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SHOHREH KARIMINEZHAD

REPETITION SUPPRESSION, A POTENTIAL BIOMARKER FOR NEUROMODULATION-INDUCED PLASTICITY

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ACADEMIC DISSERTATION

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ABSTRACT

As the cornerstone of healthcare, the use of objective biomarkers throughout the disorder diagnosis and stratification of patients can help provide a ”signature” with predictive information on the future outcome of therapeutic interventions. Since neuro-psychiatric disorders are characterized by their multifactorial complex nature of the neuro-psychiatric disorders, there is no single factor by which the treatment outcome can be reliably predicted. Yet it has been postulated that, neuroplasticity, i.e. the adaptive mechanism of the central nervous system from the behavioral level down to the cellular level, can be considered as a determinant inherent characteristic by which those individuals susceptible to neuromodulation therapies might be distinguished. Neuroplasticity has been demonstrated to associate with repetition suppression (RS); an inherent brain mechanism, manifesting as a reduction of the neural activity when an identical sensory stimulus is repeated. RS has been also quantified in the motor cortex, measured as the motor evoked potential (MEP), using transcranial magnetic stimulation (TMS).

The overall aim of this thesis was to investigate the potential of the RS as an objective biomarker for neuroplastic capacities to achieve the excitation/ inhibition balance. Since a clarification of the underpinning mechanisms can lend further validity to the predictive power of a biomarker and help gain greater mechanistic insights, the potential common modulators between the networks mediating RS and intracortical facilitation/inhibition were investigated in the first study. Our findings pointed to the existence of a potential shared inhibitory mechanism, where no interaction between RS and cortical facilitation was evident.

In the second study, the effect of the induced neuroplasticity on the RS was investigated in healthy subjects. For this purpose, RS was differentiated into two states: an initial decrement of the MEP response following the first repetition of the stimulus, and the maintenance of this reduced response following further repetitions. This probably helped to distinguish two states; the first one would reflect the efficiency of the nervous system to adapt to a novel stimulus, and the second to exhibit its capacity to store and maintain the respective information. By assessing these two states, it was demonstrated that the short-term induced plasticity resulted in MEP responses with a limited range of amplitude following the first repetition of the stimulus. This potentially implies that a mechanism exists, which is required for the storage of a short-term "automatic" memory, leading to a minimizing of the surprise reaction in the face of an intense repeated sensory stimulus. Hence, the lack

of this suggested mechanism might contribute to maladaptive patterns, leading to hypervigilance to sensory stimuli in chronic pain patients. In view of this hypothesis, the predictive power of the RS was investigated in the third study in patients with chronic pain receiving repetitive-TMS (rTMS) treatment. The results revealed the predictive value of the RS merely at the level when it had stabilized where the decrement of the neural response was maintained. These findings seem to highlight the potential of the rTMS to normalize the patterns restoring the adaptive mechanism in patients who are able to maintain the trace of a recent encountered stimulus with neither baseline hypo- nor hyper- cortical excitation.

This thesis introduces TMS-induced RS as a potential biomarker as a foundation for improved individually-based neuromodulation treatments. This might be of special interest in neuro-psychiatric disorders where a maladaption of inhibition has been suggested as the underlying pathology.

***Universal Decimal Classification**: 615.841; 577.25*
***National Library of Medicine Classification:** WL 141.5.T7, WL 307, WL 102, G11.561.638, WL 704*
***Medical Subject Headings:** Transcranial Magnetic Stimulation; Motor Cortex; Evoked Potentials, Motor; Neuronal Plasticity; Chronic pain*

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Last but not the least, I would like to thank my parents who have always stood by me and provided their constant support and love for the whole of my life. They have been my life's greatest inspiration even when I am so far away from them.

Kuopio, August 2021

Shohreh Kariminezhad

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on data presented in the following articles, referrred to by the Roman Numerals I-III.

I Kariminezhad S. et al. (2019). Interaction between repetition suppression in motor activation and long-interval intracortical inhibition. Scientific Reports, 9(1), 11543.

II Kariminezhad, S. et al. (2020). Brain response induced with paired associative stimulation is related to repetition suppression of motor evoked potential. Brain Sciences, 10(10), 674.

III Kariminezhad, S. et al. (2021). Repetition suppression of the motor cortex may predict the responsiveness to high-frequency rTMS in chronic pain. Under review.

AUTHOR'S CONTRIBUTIONS

Publication I: "Interaction between repetition suppression in motor activation and long-interval intracortical inhibition"

The author conducted the measurements with the third author, analyzed the data, interpreted the results, and prepared the manuscript.

Publication II: "Brain response induced with paired associative stimulation is related to repetition suppression of motor evoked potential"

The author conducted the measurements with the third author, analyzed the data, interpreted the results, and prepared the manuscript.

Publication III: "Repetition suppression of the motor cortex may predict the responsiveness to high-frequency rTMS in chronic pain"

The author analyzed the data, interpreted the results with the co-authors, and prepared the manuscript.

LIST OF ABBREVIATIONS

AEPs	Auditory evoked potentials
APB	Abductor policis brevis
BPI	Brief Pain Inventory
CNS	Central nervous system
CRPS	Complex regional pain syndrome
CS	Conditioning stimulus
cSP	Cortical silent period
DN	Dentate nucleus
EEG	Electroencephalography
fMRI	Functional magnetic resonance imaging
GABA	Gamma-aminobutyric acid
GPe	External globus pallidus
GPi	Internal globus pallidus
ICF	Intra-cortical facilitation
IPI	Inter-pulse interval
ISI	Inter-stimulus interval
ITI	Inter-train interval
LICI	Long-interval intracortical inhibition
LTD	Long term depression
LTP	Long term potentiation
MEP	Motor evoked potential
MRI	Magnetic resonance imaging
M1	Primary motor cortex
NMDA	N-methyl-D-aspartate
nTMS	Navigated transcranial magnetic stimulation
PAS	Paired associative stimulation
PD	PainDETECT
RAS	Reticular activating system
rMT	Resting motor threshold
RS	Repetition suppression
rTMS	repetitive transcranial magnetic stimulation
SICI	Short intra-cortical inhibition
SICF	Short-interval intracortical facilitaion
SMA	Supplementary motor area
SNr	Substantia nigra pars reticulata
ST	Sensory threshold
STDP	Spike timing-dependent plasticity
S1	Primary somatosensory cortex
S2	Secondary somatosensory cortex
TMS	Transcranial magnetic stimulation
TS	Test stimulus

TABLE OF CONTENTS

1 INTRODUCTION

An individual's survival in our ever-changing environment is influenced by three processes; learning, unlearning and relearning. This cycle, which evolution has developed to allow organisms to respond to intrinsic and extrinsic changes in an adaptive manner, is founded on a concept known as neuroplasticity. Originating from the Greek word "plastos" (meaning molded), neuroplasticity refers to an integral property of the nervous system, e.g. subserving the acquisition of new skills or a recovery phase following a mild brain injury [1–3]. By taking this into account, the disparities between the responses to the treatments in which the plasticity is induced could be partly explained by the degree to which neuroplasticity is recruited [1, 4]. This occurs through the unmasking and strengthening of the existing neural networks, or through the establishment of new networks. Although neuroplasticity is crucial in promoting a normal development and recovery, this rewiring may represent the core pathology of several neuropsychiatric disorders, for example, in the development of neuropathic pain after a spinal cord injury [5]. Hence, by defining a pre-treatment neurobiological fingerprint that characterizes the brain's capability to compensate for the lesions and maladaptive pathways, i.e. a capacity for neuroplasticity, would represent a major step towards individualized rehabilitative therapies. Adaptation is one such potential inherent brain phenomenon through which the capacity for the neuroplasticity can be determined [6]. Since evaluating the internal and external stimuli is an ongoing critical process for survival, the brain responds to an intense, novel stimulus through enhanced neural activity [7]. Although this transient heightened activity would promote the chance of withdrawal from a potential harmful stimulus, the exposure to the identical stimulus results in an attenuation of neural activity. This stimulus-specific adaptation is commonly referred to as repetition suppression (RS) [6]. RS has been well characterized across various sensory modalities using different means such as functional magnetic resonance imaging (fMRI), electroencephalography (EEG), and transcranial magnetic simulation (TMS) [8–12]. TMS is a non-invasive method that generates electrical currents in the brain by means of rapidly time-varying magnetic fields [13]. TMS is also frequently used as a therapeutic or add-on therapeutic method in neurorehabilitation where trains of stimuli are delivered to relatively focal brain regions with an inter-stimulus interval (ISI) around or less than 1s (repetitive TMS) [14]. RS has been quantified in the motor system using TMS, reflected as the reduction in the motor evoked potential (MEP) amplitude [15]. RS has been speculated to be associated with neuroplasticity in Unverricht–Lundborg type progressive myoclonus epilepsy, a disorder with impaired motor cortical plasticity [16, 17].

This thesis was conducted to develop and investigate routines of the RS stimulation-protocol for use as a biomarker to quantify the individual capacity for the required neuroplasticity, with the ultimate goal of achieving a form of patient-specific neuromodulation interventions. Furthermore, in order to broaden the understanding of the mechanisms underpinning RS, its interaction with cortical inhibition and facilitation was also studied. Alongside the necessary background, the following sections will cover the aims as well as the methodological and technical

framework of the study to help in investigating RS in healthy subjects and patients with neuropathic pain. The discussion will be made based on the above-mentioned sections and findings of the studies.

2 BACKGROUND

2.1 ANATOMIC SUBSTRATE OF MOVEMENT FUNCTION

Spreading across an area of the cerebral cortex, immediately anterior to the somatosensory cortex, the motor cortex is the main region involved in the control, planning and execution of movement [16, 18]. The motor cortex is composed of primary motor cortex (M1 or Brodmann's area 4), interconnected with non-primary motor areas, i.e. premotor cortex (lateral part of Brodmann's area 6), supplementary motor area (SMA or medial part of Brodmann's area 6), and posterior parietal cortex (Brodmann's area 8) [19] (Figure 2.1). Of these regions, the primary motor cortex can be considered as the central structure where the magnetic stimulation most readily elicits a response.
Similar to the primary somatosensory cortex (S1), the M1 has been known to have a somatotopic organization, due to the fact that different muscles are represented in different areas of the M1, with inter-individual differences in both the extent and location of motor representations [20]. This means that the representations of different muscles are in different areas of the M1, with inter-individual differences in the extent and location of motor representations [20].
Other regions involved in the motor function are located outside of the cortex, at the subcortical level, of which the basal ganglia, forming an integral network with the thalamus, and the cerebellum are of major importance. The structures of this network, which play key roles in planning and executing voluntary and involuntary movement, will be discussed in the following sections.

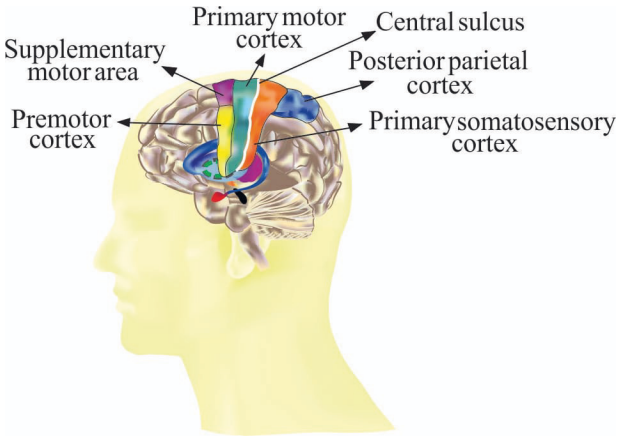

Figure 2.1: The motor cortex compromises four areas: primary motor cortex, premotor cortex, supplementary motor area, and posterior parietal cortex. In the present thesis, the focus will be on the primary motor cortex.

2.1.1 Primary motor cortex

The M1 is a six-layer convoluted sheet of neural cells, located within the precentral gyrus. With the less distal movements, represented more laterally in the M1, the M1 forms an upside-down motor map [21, 22]. Intermingled with the gamma-aminobutyric acid (GABA)-ergic inhibitory interneurons, the glutamatergic excitatory pyramidal neurons are the principle type of cells located in the motor cortex [23, 24]. Pyramidal cells of various shape and size are distributed in layers II to VI, with being the most abundant in layers II and V in the M1 (Figure 2.2). Although layer II/III pyramidal neurons are the main contributors to cortico-cortical connections, layer V pyramidal neurons are the neurons having subcortical projections. Since they send their axons down the spinal cord (pyramidal corticospinal tract) and the brainstem (pyramidal corticobulbar tract), these pyramidal neurons are believed to be involved in the control of voluntary movements [25]. While the majority of the neurons in the corticospinal tract originate from the primary motor cortex and are responsible in the movements of the torso, upper and lower limbs, other neurons extend from the non-primary motor areas as well as from the somatosensory cortex [26, 27]. These horizontal and vertical extensions into other cortical and subcortical regions provides the M1 with a dynamic structure, through which normally hidden representations of the muscles can be revealed [28].

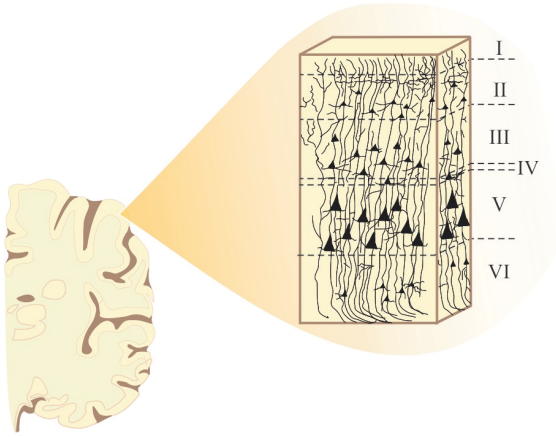

Figure 2.2: The motor cortex is a six-layered sheet of neural cells, mainly consisting of two types of cells: excitatory pyramidal cells and inhibitory interneurons. The pyramidal neurons of different size and shape are distributed across the layers II to VI, making cortical and subcortical connections.

2.1.2 Basal ganglia

The basal ganglia refers to a group of interconnected nuclei, embedded deeply in the brain. The constituent nuclei of the basal ganglia include striatum, globus pallidus, subthalamic nucleus, and substantia nigra (Figure 2.3). There is convincing evidence suggesting that the basal ganglia are not only involved in sensorimotor functions, but it significantly contributes to the neural processing involved in reward-related as well as habit formation [29, 30]. As its largest nucleus, the striatum (caudate nucleus and putamen) is a heterogeneous input nucleus through which the cortical and tha-

lamic efferent inputs project to the basal ganglia system. In addition, the striatum receives incoming information from the dopaminergic nigral region. Depending on the subtype of the dopamine receptors expressed on the striatal neurons, these signals can be either excitatory or inhibitory. The information received by the basal ganglia are transmitted to the subcortical motor areas including the pedunculopontine nucleus of brainstem by the output nuclei; these are the internal segment of the globus pallidus (GPi) and the substantia nigra pars reticulate (SNr). However, the main projection target of the output nuclei is the motor thalamus, which subsequently projects back to the cortex to facilitate/inhibit the activation of the motor system.
Two main pathways originate from the striatal neurons, i.e. direct and indirect (Figure 2.4). In the direct pathway, dopamine subtype 1 receptor (D1R)-containing striatal neurons serve as the main source of inhibition to the output nuclei [31, 32]. In turn, the evoked inhibition, reduces the tonic inhibition induced by the output nuclei on the cortex and therefore this facilitates movements. In contrast, in the indirect pathway, another type of inhibitory striatal neurons inhibit the external segment of the globus pallidus (GPe). Inhibition of the GPe results in a facilitation of the subthalamus nucleus (STN). Since the STN provides an excitatory glutamatergic projection onto the inhibitory output nuclei, its disinhibition leads to an inhibition of the movement [33, 34]. The importance of these pathways might be attributed to their role in providing the necessary feedback loop for the cortex to achieve the optimal excitation/ inhibition balance, a topic which was examined in this thesis.

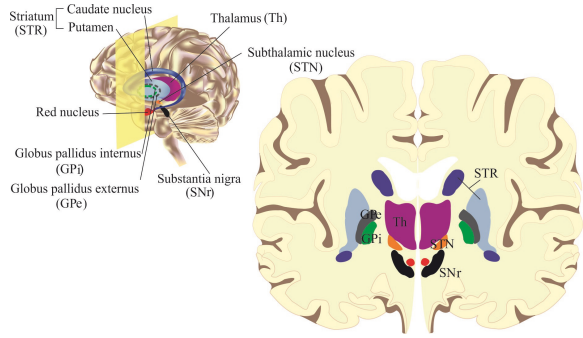

Figure 2.3: The basal ganglia. The basal ganglia is an interconnected subcortical nuclei consisting of striatum, globus pallidus, subthalamic nucleus, and substantia nigra. The basal ganglia is crucial in inhibition and facilitation of the voluntary movements through indirect and direct pathways, respectively.

