STUDIES IN SPORT, PHYSICAL EDUCATION AND HEALTH
229

Susanne Kumpulainen

Modulation of Plasticity of the Soleus Area of the Motor Cortex Using Paired Associative Stimulation

JYVÄSKYLÄN YLIOPISTO

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Esitetään Jyväskylän yliopiston liikuntatieteellisen tiedekunnan suostumuksella
julkisesti tarkastettavaksi yliopiston Ruusupuisto-rakennuksen Helena-salissa (RUU D 104)
joulukuun 11. päivänä 2015 kello 12.

Academic dissertation to be publicly discussed, by permission of
the Faculty of Sport and Health Sciences of the University of Jyväskylä,
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UNIVERSITY OF JYVÄSKYLÄ

JYVÄSKYLÄ 2015

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Editors
Taija Juutinen
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Pekka Olsbo
Publishing Unit, University Library of Jyväskylä

Cover Photo by Jussi Peltonen

URN:ISBN:978-951-39-6406-1
ISBN 978-951-39-6406-1 (PDF)

ISBN 978-951-39-6405-4 (nid.)
ISSN 0356-1070

Jyväskylä University Printing House, Jyväskylä 2015

ABSTRACT

Kumpulainen, Susanne
Modulation of plasticity of the soleus area of the motor cortex using paired associative stimulation
Jyväskylä: University of Jyväskylä, 2015, 80 p.
(Studies in Sport, Physical Education and Health
ISSN 0356-1070; 229)
ISBN 978-951-39-6405-4 (nid.)
ISBN 978-951-39-6406-1 (PDF)

Paired associative stimulation (PAS) repeatedly combines single somatosensory nerve stimuli with single transcranial magnetic stimuli to induce bidirectional changes in the excitability of the cortical projections to the target muscle. PAS and motor training have been shown to share common neural mechanisms, suggesting that PAS tests functionally relevant neuronal circuits. While PAS has been used extensively to target the hand area of the motor cortex, few studies have targeted the leg area of the motor cortex. The optimal interstimulus interval (ISI) to induce plasticity in the cortical projections to lower limbs is still not well established. Thus, the first purpose of this thesis was to define an optimal ISI to induce long-term potentiation-like plasticity in the cortical projections to the soleus muscle. Next, this PAS protocol was used as a tool to study the effect of training background on motor cortex plasticity. The functionality of the effects following PAS was evaluated in the third experiment, by quantifying fatigue resistance during a 15 s sustained maximal isometric contraction prior to and after the PAS intervention. The fourth purpose of the thesis was to place the principle of PAS in a more natural context by replacing electrical stimulation with a natural stretch reflex volley (PAS_{reflex}). The optimal ISI for the PAS intervention when targeting soleus muscle was the latency of somatosensory evoked potential plus 18 ms (P32 plus 18 ms), which resulted in an 88 ± 105% increase in amplitude of the soleus motor-evoked potential. With the optimal PAS protocol, skill trained athletes exhibited significantly greater motor cortex plasticity compared to endurance trained athletes. The reason for differential motor cortex plasticity is likely related to the different training-induced adaptations. On average, fatigue resistance did not change following PAS and consequently, the functionality of PAS was not evident. However, PAS-induced excitability changes correlated significantly with changes in fatigue resistance. The effect of PAS_{reflex} was different immediately after and 30 min following the cessation of the intervention, and thus there were most likely several different phenomena taking place in the motor cortex due to the nature of the stretch reflex. In conclusion, the findings of this thesis will help to understand the behavioral and neural signals that drive function and learning in the motor cortex.

Keywords: motor cortex, brain plasticity, paired associative stimulation, training adaptation

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ACKNOWLEDGEMENTS

This thesis was carried out in the Department of Biology of Physical Activity at the University of Jyväskylä, in collaboration with the Department of Health Science and Engineering at Aalborg University during the years 2010-2015. Both universities offered excellent facilities and I am grateful to everyone who has contributed to this thesis.

I express my deepest gratitude to my head supervisor, Professor Janne Avela, for giving me the opportunity to perform doctoral studies and for his encouraging guidance through the good and bad times. I greatly appreciate the time you have always found for me, as well as our open and relaxed discussions about science and every possible thing related to it. This has been vitally important in helping me to grow as a researcher, to find the best possible solutions and to finish this challenging journey. I also wish to express huge gratitude to my second supervisor, Associate Professor Natalie Mrachacz-Kersting, for warmly welcoming me to Aalborg University in 2011, giving me new insights into brain research and saying the right words at the right time.

I wish to address my sincere thanks to the official reviewers of this thesis, Professor Christian Leukel, PhD and Professor Michael Grey, PhD. Professor John Rothwell, PhD, is warmly acknowledged for agreeing to be the opponent in the public defense of this dissertation.

I would like to thank all my coauthors for their valuable contributions during the thesis project. Special thanks go to Professor Markus Gruber, PhD, who has had a great impact on my studies since he introduced the TMS method to us in 2007, when I was beginning my bachelor thesis. In the same project I started working with another special researcher, Jussi Peltonen, PhD, who I want to thank for sharing his scientific excellence and offering his help with all kinds of matters regardless of how long it took. I kindly thank Neil Cronin, PhD, for language revision of the original manuscripts and this thesis. In addition, I owe my sincere thanks to all the subjects who volunteered to participate in the studies, and the students who helped perform the measurements.

Also, I would like to thank the head of Department Professor Keijo Häkkinen and all of the Professors for providing an environment of great expertise, and the whole staff for fruitful collaboration and a friendly atmosphere. My amazing colleagues constantly impress me with their intelligence, kindness, craziness and willingness to help. You deserve my sincerest thanks for making research enjoyable and a lot easier. Without the laboratory and technical staff and secretaries, this work could not have been done. I kindly thank Markku Ruuskanen, Sirpa Roivas and Pirkko Puttonen for making the lab functional and Katja Pylkkänen and Minna Herpola for keeping the administrative things rolling. I lovingly thank my dear friends and relatives for listening, supporting and making my life enjoyable, also beyond science and work.

I wish to acknowledge the following institutions, which have supported our research project and my PhD work financially: Department of Biology of Physical Activity, University of Jyväskylä, Aalborg University, Finnish Cultural

foundation, Jyväskylän yliopiston tieteentekijät ry and Ellen and Artturi Nyyssönen foundation.

Finally, I dedicate my dearest thanks to my parents Olavi and Leena, my brothers Kimmo and Onni, my nephew Tino and my grandparents for their love and support throughout my life, which I will always remember.

This book is dedicated to my parents.

Jyväskylä, November 2015
Susanne Kumpulainen

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original manuscripts, which are referred to in the text by their Roman numerals.

I. Kumpulainen S, Mrachacz-Kersting N, Peltonen J, Voigt M, Avela J. 2012. The optimal interstimulus interval and repeatability of paired associative stimulation when the soleus muscle is targeted. Experimental Brain Research. Sep;221(3):241-9.

II. Kumpulainen S, Avela J, Gruber M, Bergmann J, Voigt M, Linnamo V, Mrachacz-Kersting N. 2015. Differential modulation of motor cortex plasticity in skill- and endurance-trained athletes. European Journal of Applied Physiology. May;115(5):1107-15.

III. Kumpulainen S, Peltonen J, Gruber M, Cresswell A, Peurala S, Linnamo V, Avela J. 2015. The effect of paired associative stimulation on fatigue resistance. Neuroscience Research. Jun;95:59-65.

IV. Kumpulainen S, Mrachacz-Kersting N, Peltonen J, Karczewska M, Fatela P, Mil-Homens P, Avela J. 2015 Repeated Pairing of Stretch Reflex and Transcranial Magnetic Stimulation to Induce Motor Cortex Plasticity Changes. *Submitted for publication*.

