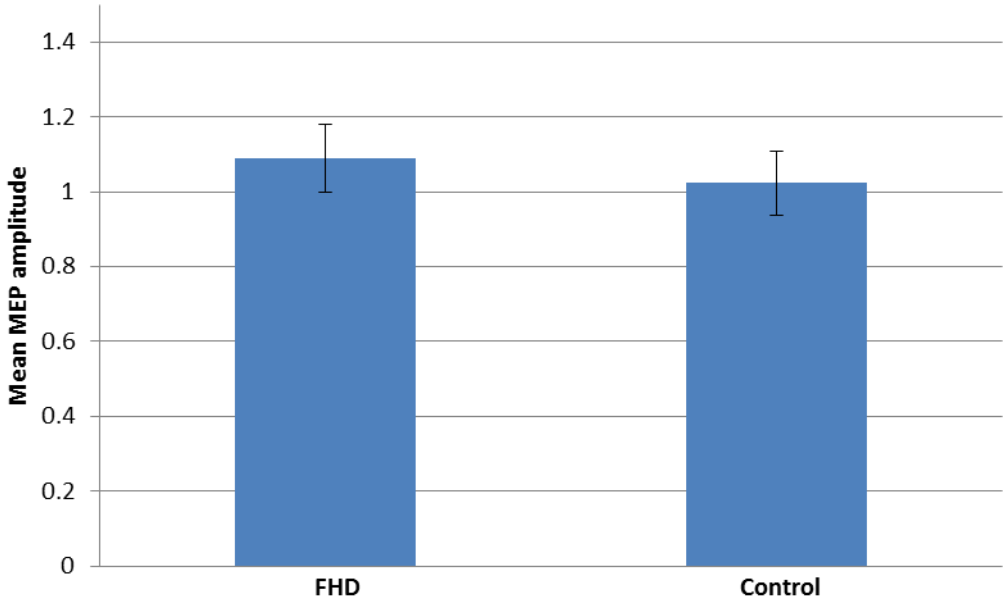


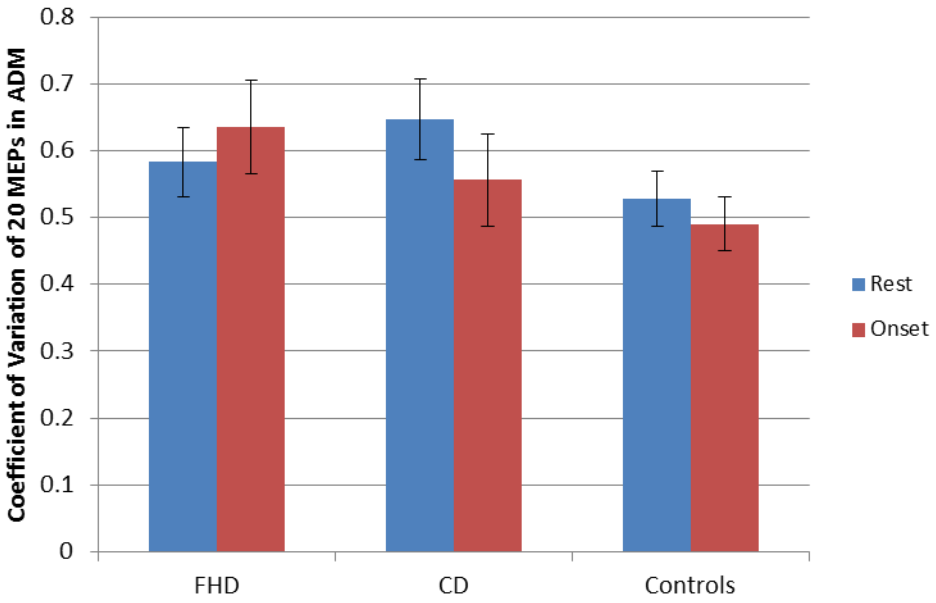

*Fig. 7.1.7: Looking for spurious correlation. Mean MEP amplitude between FHD and healthy is not significantly different.*

In addition, case wise diagnostics showed that Cook's distance was below 0.18 in all cases, confirming that there were no influential cases affecting our regression model.