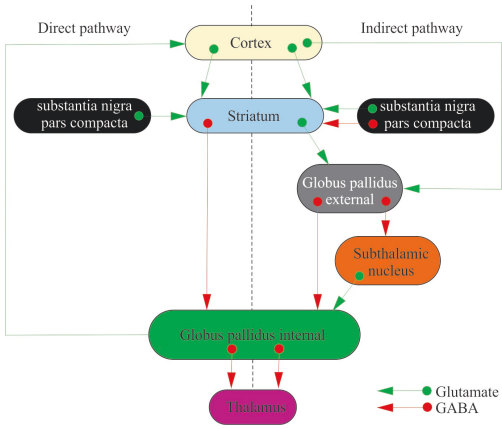

Figure 2.4: The classical model of direct and indirect pathways in the basal ganglia. In the direct pathway, the striatal neurons inhibit the output nuclei, which in turn reduces the tonic inhibition induced on the cortex, resulting in the facilitation of the movement. In the indirect pathway, the striatal neurons inhibit the GPe, leading to the facilitation of the STN. The facilitation of the STN results in the inhibition of the movement through its excitatory projection on the inhibitory output nuclei. Modified from [33]

2.1.3 Cerebellum

The recent literature has pointed to a contribution of the cerebellum in sensory and cognitive processing, extending its functional domain beyond its traditional role in motor planning and behavior [35–37]. There is growing evidence also suggesting that cerebellum possessess an established role in identifying recurrent events and their violations [38]. A fraction of the ascending projections of the dentate nucleus of the cerebellum is directed to the M1 via the thalamus, meaning that the output of the dentate nucleus (DN) is involved in the control of movement (Figure 2.5). In addition, a disynaptic projection of the DN to the striatum has been demonstrated, serving as an anatomical substrate facilitating a two-way communication between these two structures [39].

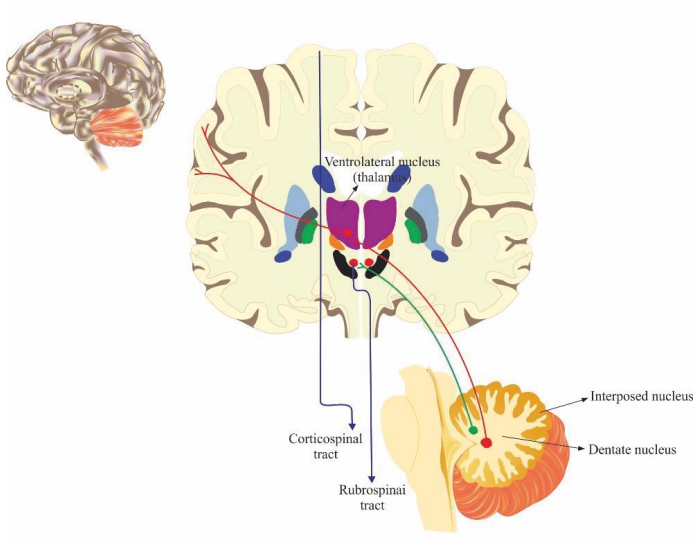

Figure 2.5: The output of the dentate nucleus in the cerebellum is mainly directed to the M1 through the thalamus. These ascending projections are primarily involved in motor control.

2.2 TRANSCRANIAL MAGNETIC STIMULATION (TMS)

2.2.1 Principles

Magnetic stimulation is based on the principles of electromagnetic induction. Accordingly, an intense, brief electric current passing through a wire loop produces a time-varying magnetic field, which in turn induces a secondary electrical current across an adjacent conductor [40] (Figure 2.6). This is described by Faraday's law, which is the fundamental physical principle behind TMS:

$$\bigtriangledown \times E = -\frac{\partial B}{\partial t} \tag{2.1}$$

where a rapidly-changing magnetic field B, gives rise to an electric field E in the brain (as the conductor), by means a coil placed over the head. The changing magnetic field around the coil could be determined according to the Biot-Savart law:

$$B(r,t) = \frac{\mu_0}{4\pi} I(t) \oint_c \frac{dl(r') \times (r - r')}{|r - r'|^3} \tag{2.2}$$

The magnitude of the TMS-generated magnetic field is of the order 1-2 Tesla near the coil, decreasing exponentially with the distance from the coil. The induced electrical field in the underlying cortex has a limited spatial distribution, i.e. from 7 mm to 3 cm, depending on stimulation parameters, coil geometry and placement [40–43]. Thus, stimulation of deep brain structures is limited with TMS and it is mostly restricted to cortical areas [44].

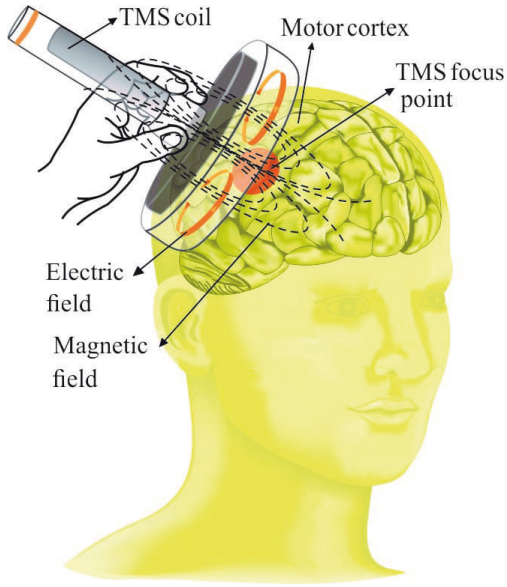

Figure 2.6: The principles underpinning TMS. An intense, brief current I generates a time-varying magnetic field B through a figure- of- eight coil, which in turn induces an electric field E in the brain.

2.2.2 Physiological basis

Cortical neurons vary in their biophysical properties such as their orientations in relation to the induced electric field. According to the current view, neural stimulation occurs more likely at those points where the electric field gradient would be predicted to trigger an action potential. Hence, the most probable sites of stimulation are either axonal terminals or bends where the electrical field is not uniform [45,46] (Figure 2.7). The primary hand area, located deep on the anterior wall of the central sulcus, contains upper motor neurons whose axons travel down the corticospinal tract, ending in the brainstem and the spinal cord. Therefore, in order to elicit the optimal motor responses in the respective muscle, the coil orientation needs to be adjusted so that the induced current in the brain is perpendicular to the precentral gyrus [42,47]. The (pyramidal) corticospinal tract is often assumed to be the major pathway through which TMS influences spinal circuitry. It originates from the cerebral cortex where its fibers (30% from the primary motor cortex) descend through the middle portion of the cerebral peduncles of the midbrain and then through the pons. In the upper region of the medulla, these fibers join together with the pyramids of the medulla, whereas in the lower region, the majority of the fibers decussate and synapse on the contralateral spinal cord (lateral corticospinal tract). The lateral corticospinal tract contributes to the control of the muscles of the limbs such as fingers [48].

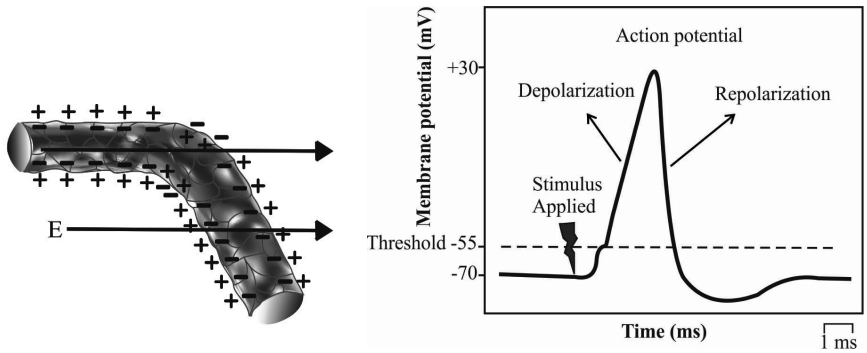

Figure 2.7: Due to required induced electric field gradient to trigger the action potential, neural stimulation occurs more likely at axonal terminations and bends where the induced E is not uniform. Modified from [49]

TMS-induced electrical field results in a number of descending volleys in the corticospinal tract, at intervals of 1-1.5 ms. While the earliest wave, referred to as a direct (D-) wave, is produced via direct activation of the layer-V pyramidal axons, the following waves (indirect (I-) waves) are elicited due to their indirect trans-synaptic activation [50,51]. At the microscopic level, the spatial and temporal summation of these descending volleys triggers a membrane depolarization and the initiation of an action potential in the lower motor neurons, leading to muscle activity [52]. The resultant muscle activity can be recorded using surface electromyography (EMG) and assessed for motor-evoked potentials (MEPs) [53] (Figure 2.8). MEPs are probed as a routine tool to assess the integrity of descending motor pathways whose measurements can provide insights into the excitability of the M1. One of the most common measures of cortical excitability that can be obtained through MEP is the motor threshold (MT). MT is commonly defined as the lowest TMS stimulus intensity that is required to elicit a reproducible MEP ($\sim$ 50 μ V) in at least half of the

10-20 consecutive trials [54,55], with generally the lowest intensity for the finger extensors and intrinsic hand muscles [54,56]. MT is believed to reflect the membrane excitability in the pyramidal neurons as has been demonstrated by changes in the MT using voltage-gated sodium channel blockers [57]. However, in contrast to the MEP amplitudes, the modulators of inhibitory and excitatory transmission such as modulators of GABAA receptors, do not affect MT [58,59]. This supports the notion that there is a difference between the mechanisms of involved in the formation of MEP and MT.

Another measure of cortical physiology is cortical silent period (cSP), i.e. the interruption of voluntary muscle contraction over a certain period of time following the application of single-pulse stimulation [60]. While the first one-third of the cSP has been suggested to be controlled by spinal cord inhibition contributions, the latter two-thirds part is entirely of cortical origin, mediated by slow GABAB receptors [61,62]. By investigating the RS in relation to SP responses, the possibility has been raised of an involvement of inhibitory GABAergic pathways in mediating RS [63].

The other commonly used measure of cortical excitability, which employs a pair of stimuli, is paired pulse stimulation. Several paired pulse protocols, each assessing a different property of cortical excitability, are available; some of those employed in this thesis will be explained in the following sections.

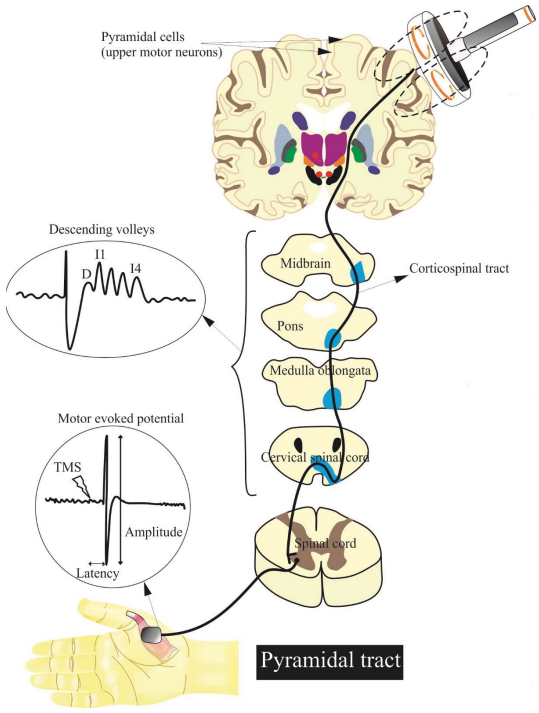

Figure 2.8: A schematic illustration of the generation of corticospinal volleys. TMS evoked facilitation leads to the generation of a number of corticospinal volleys in the pyramidal tracts, that are, direct (D-) and indirect (I-) waves. While D-waves are produced through the direct activation of layer-V neurons, I-waves are proposed to originate from their trans-synaptic activation.

2.2.3 Paired-pulse TMS

Paired-pulse TMS is another paradigm which can be used to study cortical excitability. A conditioning pulse (CS) delivered to the M1 results in a modulation of the MEP amplitude size, elicited by the subsequent test stimulus (TS) [64]. This modulation, manifesting either as decrease or an increase of MEP amplitude, reflects the activation of the inhibitory or excitatory circuits. The resultant intracortical inhibition or facilitation is determined by the inter-stimulus interval (ISI) and the intensity of both conditioning and test stimuli. Delivering the sub-threshold CS, 1-6 s prior to the supra-threshold TS, results in a reduction of the MEP amplitude compared to the isolated application of the TS, a phenomenon called short intra-cortical inhibition (SICI) [65]. In contrast, by using the same intensity but with a different ISI where the ISI is 8-20 ms increases the MEP amplitude (intra-cortical facilitation, ICF), presumably through activation of glutamatergic interneurons [65, 66]. Other paradigms using the supra-threshold CS and sub-threshold TS, with long and short ISI, increases (long-interval intracortical inhibition, LICI) and decreases the MEP amplitude (short-interval intracortical inhibition, SICF), respectively. The two paired-pulse paradigms used in this thesis are LICI and SICF.

Long-interval intracortical inhibition (LICI)

LICI, is a form of paired-pulse paradigm where a suprathreshold conditioning stimulus is followed by a suprathreshold test stimulus at an ISI of 50-200 ms, resulting in suppressed MEP response [67]. The time course of the inhibition of LICI points to a cortical contribution in the resultant decline in MEP amplitude. Alongside the time course, studies investigating descending spinal cord volleys have demonstrated that applying LICI paradigm yields the generation of suppressed I2- and I3-waves, while the early I1- and D-waves remain unaffected [68–70]. LICI can be enhanced pharmacologically using GABAB agonists, indicating that the increased activity of GABAergic inhibitory system is a plausible underlying physiological mechanism [71–73].

Short-interval intracortical facilitation (SICF)

Another well-documented TMS paired-pulse paradigm involves a suprathreshold conditioning pulse being applied prior to a subthreshold test pulse. This paradigm, referred to as SICF, produces facilitation of the resulting MEP amplitude [74–76]. Developing over ISI of 1 to 5 ms with three distinct peaks [76,77], SICF is suggested to be mediated by facilitatory I-wave interactions within the cortex [76].Furthermore, SICF has been shown to be reduced using GABA agonists, implying that there is the potential contribution of the GABAergic system in its generation [59].

2.2.4 Other pulse sequences

Although paired-pulse is one of the most widely-used pulse sequences employed in TMS stimulations, based on the required neuronal effects, pulses can also be delivered independently and repeatedly; in these cases they are, referred to as single-pulse and repetitive (rTMS), respectively. A Single-pulse sequence employs pulses with ISI of at least 3 seconds [77, 78]. In rTMS, the trains of stimuli are delivered with an ISI around or less than 1 s [79]. rTMS at frequencies higher than 5 Hz has been shown to increase cortical excitability [80], whereas the rTMS at frequencies equal to or lower than 1 Hz induces inhibitory effects [81]. In general, single-pulse

and paired-pulse TMS stimulation is employed to probe the brain function, whereas the rTMS sequence is used as a therapeutic method where the changes are required to extend beyond the stimulation period [79].

2.2.5 Waveforms

TMS pulses are commonly delivered as either monophasic or biphasic waveform. While the biphasic waveform consists of one full-sine pulse, monophasic waveform comprises half-sine pulse, with a rapid, sharp initial current and slow decay [82]. Monophasic pulses are usually applied for single-pulse paradigms while the biphasic stimuli are often used in rTMS protocols due to the lower required energy [83]. Biphasic stimuli are suggested to induce more effective, yet less focal cortical activation [84]. To provide more focal stimulation, 'figure-8' coils, consisting of two adjacent round coils, are utilized [85,86]. The currents in the adjacent coils flow in an opposite direction where they sum up at their intersection, below which there is a higher induced electric field (Figure 2.6).

2.2.6 Navigated TMS (nTMS)

Navigated TMS (nTMS) is one modality that utilizes individual's own magnetic resonance (MR) images. In doing so, the MRIs are co-registered with the subject's head through a head tracker system and an infra-red camera, resulting in online recording of the coil's position relative to the head. In comparison with the non-navigated TMS, the MRI-guided nTMS allows for highly accurate and reproducible stimulation [87,88]. These two features are achieved through the real-time estimates of the strength and orientation of the induced electric field [87,89]. One of the key applications of nTMS is in pre-surgical mapping of cortical structures in which the image-guided stimulation of the brain makes it possible to determine the functional motor/language areas with respect to the location of the tumors [88,90–93].

2.3 NEUROPLASTICITY

Neuroplasticity is a property that means that the nervous system possesses an ability to reorganize and unmask its latent neuronal connections both at a structural and a functional level [1]. This property is a critical characteristic of living organisms, enabling them to adopt to their environment. Depending on the speed of these changes, neuroplasticity can be traced back as a crucial step in the evolutionary trajectory over a long timescale, while on short timescales, it is considered as the keystone underpinning learning, memory, and the recovery from (mild) brain injuries [2,3,94]. The underlying cellular mechanisms of neuroplasticity include those leading to the formation of new networks or the arousal of dormant networks (axonal growth), those resulting in the formation (sypnaptogenesis) or elimination of synapses (synaptic pruning), and those resulting in the modulation of the efficacy of the synaptic transmission e.g. long-term potentiation (LTP) [95]. For example, although these mechanisms provide the necessary basis for a functional recovery, neuroplasticity is not always adaptive. Chronic pain is an example of a kind of maladaptive neuroplasticity arising from synaptic (LTP-like) to structural (axonal sprouting) changes [96].

In recent years, neuroplasticity has been extensively studied using different techniques such as functional magnetic resonance imaging (fMRI), electroencephalography (EEG), and non-invasive brain stimulation methods including TMS and paired associative stimulation (PAS) [97–101].