ABBREVIATIONS

AMPA	α-amino-5-hydroxy-3-methyl-4-isoxazole propionic
BDNF	Brain-derived neurotrophic factor
CONTR	Control group
EEG	Electroencephalography
EMG	Electromyography
EPSP	Post-synaptic potential
GABA	Gamma-aminobutyric acid
H_{max}	Maximal Hoffmann-reflex
H-reflex	Hoffmann-reflex
ICF	Intracortical facilitation
IO-curve	Input-output curve
ISI	Interstimulus interval
ITT	Interpolation twitch technique
LICI	long interval intracortical inhibition
LTD	Long-term depression
LTP	Long-term potentiation
MEP	Motor evoked potential
M_{max}	Maximal M-wave
MRCP	Movement-related cortical potential
MT	Motor threshold
MVC	Maximal voluntary contraction
NMDA	N-methyl-D-aspartic
PAS	Paired associative stimulation
RMS	Root mean square
RMT	Resting motor threshold
SD	Standard deviation
SEP	Somatosensory-evoked potential
SICI	Short interval intracortical inhibition
SLSR	Short latency stretch reflex
SP	Silent period
STDP	Spike-timing dependent plasticity
TMS	Transcranial magnetic stimulation

CONTENTS

1 INTRODUCTION

The ability of the brain to dynamically reorganize throughout life is called plasticity. Brain plasticity is essential for learning, memory, motor adaptation and recovery from brain injury. Several substrates have been suggested to be involved such as unmasking of latent but existing synapses, forming of new synapses and alterations in the efficacy of synapses. In particular, the latter has received increasing attention in an effort to develop non-invasive techniques to induce plasticity in the human motor cortex. The theory of activity-dependent synaptic plasticity was made famous by Donald Hebb (1904-1985). The theory is known as the Hebbian learning rule and states that "Neurons that fire together wire together." Hebb's rule still underlies the current thinking about the dynamics of synaptic plasticity and is the most probable mechanism mediating motor learning (Cooke & Bliss 2006).

Several non-invasive human brain stimulation techniques have been developed based on Hebb's postulate, to induce plasticity that lasts for minutes to hours after the intervention. The intervention protocols may involve peripheral nerve stimulation, cortical stimulation or a combination of peripheral and cortical stimulation. The latter protocol is termed paired associative stimulation (PAS) and it was first introduced by Stefan et al. (2000). PAS combines peripheral electrical nerve stimulation with cortical transcranial magnetic stimulation (TMS). Since PAS is based on Hebb's rule, the paired peripheral and cortical stimuli synchronously activate common neurons within the motor cortex and can produce long lasting changes in cortical excitability. PAS has been used as a tool to investigate motor adaptations, as well as for rehabilitation in some brain disorders (Carson & Kennedy 2013).

While PAS has been quite extensively used to investigate the plasticity of the hand area of the motor cortex, very few studies have examined the leg area. In addition, there are discrepancies between the existing PAS studies that targeted the leg area of the motor cortex. Thus, the purpose of this thesis was to examine the modulation plasticity of the soleus area of the motor cortex via a Hebbian-like mechanism. The PAS intervention was used to target soleus, as this is an important antigravity muscle during standing and a major contributor

to force production during the impact phase of walking and running (Ishikawa et al. 2005). Furthermore, the tibialis anterior muscle, where PAS effects have been demonstrated, has stronger cortical connections than soleus, suggesting that the tibialis anterior might be more susceptible to changes following PAS than the other leg muscles (Petersen, Pyndt & Nielsen 2003).

2 REVIEW OF THE LITERATURE

2.1 Brain Plasticity

The term plasticity refers to the ability of neural connections to continuously reorganize as a result of alterations in peripheral and central inputs. There are several mechanisms in the brain to induce plasticity. Rapid plastic changes can be achieved by activity-dependent synaptic plasticity; uncovering of latent or existing connections (Jacobs & Donoghue 1991), activation of existing but silent synapses (Liao et al. 1999), generalized excitability changes in postsynaptic neurons (Brons & Woody 1980), long-term potentiation (LTP) (Bliss & Lømo 1973) and long-term depression (LTD) (Lynch, Dunwiddie & Gribkoff 1977). Morphological changes like synaptogenesis, synaptic remodelling and neurogenesis are mechanisms which take more time to develop (Kleim et al. 1996). Several mechanisms can operate simultaneously, or in serial order. It has also been suggested that there is a causal relationship between the rapid and slower mechanisms (Rosenkranz, Kacar & Rothwell 2007) so that the reinforcement of pre-established pathways is a prerequisite for the formation of new pathways (Pascual-Leone et al. 2005).

2.1.1 Long-term potentiation and depression

Terje Lømo provided the first experimental evidence of LTP in the hippocampus of anesthetized rabbit's after high-frequency electrical stimulation in 1966 (Lomo 2003). Later, Bliss and Lømo together continued the experiments and published the first characterization of LTP in 1973 (Bliss & Lømo 1973). LTP refers to long lasting enhancement of synaptic transmission (Bliss & Lømo 1973) whereas LTD refers to weakening of synaptic transmission (Lynch, Dunwiddie & Gribkoff 1977), both of which persist from hours to days. Animal preparations at the cellular level suggest that LTP and LTD are the most probable mechanisms mediating memory and learning (Cooke & Bliss 2006). This is direct confirmation of the theory of synaptic plasticity, which was introduced al-

ready in the late 19th century (James 1890, p.566). In 1949, Canadian neuropsychologist Donald Hebb made the theory famous in his book "The Organization of Behaviour". The Hebbian learning rule states that when cell A reliably contributes to spiking of postsynaptic cell B, the functional strength of the synapse from A to B is increased (Hebb 1949, p.50). Thus, Hebb's theory implied many aspects of LTP such as associativity, specificity and longevity (Cooke & Bliss 2006).

LTP and LTD can be spike-time dependent. Spike-timing dependent plasticity (STDP) has been studied in a variety of animal models for twenty years (Feldman 2012). In Hebbian STDP, the timing of pre- and post-synaptic events is crucial; LTP occurs when presynaptic input precedes postsynaptic spikes by up to 20 ms and LTD occurs when postsynaptic spikes precede presynaptic input by 20-100 ms. There is a narrow transition period of 1-5 ms between LTP and LTD. Multiple pairs of pre-post spikes are needed to induce STDP. (Bi & Poo 1998; Feldman 2012.)

Many forms of LTP and LTD have been established and several factors affect their induction. The typical and most understood model of LTP and LTD is N-methyl-D-aspartic (NMDA)–dependent and occurs at excitatory glutamergic synapses (figure 1). With this model glutamate is released pre-synaptically and binds to α-amino-5-hydroxy-3- methyl-4-isoxazole propionic (AMPA) receptors. AMPA receptors become permeable to cations such as natrium and kalium and this influx generates an excitatory post-synaptic potential (EPSP). Glutamate also binds to and opens NMDA receptor channels but there is still a voltage-gated magnesium block which prevents the cation influx. Sufficient post-synaptic depolarization caused by EPSPs can release the magnesium block and allow calcium influx. Since NMDA activation depends on both pre- and post-synaptic activation, this model is consistent with Hebbian principle associativity. (Malenka & Bear 2004; Thickbroom 2007.)

Calcium is the trigger for synaptic plasticity and regulates whether LTP or LTD is induced. Calmodulin in the cell has two calcium-binding lobes. If there is a rapid increase in calcium concentration, carboxyl-lobe binding occurs and leads to LTP. Carboxyl-lobe binding triggers a kinase pathway that results in exocytosis of AMPA receptors and auto-phosphorylation of surface AMPA receptors, which increases their permeability. In this case the same amount of glutamate can produce a stronger EPSP. A slower increase in calcium concentrations leads to amino-lobe binding and LTD. Amino-lobe binding triggers phosphatase pathways that endocytose surface AMPA receptors and decrease the permeability of the surface expressed receptors. Correspondingly, more glutamate is needed to produce an EPSP. (Malenka & Bear 2004; Thickbroom 2007.)