**Variability of individual MEPs**

Given the above results of increased between subjects variability of the FHD group compared to the healthy group we went one step further to explore variability of individual MEPs within each subject. This data is not available in published studies therefore a literature review is not feasible. Nonetheless we analysed our dataset presented in this paper.

The coefficient of variation (CV) of the 20 MEPs collected in each condition (rest and onset) were compared in the two muscles and in all the groups with rmANOVA - within subject factors MOVEMENT (rest, onset) and MUSCLE (ADM , FDI) and between subjects factor GROUP (Control, FHD, CD). There was significant main effect of MUSCLE ($F(1, 51)=40.45$, $p<0.001$), a significant main effect of MOVEMENT($F(1, 51)=43.87$, $p<0.001$) a significant main effect of GROUP $F(2,51)=3.45$, $p=0.036$ and significant interaction MOVEMENTxMUSCLE $F(1,51)=45.49$ $p<0.001$. All other main effects and interactions were non-significant (Fig. 7.1.8).


Coefficient of Variation of 20 MEPs in ADM
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
FHD
CD
Controls
Rest
Onset

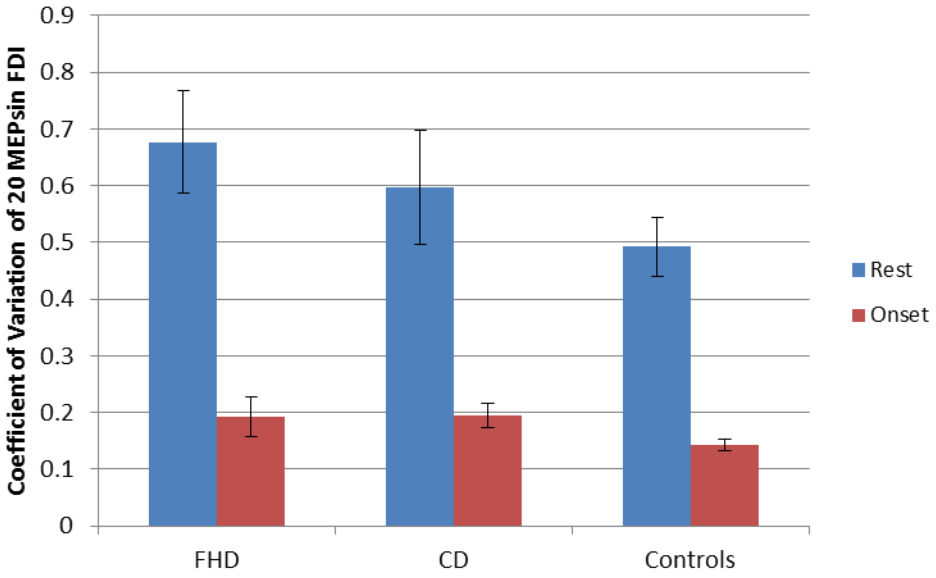

Coefficient of Variation of 20 MEPsin FDI
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
FHD
CD
Controls
Rest
Onset

*Fig. 7.1.8: Mean CVs of MEPs at rest and at the onset of the movement in all three groups. Note that CV is modified at the onset of the movement in FDI muscle when the absolute MEP amplitude is higher but not in ADM. Error bars represent SEM.*

Post hoc analysis of the interaction MOVEMENTxMUSCLE showed that CV of MEPs in the FDI muscle is reduced during activation when the absolute MEP amplitudes are increased ($p<0.001$ in FHD, $p<0.001$ in CD and $p<0.001$ in Controls) in line with previously published studies [166]. However this is not the case for ADM where the CV of MEPs where not significantly affected by movement (FHD: $p=0.39$, CD: $p=0.27$, Controls: $p=0.52$).