2.3.1 Mechanisms

The mechanisms underpinning neuroplasticity can be broadly divided into two categories: synaptic plasticity and non-synaptic plasticity [102, 103]. While both categories can evoke altered efficiency in neuronal communication, their location and mechanisms of action are different. As the paradigms used in this thesis have been mostly attributed to synaptic plasticity, this type of plasticity will be discussed here. Synaptic plasticity refers to the type of plasticity that occurs at synapses, whereas the non-synaptic plasticity occurs in areas remote from the synapses [103]. Furthermore, the synaptic plasticity typically involves changes in the release/uptake of neurotransmitters, while the non-synaptic plasticity involves the alterations in the activities of voltage-gated ion channels [102]. Although recent evidence provides some support for the role of non-synaptic plasticity in facilitating the memory and learning processes, it is synaptic plasticity that has been historically proposed as the fundamental mechanism underlying adaptation, memory and learning functions [104].

Synaptic plasticity

The pioneering research of Eric Kandel on Aplysia revealed the changes in synaptic properties following memory acquisition [105]. These findings later led to the discovery of the underlying cellular processes involved in the short-term reorganization of the nervous system, i.e. LTP [106, 107].

LTP, which refers to an increase in the synaptic efficacy, requires three essential properties to occur: 1) input-specificity, 2) associativity, and 3) cooperativity, the three main signatures of Hebb's postulate [108].

Considering the above-mentioned principle, cooperativity indicates the need for synchronous activation of multiple distinct afferent neurons in order to reach the required threshold for LTP [106], while the associativity refers to the co-occurrence of the neuronal pre- and post-synaptic activity in a spike timing dependent manner [109]. In contrast to LTP, a decrease in the synaptic efficacy results from the low frequency stimulation of excitatory afferents, a phenomenon known as long-term depression (LTD). Although the mechanisms underpinning LTP and LTD are not fully understood, the fundamental role of post-synaptic intracellular calcium influx has been demonstrated in several studies [106,110].

One main consequence of the unidirectional modification of the synaptic efficacy, evident as the Hebbian characteristic of induction of either LTP or LTD, is its inherent associated instability. This positive-feedback instability that might manifest as maximally saturated and desaturated synapses in LTP and LTD, respectively, is required to be modulated by a regulatory mechanism [111]. To achieve this type of modulation, in their mathematical model, Bienenstock, Cooper and Munro introduced a "sliding threshold" for inducing further changes in either LTP or LTD (the BCM model). The BCM model states that the magnitude and sign of the synaptic plasticity are not only influenced by the instantaneous pre- and postsynaptic activities, but also by the time-average of prior post-synaptic activity [12]. Later, the concept of 'metaplasticity' was introduced by Abraham and Bear, where the 'meta' term implies a higher-order form of synaptic plasticity, serving as a homeostatic factor [112].

Homeostatic metaplasticity

Although there is ample evidence suggesting that both LTP and LTD can serve as potential neural substrates of learning and memory, these two mechanisms pose a number of challenges to the neuronal networks, including a runaway effect. Excessive weighting of synaptic strength towards either a floor or ceiling level, hinders further synaptic modifications in the same direction. Hence in order to maintain the dynamic neural activity around a physiologically given ”set point”, the synaptic plasticity is dynamically influenced by the prior synaptic activity. As mentioned earlier, the BCM model proposed that there should be a sliding threshold in order to reach the required balance between the synaptic modifications and stabilization by recruiting two key principles. First, the change of synaptic efficacy varies as a nonlinear function of the postsynaptic activity. Consistent with this proposal, LTD is induced by a low level of postsynaptic activation while LTP is induced by a high level of postsynaptic activation, such as high-frequency stimulation. Second, the crossover point at which LTD is converted to LTP, termed the modification threshold (θ_m), is not fixed and changes as a function of the time-average of prior postsynaptic activity (metaplasticity). In accordance with the homeostatic function of the metaplastic regulatory mechanism, experimental studies on the visual cortex of rats revealed a significant difference in the required threshold to induce LTP or LTD, depending on their exposure to the darkness or light during their early developmental period [113]. Enhancement of LTP induction and a reduction of LTD induction were observed in the rats reared in darkness. These findings are in line with the hypothesis which states that a sliding synaptic modification threshold is required with synaptic weights in neural networks in order to achieve a stable equilibrium [114].

Although it is not yet clear how the finely tuned properties of neural networks can result in functional outputs without succumbing to either hypo- or hyper-

activity, a failure to achieve this optimal balance can cause instability and abnormal states, as seen in epilepsy. The failure of this metaplastic regulatory mechanism has been also postulated as the pathophysiological basis of several other neuropsychiatric disorders such as schizophrenia, depression and chronic pain [115–117].

2.3.2 Paired associative stimulation (PAS)

Paired associative stimulation (PAS) is a well-established neuromodulation paradigm, widely used to induce short-term, topographically specific plasticity in the human motor cortex [101,118,119]. In addition, the paradigm can provide a unique perspective with which to investigate several neuropsychiatric disorders where maladaptive plasticity contributes to the pathophysiology [120,121]. The paradigm uses electrical median nerve stimulation paired with TMS cortical stimulation. The intensity employed to stimulate the median nerve is commonly set at three times the perceptual sensory threshold [118, 122, 123]. This intensity corresponds to the required intensity at which ipsilateral MEPs are generated, but is subthreshold for stimulation of the contralateral M1 [96, 124–126]. In contrast, the TMS intensity is adjusted to generate MEPs of 1 mV to evoke an action potential in the contralateral corticospinal tract [118]. The median nerve-induced antidromic volleys and TMS-induced descending volleys are timed to coincide at the motor cortex, inducing bidirectional LTP-like and LTD-like plasticity in M1 [119, 127]. This bidirectional plasticity is determined by the timing between the stimuli, suggesting that PAS-induced plasticity is a type of spike timing-dependent plasticity (STDP) [128]. PAS can lead to elevated cortical excitability (LTP-like plasticity) (Figure 2.9), evident as an increased MEP amplitude, where the median nerve stimulation precedes the TMS stimulation with an ISI of up to 35 ms [123, 127]. In contrast, an ISI of approximately 10 ms induces a depression of the MEP amplitude (LTD-like plasticity) [118]. The PAS-induced plasticity is reversible. Although the MEP amplitudes remain elevated for approximately 60 minutes following facilitatory PAS, it has been shown that it reverses within 24 hours [118]. Pharmacological studies have demonstrated that both LTP-/LTD-like plasticity, induced with PAS, can be blocked by treatment with an N-methyl-D-aspartate (NMDA) receptor antagonist [129]. Furthermore, the inhibitory PAS did not induce LTD-like plasticity when nimodipine, i.e. an L-type calcium channel antagonist, was administered [130]. These convergent findings point to synaptic modification as a plausible underlying mechanism underpinning PAS, i.e., synaptic modification.

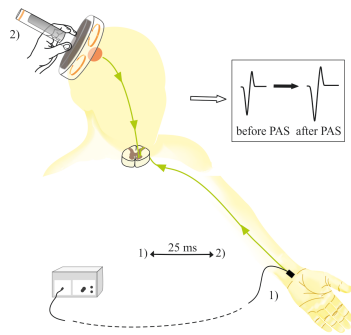

Figure 2.9: A schematic illustration of facilitatory PAS. TMS stimulation is paired with peripheral median nerve stimulation with an ISI of 25 ms. This results in an LTP-like plasticity, manifesting through the heightened MEP amplitude.

2.4 REPETITION SUPPRESSION

The presence of a sudden and intense sensory stimuli results in an enhanced cortical arousal, known as an arousal reaction; this phenomenon is commonly assessed using auditory stimuli and the resultant auditory evoked potentials (AEPs) [131, 132]. An arousal reaction has been shown to originate in the brainstem reticular activation system (RAS), a component of the reticular formation [133, 134] (Figure 2.10). While the slow (tonic) arousal reaction is mediated by the lower parts of the RAS, the upper portions mediate the rapid (phasic) arousal reaction [135]. RAS also plays a key role in the regulation of muscle tone both during sleep (suppression of muscle tone) and wakefulness (mediating arousal to help with the fight or flight response) [136, 137]. The reticular formation is a set of interconnected nuclei along with their fibers present in the brainstem, with afferent connections from the cortex, thalamus, sensory pathways, and the spinal cord, which sends its outputs throughout the nervous system. The reticular formation gives rise to a descending pathway called the reticulospinal pathway, which projects to the spinal cord. The reticulospinal pathway is composed of two components: the pontine (medial) reticulospinal tract, and the medullary (lateral) tracts; these terminate either directly or indirectly (through synapsing with the interneurons in the spinal gray matter) on motoneurons (Figure 2.10). These pathways mediate the startle reflex, i.e. an involuntary motor reaction triggered by an unexpected sensory stimulus [138–140].

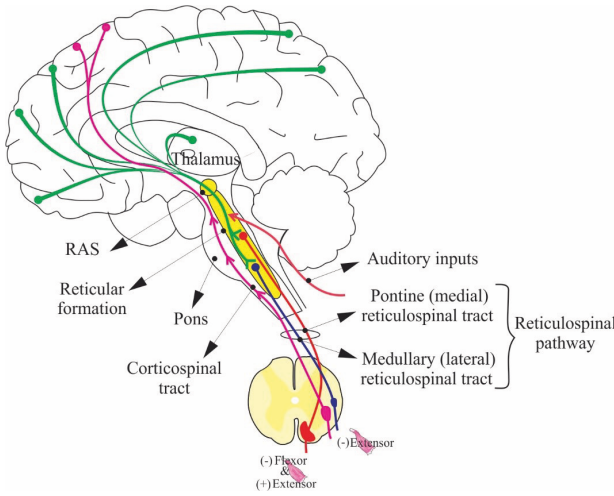

Figure 2.10: Reticular activating system (RAS). RAS is a component of the reticular formation, i.e. a complex network of nuclei located throughout the brainstem. RAS mediates an arousal reaction in response to surprising sensory stimuli and helps to regulate the muscle tone. Moreover, the reticular formation gives rise to the reticulospinal pathway through which the startle reflex is mediated.

While the presence of a surprising stimulus causes an arousal response, presenting the same stimulus within a relatively short time results in an attenuation of the response, and with the second and further repetitions, the arousal effect tends to disappear. This phenomenon, i.e. a reduction of the arousal reaction with repeated stimulation, is called repetition suppression (RS) and is commonly encountered in a range of sensory modalities [6, 9, 141, 142].

RS, has been rather widely studied in the auditory system. These studies have been mainly conducted in the cortex and the reduction has been commonly attributed to neural fatigue (lower firing rate) [143, 144] and neural sharpening (fewer neurons responding) [145]. However, some investigators have suggested the possibility that the RS does not primarily occur in the cortex and instead originates from subcortical areas [146, 147].
RS was initially perceived merely as an expression of a bottom-up mechanisms, while the more recent theories have also incorporated a role for top-down mechanism as well, such that a feedback loop would be involved [148, 149].
In addition, RS has been demonstrated in the motor cortex with a TMS stimulation train of 4 stimuli, where the first pulse elicits the motor response with the highest amplitude compared to the following responses [15, 63] (Figure 2.11).
This TMS-induced RS is thought to be evidence of the adaptive behavior of the motor system to external stimuli. As RS has been mainly investigated using other modalities in other cortices, the underlying mechanism for this type of RS has remained elusive. As the main locus of the TMS influence has been suggested to be mediated through the corticospinal tract, the observed inhibition in the TMS-induced RS could be a contributor to the multi-synaptic pathways including thalamus, basal ganglia, and interconnected cortical areas with one potential pathway mediating RS being the thalamocortical pathway. The activation of the interconnected sensory areas following the enhanced cortical excitation of the M1 (in response to the first intense stimulus), might result in the activation of the ventral posterior lateral nucleus of the thalamus which receives inputs from the somatosensory inputs. In turn, this creates an inhibitory loop to strive to reach an excitation/inhibition balance.
As mentioned earlier, the ponto-medullary reticular formation, giving rise to the reticulospinal tract plays a key role in mediating the startle reflex [150, 151]. Some cortico-reticular tracts arising from the M1 make collateral connections with the corticospinal tract [152]. This can lead to the indirect activation of the reticular formation when an intense TMS pulse is applied over the M1 as the main and primary locus of activation is the corticospinal tract. However, the feedback from this tract probably does not contribute to the RS in the motor system [153].
At a cellular level, the RS might be partially explained by a reduced firing rate due to the prolonged hyperpolarization of the neurons following the first stimulation. This reduced firing rate has been demonstrated to be linked to the intrinsic membrane mechanisms through activated calcium- and/ or sodium ion channels [154–156]. However, these mechanisms cannot fully explain the RS and this phenomenon has been also proposed to be mediated partially through synaptic depression [157–160]. Nonetheless, synaptic depression cannot fully explain the TMS-induced RS as the synaptic depression has a time span from a hundred of milliseconds to tens of seconds, a duration which is not totally in line with the longest recovery time between RS trials. Alterations of synaptic efficacy in intrinsic and extrinsic connections have been suggested as an alternative possible cellular mechanism behind stimulus-specific adaptation [161, 162]. The increased activity of inhibitory GABAergic interneurons is another potential candidate that has been postulated as the underlying mechanism [63].

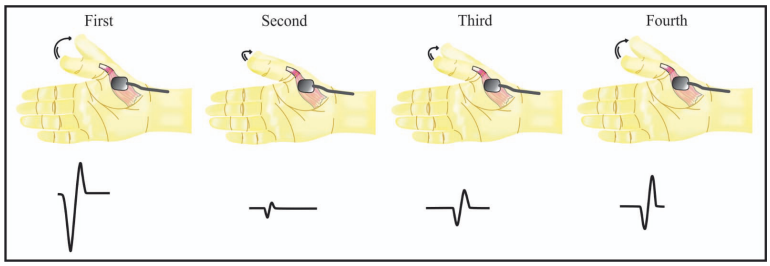

Figure 2.11: A schematic illustration of TMS-induced RS, manifesting through an attenuated MEP amplitude. The initial MEP amplitude appears as its largest in response to the first TMS stimulus. However, delivering further identical TMS stimulus results in an attenuation of the MEP response which does begin to recover following the third stimulus.

3 AIMS OF THE THESIS

The central aim of this thesis was to investigate the applicability of a novel potential biomarker for quantifying the capacity for neuroplasticity at the individual level, as an important determinant of the individual's response to neuromodulation therapies. Moreover, a better understanding of the mechanisms governing the neuroplasticity through its interaction with adaptation could help to clarify the underlying pathophysiology of neuropsychiatric disorders in which the facilitation/inhibition balance has been disturbed.

The specific aims of the thesis were:

I To study the interaction between RS and neural facilitatory/inhibitory characteristics.

II To estimate two different aspects of individual neuroplastic capacity, one related to the immediate adaptation in the face of the first repetition of a novel stimulus and one investigating the restoration of information in response to the repeated stimuli, through RS following PAS short-term induced plasticity in healthy subjects.

III To assess the potential of RS as a biomarker of neuroplasticity in patients with chronic pain and thus to consider whether the patient would be susceptible/immune to induced neuroplasticity via rTMS.

4 METHODS

4.1 SUBJECTS

The detailed study-specific demographic information of the subjects are demonstrated in the Table 4.1. In studies I and II, neurotypical volunteers were recruited from University of Eastern Finland and Kuopio University Hospital. The subjects in study III were recruited from Kuopio University Hospital and Helsinki University Hospital; they were patients with drug-resistant neuropathic pain or complex regional pain syndrome (CRPS) type I or II. Written informed consents were provided by all subjects prior to the studies. All tests were conducted in accordance with the Declaration of Helsinki, abiding by the safety guidelines for the application of TMS [163]. The research ethics committee of the Kuopio University Hospital reviewed and approved all studies.

Table 4.1: Background data of the study population

Study	Subjects	Gender (Female/ Male)	Age (range)
I	8	2/6	22-42
II	16	9/7	22-42
III	21	14/7	25-87

4.2 NAVIGATED TMS

Structural T1-weighted MRIs were acquired using a 1.5 T or 3T clinical MRI scanner (Philips Achieva, Philips, Eindhoven, The Netherlands or GE Signa, GE Healthcare, Chicago, IL, USA, or Siemens Skyra, Siemens Healthcare, Erlangen, Germany) prior to the experiments. The MRI data were further utilized in the individual MRI-guided navigated TMS (nTMS) examinations (NBS System 4.3 or NBS System 5, Nexstim Plc, Helsinki, Finland) with an air-cooled figure-of-eight coil and a biphasic waveform. The measurement was initiated by determining the cortical abductor pollicis brevis (APB) "hotspot". The hotspot is defined as the cortical site where the MEPs of maximal amplitude are elicited with a minimal stimulator output intensity. Once the hotspot had been located, the resting motor threshold (rMT) was determined using a system-integrated iterative threshold assessment tool [164].

4.3 REPETITION SUPPRESSION PARADIGM

The RS paradigm, consisting of twenty trains of four single TMS stimuli with an ISI of 1 s and an inter-train interval (ITI) of 17 s [165] was administered over the APB hotspot. The stimuli were delivered either at 120% rMT (studies I and II) or at 110% rMT (study III). The RS paradigm, employed in all studies, was of a single pulse form, with the sequence lasting approximately 6 minutes. However, in study I, two paired-pulse RS paradigms were also applied, that is RS-LICI and RS-SICF.