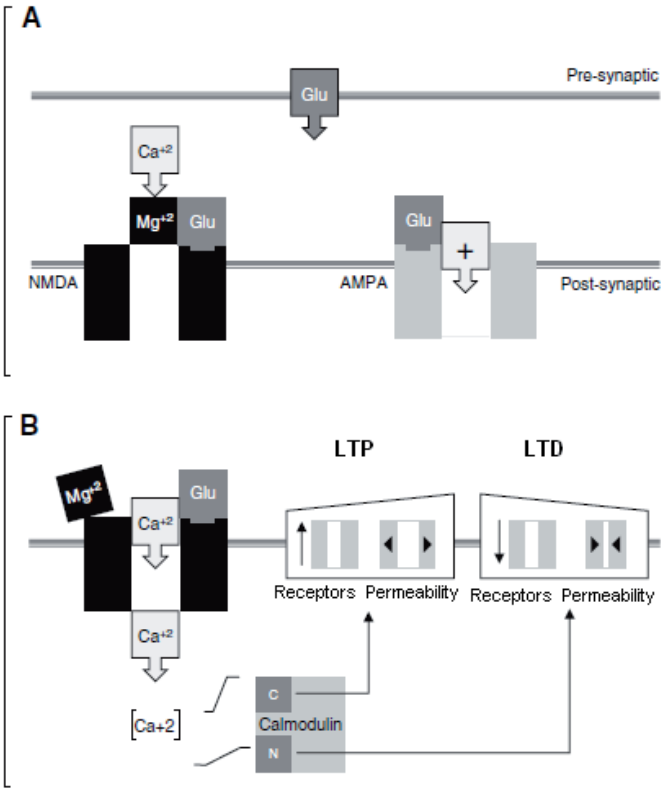

FIGURE 1 A model of activity dependent LTP and LTD mechanisms. A: Pre-synaptic glutamate release which then binds to post-synaptic NMDA and AMPA receptors. B: Sufficient EPSP releases the voltage-gated magnesium block and allows calcium influx. Calcium regulates whether LTP or LTD is induced (Modified from Thickbroom 2007).

2.1.2 Plasticity of the primary motor cortex

The main role of the primary motor cortex is to control voluntary movements but it is also fundamentally important in motor learning; voluntary movements are produced by distributed neuronal networks, which are capable of reorganizing during acquisition or training of motor skills (Sanes & Donoghue 2000). There are six distinct layers of neurons in the motor cortex. The main output cells are large pyramidal cells in layer V. The corticospinal track originates from these pyramidal cells and is the only descending motor pathway that makes monosynaptic connections with spinal motor neurons. (Weber & Eisen 2002.)

The association between motor skill learning and LTP in the rat motor cortex was first suggested by Rioult-Pedotti et al. (1998), who demonstrated that motor cortex LTP/LTD occurs in horizontal pathways spanning layers II-III. These connections have glutamergic synapses and are regulated by feed-forward GABA inhibition (Keller 1993; Hess, Aizenman & Donoghue 1996).

Motor cortex LTP has been shown to be NMDA-dependent (Hess, Aizenman & Donoghue 1996). Adult motor cortex LTP seems to be more restricted than LTP induction in the hippocampus because it fails if transient inhibition is not reduced or vertical pathways are not activated (Hess, Aizenman & Donoghue 1996). This vertical activation probably includes thalamocortical neurons and a mixture of other input and output neurons. Unlike LTP, LTD does not need a specific context to appear in the motor cortex (Hess & Donoghue 1996). Through LTP and LTD the motor cortex connections are continuously modified as a result of appropriate activity patterns and this can lead to functional reorganization of motor representations of muscles (Sanes & Donoghue 2000).

2.2 Training-induced plasticity of the motor cortex

There is now extensive evidence that different types of exercise produce experience-specific alterations in the corticomotoneuronal system (Adkins et al. 2006). For example, following skill training, synaptogenesis and reorganization of movement representations within the motor cortex have been reported (Kleim et al. 1996; Nudo et al. 1996), whereas after endurance and strength training the synapse number in the motor cortex is not altered (Remple et al. 2001; Kleim, Cooper & VandenBerg 2002). Skill and endurance training have been shown to increase the quantity of neurotrophic factors such as brain-derived neurotrophic factor (BDNF) in the motor cortex (Klintsova et al. 2004). The main difference between these training types is the way limb muscles are used; skill training requires learning of new coordinated patterns continuously and progressively while the coordination pattern of endurance and strength training does not change considerably throughout the training period. The number of synapses within the motor cortex is important since expansion of movement representations has been shown to parallel motor learning (Monfils, Plautz & Kleim 2005).

2.2.1 Adaptations to skill training

Skill training is defined here as the acquisition and subsequent refinement of novel movement sequences such as those in dance, gymnastics, and figure skating (Adkins et al. 2006). There is convincing evidence from animal in vivo/in vitro and human studies that skill training increases synaptic strength, synaptogenesis and cortical map reorganization within the motor cortex (Monfils, Plautz & Kleim 2005). These adaptations drive the acquisition and performance of skilled movements. The time course of plasticity changes within rat motor cortex during motor skill learning is presented in figure 2.

Animal studies have shown that synaptic changes and map reorganization after a skill training intervention occur in the specific motor areas that control the muscle groups used in the task (Nudo et al. 1996; Kleim, Cooper & VandenBerg 2002).

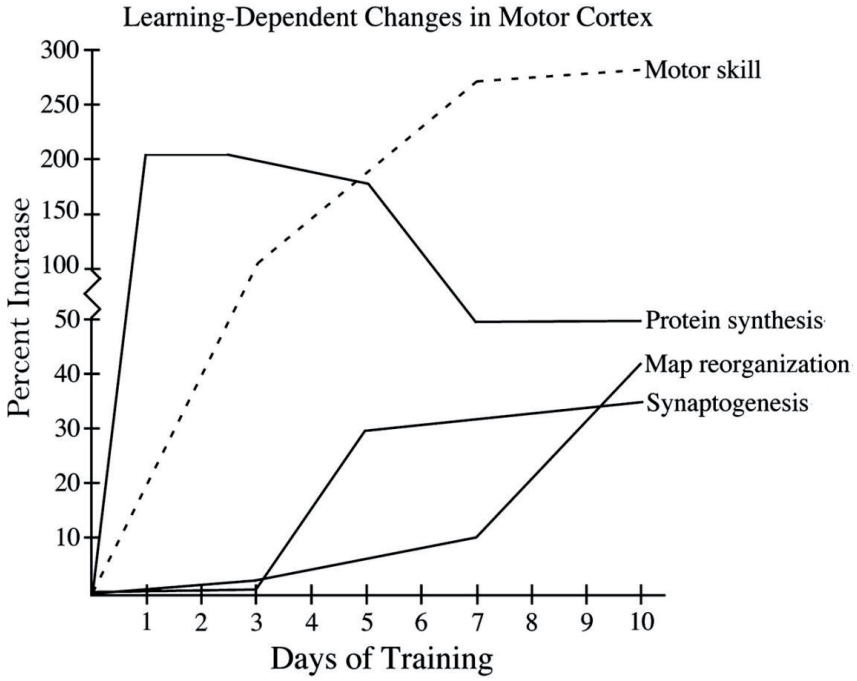

FIGURE 2 Time course of plasticity changes within rat motor cortex during motor skill learning. Measures are expressed as percentage of control or pre-training values. Motor skill level improves rapidly over the first few days of training. This is initially due to an increase in protein synthesis and LTP and later due to an increase in synapse number and motor map reorganization (Modified from Adkins et al. 2006).

The TMS technique has revealed comparable adaptations in the human motor cortex (Adkins et al. 2006). TMS applied over the primary motor cortex elicits descending volleys in the corticospinal track and the resultant efferent response recorded with electromyography (EMG) from the target muscle is called a motor-evoked potential (MEP) (Weber & Eisen 2002). Pascual-Leone et al. (1995) found enlarged finger representation areas using TMS and also improved performance following five days of skilled piano training in human subjects. These adaptations did not occur in the contralateral hand or in the control group, which did not have any piano practice. An additional control group was allowed to play the piano with the same amount of practice time but without specific skill learning instructions. This group showed similar but significantly smaller changes in the cortical representation areas than those which were observed in the skill training group. This suggests that the extent of cortical map changes depends on the demands of the trained task.

Several cross-sectional studies have also demonstrated training-specific adaptations in the areas of the motor cortex that have direct projections to task-related muscles. Highly trained Olympic badminton players exhibit higher MEP amplitudes and shifts in the cortical motor maps in the playing hand

compared to their unskilled hand or to unskilled players (Pearce et al. 2000). Elite volleyball players have significantly larger and more overlapping representations of the medial deltoid and carpi radialis muscles when compared to runners (Tyč, Boyadjian & Devanne 2005). Volleyball players have larger map areas for the dominant muscles while there are no differences in the motor maps for dominant versus non-dominant muscles of runners. Similar results have been observed in the leg area of the motor cortex. Highly skilled figure skaters have a larger representation area for the tibialis anterior muscle when compared to non-trained controls (Vaalto et al. 2013). In addition to cortical mapping studies, Rosenkranz et al. (2007) used PAS as a tool (PAS - introduced in detail in chapter 1.3) to investigate motor cortex plasticity of professional musicians. Musicians showed higher sensitivity towards induction of LTP-like plasticity compared to non-musicians when applying PAS to target task-related hand muscles.