### 7.1.3. Discussion

In contrast to the general assumption that SI is abnormal in FHD patients we found that mean SI in FHD patients was similar to healthy controls. We also examined for the first time patients with CD, and again found SI to lie in the normal range. A detailed analysis of the previous literature showed that our data fall within the range of those reported previously, thus suggesting that if a difference between patients and healthy volunteers indeed exists, the effect is not large (Cohen's $d=0.80$) and requires larger sample sizes ($n=26$ per group) to be demonstrated with any certainty. We further showed that larger sample sizes are required due to increased variability of SI measurements in the FHD

group. Results of the second review of the literature expanded this finding further, showing that increased variability of the FHD group is not only restricted to SI but it is present even when single MEP amplitudes are measured at rest , as widely evidenced in the literature. Finally, we showed that intrinsic variability of MEP amplitude in hand muscles, whether at rest or associated with movement, was higher in dystonia patients than in healthy participants. Such increased variance may be a contributing factor to the variability in estimation of SI.

Although this is not the first time that corticospinal excitability in a surround muscle has been found to be suppressed during movement in patients with FHD [167], our study apparently contradicts most of the other published studies on SI in patients with FHD. However, from the above literature review it is clear that the existing evidence on SI is limited. An important factor that may be responsible for this disagreement is different statistical methods used in different studies. Previous studies mainly performed statistics on normalised MEP amplitudes (except for [36] which used non-parametric tests), while we used the raw MEP data.

Thus, the question as to whether mean SI is different between patients and controls still remains unanswered. Sample size calculations showed that a larger sample size (26 participants) is needed in order to reliably approach this issue. But what would a between group difference really mean? The mean SI ratio in all published studies in FHD (total N=68) is 119% with SD 68% and in healthy subjects (total N=214) is 78%,

with SD 25%. Assuming a normal distribution, the probability of a patient with FHD having SI within 2 standard deviation of the mean SI in healthy subjects is 46%. Therefore a simplistic comparison of the means and a dichotomy that FHD patients have abnormal SI would be misleading since a large proportion of patients has inhibition similar to healthy subjects. Equally a conclusion that SI in this group is normal would also be misleading because clearly there is some abnormality in the neurophysiological profile of FHD groups.

Assuming that simple comparisons of SI ratios between groups are not significantly contributing to our understanding of the pathophysiology of FHD, and given the lack of significant differences between groups, we attempted to look into the variability profiles of the groups. Interestingly we found the increased variability in corticospinal excitability in patients with dystonia at three different levels: 1. Between subject variability of SI ratios; 2. Between subject variability of the mean MEP amplitudes at rest; and 3. Within subject MEP variability in individual patients. We acknowledge that TMS techniques are subject to high variability in general but the systematic differences between the groups possibly represents a true physiological difference. We believe that within and between subjects variability might have common origins and perhaps represent a general instability of the motor system in patients with FHD. It is also remarkable that although FHD symptoms manifest only during action, increased variability is not only present during movement but also at rest. This finding is in agreement with previously published imaging studies [168, 169] and electrophysiological studies with recordings at rest [51, 53, 170] and it may be related to

abnormalities in motor network connectivity in patient with FHD. Further studies on the spatial and temporal patterns of variability in these patients may provide valuable clues about its origins in the nervous system.

What are the implications of increased variability for measurement of SI? Here we describe a systematic difference in variability of the MEP amplitudes between normal and dystonic groups which may be important for the statistical tests for group comparisons. In particular ANOVA requires the assumption of equal variance to be fulfilled. ANOVA has been used in numerous previous electrophysiological studies and commonly authors use normalisation methods to overcome this obstacle. With this study we highlight that authors should expect to find between groups' differences in variability measures and that extra attention should be paid for the selection of the appropriate statistical methods. The same principle applies to other inhibitory paradigms tested with TMS where within group comparisons are made (e.g. SICI, LICI, IHI, etc).