A subthreshold pulse was preceded by a suprathreshold pulse at an inter-pulse interval (IPI) of 1.4 ms in RS-SICF [166], where a suprathreshold pulse was followed by a suprathreshold one at an IPI of 100 ms in RS-LICI (Figure 4.1) [167,168].

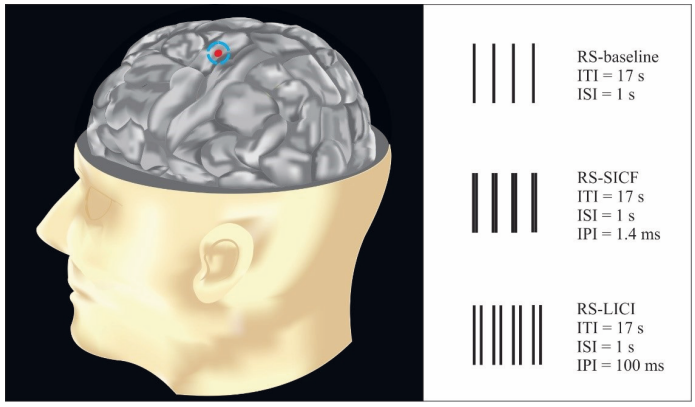

Figure 4.1: The repetition suppression (RS) paradigms were administered over the abductor pollicis brevis (APB) hotspot, i.e. the APB representation site where the largest motor responses were induced with the smallest stimulator intensity, in studies I-III. The RS-baseline paradigm, consisting of four single pulses with an inter-stimulus interval (ISI) of 1 s and inter-train interval (ITI) of 17 s were used in all studies. However, the paired-pulse RS-SICF and RS-LICI paradigms were only used in study I with inter-pulse intervals of 1.4 ms and 100 ms, respectively.

4.4 ELECTROMYOGRAPHY

TMS-induced responses were recorded using an integrated EMG system at a sampling frequency of 3 kHz. A pair of disposable Ag-Cl electrodes was utilized with the active electrode placed over the belly of the APB muscle and the reference electrode over the joint distal to the active electrode. The recorded MEPs were recorded by triggering the EMG signal with TMS, and were processed offline in Matlab (R2017b, R2018b, MathWorks Inc., Natick, MA, USA). MEPs occurring in the resting muscles with peak-to-peak amplitude lower than 50 μV were not considered as responses.

4.5 PAIRED ASSOCIATIVE STIMULATION

In study II, one hundred eighty single-pulse stimuli were delivered over the right median nerve at an intensity of 300% of the sensory threshold (ST) during a PAS intervention [169]. ST is defined as the minimum stimulus intensity sensed by the subject. When measuring ST, a bipolar stimulation electrode was placed over the median nerve and the current was adjusted so that the subject could sense the stimulus. The pairing of the TMS and peripheral stimuli at the hotspot was performed using a self-built triggering and delayer device. The peripheral stimulation of the median nerve was performed (Digitimer model DS7A, Digitimer, Welwyn Garden City, Herts, UK) at a frequency of 0.2 Hz, and 25 ms prior to TMS stimulation at

120% of rMT to induce an LTP-like plasticity effect. RS was applied before the PAS and at three different time points, i.e. 0, 10, and 20 minutes after PAS.

4.6 REPETITIVE TRANSCRANIAL MAGNETIC STIMULATION

In study III, patients with chronic pain received 10 Hz rTMS treatment on either 5 or 10 consecutive days. The number of sessions was determined by the prior outcome, meaning that those patients who had experienced an analgesic effect from rTMS before, received 5 more sessions. The primary treatment target area was M1. However, if no analgesic effect was observed, the treatment target was switched to the secondary somatosensory cortex (S2). The rTMS treatment was administered in two different centers: Kuopio University Hospital and Helsinki University Hospital. The protocol used in Kuopio Hospital consisted of a total number of 2,400 stimuli delivered as trains of 6 s with ITIs of 24 s, whereas the protocol used in the Helsinki center consisted of a total number of 3,030 stimuli administered in trains of 10 s with ITIs of 20 s. RS was applied on the first session before administering the rTMS treatment.

4.7 OUTCOME OF REPETITIVE TRANSCRANIAL MAGNETIC STIMULATION

The treatment outcome in study III was determined based on two questionnaires: 1) Brief Pain Inventory (BPI) [170,171] and, 2) painDETECT (PD) [172]. The patients filled in the questionnaires during the first and last sessions, prior to receiving rTMS. Subsequently, the scores were compared to evaluate the outcome. BPI is composed of five items; the first four items indicate the pain intensity on an 11-point scale (0 = no pain to 10 = worse pain ever). The mean of the last item which itself consists of seven sub-items is used to rate the extent to which the pain has interfered with daily activity (QoL score), with higher score representing greater interference.

The nine-item version of PD questionnaire (with total score ranging from 0-38) is composed of seven sensory items, one pain-course pattern item, and one pain radiation item. To assess the improvement of the neuropathic component of the pain, the scores on seven sensory items were also calculated separately (ranging from 0-35). The decrease in scores calculated from the items rating the pain intensity in both questionnaires were used to evaluate if there had been an improvement of the intensity component. However, as it has been proposed that the changes in pain intensity, which are mainly addressed in most questionnaires, are not sufficient for determining the analgesic effect, the QoL score was also taken into account [171,173]. Therefore, the QoL score was regarded as an indicator for experiencing the analgesic effect when a decrease in intensity score (in either BPI intensity score or seven-item PD) was accompanied by a decrease in QoL. This score was labeled as the "combined score" to identify those prone to benefit from the treatment.

4.8 STATISTICAL ANALYSIS

In all studies, the MEP data were first averaged over all the trains on the basis of their stimulus order within a train, per subject. In study I, a repeated measures ANOVA with "order of the stimulus" (first, second, third, and fourth), as the fixed effect was employed to investigate the general RS effect. When assessing the main effect of

two factors, that is the paradigm (RS-baseline, RS-LICI, and RS-SICF), and the order of the stimulus, and their interaction, a two-way repeated measures ANOVA was applied. Tests were conducted on both absolute and normalized MEPs by dividing the MEPs within a trial by the values of the first one.

When assessing the RS in studies II and III, RS was subdivided into two components and assessed for the MEPs. The first component, termed as "dynamic" was the ratio of the second MEP to the first one within the RS trains. The second component, "stable" was calculated as the mean of the second, third, and fourth stimuli (Figure 4.2).

When evaluating the effect of the PAS on the RS, a non-parametric Wilcoxon signed rank test was utilized, for each individual subject. The RS immediately after the PAS was compared with that before the PAS and the subjects who demonstrated a statistically significant increase in MEP amplitudes were classified as the "LTP-like group", whereas those showing a significant decrease were classified as the "LTD-like group". Dynamic and stable components of the RS were both evaluated at different time points (before, and 0, 10, and 20 minutes after PAS), and between two groups. The change of components at different time points was evaluated with the Friedman test; Mann-Whitney U test was applied in the comparison of components between the group.

In the statistical analysis of the results in study III, area under the curve (AUC) and accuracy analysis of receiver operating characteristic (ROC) curve were employed. Different measures of the RS (dynamic and stable components) and changes in consecutive MEP amplitudes (from second to the fourth) within the stable component were used to evaluate their ability to identify those individuals likely to benefit. When attempting to differentiate those patients likely to benefit from those who would not, the cut-off value for the outcome scores (combined impact score and PD intensity) was determined and optimized by running the ROC analysis so that the AUC would be maximized. This step was performed using all scores (from the minimum to the maximum score) as it was considered that each group (those prone to benefit vs. not prone to benefit) included at least 5 patients. When using the optimized ROC curves, the cut-off values for the RS measures of interest were calculated by finding the point closest to the upper left corner of the curve.

A p-value of <0.05 indicated statistical significance and all statistical analyses were conducted using SPSS (v. 25.0, SPSS Inc., IBM Company, Armonk, NY, USA) and Matlab (R2017b, R2018b, MathWorks Inc., Natick, MA, USA).

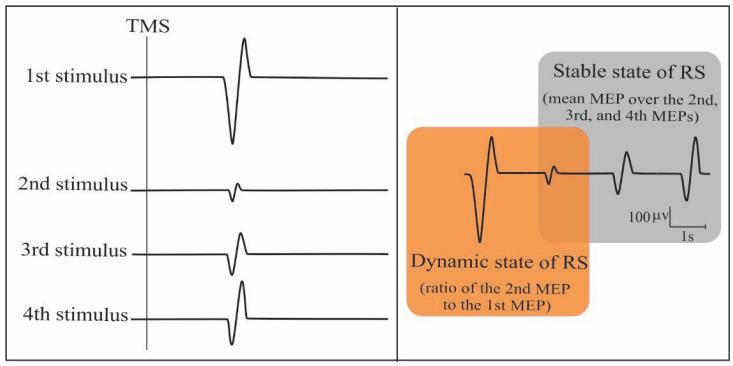

Figure 4.2: The RS paradigm was assessed for and subdivided into two components, each believed to reflect one aspect of neuroplasticity. The dynamic component was the ratio of the second TMS-evoked response (second MEP) to the first response, whereas the stable component was the mean response of the second, third, and fourth responses.

5 RESULTS

5.1 INTERACTION BETWEEN REPETITION SUPPRESSION AND THE CHARACTERISTICS OF NEURAL FACILITATION/ INHIBITION

In study I, the typical and clear TMS-evoked RS phenomena were observed ($F(3,56) = 19.24, p = 0.004$), meaning that the second, third, and fourth induced MEPs were significantly lower than the first induced response. When combining RS with the SICF, this absolute RS effect remained unaffected ($p > 0.5$). The main effect of the SICF appeared as an increase observed in all MEPs, with a common offset, meaning that the first, second, third, and fourth MEP amplitudes experienced the same level of increase ($F(3,56) = 7.22, p = 0.031$) for the absolute MEPs and $F(3,56) = 8.34, p = 0.023$ for the normalized MEPs) (Figure 5.1). A significant increase of the MEP amplitudes was demonstrated using post-hoc paired-samples t-test ($p < 0.05$ in the first, second, third, and fourth stimuli).

However, combining the RS with LICI revealed a non-linear interaction effect ($F(3,56) = 4.12, p = 0.081$ for the absolute MEPs and $F(3,56) = 15.04, p = 0.006$ for the normalized MEPs). A pairwise comparison of the stimuli revealed that the amplitude of the third MEP in RS-LICI was significantly higher than that in RS-baseline (Figure 5.1) ($p = 0.019$ for the absolute MEPs and $p < 0.001$ for the normalized MEPs).

5.2 INVESTIGATING REPETITION SUPPRESSION WITH RESPECT TO THE SHORT-TERM INDUCED PLASTICITY

The dynamic and stable components of the RS were compared before and immediately after the PAS. With regard to the dynamic component of the RS, out of sixteen subjects, eleven exhibited no significant change ($p > 0.1$), while one showed significantly milder ($p < 0.05$), and four displayed a significantly stronger change ($p < 0.05$) after PAS (Figure 5.2). The stronger change appeared as a larger drop from the first MEP amplitude to the second one, indicative of greater suppression.

When considering the stable component of the RS, fourteen subjects demonstrated a significant change (either as an increase or a decrease) ($p < 0.05$), with six subjects showing increased stable RS (classified as the LTP-like group). The increased stable RS appeared as the increase in the mean amplitude calculated over the second, third, and fourth stimuli. Eight participants exhibited a decreased MEP amplitude in this component (classified as LTD-like group) ($p < 0.05$). With the exception of one subject with delayed LTP-like plasticity (after 20 minutes), no change in this trend of change was observed in subjects.

In addition, a comparison of both components between the two groups before applying the PAS, revealed a significant difference ($p < 0.05$). However, applying the PAS revealed only a statistically non-significant difference in the stable component between the two groups, leading to an overall tendency towards a common level of the stable component (Figure 5.3).

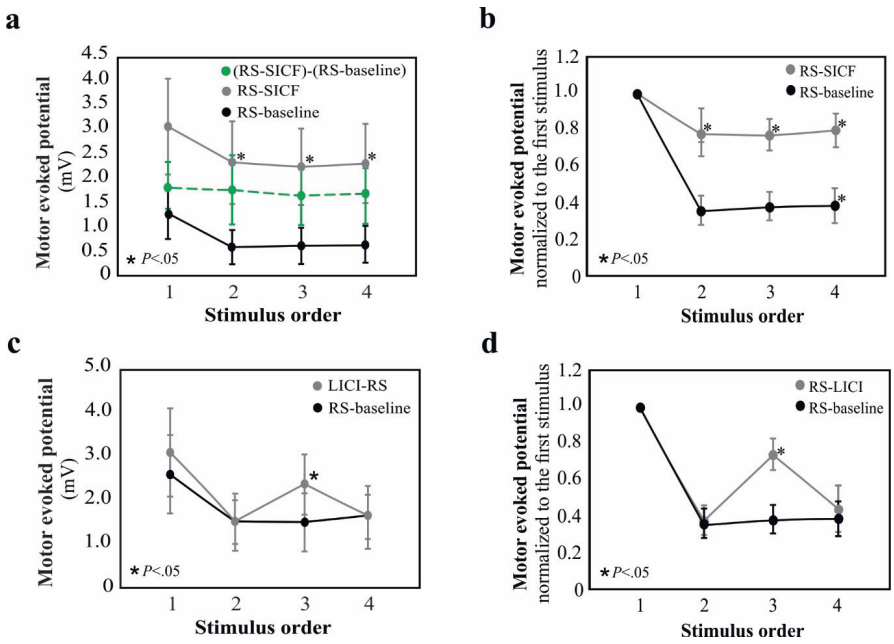

Figure 5.1: (**a**) Absolute MEP amplitudes and (**b**) normalized MEP amplitudes (mean ± standard error) across all participants subjected to the RS-baseline and RS-SICF paradigms. Combining RS and SICF revealed that I1-timed SICF increased the MEP amplitudes with a common offset, while the RS effect remained unchanged. (**c**) Absolute MEP amplitudes and (**d**) normalized MEP amplitudes (mean ± standard error) across all subjects with the RS-baseline and RS-LICI paradigms. Combining RS and LICI (RS-LICI) resulted in a non-linear interaction in which the amplitude of the third TMS-induced response was significantly higher than that in the baseline paradigm. An asterisk indicates that there were significant differences in the pair-wise comparisons ($p < 0.05$).

5.3 REPETITION SUPPRESSION IN PREDICTING THE RESPONSIVENESS TO HIGH-FREQUENCY RTMS IN CHRONIC PAIN

The optimal cut-off points for the ROC curve analysis of the parameters of interest have been demonstrated in Table 5.1. The patients experiencing a benefit from the rTMS were distinguished from those who reported no pain relief by the use of the ROC curve. The AUC illustrated the predictive power for discrimination of these two groups through the stable component of the RS (based on the combined impact score) and the change from the second to fourth MEP amplitudes at the suppressed level of the RS (based on the PD intensity score) (Figure 5.4). Our results showed high predictive power of the stable component with AUC of 0.912 (accuracy = 0.889) which decreased to 0.818 (accuracy = 0.882) for the MEP change. Furthermore, the optimal cut-off point was found to be 323 μV for the stable component of the RS, and 87 μV for the MEP change. This meant that the minimum MEP amplitude of the stable component and the that minimum change of MEPs at the stable level in patients showing benefit was 323 μV and 87 μV, respectively, distinguishing them from those who experienced no pain relief.

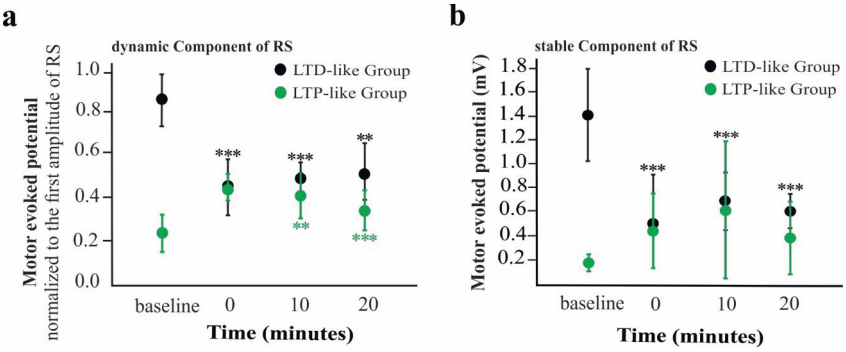

Figure 5.2: Changes of the two investigated components of the RS: (**a**) dynamic (mean $\pm$ standard error) and (**b**) stable (mean $\pm$ standard error), across the subjects from LTP-like and LTD-like groups, before (baseline) and after (at 0, 10, and 20 minutes) the induction of short-term plasticity with PAS. In the LTD-like group, the dynamic component appeared mild (less suppression) at baseline, changing towards a stronger suppression following the induction of plasticity. However, a stronger suppression was observed at baseline in LTP-like group which recovered following the application of applying PAS. Similar trends were also observed in both groups in the stable component of the RS. An asterisk indicates significant differences in the pairwise comparisons between time points ($p < 0.01$ for *** and $p < 0.05$ for **).