2.2.2 Adaptations to endurance / repetitive training

Endurance training entails the repetition of the same movement sequence aiming to increase the capacity for continued motor output such as in running, cycling and cross-country skiing (Adkins et al. 2006). Endurance training has been shown to enhance cognitive and neural plasticity in several brain regions including the cerebellum, hippocampus and cerebral cortex (Kramer & Erickson 2007; Thomas et al. 2012). However, endurance training does not alter the synapse number in the task-related areas of the motor cortex (Kleim, Cooper & VandenBerg 2002).

Motor map changes have not been observed in rats trained to continuously reach for an unattainable pellet (Kleim et al. 2004), nor in squirrel monkeys trained to retrieve food pellets from a large well despite 13,000 digit flexions during the course of training (Plautz, Milliken & Nudo 2000). In addition, 30 days of running exercise did not alter motor maps in rats, although running increases angiogenesis in the motor cortex (Kleim, Cooper & VandenBerg 2002). Endurance training increases blood flow to the motor cortex, which has been shown to be accompanied by increased quantities of neurotrophic factors that regulate neuronal activity, as well as facilitation of the survival and differentiation of neurons (Vaynman & Gomez-Pinilla 2005; Erickson et al. 2012).

Although endurance training creates this optimal environment for synaptogenesis, there has been no documented synaptogenesis in task-related areas of the motor cortex, which is likely related to a lack of task complexity (Adkins et al. 2006). Improved cognitive learning, but not motor learning, has been shown in endurance-trained rats when the motor learning task was task-specific to running (Wikgren et al. 2012). Cirillo et al. (2009) used PAS to investigate plasticity of task-unrelated areas of the motor cortex in endurance-trained subjects. They found heightened motor cortex plasticity in endurance-trained subjects compared to sedentary controls when using PAS to target abductor pollicis brevis muscle. Contrary to skill training, motor cortex plasticity of task-related areas after long-term endurance training has not been investigated in humans.

2.2.3 Acute effects of skill and endurance exercise

In addition to the long-term effects following skill and endurance training, acute effects have been shown to differ in the motor cortex. Perez et al. (2004) investigated the effect of a 32 min period of skill, repetitive, and passive training involving the ankle muscles on leg motor cortical excitability with TMS. They found an increase in the excitability of the cortical projections to the tibialis anterior muscle only after skill training. Similarly, Ziemann et al. (2004) found increases in MEP amplitude in the abductor pollicis brevis muscle after 30 min of skilled but not unskilled thumb exercise. These learning-induced increases in MEP amplitude were attributed to LTP-like plasticity in the motor cortex.

In an effort to understand how learning affects synaptic plasticity, Ziemann et al. (2004) investigated the acute effect of these thumb exercises on motor cortex plasticity using PAS interventions. Skilled thumb exercise prevented subsequent PAS-induced LTP-like plasticity while it enhanced LTD-like plasticity. Unskilled thumb exercise did not affect subsequent PAS-induced plasticity. Stefan et al. (2006) found similar results, showing that PAS-induced LTP-like plasticity was abolished after skilled thumb exercise and LTD-like plasticity remained unchanged. These results are in line with animal studies and the theory of homeostatic metaplasticity, which proposes that the threshold for LTP/LTD induction is flexible and depends on the recent history of synaptic plasticity (Abraham 2008). Rioult-Pedotti et al. (2000) showed that motor skill training occluded subsequent experimentally-induced LTP plasticity and enhanced LTD plasticity in rat motor cortex. The authors proposed that motor skill learning strengthened synaptic connections up to the point of saturation and therefore additional LTP was occluded. On the contrary, motor skill learning increased the range for synaptic weakening, LTD plasticity. Thus, homeostatic metaplasticity is an important concept for maintaining overall synaptic weight within the physiological range (Abraham 2008).

Rosenkranz et al. (2007) measured the effect of the same skilled thumb exercise on human motor cortex plasticity over five consecutive days. On day one motor cortex excitability was increased after the exercise and PAS-induced LTP-like plasticity reversed to LTD-like plasticity. On day five, neither motor cortex excitability nor PAS-induced LTP-like plasticity were affected by the skilled thumb exercise. The authors suggested that new synaptic connections might have formed on day five that allow LTP susceptibility to be restored. Contrary to skill training, PAS-induced LTP plasticity was enhanced after 20 min moderate-intensity endurance exercise (Singh, Neva & Staines 2014). Recently Mang et al. (2014) showed that a single bout of high-intensity endurance exercise enhanced PAS-induced LTP plasticity and motor learning in task-unrelated upper limb muscles. It can be concluded that the effect of short-term history of synaptic activity on motor cortex plasticity depends on the type of activity.

2.3 Paired associative stimulation to induce Hebbian-like plasticity

The PAS intervention was first introduced by Stefan et al. (2000), who targeted the hand area of the motor cortex. PAS is based on the Hebbian learning rule and it combines electrical stimulation of a peripheral nerve innervating the target muscle followed by TMS over the contralateral motor cortex of the representative area of the same muscle (Stefan et al. 2000). PAS-induced excitability changes can be quantified by MEPs, which are recorded with electromyography (EMG) from the target muscle and elicited with TMS. Mrachacz-Kersting et al. (2007) were the first to introduce a comparable PAS intervention for the lower limb muscles (figure 3). PAS interventions consist of 90-360 pairs of stimuli delivered at a rate of 0.05-0.50 Hz (Carson & Kennedy 2013).

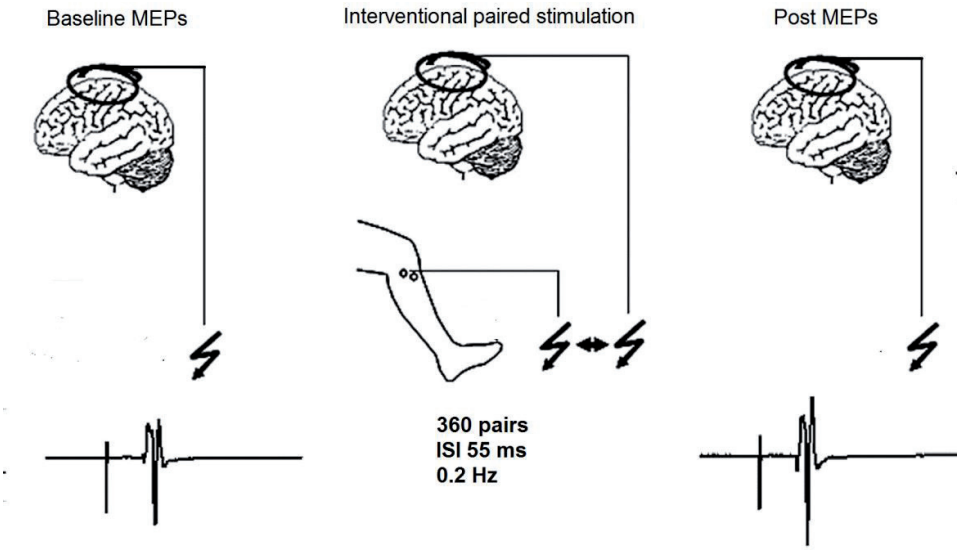

FIGURE 3 The experimental design by Mrachacz-Kersting et al. (2007). The test MEP amplitudes were elicited by single-pulse TMS before and after the intervention in tibialis anterior muscle. The greatest increase in MEP amplitude was observed with an interstimulus interval (ISI) of 55 ms (Modified from Mrachacz-Kersting et al. 2007).

2.3.1 PAS-induced plasticity

Depending on the order of the electrical stimulation and TMS, PAS can produce either LTP-like plasticity (PAS_{LTP}) or LTD-like plasticity (PAS_{LTD}) at the target synapse. When a weaker electrical stimulus eliciting an excitatory post synaptic potential precedes a stronger transcranial magnetic stimulus that elicits an action potential, PAS_{LTP} occurs at the target synapse. If the stimuli arrive in reverse order, PAS_{LTD} occurs (Ziemann et al. 2008). Thus, PAS resembles STDP in

the human cortex (Muller-Dahlhaus, Ziemann & Classen 2010). As in animal preparations at the cellular level, PAS shows LTP plasticity properties such as rapid onset, associativity, duration, specificity, calcium channel and NMDA-receptor dependence (Cooke & Bliss 2006; Ziemann et al. 2008).