As a further question over the usefulness of FHD as a model for the hypothetical behavioural consequences of abnormal SI, we failed to find any correlation between clinical severity of dystonia and SI. Other electrophysiological parameters (i.e. SICI, response to PAS, SP) have been found to be 'abnormal' but are not directly related to clinical manifestation. However, SI is commonly presented as measure that is directly causally linked to abnormal motor output in dystonia. Patients with focal hand dystonia

have variable phenotypic presentations, therefore the balance of excitability between active and surround muscles may significantly differ between individual patients. In this study, we found that a proportion of patients had normal SI, but this result should not be generalised and cannot be interpreted as presence of normal motor output in these patients. Most likely, the phenotypic expression of the motor abnormality in these patients was such that it could not be captured by the particular paradigm used here. Perhaps development of more detailed paradigms tailored specifically to the phenotypic expression of individual patients, would be more efficient to identify the abnormality without the "dilution effect" caused by phenotypic variability. In addition, more precise clinical scales or kinematic studies (able to capture the exact finger abnormalities) or experiments with clusters of patients with similar clinical symptoms could finally provide support for the association between SI and the motor performance.

With regards to the CD group, we found that these patients had SI comparable to the healthy group. This is an interesting finding given that other inhibitory networks within the motor cortex have been found to be normal in those patients [171-173]. The within subject variability of MEPs was also found to be increased in this group. This is the first time SI is described in this group therefore more studies are needed to draw firm conclusions.

This study is limited by the fact that there is high variability of the baseline MEP measurements – see discussion above. However our results are comparable to published

literature and serve the purposes of this particular study. In addition, we chose the TMS intensity as the intensity to evoke MEPs with average peak-to peak amplitude of approximately 1mV-1.5mV at rest in the ADM muscle." While this is common, it probably not optimal because the "1 mV standard" may have a variable position on an Input Output curve and thus a variable response to a change in excitability. Other authors have suggested alternative techniques such as to set the test stimulus intensity to produce 50% of the maximal MEP amplitude at rest[174]. Finally we followed the design of previous studies and therefore we grouped data from patients with WC and MD. However, there is evidence of pathophysiological differences between these two conditions[175] therefore future studies may need to further explore differences between MD and WC with regards to SI.

We studied SI in patients with two different types of focal dystonia and we found that their SI profile is similar to healthy participants. In addition we found that patients with FHD have more variable neurophysiological profiles, which is further confirmed by review and analysis of previously published studies. We believe that these data call for a reappraisal of the role of SI in the pathophysiology of dystonia, in particular the proposal that it relates directly to motor performance deficit in dystonia. This reappraisal needs also to consider how motor SI relates to motor performance in general, and if it can indeed be shown to be crucial for individuation of single finger movements.

# Chapter 8. GENERAL CONCLUSIONS AND FUTURE STUDIES

We studied the phenomenon of SI in 4 distinct axes. We firstly characterised SI in a large cohort of normal subjects, we then attempted to find its origins in the cerebellum, we modified it by means of peripheral stimulation and finally we tested it in two groups of dystonic patients. The findings of this thesis provide significant insight in the mechanisms of SI although its enigmatic nature yields further research.

As discussed above the most prominent evidence about SI in this thesis is its very presence. In Chapter 4 we measured SI in a large cohort of healthy participants and explored its relationship with EMG activity in the active and surround muscles. We found strong evidence of presence of SI in the ADM muscle at the onset of FDI contraction. Interestingly the analysis of the EMG signals showed increased EMG activity in the surround muscles at the onset of FDI contraction despite reduction of corticospinal excitability measured with TMS. This finding firstly provides evidence that MEPs and EMG are modulated in opposite directions at the onset of movement and secondly suggests that EMG signal measured in surround muscles has subcortical origins in contrast to SI which probably has cortical origins as it has been postulated in the past. These results open the field for further exploration of inter and intracortical neural connections that drive SI and its relationship to finger kinematics.