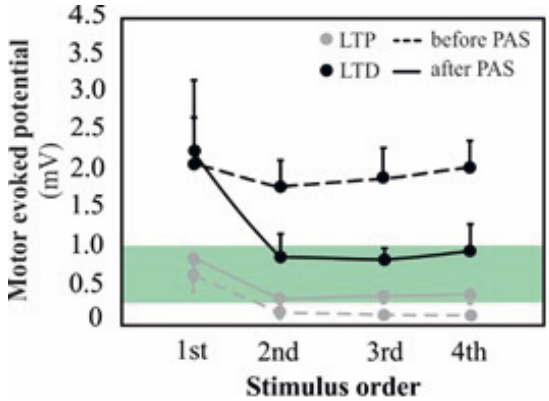

Figure 5.3: RS before and immediately after PAS in LTD-/LTP-like groups. Although the MEP amplitudes differ significantly between the groups before PAS at all four stimuli, this difference decreased with the second, third, and fourth stimuli (stable RS component) after PAS was applied. In other words, RS exhibited a tendency towards a neural response, reflected in MEP amplitudes, with low variation in the stable component of the RS (the green band).

Table 5.1: AUCs and optimal cut-off points for those prone to benefit vs. those not prone based on the parameters of interest.

Parameters	Accuracy (AUC)	optimal cut-off for change in score	responders/non-responders**
BPI impact & intensity*		N.A.	11/6
Stable state of RS	0.882 (0.697)		
Change of stable state	0.882 (0.818)		
Dynamic state of RS	0.470 (0.409)		
BPI intensity			
Stable state of RS	0.889 (0.785)	0.0 points	13/5
Change of stable state	0.833 (0.818)	-0.2 points	11/7
Dynamic state of RS	0.889 (0.833)	-1.2 points	6/12
BPI impact			
Stable state of RS	0.765 (0.657)	-0.8 points	10/7
Change of stable state	0.882 (0.767)	-0.1 points	12/5
Dynamic state of RS	0.588 (0.545)	-0.7 points	11/6
PD intensity			
Stable state of RS	0.889 (0.912)	-1 points	8/10
Change of stable state	0.778 (0.700)	-1 points	8/10
Dynamic state of RS	0.944 (0.877)	-2 points	5/13
PD score			
Stable state of RS	0.667 (0.575)	-2 points	8/10
Change of stable state	0.611 (0.554)	-3 points	5/13
Dynamic state of RS	0.722 (0.725)	-2 points	8/10

** Reduction of scores required in BPI impact and either BPI intensity score or PD intensity score.*

*** patients with incomplete follow-up information were not included in the analysis.*

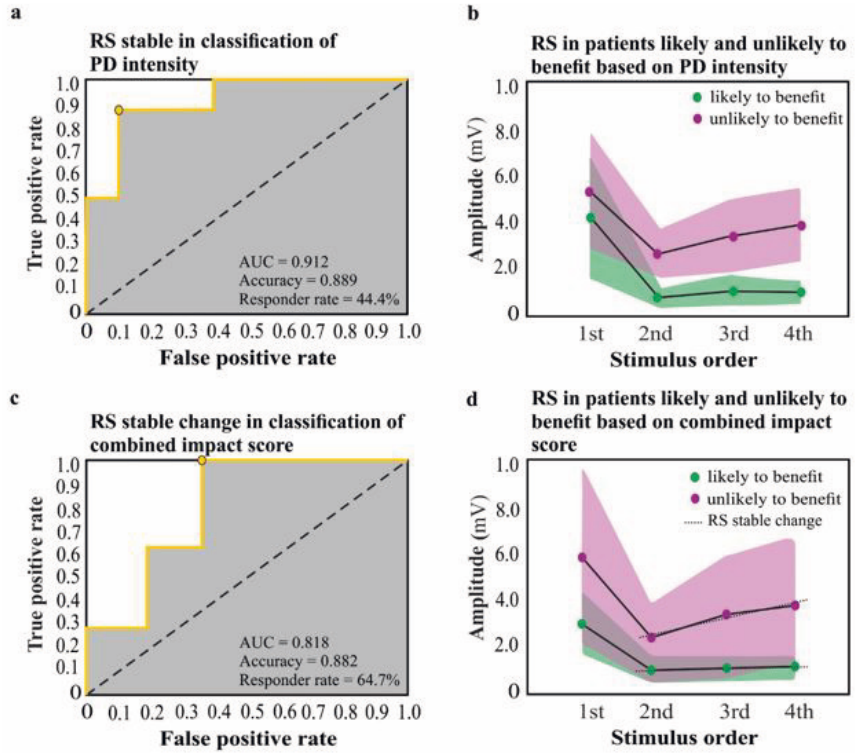

Figure 5.4: (**a**) Receiving operating characteristic (ROC) curve to identify the responders vs. non-responders based on the stable state of the RS. The responders were defined as those showing a decrease in PD intensity score in the last treatment session. (**b**) MEP amplitude responses (mean $\pm$ standard error) of RS at baseline level, between the patients who responded and those who did not respond to rTMS. (**c**)The ROC curve to discriminate the patients showing an analgesic effect in response to rTMS from those who did not, based on the change in the stable state of RS throughout the second to the fourth evoked responses. An analgesic effect was assessed via the changes in intensity scores together with the intervention score (the decrease in both scores was assessed via as being indicative of analgesia). (**d**) MEP amplitude (mean $\pm$ standard error of RS, within each cluster of patients before the initial rTMS session based on the PD intensity score. The red dot in figures A and C represents the optimum threshold to achieve the maximum AUC. PD, painDETECT. AUC, area under the ROC curve.

6 DISCUSSION

Investigators have made considerable progress in the field of biomarkers, despite the fact that the development of the biomarkers in the central nervous system (CNS) has proved challenging [174, 174–178]. In this thesis, we showed the utility of the TMS-induced RS as a biomarker of neuroplasticity, which is a fundamental feature of the CNS. A wide number of modalities exist for evaluating neuroplasticity e.g. fMRI, PET, EEG and TMS, each examining one aspect of the neuroplasticity [97, 179–181]. However, as each treatment intervention works through its own distinctive mechanism, each biomarker needs to be evaluated in the context of restorative therapy. With respect to neuromodulation therapies, several TMS parameters and paradigms such as MT and LICI have been investigated as potential biomarkers for TMS outcomes [181, 182]. Due to the advances in TMS-induced RS, we could investigate the neuroplastic changes after applying PAS in healthy subjects and rTMS in patients with chronic pain. The findings that the state of the brain before applying the interventions, reflected through the stable state of the RS, could modulate the PAS effect, and affect the benefit from rTMS plausibly indicate the potential clinical feasibility of TMS-induced RS as a predictor of the clinical response. Although a few questions were answered regarding its applicability in this thesis, further studies investigating a larger population of patients will be required.

6.1 INTERACTION BETWEEN REPETITION SUPPRESSION AND THE NEURAL FACILITATORY/ INHIBITORY CHARACTERISTICS

The mechanistic insight gained in this thesis revealed that the typical TMS-induced RS that manifests as a decrease in the amplitude of the motor response is disrupted in the presence of LICI. The findings from this study suggest that unlike the RS and LICI, which might modulate the neural responses through a shared inhibitory mechanism, RS and I1-wave timed SICF shows no such interaction. Although the exact underpinning mechanism of this interaction remains unknown, activation of the thalamo-cortical loop can be speculated to be the most likely candidate.

The inhibitory phenomena observed in LICI are thought to reflect the GABAB-receptor mediated inhibitory post-synaptic potential (IPSP), occurring primarily at cortical level [183–185]. However, the neuronal mechanism underlying the inhibition observed in RS has remained elusive. While in early works, RS had been often portrayed merely as an expression of bottom-up mechanisms [186, 187], the more recent theories have supported the posssibility of top-down influences, mediated through optimized synaptic efficacy [188]. The waning of neural activity in response to the repeated exposure to the stimulus may have also contributed to the enhanced activity of the GABAergic inhibitory system at the thalamo-cortical level [63]. The TMS-evoked RS results in an involuntary motor response which is at its largest following the delivery of the first stimulus and then vanishes with further repetitions of the stimulus. As such, it has been demonstrated that the tightly interconnected somatosensory areas provide inhibitory feedback to the M1 following the initial involuntary movement [189]. Subsequently, it was speculated

that to prevent further inhibition and maintain its homeostasis, the brain activates a negative feedback loop in order to weaken the inhibitory tone, after the first repetition of the stimulus, leading to a minor recovery, manifesting as the tendency of the depressed neural response to increase, i.e. to disinhibition. Furthermore, the delivery of identical stimuli employs the same feedback loop to restore the disinhibition/inhibition balance. The activation of this feedback loop might tap into the LICI at the cortex and result in the observed interaction found in this study. As LICI is an intra-cortical mechanism, this candidate mechanism might be the most likely to be correct [65,190]. However, Daskalakis et al. have suggested that the cerebello-thalamo-cortical pathway is affected at the motor thalamus following the delivery of the conditioning stimulus of the LICI [191]. This also raises speculations on the interactions site of the RS and LICI at the sub-cortical level.

Furthermore, our findings revealed that the RS is preserved and its inhibitory modulation is not affected by I1-wave timed SICF, where all the MEPs were facilitated with a common offset. This was in contrast with our findings when we combined RS with LICI, where the RS was affected. The facilitatory I1-wave is suggested to originate from the monosynaptic excitatory cortico-motoneuronal pathway and this leads to the indirect activation of pyramidal tract neurons [77,192–194]. As the TMS-induced I-wave volleys appear at an interval of 1-1.5 ms, it has been suggested that setting the IPI of the SICF at an interval so that the I2-wave of the conditioning stimulus can target and synchronize with the I1-wave of the test stimulus, could be one way to enhance the TMS effect [195]. Our results also confirmed this enhanced effect where no interaction occurred in inhibitory networks. The findings from this study might be of major significance since gaining a mechanistic insight into the RS could confer on it further validity to be used as a way of investigating disorders in which the inhibitory system has been disrupted due to the existence of a potential common pathway.

6.2 ESTIMATING NEUROPLASTIC CAPACITY VIA REPETITION SUPPRESSION FOLLOWING SHORT-TERM INCLUDE PLASTICITY

In study II, the effect of the PAS-induced neuroplasticity on the RS was investigated. For this purpose, the RS was subdivided and assessed for two components: 1) immediate adaptation of TMS-evoked motor response following the first repetition of the stimulus ("dynamic"), and 2) maintenance of this response with further repetition of the stimulus ("stable"). Our findings demonstrated that the induced plasticity resulted in a common tendency of the stable component to reside within a limited range, irrespective of the dynamic component. This means that the MEP amplitudes appeared with a low variation in response to the first and later repeated TMS pulses, after PAS.

The typical RS is often portrayed as the decrease in the amplitude of the neural response following the first stimulus repetition. Although the exact underlying mechanism of action is still obscure, the role of the prior expectation has been postulated as a candidate mechanism [196,197]. Accordingly, it does seem that the brain is an adaptive system that encodes a generative model of the world and based on this, it seeks to make predictions [196]. The discrepancy between the predictions and the upcoming stimuli results in an error-signal which is eliminated with stimulus repetition, as the stimulus now matches the expectation. The key theme that emerges here can explain the dynamic component of the RS. It seems reasonable to

assume that the brain seeks to maintain the information with regard to the recently processed stimulus over a timescale of seconds, as the decreased motor excitability (reflected as decreased MEP amplitudes of the stable component) is sustained over the second and further stimulus repetition with an ISI of 1 s [198]. A previous study conducted by Pitkänen et al. supports this notion by demonstrating that RS could not occur with an ITI less than 3 s [199]. The existence of this kind of potential "automatic memory" might contribute to the modulation of a short-lasting form of synaptic plasticity. Short term synaptic plasticity might contribute to the capacity of the brain to translate transient experiences into persistent memories [200, 201]. Hence, assessing the stable component of the RS could be of major importance as the lack of this component might imply that the brain was incapable of holding the trace of a recently processed stimulus, preventing minimizing of the surprise effect, as observed in patients with progressive myoclonus type 1 [16].

On the other hand, the PAS with an ISI of 25 ms is believed to induce an LTP-like plasticity [101,123]. However, LTP-like plasticity may provide a means to reverse LTP where the synapses are saturated in order to preserve the neuronal stability and provide the required facilitation/inhibition balance [202]. Hence, as demonstrated in study II, induction of plasticity using PAS, resulted in LTD-like plasticity in some subjects in whom the baseline stable state was high. Our findings also demonstrated that despite this discrepancy, all subjects had a tendency towards a common level of motor cortical excitation, reflected as MEPs with a low variation in the stable component, in healthy subjects. As the post-PAS excitability enhancement/ decrement has been attributed to a modificantion of synaptic efficacy [203, 204], the degree of the required modification for the motor responses to reside within a given range which is neither hyper- nor hypo- might be explained by updating of the synaptic efficacy. Thus, assessing the RS from the two above-mentioned aspects provided us with the information about a form of short-term synaptic plasticity which is essential not only in processing the information, but also in restoring that information over a short timescale.

6.3 ASSESSING THE POTENTIAL OF REPETITION SUPPRESSION AS A BIOMARKER OF NEUROPLASTICITY IN INDIVIDUALS WITH CHRONIC PAIN IN HOW THEY WILL RESPOND TO RTMS

By investigating the RS in patients with chronic pain who had received high frequency rTMS, the predictive power of the stable component of the RS was revealed as it helped to identify those individuals who would potentially benefit from the treatment. Although the findings do not make it possible to pinpoint the precise details of the underpinning factors contributing to explaining why some patients experience a benefit, the synaptic efficacy modifications might be one key contributor [6]. The unidirectional modification of the synaptic efficacy has been demonstrated in hyperalgesia, i.e. the abnormal enhanced sensitivity to pain [1, 2]. This unidirectional modification might leave the synaptic strength with a ceiling/floor level, which in turn prevents further modification and leads to an excessive expression of LTP/LTD. In our study, the significantly higher pre-rTMS neural response in those individuals reporting no analgesic effect, at a stable level of the RS, might reflect a saturated LTP in the brain networks mediating pain processing. This might be explained by potentially shared underlying networks between the pain processing networks and the RS, primarily composed of the thalamus and the somatosensory

cortex [3,5]. The saturated network possibly hinders rTMS when it strives to regulate synaptic plasticity to help with sustaining the cortical excitation within a functional dynamic range. It seems that the prerequisite for this "functional dynamic range" has been reflected through the baseline MEP responses with amplitudes which are neither lower nor higher than certain set points. In addition, on a smaller scale, the saturated LTP might be reflected in another aspect of the stable component of the RS in those patients reporting no benefit i.e. the faster recovery from the second to the fourth MEP responses (compared to those who had a benefit). This finding potentially implies the existence of an automatic memory that had been also observed in study II. The fast recovery of the repeated stimulus probably may well be indicative of the lack of deficiency in the mechanisms employed to hold the memory trace of the recently encountered stimulus, resulting in processing of the repeated stimulus as a novel one. The hypervigilance to sensory stimuli in chronic pain patients might be the result of this deficiency.
These findings are compatible with the concept in which the development of chronic pain has been attributed to a loss of inhibition mainly at the thalamic level, leading to disrupted pain suppression [5,45]. The extent to which the patients could benefit from rTMS, might be determined by how well synaptic plasticity could be recruited to help with restoring the balance between inhibition and disinhibition. This might be the underlying reason for the patients with mild recovery of the repeated stimuli and lower suppressed MEP amplitudes seemed to benefit more, as their less saturated inhibition tone allows more room for the required modifications at the synaptic level, where the high frequency rTMS unleashes more inhibitory tone [6].

Considering all three of the conducted studies, certain limitations need to be acknowledged as TMS-induced RS is novel technique and only one thesis has investigated the TMS-induced RS in the motor system. This results in the formation of some open-ended questions that have not yet been examined and will need to be addressed in future studies.
One possible limitation is that the underlying mechanisms and locus of action of TMS-induced RS, at both the macroscopic and microscopic levels, are not totally understood. Some of the answers might be provided by investigating the effects of TMS-induced RS especially at the cellular level, e.g. through pharmacological interventions as these could provide vital evidence for its potential utility as a biomarker. Furthermore, it may be possible to gain new insights by means of assessing the interaction of RS with other facilitatory or inhibitory neural phenomena such as SICI and ICF as these are mediated through different mechanisms other than the paradigms employed in this thesis.
In addition, investigating the RS in a wider range of neuropsychiatric disorders which are known to be characterized by disturbed neuroplasticity could help to evaluate the validation of RS as a biomarker for neuroplasticity.

7 SUMMARY AND CONCLUSIONS

The effect of the stimulus repetition on the neural activity, i.e. RS, has been widely-studied in neuroscience. RS is often portrayed as the immediate attenuation of neural activity in response to the repetition of an intense stimulus. In this thesis, two aspects of the TMS-induced RS were investigated for the first time. One aspect was assumed to reflect the capacity of the brain to undertake a fast and stimulus-specific adaptation, occurring following the first repetition of a stimulus. The other aspect might be reflecting a short-term synaptic plasticity that is dependent on a change of synaptic efficacy resulting in a form of short-term synaptic plasticity. This potential candidate allows the brain to hold a memory trace of a recently processed stimulus over a timescale of seconds, reflected as the maintained suppressed motor response for the second and later responses. Hence, these aspects might hold the potential to act as an estimate of plasticity.

The main findings of this thesis were:

1. A typical RS was disrupted when combined with LICI, implying that there are interacting inhibitory mechanisms.

2. RS was facilitated when combined with the I1-wave timed SICF while its absolute effect remained unaffected.

3. The induction of short-term plasticity in healthy subjects, resulted in a tendency towards a common suppressed in the M1 excitation following the repetition of the first stimulus, irrespective of the cortical excitation before applying the PAS.