2.3.2 Interstimulus interval (ISI)

The direction of PAS-induced effects critically depends on the interstimulus interval (ISI) of the paired stimuli. To induce LTP-like plasticity the electrical stimulus should reach the motor cortex before the application of TMS. The time it takes for the afferent volley generated by the electrical stimulation to arrive at the motor cortex can be estimated from the latency of the somatosensory evoked potential (SEP) and the central processing time.

2.3.2.1 ISI for upper limb muscles

Afferent inputs elicited by median nerve stimulation reach the primary somatosensory cortex at the latency of the N20 component of SEP recorded with electroencephalography (EEG). N20 refers to the negative peak at a latency of approximately 20 ms (Allison et al. 1991). The minimum central processing time is reported to be approximately 2-4 ms for hand muscles (Goldring, Aras & Weber 1970). Based on these observations, the optimal ISIs to induce LTP/LTD-like plasticity in the motor cortex of the hand area has been established to be constant at intervals of 25 ms and 10 ms respectively (Stefan et al. 2000; Wolters et al. 2003). The optimal ISI has also been established based on the individual SEP latency only. Significant PAS_{LTP} is induced when the ISI is longer (2 ms) than the SEP latency and PAS_{LTD} when the ISI is shorter (5 ms) than the SEP latency (Müller-Dahlhaus et al. 2008; Jung & Ziemann 2009; Lu et al. 2009; Heidegger, Krakow & Ziemann 2010; Korchounov & Ziemann 2011; Voytovych, Kriváneková & Ziemann 2012). Hamada et al. (2012) suggested that the somatosensory stimulus arrives via the rapid dorsal column-medial leminiscal route with an ISI of 21.5 ms and via a longer pathway involving the cerebellum with an ISI of 25 ms when the median nerve is stimulated.

2.3.2.2 ISI for lower limb muscles

The optimal ISI to induce LTP/LTD-like plasticity in the leg area of the motor cortex is not well established since the available data are heterogeneous. The same ISIs have been found to induce significant PAS_{LTP} (Prior & Stinear 2006; Jayaram, Santos & Stinear 2007; Roy, Norton & Gorassini 2007) and significant PAS_{LTD} (Stinear & Hornby 2005; Mrachacz-Kersting et al. 2007; Jayaram & Stinear 2008) in different studies targeting the tibialis anterior muscle (table 1).

The reasons for these discrepancies and the differences compared to PAS when targeting hand muscles are still unclear (Ziemann et al. 2008). Studies where PAS is applied during walking (Stinear & Hornby 2005; Prior & Stinear 2006; Jayaram, Santos & Stinear 2007; Jayaram & Stinear 2008) might not be comparable with the studies where PAS is applied during sitting because different conditions in the muscle can affect the length of the SEP latency as well

as the central processing time (Duysens et al. 1990; Brooke et al. 1997). However, one deficiency in previous studies of leg muscles is the definition of ISIs, which varies widely. For example, the ISI has been determined based on the MEP latency (29-33 ms) in the tibialis anterior muscle (Stinear & Hornby 2005; Prior & Stinear 2006; Jayaram, Santos & Stinear 2007; Jayaram & Stinear 2008), and the common peroneal nerve SEP latency (42 - 49 ms, peak N34) (Mrachacz-Kersting et al. 2007). All of these studies suggest that adding 5-6 ms to account for a central processing delay results in the optimal ISI to induce PAS_{LTP}. The optimal ISI to induce PAS_{LTD} in the leg area has not been studied but an ISI of 20 ms is mostly used.

TABLE 1 Summary of PAS studies done on the leg area of the motor cortex. Positive interstimulus interval (ISI) values mean that the peripheral stimulus was delivered first. The letter P in the table refers to LTP-like plasticity changes and D refers to LTD-like plasticity changes obtained in the studies with the particular ISI. TA refers to tibialis anterior and SOL to soleus muscle.

PAS studies			**ISI (ms)**												
Authors	Condition	M	-40	-25	0	5	10	20	35	40	45	50	55	60	Based
Mrachacz-Kersting et al.(2007)	PAS	TA								D	P	P	P		N34
Roy et al. (2007)	PAS	TA	P	P	P			P	P						MEP
Stinear and Hornby (2005)	PAS+walk	TA						D	P						MEP
Prior and Stinear (2006)	PAS+walk	TA								P					MEP
Jayaram et al. (2007)	PAS+walk	TA							P	P					MEP
Jayaram and Stinear (2008)	PAS+walk	TA						D							MEP
Poon et al. (2008)	One pair	SOL				P	D	D	D	D		P	P	P	-
Roy and Gorassini (2008)	One pair	SOL							D	D	P	P			-

None of the previous studies have defined the ISI based on the occurrence of the first negative peak (P32) of the lower limb SEP, which reflects activation of the primary cortical somatosensory receiving area (Vas, Cracco & Cracco 1981; Desmedt & Bourguet 1985). This corresponds to the N20 component of the median SEP (Vas, Cracco & Cracco 1981; Desmedt & Bourguet 1985; Pelosi et al. 1988), which has been used in PAS studies of the hand area (Müller-Dahlhaus et al. 2008; Jung & Ziemann 2009; Lu et al. 2009; Heidegger, Krakow & Ziemann 2010; Korchounov & Ziemann 2011; Voytovych, Kriváneková & Ziemann 2012). A definition of the ISI based on the latency of P32 would also help in the planning of optimal PAS protocols for the lower limb muscles.

2.3.3 Interaction of cortical and sensory stimulation

In PAS protocols, TMS is normally delivered through a figure-of-eight coil with posterior-to-anterior directed current. The intensity is adjusted to generate MEPs with a peak-to-peak amplitude of 1 mV. Alternatively, a certain percentage e.g. 120% of resting motor threshold (RMT) is used (Carson & Kennedy 2013). With this setup TMS activates cortico-cortical connections of layers II and

III, which project onto pyramidal track neurons of layer V (Di Lazzaro & Ziemann 2013). Implanted electrode recordings from the cervical epidural space have revealed that MEPs consist of a series of descending corticospinal volleys, I1-I4- waves with progressively declining amplitude, at 1.5 ms intervals (Di Lazzaro et al. 1998; Di Lazzaro et al. 2001).

The intensity of electrical stimulation is a certain percentage above motor threshold (MT). It has been shown that cutaneous afferents contribute to the PAS effect (Stefan et al. 2000), while it has also been proposed that muscle afferents could be the principle afferent route mediating PAS-induced plasticity (Carson & Kennedy 2013). The arrival of the electrical stimulation to the somatosensory cortex can be estimated from the latency of SEP as mentioned earlier. However, the exact route is not entirely known but afferent information via median nerve stimulation probably reaches the motor cortex somatotopically via corticocortical connections from the somatosensory cortex (Stefan et al. 2000; Classen et al. 2000).

Wolters et al. (2003) suggested that the excitatory interneuron receiving the afferent input could also be among the interneurons activated by TMS. Thus, following the intervention TMS could probe the PAS-induced modulation of excitability. However, in the absence of invasive neuronal recordings, any hypothesis about the precise nature and location of cellular events resulting in this timing-dependent plasticity remains speculative (Wolters et al. 2003).

2.3.4 Origin of PAS-induced changes

A variety of techniques have been employed to identify the location of PAS-induced changes. A lack of changes at the spinal level has been shown with F-waves, electrical brainstem stimulation (Stefan et al. 2000; Wolters et al. 2003) and Hoffmann-reflexes (H-reflex) (Mrachacz-Kersting et al. 2007; Roy, Norton & Gorassini 2007). However, parallel changes in H-reflex and MEPs following PAS-induced LTP-like plasticity have also been observed (Meunier et al. 2007; Lamy et al. 2010). Lamy et al. (2010) demonstrated that H-reflex facilitation was due to decreased presynaptic Ia inhibition. This could be caused by the alteration of descending inputs to presynaptic terminals or changes in presynaptic networks at the spinal level. Di Lazzaro et al. (2009) investigated the level at which PAS produces its effects more directly by recording corticospinal descending volleys before and after PAS. They showed that PAS increased the size of later I-waves while the earliest I1-wave was unaffected. I-waves are generated by trans-synaptic activation of pyramidal track neurons. Thus, the authors concluded that PAS effects are cortical in origin. Kujirai et al. (2006) used different TMS approaches to examine cortical elements of PAS-induced effects and also reported that the later I-waves were facilitated after PAS.