In our pursuit to identify structures with modulatory function to SI in the motor system, we explored the role of cerebellum in finger movement individuation in two different studies. We failed twice to find evidence of muscle specific modulation of cortical excitability driven by the cerebellum via the cerebello-thalamo-cortical pathway. This finding can be interpreted in two ways, either that the cerebellum is not involved in modulation of SI or that cerebellar stimulation was ineffective in modulating cerebellar output. Regarding the first possibility, although there is no direct evidence of a potential link between cerebellum and SI, it is known that the cerebellum plays important role on the modulation of motor output. In particular, it has been shown that the timing of the triphasic agonist-antagonist pattern at the onset of voluntary ballistic movement is largely controlled by the cerebellum [176-178]. Interestingly, this modulation of motor output takes place at the onset of voluntary movement, when SI is present, suggesting that there is a rationale to explore if SI is modulated by the cerebellum. On the other hand, the two types of cerebellar stimulations used in this study (TMS and TDCS) are very crude and although they have been used for many years in neurophysiological studies, their underlying mechanism is not entirely clear. From the above, it is difficult to conclusively determine whether or not the cerebellum plays a modulatory role in SI. In order to address this issue further, it would be interesting to investigate SI in patients with isolated cerebellar abnormalities (stroke/degeneration). Impairment of SI in this group would provide indirect evidence that cerebellum is indeed involved in generation or modulation of SI.

Although no change of SI was found to be driven by the cerebello-thamo-cortical pathway, we coincidentally found that excitability in that pathway is modulated equally in both active and surrounding muscles in a non-muscle specific manner at the onset of a brief finger movement. Although this outcome may not be directly relevant to SI, it actually provides evidence about another puzzling phenomenon in the motor system, the enhancement of the excitability of the monosynaptic reflex pathway when the Jendrassik manoeuvre is performed. We did not further explore this effect as it exceeded the focus of this thesis on SI.

At the time we designed the two next projects described in Chapter 6, previously published studies had provided evidence of impairment of SI in patients suffering from focal hand dystonia. An almost reflexive response to these results is to attempt to normalise SI in these groups of patients. The incentive was based in the hypothesis that if loss of SI were related to the symptoms of patients with dystonia, then it would be possible to relief symptoms by restoring the normal strength of SI. Indeed, the results of our studies in healthy population were very exciting. We showed that SI is not hard-wired and that it can be modulated with peripheral stimulation. In particular, SI can adapt according to sensory feedback and that these adaptive changes are retained for a short period after the end of training. In addition, PAS21.5 protocol can artificially increase SI in hand muscles by changing the balance of corticospinal excitability in the intrinsic muscles of the hand. We discuss several hypotheses about the underlying mechanism of these results in the individual chapters but the bottom-line is that these

results opened up the possibility of modification of SI in patients where SI was supposingly impaired.

In the next and final study of this thesis we recruited a cohort of patients with focal cervical dystonia and focal hand dystonia. We based the design and hypothesis of the study on evidence provided by previously published studies. However, we surprisingly failed to find evidence of impaired SI in patients with focal hand dystonia. Further exploration of our results and review of the literature showed that a major fault of all studies (including ours), was inadequate statistical power. A larger number of subjects were required to adequate power the studies, because of increased variability of SI in the patient population. Although this is a major limitation for any conclusion about the mean SI in patients with dystonia, the fact that SI was found to be more variable in patients with FHD is noteworthy on its own. Increased variability may have important implications in the design and interpretation of future studies and may indeed be related to pathophysiological mechanisms of dystonia.

***Further studies***

The studies described above provided significant evidence about the phenomenon of SI but they also generated further questions. Here we describe the hypotheses and design of studies that could certainly provide more evidence about SI, based on the results of this thesis.

1. Movement kinematics and SI

In the first study described in Chapter 4 we showed that MEPs and EMG in the surround muscles are modulated differentially (MEP decreases and EMG increases) at the onset of a brief finger movement. This result raises the question how cortical modulation of corticospinal excitability is related to the final motor output. The traditional paradigm for assessment of SI (which was also used in this thesis) does not allow measurement of movement kinematics, as the surround fingers do not move at all during the motor task. However, a different paradigm with increasing involvement of the surround finger in the task and simultaneous measurement of SI would allow correlations between movement kinematics and SI. The strength of such correlation will provide direct evidence on the role of SI in defining the kinematic parameters of finger movements. We hypothesize that SI will become less strong as the surround finger becomes increasingly involved in the movement.