4. The maintenance and amplitude of the suppressed responses in the RS might serve as a good predictor to identify positive outcomes in patients experiencing chronic pain.

To conclude, this thesis has investigated the potential of RS as a novel biomarker of abnormal adaptation to external stimuli has and provided a means to estimate a patient's potential for recovery. The preliminary studies on the feasibililty of applying RS as a novel biomarker of neuroplastic capacity suggest that there is a clear potential for even some clinical applications. This proposal will depend on whether or not future studies confirm the findings emerging from this thesis in clinical trials with larger populations of patients. In addition, it can be concluded that the RS indeed distinguished neuroplastic changes in the experimental setting in healthy volunteers, providing a novel tool for basic neuroscience research.

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Interaction Between Repetition Suppression in Motor Activation and Long-interval Intracortical Inhibition

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Interaction between repetition suppression in motor activation and long-interval intracortical inhibition

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Repetition suppression (RS) is the adaptation of the neural activity in response to a repeated external stimulus. It has been proposed that RS occurs at the thalamo-cortical level, hence activating a feedback loop to the cortex in order to counteract with the repeated motor cortical activation. In this study, to elucidate the common modulators between the RS and the inhibitory/facilitatory cortical networks, two TMS paradigms were applied, i.e. the characteristic long-interval intracortical inhibition (LICI) and the I1-wave timed short-interval intracortical facilitation (SICF). Since LICI is a local intracortical inhibitory phenomenon affecting cortical excitation over a long interval like the RS, the interaction between RS and LICI was tested. As the I1-wave timed SICF is likely not affected by inhibitory modulation, the appearance of the RS with respect to SICF was investigated. Non-linear interaction between LICI and RS was observed, while I1-wave timed SICF facilitated all MEP responses of RS by a common offset still preserving the RS. These findings implicate that the underlying mechanism for the observed interaction is likely contributed to the activation of the negative thalamo-cortical feedback loop represented by the RS, most likely at the cortical level.

The primary motor cortex is a highly organized, five-layered convoluted sheet of neural cells, located on the precentral gyrus of the cerebral cortex. In the motor cortex, intermingled with the inhibitory GABAergic interneurons, the excitatory glutamatergic pyramidal neuron is the principal cell type[1,2]. Pyramidal cells are most abundant in layers III and V. However, their horizontal and vertical extensions into other layers provide the motor cortical networks with a flexible synaptic organization[3]. This organization provides a cortical platform for neuroplasticity in the primary functions, such as movements. Neuroplasticity is a crucial characteristic in the central nervous system, enabling recruitment of the neuronal connections to adapt, as well as to maladapt, to modified requirements. Neuroplasticity is also mediated by the inhibitory GABAergic interneurons[4].

Transcranial magnetic stimulation (TMS) is a non-invasive technique that allows us to study the facilitatory and inhibitory cortical networks by means of time-varying magnetic fields[5]. TMS-evoked facilitation results in a number of cortico-spinal descending volleys in the pyramidal tracts: direct (D) and indirect (I) waves. D-waves, produced via direct activation of the pyramidal neurons of layer V, are the earliest of these descending volleys. While the origin of later I-waves is contributed to polysynaptic connections between the neurons in layer II/III and those in layer V, I1-wave is proposed to originate from the monosynaptic excitatory cortico-cortical projections on the cortico-spinal fibers[6]. Supporting the notion that the GABAergic inhibitory system is not involved in the formation of the I1-waves, insensitivity of these waves to $GABA_A$ agonists has been demonstrated[7]. To characterize the I-waves, short-interval intracortical facilitation (SICF) is a well-documented paired-pulse TMS paradigm. There is convergent evidence that the facilitatory interaction of the paired pulses in SICF occurs primarily at the cortical level[8]. SICF has been conventionally demonstrated using monophasic waveform[8]. However, it has been recently evoked applying biphasic waveform[9,10]. Repetition suppression (RS) refers to the adaptation of the neural activity in response to repeated external stimuli[11,12]. The recovery of the habituated response after the heightened initial response implies that the RS functions to prevent the brain from overreaction to a novel stimulus[13]. Impairment of the RS has been demonstrated previously in progressive myoclonus epilepsy and schizophrenia[14,15]. It has been suggested the RS occurs at the thalamo-cortical level, following TMS[14,16]. This phenomenon has been demonstrated in the motor system as a decrement in the amplitudes of the subsequent motor evoked

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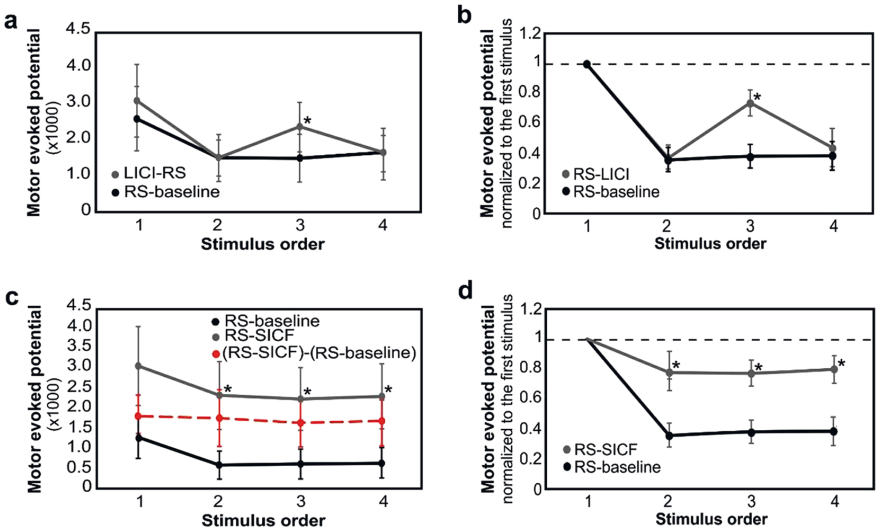

Figure 1. (**a**) Absolute MEP amplitudes and (**b**) normalized MEP amplitudes (*mean* ± *standard error*) across all participants within the trains during RS-baseline and RS-LICI paradigms. The third MEP amplitude in RS-LICI paradigm was significantly higher than that in the RS-baseline paradigm. (**c**) Absolute MEP amplitudes (*mean* ± *standard error*) across all participants within the trains during the RS-baseline and RS-SICF paradigms. MEPs in RS-SICF paradigm are increased by a common offset while the absolute RS effect remains unchanged. The dashed red line marks the MEPs of RS-SICF subtracted from those of RS-baseline. (**d**) Normalized MEP amplitudes (*mean* ± *standard error*) across all participants within the trains during RS-baseline and RS-SICF paradigms demonstrating an increase of the normalized MEPs in the RS-SICF paradigm. The MEPs were averaged over all trains. Normalization of MEPs were performed with respect to the mean MEP amplitude induced by the first stimulus (dashed line). Asterisk indicates significant differences for pairwise comparisons ($p < 0.05$).

potentials (MEP) following the initial one[16,17]. The decline in the MEP amplitudes indicates the adaptive behavior of the motor system. Adaptation has been associated with neuroplastic changes in the nervous system[18], and therefore as an objective biomarker to assess the neuroplastic abilities. Several mechanisms have been postulated regarding the neurophysiological basis of the inhibitory phenomenon observed in the RS[11]. One such underlying mechanism might contribute to the increased activity of GABAergic inhibitory system, a mechanism that is also known to mediate long-interval intracortical inhibition (LICI)[19,20]. LICI is an intracortical inhibitory phenomenon that is demonstrated as suppression of neural activity, when a suprathreshold stimulus is followed by a second suprathreshold stimulus at an inter-stimulus interval (ISI) of 50–200 ms[20]. The inhibition time course of LICI and the pharmacological studies reflect the cortical origin and activation of the $GABA_B$ inhibitory receptors in this phenomenon[21,22]. In this study, we applied two stimulation paradigms with the RS: (1) SICF interaction in I1-wave and (2) LICI, to test the effects of immediate cortical facilitation and a cortical long-interval inhibitory modulation on the RS. The appearance of RS in relation to facilitatory I1-wave timed SICF, was applied as it is suggested to be unaffected by inhibitory modulation[23,24], while LICI is known to be mediated by $GABA_B$[25–27]. We hypothesized that I1-wave timed SICF would facilitate all MEP responses in the RS without affecting the inhibitory modulation, while the interaction of the LICI and RS was hypothesized to occur through the RS affecting the cortical inhibitory function through the thalamo-cortical feedback.

Results

The typical main effect of RS was observed in RS-baseline paradigm ($F(3,56) = 19.24$, $p = 0.004$), where the second, third and fourth MEP amplitudes were significantly lower than the first one ($p < 0.001$, $p = 0.001$, $p = 0.002$ for normalized MEPs, respectively). No difference in MEP amplitude was observed between the second and third, third and fourth, and fourth and second stimuli ($p > 0.1$). Further, to show the inhibitory effect of LICI and facilitatory effect of SICF at baseline, a Wilcoxon signed-rank test revealed a significant inhibition, on average of 92%, induced by the LICI paradigm ($p = 0.012$), and a paired samples t-test indicated facilitation effect on MEPs (273% on average), induced by the SICF paradigm ($p = 0.013$).

As opposed to MEP amplitude decline in the RS-baseline, the RS interacted with the LICI when combining the RS and the LICI paradigms, demonstrated as "Paradigm*Stimulus Order" interaction effect ($F(3,56) = 4.12$, $p = 0.081$ for the absolute MEPs and $F(3,56) = 15.04$, $p = 0.006$ for the normalized MEPs) (Fig. 1). This non-linear modulatory effect was observed as an increase in normalized MEP amplitudes during RS-LICI (Fig. 1a). *Post hoc* paired t-test revealed that the third MEP amplitude within RS-LICI trains was significantly higher than that in

RS-baseline trains ($p = 0.019$ for the absolute MEPs and $p < 0.001$ for the normalized MEPs). No increase in the second and fourth MEP amplitudes was observed ($p > 0.1$). The Main effects of "Paradigm" and "Stimulus Order" on MEPs were also investigated ($F(3,56) = 4.12$, $p = 0.082$ (Paradigm) and $F(3,56) = 3.10$, $p = 0.127$ (Stimulus Order) for the absolute MEPs and, $F(3,56) = 38.44$, $p = 0.001$ (Paradigm) and $F(3,56) = 5.32$, $p = 0.054$ (Stimulus Order) for the normalized MEPs).

Interaction between the RS and the SICF were observed through a combined RS-SICF paradigm. Using the absolute MEPs illustrates the common offset added to the RS by SICF clearly. Main effect of "Paradigm" revealed that the RS-SICF paradigm increased the MEP amplitudes by a common offset ($F(3,56) = 7.22$, $p = 0.031$) for the absolute MEPs and $F(3,56) = 8.34$, $p = 0.023$ for the normalized MEPs), while the absolute RS effect remained unchanged ($p > 0.5$) (Fig. 1), as hypothesized. The main effect of "Stimulus Order" on MEPs was also demonstrated ($F(3,56) = 16.62$, $p = 0.005$ for the absolute MEPs and $F(3,56) = 5.53$, $p = 0.048$ for the normalized MEPs). *Post hoc* paired-samples t-test revealed the significant increase of the MEP amplitudes within the RS-SICF trains ($p = 0.013$, $p = 0.044$, $p = 0.031$ and, $p = 0.031$ for the absolute MEPs, in the first, second, third and, fourth stimulus, respectively and $p = 0.050$, $p = 0.034$, $p = 0.030$ for the normalized MEPs in the second, third and, fourth stimulus, respectively).

Discussion

The findings from the present study suggest that combining the RS and LICI causes the RS to be overridden by the interaction with LICI, hence suggesting an interacting mechanism at the cortex between the two inhibitory phenomena. Since the ISI at which LICI occurs was 100 ms in our study, the time course at which LICI persists is over 100 ms following the first stimulus. On the other hand, the ISI between the consecutive pulses in the RS paradigm, 1 s, confirms its inhibitory action around 1 s. Hence, the relatively long time course of these two inhibitory phenomena raises the possibility of their interaction.

The RS is demonstrated by characteristic immediate drop in the MEP amplitude after the first stimulus, and a slight recovery of amplitudes may be observed towards the baseline[28]. The immediate drop is considered to occur to prevent meaningless movements by increase of the intracortical inhibition after a movement executed without prefrontal planning[17]. A previous study reported that in the active muscles the TMS-induced silent periods increase over time during the RS, suggesting that cortical inhibition is becoming stronger with repeated stimuli[19]. Hence, the suppression via cortical inhibition is strengthened after each repeated stimulus perhaps to balance the drive towards homeostatic baseline, which could be reflected as the observed gradual recovery in responses during RS, demonstrating an ongoing chance in the inhibition/excitation balance. In progressive myoclonus type 1 (EPM1) the inhibitory tonus is impaired[29], and the observed abnormal RS is weaker[14]. As the inhibitory tonus is prevailing and saturated, disabling the maintained RS and enabling an immediate recovery in adolescent patients, the mechanism described above could be a viable explanation for the observed recovery. Although several mechanisms have been proposed, the underlying neurophysiologic basis of the RS has remained elusive[11]. The heightened amplitude of the initial response as compared to the consecutive responses can be explained by the arousal effect; an effect originated in the brainstem ascending reticular activation system (RAS) in response to novel stimuli[30,31]. A potential model explaining the attenuation of the consecutive responses is the Sharpening model. According to this model, the population size of the firing neurons is optimized to a maximal capacity of the target organ by a feedback loop as the stimulus is repeated[12,30,32].

Another plausible underlying physiological mechanism might contribute to the increased activity of the GABAergic inhibitory system[19], a mechanism that is mediating the LICI[22]. Apart from the production mechanisms of these two inhibitory phenomena, it has been suggested that they occur at different levels of the central nervous system. The RS is thought to occur at the thalamo-cortical level, while the LICI originates at the cortical cortical level[7,14,16,20]. Therefore, most likely, RS is ruled out by LICI at the cortex, tapping into the thalamo-cortical feedback loop that is activated by the RS. To elaborate on this, one possible explanation for the underpinning mechanism of the observed interaction between the RS and LIC is the activation of the thalamo-cortical feedback loop. We speculate that similar to the baseline measurement, the negative feedback from the cortical areas such as tightly interconnected sensory areas close by may increase the GABAergic intracortical inhibition after the first stimulus, which in turn causes the suppression of the second MEP amplitude. To restore the balance between the inhibitory and excitatory states of the motor network, the negative feedback loop weakens the thelamo-cortical inhibition and disinhibition dominates, following the second stimulus, resulting in a greater MEP during the third stimulus. During the third stimulus, the feedback loop is attenuated again, allowing for a greater response to occur. Higher response causes the enhancement of the function of the negative feedback loop following the third stimulus, resulting in suppression of the fourth response. Even though, our results cannot directly pin-point the thalamo-cortical pathway as the interaction locus for RS and LICI, this possibility cannot entirely be ruled out either. Animal studies have demonstrated activation of the inhibitory thalamic reticular nuclei following stimulation of the cerebral cortex[33,34]. This activation can result in inhibition of the cerebro-thalamo-cortical pathway. It has been suggested that the conditioning stimulus of the LICI may affect the thalamo-cortical pathway at the thalamus[35]. Therefore, there is a possibility that the interaction site is at the subcortical level.

We observed that I1-wave timed SICF facilitated all MEP responses without modulating the absolute RS, meaning the inhibitory effect appeared not to affect the I1-wave SICF interaction. There is growing evidence on the stability of I1-wave, making it implausible to be modulated by inhibitory and excitatory mechanisms[23,24,36]. Setting the time interval between the two consecutive pulses in SICF in such manner that I1-wave is targeted can enhance the effects of TMS by influencing the absolute momentary motor cortical activation, probably via synchronization of I2-wave in conditioning pulse with I1-wave in test pulse. Facilitatory effect of the I1-wave timed paired-pulse TMS has been recently demonstrated with biphasic TMS waveform[10]. In accordance with these data, our finding also suggests facilitation effect observed, using I-wave-timed paired-pulse TMS, where the inhibitory

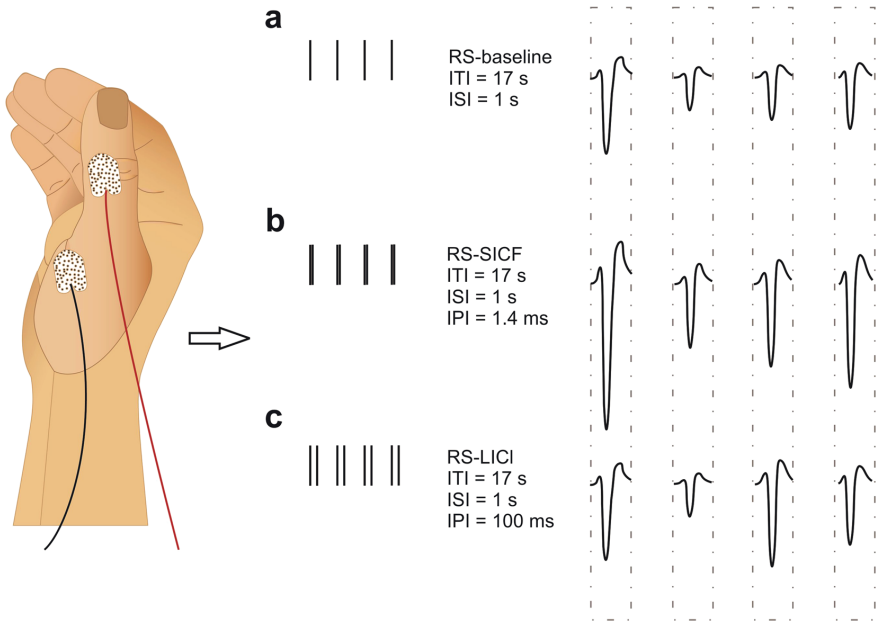

Figure 2. Schematic figure of three TMS stimulation paradigms to study RS via MEPs of target APB muscle. (**a**) In baseline paradigm for RS, twenty trains of four single biphasic pulses were applied. (**b**) In combining SICF and RS (RS-SICF), twenty trains of four biphasic paired pulses at an IPI of 1.4 ms were used to induce the SICF. (**c**) In combining LICI and RS (RS-LICI), twenty trains of four paired pulses with IPI of 100 ms were utilized to induce LICI. ITI of 17 s and ISI of 1 s were utilized in all paradigms.