2.3.5 Neuronal mechanisms of PAS

Studies combining PAS with drugs affecting the central nervous system have revealed that PAS-induced plasticity is influenced by the glutamatergic system,

GABAergic system and voltage-gated ion-channels which are considered as drivers of neuroplastic adaptations. In addition, modulators of plasticity such as the dopaminergic system, cholinergic system and adrenergic system affect PAS. (Nitsche et al. 2012.)

Intracortical neural circuits, which are influenced by PAS, can be investigated with paired pulse TMS. By adjusting the intensity and ISI of these two pulses, it is possible to investigate inhibitory and facilitatory circuits in the motor cortex (Kujirai et al. 1993; Ziemann, Rothwell & Ridding 1996). After PAS, $GABA_A$ receptor-mediated short-interval intracortical inhibition (SICI), $GABA_B$ receptor-mediated long-interval intracortical inhibition (LICI), and glutamergic interneuron-influenced intracortical facilitation (ICF) have been investigated (Carson & Kennedy 2013). After PAS_{LTP} there are no changes in SICI (Stefan et al. 2002; Quartarone et al. 2003; Rosenkranz & Rothwell 2006; Sale, Ridding & Nordstrom 2007; Sale, Ridding & Nordstrom 2008; Cirillo et al. 2009; Russmann et al. 2009; Di Lazzaro et al. 2011; Elahi, Gunraj & Chen 2012; Schabrun et al. 2013) nor ICF (Di Lazzaro et al. 2011; Elahi, Gunraj & Chen 2012; Sale, Ridding & Nordstrom 2007; Roy, Norton & Gorassini 2007) while LICI is decreased only when PAS is applied using moderate intensities (MEP of 0.5 mV) (Russmann et al. 2009; Meunier et al. 2012). After $PAS_{LTD,}$ SICI is reduced (Russmann et al. 2009; Di Lazzaro et al. 2011), ICF is unchanged (Di Lazzarro et al. 2011) and LICI is increased with moderate intensity PAS (Meunier et al. 2012) and decreased with higher intensity (MEP of 1 mV) PAS (De Beaumont et al. 2012).

When TMS is delivered during voluntary contraction, silencing of ongoing EMG- the silent period (SP) can be observed after the MEP. The later part of the SP is cortical in origin and probably mediated by $GABA_B$ receptors (Ziemann 2004). Several studies have reported an elongation of the SP after PAS_{LTP} (Stefan et al. 2000; Stefan, Wycislo & Classen 2004; Sale, Ridding & Nordstrom 2007; Sale, Ridding & Nordstrom 2008; Cirillo et al. 2009; De Beaumont et al. 2012; Elahi, Gunraj & Chen 2012) with only one study reporting no change (Di Lazzaro et al. 2011). While others measured SP during slight muscle contraction levels of 10-20% of the maximal voluntary contraction (MVC), Ziemann et al. (2011) measured SP at a contraction level of 50% of MVC as recommended by Säisänen et al. (2008), which is probably the reason for the different results. PAS_{LTD} has been shown not to influence the SP (Di Lazzaro et al. 2011, De Beaumont et al. 2012).

Short (SAI) and long latency afferent inhibition (LAI) can be investigated when the ISI between the peripheral afferent stimulus and TMS is appropriate. Both are influenced by GABAergic inhibitory interneurons and SAI has been reported to interact with SICI and LAI with LICI (Carson and Kennedy 2013). SAI has been shown to be unaffected by PAS protocols (Stefan et al. 2002; Di Lazzaro et al. 2011; Hamada et al. 2012; Elahi, Gunraj & Chen 2012; Schabrun et al. 2013). LAI (ISI of 150 ms) has been shown to decrease with moderate intensity PAS_{LTP} (Russmann et al. 2009, Meunier et al. 2012). With a longer ISI (240 ms), LAI has been shown to increase after PAS_{LTP} and decrease after PAS_{LTD}. Thus, it seems that $GABA_B$ mediated projections play a role in the development of

PAS_{LTP} but the underlying mechanisms are still not fully understood (Carson & Kennedy 2013).

2.3.6 Factors affecting PAS-induced plasticity and limitations

Several factors have been identified which affect PAS-induced plasticity in healthy subjects and cause variability in the results between studies, between subjects and also between sessions in the same subject. One explanation for variability between studies is related to the differences in the PAS protocols. Intensity, direction of the current, amount of stimuli, rate of stimulation, target muscle and the state of the target muscle vary between these studies. The effects of protocol variation have not been systematically addressed yet (Ziemann 2008, Carson & Kennedy 2013). In addition, considerable intra- and inter-individual variability has been reported in PAS-induced plasticity when the hand areas have been targeted (Fratello et al. 2006; Sale, Ridding & Nordstrom 2007; Ridding & Ziemann 2010; López-Alonso et al. 2014). The repeatability of PAS of the leg area has not been measured previously.

2.3.6.1 Intra-individual variability

The attention level of the subject (Stefan, Wycislo & Classen 2004), recent neuronal activity (Ziemann et al. 2004; Stefan et al. 2006) and time of day (Sale, Ridding & Nordstrom 2007) have been reported to be determinants of intra-individual variability of PAS-induced effects. Stefan et al. (2004) showed that PAS-induced plasticity was maximal when the subject visually and kinesthetically concentrated on the target muscle, and plasticity was occluded when the subject's attention was diverted from the target muscle by a competing cognitive task. PAS-induced LTP-like plasticity is also prevented by prior motor learning (Ziemann et al. 2004, Stefan et al. 2006) but enhanced by prior endurance training (Singh, Neva & Staines 2014; Mang et al. 2014). Sale et al (2007) demonstrated that PAS was more effective and reliable when applied during the afternoon compared to the morning. They suggested that the reason could be the circadian rhythms in hormones and neuromodulators which are known to influence neuroplasticity, and showed later that circulating levels of cortisol were negatively correlated with the effectiveness of PAS (Sale, Ridding & Nordstrom 2008). Intra-individual variability can be reduced by careful planning of the experimental design and thus taking into account these factors.

2.3.6.2 Inter-individual variability

Inter-individual variability cannot be manipulated because it has been associated with differences in brain anatomy (Conde et al. 2012; Huber et al. 2008), genes (Cheeran et al. 2008), age (Müller-Dahlhaus et al. 2008; Fathi et al. 2010) (Muller-Dahlhaus 2008; Fathi 2010) and training background (Cirillo et al. 2009). Due to the dissimilarities in brain anatomy, TMS can preferentially activate different neuronal circuits with variable delays in different subjects (Sakai et al. 1997) causing inconsistency in the PAS-induced effects (Huber et al. 2008). In addition, a significant positive correlation has been found between PAS-

induced effects and the thickness of the sensorimotor cortex (Conde et al. 2012). It was suggested that a thicker sensorimotor cortex likely provides better processing resources and thereby enhanced synaptic efficacy. Genetic polymorphisms of neurotrophins can influence the induction of plasticity (Ridding & Ziemann 2008). However, there is only one BDNF polymorphism, which has been shown to limit PAS-induced motor cortex plasticity (Cheeran et al. 2008). Younger subjects exhibit larger plasticity changes compared to elderly subjects (Müller-Dahlhaus et al. 2008; Fathi et al. 2010). Cirillo et al. (2009) showed that endurance trained subjects exhibited higher motor cortex plasticity in a task-unrelated abductor pollicis brevis muscle when compared to sedentary controls. The wide variability of PAS-induced effects limits its use in research and rehabilitation settings (López-Alonso et al. 2014).

2.3.7 Functionality of PAS

PAS and motor training have been shown to share common neural mechanisms, suggesting that PAS tests functionally relevant neuronal circuits (Ziemann et al. 2004; Stefan et al. 2006; Rosenkranz, Kacar & Rothwell 2007; Jung & Ziemann 2009). In addition, the association between motor skill learning and PAS_{LTP} has been demonstrated by Frantseva et al. (2008). However, very few studies have shown the functionality of PAS-induced excitability changes in healthy subjects (Jung & Ziemann 2009; Rajji et al. 2011). Ziemann et al. (2009) investigated learning of rapid thumb flexion movements following PAS. Right after PAS, learning was enhanced in the PAS_{LTD} group and less enhanced in the PAS_{LTP} group. At a 90 min delay, learning was enhanced only after PAS_{LTD} whereas PAS_{LTP} depressed learning. Raiji et al. (2011) found that learning of a rotatory pursuit task was enhanced 1 week after PAS_{LTP} but not 45 min after. The authors suggested that this was due to the complexity of the learning task.