2. Motor learning and SI

A dominant assumption throughout the SI literature is that the strength of SI is directly related to motor performance. This hypothesis is mainly derived by the notion that SI is impaired in patients with dystonia (although this remains under question according to

evidence presented above) and no direct evidence exist to support this assumption. Therefore it remains unclear if recruitment of SI circuits in the motor system is really beneficial to movement. A direct way to disentangle this problem is to measure modulation of SI as subjects learn a motor task. We hypothesise that SI will be strongest during initial exposure to the task and as the subjects continue to learn the task, movement kinematics will improve and SI will decrease.

3. Motor SI in large cohort of dystonic patients

A key result of the study described in Chapter 7 was the sample size calculations for adequate power of the statistical tests for comparison of SI between patients with dystonia and normal controls. All previous studies were found to be underpowered therefore the credibility of the published results remains under question. At this point a larger study with 26 subjects per group is necessary to give a valid answer to the question if SI is impaired in dystonic patients. Such a study will allow planning for further exploration of modulation of SI with pharmacologic agents or brain stimulation protocols as a treatment of FHD (See below).

4. Modulation of SI in patients with dystonia.

If an adequately powered study shows that SI is indeed impaired in patients with FHD, the methods described in Chapter 6 would constitute great tools for its modification. We showed that timed vibration in a non active muscle can be effectively modify the strength of SI by changing the sensorimotor associations through an adaptive process driven by introduction of sensory imbalance between active and non active muscles. In addition, we show that enhancement of corticospinal excitability with the use of paired associative stimulation protocol can also lead to modulation of the strength of SI. Both surround muscle vibration and PAS protocol can be used in future studies as potential tailored treatments for patients with FHD.

# Chapter 9. APPENDIX 1: Publications during PhD period related to this thesis

1: Belvisi D, Kassavetis P, Bologna M, Edwards MJ, Berardelli A, Rothwell JC. Associative plasticity in surround inhibition circuits in human motor cortex. Eur J Neurosci. 2014 Oct 7.

2: Kassavetis P, Sadnicka A, Saifee TA, Belvisi D, van den Bos M, Pareés I, Kojovic M, Rothwell JC, Edwards MJ. Motor 'surround inhibition' is not correlated with activity in surround muscles. Eur J Neurosci. 2014 Aug;40(3):2541-7.

3: Sadnicka A, Kassavetis P, Saifee TA, Pareés I, Rothwell JC, Edwards MJ. Cerebellar transcranial direct current stimulation does not alter motor surround inhibition. Int J Neurosci. 2013 Jun;123(6):425-32.

4: Kassavetis P, Saifee TA, Sadnicka A, Pareés I, Kojovic M, Rothwell JC, Edwards MJ. Adaptation of surround inhibition in the human motor system. Exp Brain Res. 2012 Oct;222(3):211-7.

5: Kassavetis P, Hoffland BS, Saifee TA, Bhatia KP, van de Warrenburg BP, Rothwell JC, Edwards MJ. Cerebellar brain inhibition is decreased in active and surround muscles at the onset of voluntary movement. Exp Brain Res. 2011 Mar;209(3):437-42.

## Chapter 10. APPENDIX 2: Other publications during PhD period

1: Belvisi D, Kassavetis P, Bologna M, Edwards MJ, Berardelli A, Rothwell JC. Associative plasticity in surround inhibition circuits in human motor cortex. Eur J Neurosci. 2014 Oct 7.

2: Sadnicka A, Patani B, Saifee TA, Kassavetis P, Pareés I, Korlipara P, Bhatia KP, Rothwell JC, Galea JM, Edwards MJ. Normal motor adaptation in cervical dystonia: a fundamental cerebellar computation is intact. Cerebellum. 2014 Oct;13(5):558-67.

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