RS remains unchanged, i.e., the observed effect in the normalized MEPs was explained fully by the common offset added by the SICF to all absolute MEP responses in the RS paradigm.

Detrimental parameters that need to be taken into account to optimize the probability of the occurrence of the required paradigms, e.g. the RS and LICI, are the intensity, the location of the stimulus and the direction of the induced current, and ITI[28,37–40]. In the current study, ITI of 17 s was employed in all RS paradigms, since it has been depicted that RS effect is more pronounced at this ITI, probably due to more time to recover. No carry-over effects from trial to trial have been observed at this ITI[28]. Similarly, we optimized the parameters for LICI and SICF protocols to optimize the inhibitory and facilitatory effects confirmed as successful by our baseline measurements[9,10,19]. Altering the applied parameters for the SICF and LICI could have altered the results.

In conclusion, in agreement with our hypothesis, the SICF did not interact with the RS, but facilitated all responses in the RS train. Again, in agreement with our hypothesis, the LICI demonstrated an interaction effect with RS, exhibiting a non-linear interaction partly reducing the common RS effect potentially interfering with the RS at the cortical level.

Methods

Subjects. Eight healthy right-handed volunteers with no history of neurological disorders were recruited in this study (6 males, aged 22–42 years). Written informed consents were obtained from all participants. The study was approved by the research ethics committee of the Kuopio University Hospital (256/2017). The study was performed in accordance with the Declaration of Helsinki abiding the safety guidelines for TMS applications[41].

Transcranial magnetic stimulation (TMS). The measurements were performed using a customized NBS System 4.3 (Nexstim Plc., Helsinki, Finland) with an air-cooled figure-of-eight coil. Prior to the study, structural T1-weighted magnetic resonance images (MRIs) were acquired with a 3T MRI scanner (Philips Achieva 3.0T TX, Philips, The Netherlands). MRI data were further utilized in neuronavigation. The initial step in stimulation procedure was to determine the abductor pollicis brevis (APB) muscle "hotspot", i.e. the cortical site capable of eliciting the maximal contralateral muscle response with minimal stimulator output intensity. Once the hotspot was identified, the resting motor threshold (rMT) was determined using system-integrated iterative threshold assessment tool[10]. The subsequent TMS stimuli were administrated over the APB hotspot at an intensity of 120% rMT during three different paradigms used during this study: RS-baseline, RS-SICF and RS-LICI, utilizing the biphasic waveform (Fig. 2). All RS paradigms included twenty trains of four trials. Four trials have been demonstrated to evoke a reliable RS effect[14,28]. The trials consisted of the stimuli either from a single-pulse form (RS-baseline) or a paired-pulse form (RS-SICF and RS-LICI). In RS-SICF a suprathreshold pulse was followed by a subthreshold one

at an inter-pulse interval (IPI) of 1.4 ms[10], whereas in RS-LICI, a conditioning suprathreshold pulse was delivered 100 ms before a suprathreshold test pulse[42]. An inter-stimulus interval (ISI) of 1 s within the trials and inter-train interval (ITI) of 17 s maintained throughout all the paradigms[28]. For confirming the induction of the LICI, baseline measurements were also conducted prior to the RS experiments applying 20 trials of paired pulses at 120% rMT at IPI of 100 ms at the ITI of about 5 s and comparing the first response amplitude to the second one in the stimulus pairs[43]. The occurrence of SICF was verified at the first stimuli of the RS-SICF protocol by comparing those to the first stimuli of the RS-baseline protocol.

Electromyography (EMG). EMG responses, i.e. MEPs, were recorded via an integrated EMG system at the sampling frequency of 3 kHz. A pair of disposable Ag-Cl electrodes were utilized with the active electrode placed over the belly of the APB muscle and a reference electrode placed over the joint distal to the active electrode (Fig. 2). The MEPs were recorded by triggering the EMG signal with TMS, and were processed offline in Matlab (version R2017b, MathWorks Inc., Natick, MA, USA). The timing and intensity of the stimuli within the protocols was set with the integrated navigation and recording system software at a millisecond precision. The IPI for the paired-pulses can be adjusted at a 0.1 ms precision. MEPs occurring in the resting muscles with peak-to-peak amplitude greater than 50 μV, were considered as MEPs.

Statistical analysis. MEP data in each paradigm were first averaged over all the trains on the basis of their stimulus order in a train, for each subject. First, to assess the RS effect in the baseline condition, repeated measures ANOVA with "Stimulus Order" within a train (first, second, third and, fourth) as fixed effect was applied. Also, to evaluate the inhibition effect in LICI baseline, Wilcoxon signed-rank test was conducted to compare the test pulses against the conditioning ones. A two-way repeated measures ANOVA with "Paradigm" (RS-baseline, RS-LICI and, RS-SICF) and "Stimulus order" (first, second, third and, fourth) as within-subject factors was employed to evaluate the influence of the paradigms and stimulus order as main effects, as well as their interaction effect. Further, *Post-hoc* tests were performed to assess the effect of the paradigms on MEPs at each stimulus order. All tests were conducted on both absolute and normalized MEPs. Normalization was performed by dividing all average MEP amplitudes within the trials with the average amplitude of the first MEP. *Post-hoc* comparisons were conducted using paired *t*-test with Bonferroni correction. A p-value of <0.05 indicated statistical significance. Statistical analyses were conducted using SPSS (v. 25.0, SPSS Inc., IBM Company, Armonk, NY, USA) and Matlab (version R2017b, MathWorks Inc., Natick, MA, USA).

Data Availability

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

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Author Contributions

P.J. and J.K. designed the experiments; S.K., P.J. and L.S. conducted the experiments; S.K. conducted the formal analysis of the data; S.K. wrote the original manuscript; P.J., J.K. and L.S. and M.K. participated in editing the manuscript. All authors reviewed the manuscript.

Additional Information

Competing Interests: P.J. has received consulting fees and travel support from Nexstim Plc. J.K. is employed part-time by Nexstim Plc, manufacturer of navigated TMS systems. S.K., L.S., and M.K. declare no conflict of interest.

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Brain Response Induced with Paired Associative Stimulation Is Related to Repetition Suppression of Motor Evoked Potential

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Abstract: Repetition suppression (RS), i.e., the reduction of neuronal activity upon repetition of an external stimulus, can be demonstrated in the motor system using transcranial magnetic stimulation (TMS). We evaluated the RS in relation to the neuroplastic changes induced by paired associative stimulation (PAS). An RS paradigm, consisting of 20 trains of four identical suprathreshold TMS pulses 1 s apart, was assessed for motor-evoked potentials (MEPs) in 16 healthy subjects, before and following (at 0, 10, and 20 min) a common PAS protocol. For analysis, we divided RS into two components: (1) the ratio of the second MEP amplitude to the first one in RS trains, i.e., the "dynamic" component, and (2) the mean of the second to fourth MEP amplitudes, i.e., the "stable" component. Following PAS, five subjects showed change in the dynamic RS component. However, nearly all the individuals (n = 14) exhibited change in the stable component ($p < 0.05$). The stable component was similar between subjects showing increased MEPs and those showing decreased MEPs at this level ($p = 0.254$). The results suggest the tendency of the brain towards a stable state, probably free from the ongoing dynamics, following PAS.

Keywords: repetition suppression; neuroplasticity; transcranial magnetic stimulation; paired associative stimulation

1. Introduction

Owing to its dynamicity, the brain responds to an intense, novel stimulus with enhanced, transient neural activity. This rapid response, referred to as a startle, is considered to play a critical function in promoting survival [1]. However, exposure to a higher number of identical sensory stimuli yields attenuation of neural activity in the responding network, a phenomenon known as repetition suppression (RS) [2]. RS has been well-characterized across several brain regions, employing various stimulus categories and modalities [3–6]. In the motor system, RS has been demonstrated as a decrement in the amplitude of motor-evoked potentials (MEPs) when transcranial magnetic stimulation (TMS) is applied to an optimal motor cortex location [7,8]. Although it has been suggested that the attenuation observed in RS may serve to provide an energy-efficient neuronal information processing [9], the exact mechanisms underlying RS have remained elusive. RS was initially portrayed merely as an expression of bottom-up mechanisms [2,3,10,11]. However, more recent theories have emphasized the role of top–down mechanisms within a predictive coding scheme, relying on iterative

comparison between prior expectations and sensory inputs [12]. Interestingly, RS of MEPs have been demonstrated to be closely associated with neuroplasticity [13].

Neuroplasticity is considered one of the key mechanisms that grants living organisms the ability to adapt and respond flexibly in the face of changing environmental demands [14]. Depending on the speed of these changes, neuroplasticity can take different forms and occur at different timescales. Neuroplasticity is considered the keystone of learning, memory, and recovery from (mild) brain injuries [15–17]. Aberrant neuroplasticity has been put forth as the pathophysiological basis of several neuropsychiatric disorders, such as schizophrenia, depression, and chronic pain [18–20]. Long-term potentiation (LTP) consists of persistent synaptic activity, which is often considered as the cellular basis in the mediation of these functions [21].

Currently, TMS provides the opportunity to study neuroplasticity at the system level, ranging from synaptic plasticity to network-level plasticity [22]. The shifts towards either elevated excitation or diminished inhibition have been proposed as potential underlying mechanisms of neuroplasticity, with the short-term plasticity most likely mediated by the reduction of GABAergic inputs onto excitatory synapses [23].

A well-established and widely used TMS paradigm to induce short-term, topographically specific plasticity in the motor cortex is paired associative stimulation (PAS), in which electrical peripheral nerve stimulation is paired with cortical stimulation [24,25]. If the peripheral input precedes the cortical stimulation, PAS can lead to elevated cortical excitability that manifests itself via an increase in the MEP amplitude (LTP-like plasticity) [26,27]. By contrast, if the order of the arrival of inputs is reversed, depression of cortical excitability is likely to occur (long-term depression (LTD)-like plasticity) [24]. Due to its dependency on timing, PAS has been suggested to induce spike-timing dependent plasticity [28].

In the present paper, to investigate neuroplastic effects induced with PAS, RS is hypothesized to represent the interplay of two states: (1) one reflecting the efficient processing of a novel input, "dynamic RS", indexed by the initial decrement from the first amplitude to the second one, and (2) one reflecting the overall cortical excitability free from the ongoing dynamics, "stable RS". Stable RS, described here as the suppressed amplitude level of the second to the fourth MEPs within the RS trials, might potentially display the capacity of the brain to maintain the processed input as an initial "memory trace". We investigated the dynamic and stable RS prior to and following a common PAS-LTP protocol [24]. We hypothesized that the brain would demonstrate a trend towards a state with low variation in MEP amplitude, which we consider the target level of neuronal network excitability as it is independent from reactive dynamics within the network. As an implication, for long-term neuroplastic effects, the modulation of this stable level could potentially be targeted by neuromodulation, and to create optimal conditions for adaptive neural changes.

2. Materials and Methods

2.1. Subjects

Sixteen healthy, right-handed volunteers with no history of neuropsychiatric disorders participated in this study (seven male, age range: 22–42 years, mean ± SD: 30 ± six years). All subjects provided a written informed consent prior to the experiment. This study was approved by the research ethics committee of the Kuopio University Hospital (256/2017).

2.2. Transcranial Magnetic Stimulation (TMS)

To enable neuronavigation for TMS, structural T1-weighted magnetic resonance images (MRIs) were obtained with a 3T MRI scanner (Philips Achieva 3.0T TX, Philips, Eindhoven, The Netherlands) with the following parameters: repetition time (TR) = 8.2 ms, echo time (TE) = 3.7 ms, flip angle = 8°, voxel size = $1 \times 1 \times 1$ mm^3. TMS was conducted using NBS System 4.3 (Nexstim Plc, Helsinki, Finland) with an air-cooled figure-of-eight coil and biphasic pulses.

The stimulation procedure was initiated by locating the optimal motor representation of the right abductor pollicis brevis (APB) muscle, i.e., APB "hotspot", with the corresponding optimized coil orientation. The hotspot was defined as the cortical site repeatedly eliciting the greatest peak-to-peak MEP responses compared to adjacent stimulation sites. Once the hotspot was determined, the resting motor threshold (rMT) was identified at this cortical site using a system-integrated iterative threshold assessment tool [29]. In the RS paradigm, trials of four TMS stimuli were applied over the APB hotspot, with an inter-stimulus interval (ISI) of 1 s, at an intensity of 120% rMT. The RS paradigm, comprising 20 trials of four single biphasic TMS pulses, was employed with an inter-train interval (ITI) of 17 s [30], before (RS-baseline) and immediately (0 min), 10 min, and 20 min after the PAS intervention (Figure 1).

We recorded MEPs via an integrated electromyography (EMG) system (Nexstim Plc) at a sampling frequency of 3 kHz. A pair of disposable Ag–Cl electrodes was utilized, with the active electrode over the belly of the APB muscle while the reference electrode was placed over the joint distal to the active electrode (Figure 1). The MEP data were processed offline in MATLAB (version R2017b, MathWorks Inc., Natick, MA, USA), and only the MEPs with no preceding muscle activation and peak-to-peak amplitude greater than 50 μV were included as responses.

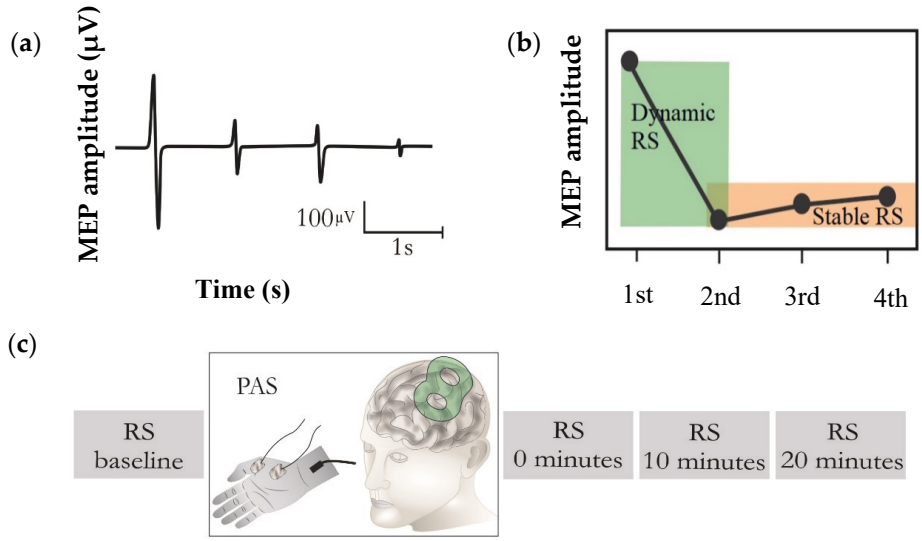


Figure 1. Repetition suppression (RS) paradigm. (**a**) Typical RS. Motor evoked potentials (MEPs) were recorded during four identical TMS pulses. (**b**) The RS paradigm was divided into two components for the analysis: "dynamic RS", i.e., the ratio of the second MEP to the first one, and "stable RS", i.e., the mean of the second, third, and fourth MEPs. (**c**) RS applied before (baseline) and after PAS intervention (at 0 min, 10 min, and 20 min). In the PAS intervention, electrical stimulation of the median nerve-innervated APB muscle was delivered prior to TMS at an ISI of 25 ms to generate plasticity.

2.3. Paired Associative Stimulation (PAS)

PAS consisting of 180 single stimuli was applied over the right median nerve at an intensity of 300% of the sensory threshold (ST) [31]. A bipolar stimulation electrode was placed over the median nerve, and the ST was measured by adjusting the stimulation current until the subject indicated sensation of the stimulus. The pairing with TMS at the APB hotspot was implemented with a self-built triggering and delayer device. To generate a plasticity effect, median nerve stimulation at a frequency of 0.2 Hz was delivered 25 ms prior to TMS [24], with the TMS pulses delivered at 120% of rMT. The median nerve stimulation was conducted using a constant-current electrical stimulator (Digitimer model DS7A, Digitimer, Welwyn Garden City, Herts, UK), using a rectangular pulse form (0.2 ms, maximum voltage of 300 V).