The functional relevance of PAS could probably be seen during fatiguing exercises since it is well known that both central and peripheral factors contribute to the development of fatigue (Gandevia 2001). Fatigue can be defined as any exercise-induced reduction in the ability of a muscle to generate maximal force or power (Gandevia 2001). Central fatigue refers to processes proximal to the neuromuscular junction and peripheral fatigue to processes at or distal to it (Gandevia 2001). The relative contribution of the central and peripheral components depends on the intensity and duration of the fatiguing exercise. Short maximal sustained contractions have been shown to have a substantial central contribution to the development of fatigue (Gandevia et al. 1996; Hunter et al. 2006; Hunter et al. 2008; Lentz & Nielsen 2002; Szubski, Burtscher & Loscher 2007; Taylor et al. 1996; Taylor, Butler & Gandevia 1999). Central fatigue has been defined as a progressive reduction in the voluntary activation of a muscle during exercise and it can originate at both spinal and supraspinal levels (Gandevia 2001). Previous studies suggest that central fatigue at least partially originates from inadequate cortical drive to the motorneurons (Gandevia 2001; Hunter et al. 2006; Hunter et al. 2008).

Milanovic et al. (2011) examined the effect of PAS_{LTP} on a fatigue test involving sustained isometric contractions at 50% of MVC and they did not find any effect. However, they used the duration of submaximal contraction as an indication of fatigue, which may involve a greater peripheral component of fatigue compared to short maximal contractions (Lentz & Nielsen 2002). Endurance time and changes in maximal capacity to generate force provide information about different processes induced by the exercise (Vøllestad 1997). Thus, endurance time may not be affected by motor cortex excitability changes. So far, the effect of PAS on shorter maximal sustained contractions has not been investigated.

3 AIMS AND HYPOTHESIS

While plasticity of the upper limb area of the motor cortex has been extensively investigated, studies concerning plasticity of the lower limb area of the motor cortex are limited. In addition, there are discrepancies in the results between the previous PAS studies targeting the lower limb area. Therefore, the purpose of this study was to investigate the modulation of plasticity of the lower limb area of the motor cortex via Hebbian-like mechanism. To achieve this task, the thesis had four specific aims:

1. To define the optimal PAS protocol to induce LTP-like plasticity in lower limb muscles based on individual SEP latencies. The PAS intervention was used to target soleus muscle as this is an important antigravity muscle during standing and a major contributor to force production during the impact phase of walking and running (Ishikawa et al. 2005). Due to the diverse results of previous PAS studies targeting the leg representation in the motor cortex, repeatability of the optimal PAS protocol was also evaluated. It was hypothesized that intra-individual variability could be reduced by careful planning of the experimental design and thus taking into account factors such as attention level of the subject, recent neuronal activity and time of day (I).

2. To use the optimal PAS protocol as a tool to study the effect of training background on plasticity of the lower limb area of the motor cortex. Motor cortex plasticity was compared between skill- and endurance-trained athletes. PAS intervention was used to induce LTP-like plasticity in the cortical projections to the soleus muscle as this is a training-relevant muscle for both athlete groups. It was hypothesized that due to the increased number of synapses induced by skill training, skill athletes would show a higher degree of plastic changes in the motor cortex when compared to endurance athletes (II).

3. To further understand the effects of PAS, the functionality of PAS-induced effects was examined. Functionality was measured with fatiguing exercise of the plantar flexor muscles. Since PAS can be used to systematically alter the responsiveness of neurons in the primary motor cortex, the effect of two different PAS interventions on fatigue resistance during 15 s sustained maximal isometric contractions was investigated. It was hypothesized that after $PAS_{LTP,}$ fatigue resistance would increase whereas after PAS_{LTD} fatigue resistance would decrease (III).

4. To modify the traditional PAS protocol to allow a more natural afferent volley to be combined with TMS (PAS_{reflex}), and consequently to reduce the variability in plasticity. In the PAS_{reflex}, the synchronous electrical stimulation of afferents was replaced by a more asynchronous burst-like activity induced by natural fast muscle stretch. It has been shown that the different types of afferent stimulation affect corticomotor organization in specific ways (Rosenkranz & Rothwell 2003; Rosenkranz & Rothwell 2006; Rosenkranz & Rothwell 2012). Stretch reflex was used because natural proprioceptive input might optimize LTP-like plasticity in the motor cortex (Pavlides, Miyashita & Asanuma 1993). Moreover, the asynchronous reflex volley likely recruits the cortical structures at slightly different latencies, allowing more flexibility for the optimal ISI to induce LTP-like plasticity. Thus, it was hypothesized that the stretch reflex combined with TMS would reduce the variability of the PAS-induced effects (IV).

4 METHODS

4.1 Subjects

The thesis consists of four original manuscripts, each of which is based on a separate experiment (I-IV). A total of 100 healthy subjects volunteered for the experiments. Descriptive characteristics of the subjects of the four experiments are presented in table 2.

TABLE 2 Descriptive characteristics of the subjects (mean ± standard deviation).

Experiment	Group	N	Gender (m / f)	Age (years)	Height (cm)	Weight (kg)
I	Optimal ISI	8	2 / 6	25 ± 3	170 ± 5	64 ± 9
	Duration	8	1 / 7	22 ± 2	173 ± 7	76 ± 10
	Repeatability	8	5 / 3	25 ±2	173 ± 8	70 ± 12
II	Skill	15	3 / 12	23 ± 4	166 ± 7	60 ± 10
	Endurance	15	5 / 10	26 ± 4	172 ± 8	62 ± 8
	Control	8	0 / 8	21 ± 1	171 ±8	73 ± 13
III	PAS_{LTP}	15	6 / 9	25 ± 4	168 ± 11	62 ± 10
	PAS_{LTD}	15	6 / 9	25 ± 4	168 ± 7	63 ± 7
IV	PAS_{reflex}	14	8 / 6	27 ± 6	177 ± 6	75 ± 13
	PAS_{contr}	9	4 / 5	28 ± 4	173 ± 7	70 ± 7

None of the subjects had any history of neuromuscular or orthopedic diseases and all subjects were naïve to the experiments. Before testing, subjects were informed about the procedures and gave written consent. The study was approved by the ethics board of the University of Jyväskylä and was performed in conformity with the latest revision of the declaration of Helsinki. The partici-

pants were asked not to perform any physical activities on the measurement day to avoid any possible interference with the PAS protocol (Ziemann et al. 2004). In addition, different interventions/groups in each experiment were measured at comparable times of day.

In experiment II, the subjects were highly trained skill athletes and highly trained endurance athletes. The skill group had trained skill sports on average for 14 ± 4 years, 5 ± 1 times and 8 ± 2 hours per week up to the date of the measurements. Eleven participants were dancers, two were gymnasts and two were figure skaters. The dancers represented a variety of different genres including ballet, jazz and modern dance. Participants in the endurance group had trained endurance sports on average for 12 ± 4 years, 7 ± 2 times and 10 ± 2 hours per week up to the date of the measurements. Eight participants were cross-country skiers, four were orienteerers and three were long-distance runners. All subjects in the endurance group and ten in the skill group trained for competitions. All non-athletes from experiment I were selected and treated as a control group in the analysis of experiment II. The selected control group did not attend any formal training and did physical exercise less than 3 hours per week. In experiment IV seven subjects participated in both experiments on different days separated by at least four days.

4.2 Experimental design

In all experiments, participants were positioned in a custom built ankle dynamometer (University of Jyväskylä, Finland) with the hip at 110°, the knee in an extended position at 180°, the ankle at 90° and the right foot resting on a force pedal (figure 4). A seat belt restricted movement of the upper body and straps secured the right leg and foot. Hands were resting in the lap during all measurements. After the positioning procedure, the participants performed three maximal isometric plantarflexions with a 3 min rest interval between trials (I, II, III). The highest force value was considered as MVC. Motor cortex excitability was assessed by soleus MEPs, elicited with TMS before and after different PAS interventions. Spinal excitability was also measured before and after the interventions. As the force-time curve was displayed on the screen in front of the subjects, they were able to reach their target level with an accuracy of ± 2% during the active conditions. Before the PAS interventions, the latency of SEP was measured in order to determine the individual ISI for the PAS interventions (I, II, IV).