2.4. Statistical Analysis

The MEP amplitudes of each subject were first averaged based on their ordinal position in a trial, i.e., the first, second, third, and fourth. To evaluate the dynamic component of RS, the average of the second stimulus MEP amplitudes was divided by the average of the first stimulus MEP amplitudes. Further, to assess the stable component of RS, the mean of the averaged responses was computed over the second, third, and fourth stimuli per subject.

Considering the inherent heterogeneity of the neurophysiological characteristics, the analysis for identifying significant PAS-effects was initially performed at the individual level using the non-parametric Wilcoxon signed rank test. Individuals with a statistically significant increase in MEP amplitudes at a stable level at 0 min were identified as those showing LTP-like plasticity as an immediate response to PAS (as a higher MEP amplitude is considered as an index of elevated cortical excitability), and clustered as the "LTP-like group". In addition, individuals with decreased MEP amplitudes at a stable level were considered as those exhibiting LTD-like plasticity as an immediate effect to PAS and clustered as the "LTD-like group".

To test the change of the dynamic RS and stable RS over a time course of 20 min, the Friedman test was employed. Post hoc comparisons were performed using the Wilcoxon signed rank test.

A comparison of the two clusters prior to and following PAS was made using the Mann–Whitney U test. Statistical analysis was conducted using SPSS (v. 25.0, SPSS Inc., IBM Company, Armonk, NY, USA) and MATLAB (version R2017b, MathWorks Inc., Natick, MA, USA), and $p < 0.05$ indicated statistical significance.

3. Results

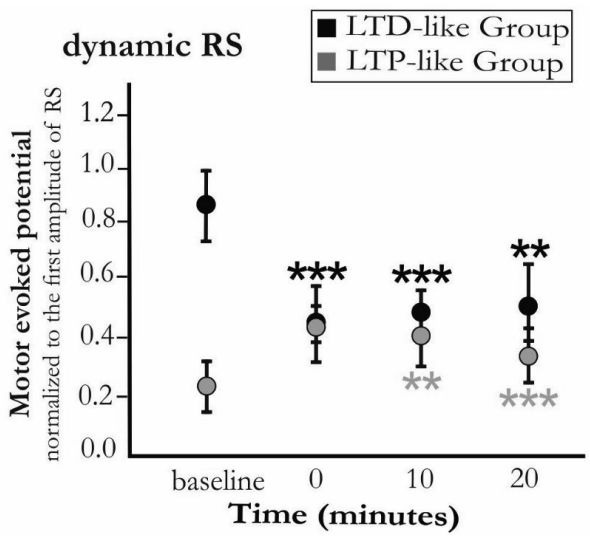
Eleven subjects showed no significant change of dynamic RS following PAS ($p > 0.1$). Only one subject exhibited significantly milder dynamic RS (a lower drop from the second MEP to the first one) ($p < 0.05$), and four subjects showed significantly stronger dynamic RS (a higher drop from the second to the first MEP) ($p < 0.05$) (Figure 2a).

Fourteen subjects exhibited a significant change at stable RS (Figure 2b). The stable RS levels were significantly higher in six subjects immediately following PAS compared to those before PAS ($p < 0.05$). This heightened post-intervention MEP amplitude was assumed to be linked to LTP-like plasticity. However, eight subjects demonstrated significantly diminished MEP amplitudes at stable RS (i.e., the LTD-like group) ($p < 0.05$), and two subjects showed no significant change ($p > 0.1$). A non-parametric Friedman test revealed no change in the trend over the time of measurement following PAS (0, 10, and 20 min). One subject demonstrated delayed LTP-like plasticity at 20 min after exhibiting no effect at earlier time points.

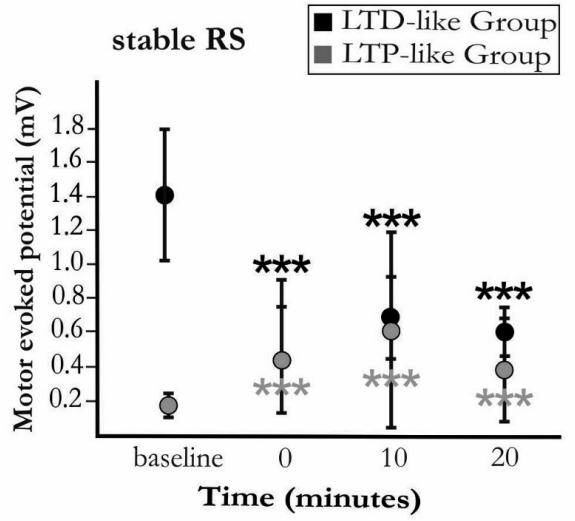
Furthermore, a Mann–Whitney U test revealed that the dynamic and stable RS at the baseline was significantly higher in the LTD-like group compared to the LTP-like group ($p < 0.05$). Following PAS, no significant difference in these two components was observed between the two groups ($p > 0.1$) (Figure A1).

The STs were 2.1 ± 0.5 mA, rMTs were 35 ± 8%-maximum stimulator output (MSO) and MEP latencies were 22.8 ± 1.7 ms. No difference in rMT ($p = 0.845$), ST ($p = 0.244$), and MEP latency ($p = 0.825$) was observed between the two groups.

The low between-group and high within-group homogeneities were observed at stable RS prior and following the PAS, respectively (Figure 3).

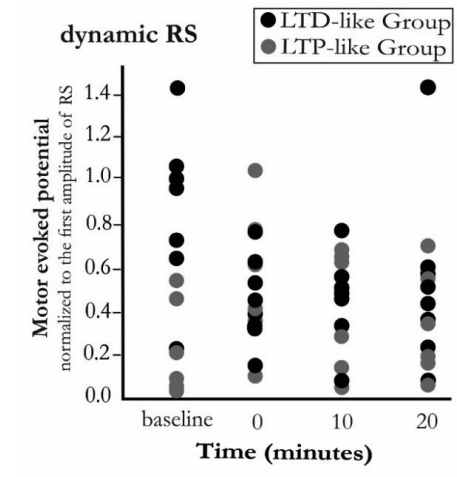


(**a**)

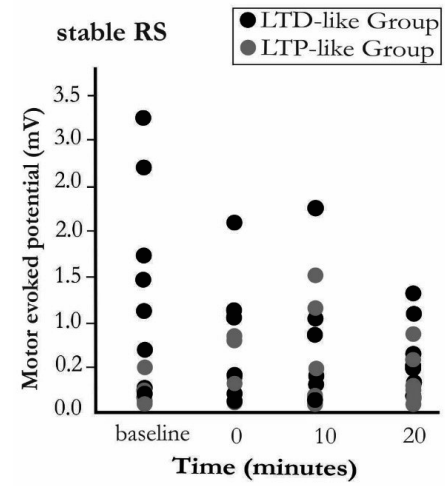


(**b**)

Figure 2. Changes in (**a**) dynamic component (mean ± standard error) and (**b**) stable component of RS (mean ± standard error), across the subjects from two clusters, within the trials before (baseline) and at 0, 10, and 20 min after the induction of short-term plasticity with PAS. Although dynamic RS was mild in the LTD-like group at baseline, a trend towards a stronger suppression and low variability at this level was observed in the LTD-like group following PAS. However, this trend in dynamic RS was towards recovery in the LTP-like group, as this component became milder with time. Similar trends, i.e., sustaining of the suppression and recovery from it, were also observed in stable RS after PAS in the LTD-like group and the LTP-like group, respectively. An asterisk indicates significant differences for pairwise comparisons between time points ($p < 0.01$ for *** and $p < 0.05$ for **).



(**a**)



(**b**)

Figure 3. Scatter plot indicating (**a**) the dynamic RS component and (**b**) the stable RS component in all subjects. The low between-group and high within-group homogeneity can be observed in stable RS following applying the PAS intervention, whereas this is not evident for the dynamic RS.

4. Discussion

Our study investigated two distinct components of RS as a measure of neuroplasticity: (1) immediate changes in motor response upon the first repetition (”dynamic RS”) and (2) the suppressed level of RS (“stable RS”). Surprisingly, induction of plasticity with PAS with a 25 ms ISI resulted in different trends whereof one was rather LTD-like. Irrespective of such a discrepancy, the brain demonstrated an overall tendency towards a common level in stable RS following PAS intervention (Figure A2).

Minimizing the surprise encountered in the face of a novel stimulus is the principle behind the free energy principle [12]. According to this principle, to maintain its integrity, any adaptive biological system, like the brain, seeks to minimize its free energy [32]. It has been proposed that minimizing the free energy rests on either changing the top–down predictions, which are the conceptual internal models, or the bottom–up predicted sensory inputs [32]. In this regard, the dynamic RS depicts an

update of the predictions in response to a twice repeated stimulus, ensuring an efficient sensory processing in a known environment. In other terms, the attenuation of the stimulus-evoked motor response upon the first repetition of the stimulus reduces the prediction errors originating from the incoming sensory information. In this respect, the suppressed level of the MEPs during the stable, suppressed part of RS may reflect a level of cortical excitability that is relatively free from ongoing dynamics in cortical excitability, which exhibits as a characteristically high intra-individual variance in the MEPs and may affect the sensitivity of MEPs to reveal longitudinal changes in excitability due to long-term neuromodulation and -plasticity.

RS has been demonstrated to last over short timescales in the visual and auditory systems, indicating a memory trace of the recently viewed or heard stimulus [33]. This short-term storage of information is reflected in our findings in the stable RS. A potential explanation for the observed stable RS might go back to the existence of a short-term internal representation of the perceived involuntary movement ("automatic memory"). Evidence consistent with this postulate is the lack of RS while an ITI of less than 3 s was employed in a TMS study, with the RS being more pronounced with longer ITIs [30]. Apart from the initial motor response, the subsequent responses elicited by TMS are modulated by sensory feedback, i.e., their magnitudes are controlled by the sensory inputs onto the motor neurons. The brain embodies a dynamic interconnected hierarchal processing organization that enables the reciprocal influence of current and past information. A plethora of positive and negative-feedback connections at both the cellular and network levels is central to sustaining the encoded sensory information on a timescale of seconds [34,35]. Hence, to maintain the automatic memory over a short timescale in RS, a negative feedback probably needs to be provided via recruiting inhibitory pathways to sustain the underlying neural activity. These pathways include the intracortical sensory areas and subcortical areas, among which the basal ganglia and thalamus play a key role. It has been demonstrated via RS that this stable state cannot be achieved in patients with progressive myoclonus type 1, who have impaired neuroplasticity in the thalamo-cortical connections [13,36].

Both dynamic and stable RS might reflect alterations in synaptic efficacy. The persistent changes in synaptic efficacy serve as a window into the formation of synaptic plastic changes, a candidate mechanism through which PAS works [26]. If the neuronal network is provided with only a positive feedback loop, that is, the spiking activity of the presynaptic neuron is correlated with the spiking activity of the postsynaptic neuron, its stability gets disrupted. In fact, this unidirectional process reduces the threshold for the presynaptic neuron to stimulate the postsynaptic neuron, thus precluding the stability and reversibility of the system. To counteract this instability and to tune the neuronal activity within a functional dynamic boundary, the brain employs an array of homeostatic mechanisms [37]. Homeostatic plasticity provides the necessary negative feedback loop to prevent the neural circuits from hyper- or hypo-activity.

A well-established proposed mechanism for homeostatic plasticity is the Bienenstock, Cooper, and Munro (BCM) model [38]. This model assumes a bidirectional synaptic plasticity, where the threshold for LTP/LTD induction varies as a function of the dynamic state of the brain. Considering this model, the more excitable the corticospinal pathway is, the more capacity for inhibition may be required. This can in part explain the reversal of the LTP-like plasticity effect to LTD-like plasticity in individuals showing higher pre-intervention MEP amplitudes (baseline). The degree of the modifications of neuronal plasticity depends on updating the synaptic efficacy. Thus, assessing the RS in the mentioned terms, i.e., are dynamic RS and stable RS, can provide us with information on how the alteration in synaptic efficacy following PAS can be reflected in RS.

A few limitations need to be acknowledged in this study. First, we applied the PAS paradigm using a fixed ISI of 25 ms. Inter-individual variability in responses has been reported for PAS due to non-optimized timing of the peripheral stimulus [39]. The potential decrease in this variability might have been achieved by employing an individualized ISI [40]. However, we did not measure individual sensory evoked potential to optimize PAS for LTP-like effects. This was by design to enable more inter-individual latency variance in the induced PAS effect, and to make the sessions shorter

for the subjects. Second, in spite of having a sample size within the range of other studies in this field, the number of subjects was still small to account for generalization in large populations or in patients. We consider this a successful proof-of-concept study, but for application in patient groups, a larger-scale trial is required considering more inter-individual heterogeneities. Thirdly, we identified the PAS effect from the suppressed responses of RS (stable RS) to avoid the dynamicity of causing variance in the identification of the plastic effects, as we observed in the case of the first responses in the RS trials. This is not common practice with PAS. However, since no previous studies have been conducted with PAS in relation to RS, we had no point of reference.

Author Contributions: P.J. and J.K. designed the experiments; S.K., P.J. and L.S. conducted the experiments; J.R. performed MRI imaging; S.K. conducted the formal analysis of the data; S.K. wrote the original manuscript; P.J., J.K., L.S., J.R., and M.K. participated in editing the manuscript. All authors have read and agreed to the published version of the manuscript.

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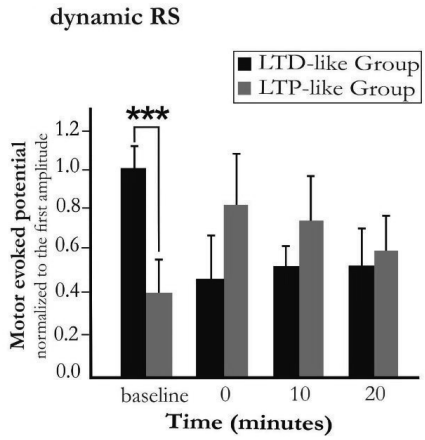
Conflicts of Interest: P.J. has received consulting fees and travel support from Nexstim Plc and has an unrelated patent with Nexstim Plc. J.K. is employed part-time by Nexstim Plc, manufacturer of navigated TMS systems. S.K., L.S., J.R., and M.K. declare no conflict of interest.

Appendix A

Figure A1, Comparison of LTD-like group and LTP-like group prior (baseline) and following PAS (at 0, 10, and 20 min).

Figure A2, RS at 0, 10, and 20 min after PAS in LTD-like and LTP-like groups.



(**a**)

Figure A1. *Cont.*

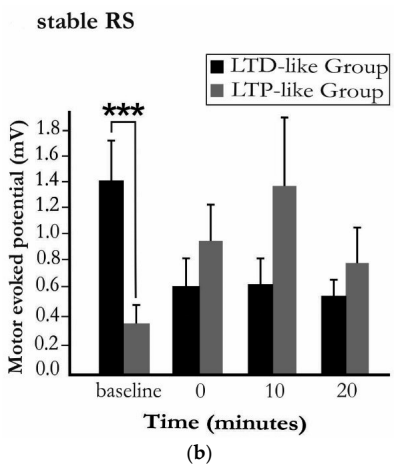


Figure A1. (**a**) Normalized MEP amplitude (mean ± standard error) at dynamic level of RS and (**b**) MEP amplitude (mean ± standard error) at stable level of RS, across the subjects from the LTP/LTD-like groups within the trials before (baseline) and at 0, 10, and 20 min after the induction of short-term plasticity with PAS. Subjects in the LTD-like group exhibited significantly higher pre-intervention MEP amplitudes (baseline) in dynamic RS ($p = 0.005$), and stable RS ($p = 0.007$). No significant difference was observed between the two clusters following PAS in either component. *** indicates significant differences for pairwise comparisons ($p < 0.01$).

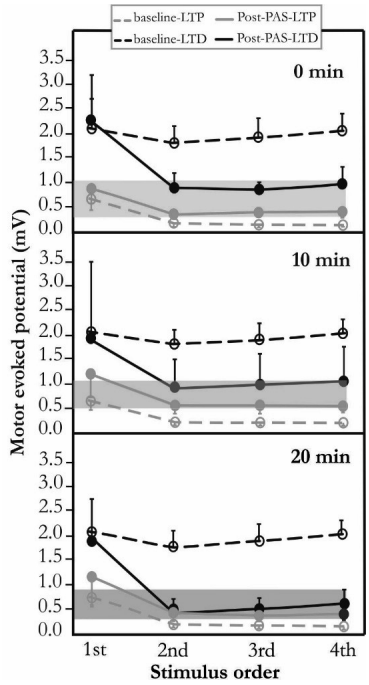


Figure A2. RS at 0, 10, and 20 min after the induction of short-term plasticity with PAS (post-PAS). Effects of LTP/LTD-like plasticity were measured with respect to "baseline" pre-intervention neural activity. Irrespective of the dynamic state of RS, the brain shows a tendency towards a suppressed amplitude level of the second to the fourth MEPs within trials upon repetition of the stimulus. This static status was maintained within a narrow range in two groups after PAS.

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III

Repetition Suppression of the Motor Cortex May Predict the Responsiveness to High-frequency rTMS in Chronic Pain

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Manuscript

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Repetition suppression (RS) refers to the diminished neural responses to repeated exposure of sensory stimuli. In this thesis, RS was studied in the motor system with transcranial magnetic stimulation as a potential biomarker of neuroplasticity, i.e. the ability of the brain to adapt to our changing environment. The studies of this thesis suggest that there is potential for RS to be used as a biomarker of induced neuroplasticity.

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