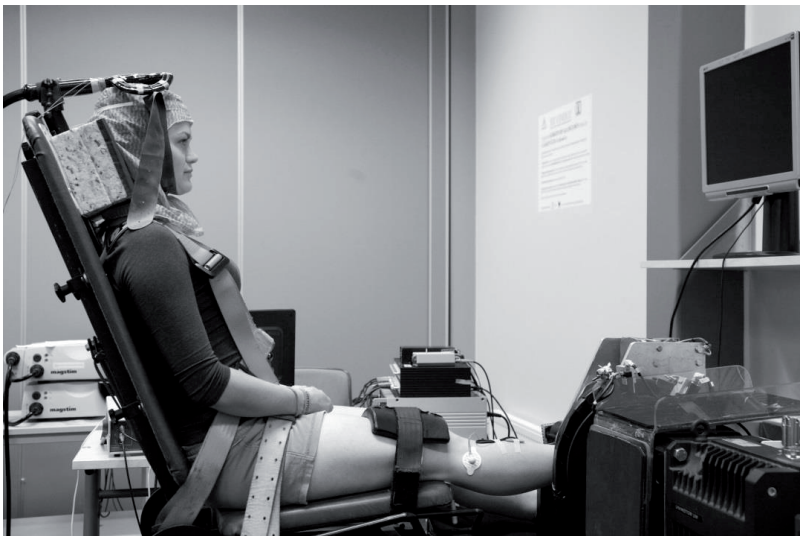

FIGURE 4 Measurement setup in the ankle dynamometer. In all experiments, participants were positioned in a custom built ankle dynamometer with the hip at 110°, the knee in an extended position at 180°, the ankle at 90° and the right foot resting on a force pedal.

4.2.1 Experiment I

Eight subjects participated in the first part of experiment I to define the optimal ISI for PAS targeting the soleus muscle. Four different PAS interventions (ISI = SEP latency + either 6 ms, 12 ms, 18 ms, or 24 ms) were tested in each subject separated by at least three days. Ten MEPs were recorded at 120% RMT before and 5 min after the PAS protocol while the soleus muscle was relaxed or active. Active MEPs were recorded while subjects maintained a small plantar-flexion corresponding to 5% of their MVC.

The duration, specificity and possible site of origin of the optimal PAS intervention (SEP latency + 18 ms) were investigated in another eight subjects. The input-output curve (IO-curve) of the soleus MEP was measured before, immediately after and 30 min following the PAS intervention. In addition, ten trials at 120% RMT of the tibialis anterior muscle were recorded at the optimal site for eliciting a MEP in tibialis anterior before and immediately after PAS. To identify potential changes in spinal excitability, the soleus H-reflex curve was measured before and immediately after PAS.

Another eight subjects attended the repeatability test, which included two separate sessions (at least three days apart) to test the effectiveness and repeatability of the optimal PAS. Otherwise the experimental design corresponded to the first part of the experiment.

4.2.2 Experiment II

In experiment II, possible exercise-specific effects on motor cortex plasticity were compared between 15 skilled and 15 endurance-trained athletes. Ten MEPs were elicited in the resting soleus muscle before and five minutes after PAS. To identify potential changes at the spinal level, ten soleus short latency stretch reflexes (SLSR) were mechanically elicited and compared between the groups using imposed ankle joint rotations.

4.2.3 Experiment III

Experiment III was designed (figure 5) to measure the functional relevance of PAS. The upper panel of Figure 5 represents the main protocol and the lower panel the additional procedures for sub-groups. Two PAS interventions were tested. There were 15 subjects in the PAS-induced LTP-like plasticity group (PAS_{LTP}) and 15 in the LTD-like plasticity group (PAS_{LTD}). MEPs were elicited ten times to passive muscle and five times to active muscle at 20% of MVC and 50% of MVC before and twice after the PAS intervention; immediately after (post0) and 15 min after (post15) PAS. The fatigue resistance tests were performed before and after all TMS measurements. To test for changes at the spinal level, ten H-reflexes were measured in sub-groups of six subjects from both PAS_{LTP} and PAS_{LTD} groups before, immediately after and 15 min after PAS. Fatigue, MEP and PAS procedures were conducted so that fatigue would not affect MEPs or the PAS intervention. Test-retest repeatability of fatigue resistance was determined by repeating the test prior to PAS intervention in ten subjects. There was a mandatory break of 10 min between the test and the retest to reduce the possibility of any fatigue effect.

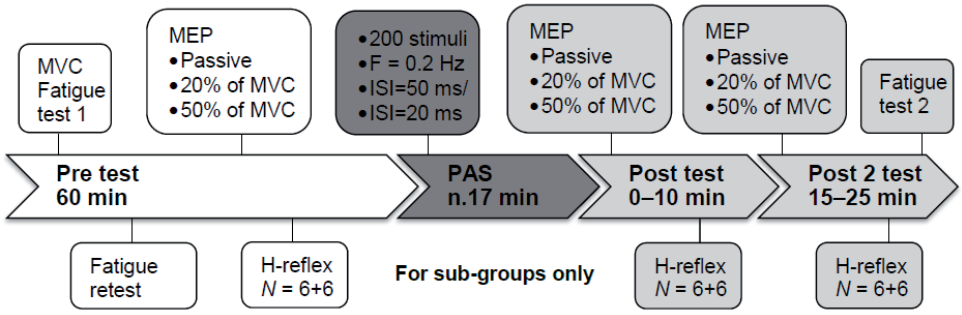

FIGURE 5 Experimental protocol III. The upper panel represents the main protocol and the lower panel additional procedures for sub-groups only. Two different PAS interventions were applied to induce LTP-like plasticity (ISI = 50 ms) and LTD-like plasticity (ISI = 20 ms). Excitability measures were made before, immediately after and 15 min after PAS. Fatigue resistance test was performed before and after all excitability measurements.

4.2.4 Experiment IV

In experiment IV, the PAS intervention was modified (PAS_{reflex}) by replacing synchronous electrical stimulation with a more asynchronous natural stretch reflex. There were 14 subjects in the PAS_{reflex} group and 9 subjects in the control PAS_{contr} group, which underwent a similar intervention but without TMS. The IO-curve of resting MEPs and H-reflex recruitment curves were measured before, immediately after (post0) and 30 min following (post30) the interventions.

4.3 Measurements and analysis

4.3.1 Recordings and data collection

For EMG measurements, a pseudo-monopolar electrode placement protocol (Hoffman et al. 2009) was used where one surface electrode of a pair was placed on the right soleus and the other over a bony surface of the tibia. A ground electrode was placed on the head of the tibia (I, II, IV) or on the lateral malleolus (III). The pseudo-monopolar setup allowed MEPs of higher amplitude to be recorded, which in turn also decreased the intensity of the stimulus needed to evoke a detectable MEP. In addition, bipolar electrodes were placed on the antagonist tibialis anterior muscle in experiment I and on soleus muscle in experiment II for SLSR recordings to minimize the noise generated by the perturbations of the ankle dynamometer. Prior to electrode placement, the skin was shaved, abraded and cleaned with alcohol to reduce resistance below 5 kΩ. EMG signals were amplified (100 x for pseudo-monopolar and 1000 x for bipolar configuration), band-pass filtered (10-1000 Hz) and sampled at 5 kHz (Neural Systems NL 900D and NL 844, Digitimer Ltd., Hertfordshire, UK). The EMG signals were collected with Spike2 software (CED, Cambridge, UK) via 16-bit AD converter (CED power 1401, Cambridge Electronics Design Limited, UK) and stored for later analysis. Commercially available software (Spike2, CED, Cambridge, UK) was used for all offline analyses.

4.3.2 Electrical stimulation

An electrical rectangular pulse with a duration of 1 ms was delivered to the posterior tibial nerve using a constant-current stimulator (DS7AH, Digitimer Ltd., Hertfordshire, UK). The optimal site for stimulation was located, where the highest M-wave amplitude at submaximal current was elicited in the soleus muscle. A circular cathode with a pickup area of 77 mm2 (Unilect short-term ECG Electrodes, Ag/AgCl, Unomedical Ltd., UK) was placed over the tibial nerve on the popliteal fossa and an oval shaped 5.08 cm x 10.6 cm anode (V-trodes neurostimulation electrodes, Mattler Electronics corp., USA) was placed above the patella.

4.3.3 Somatosensory-evoked potential

The latency of the somatosensory-evoked potential elicited from the tibial nerve stimuli was measured (I, II, IV) with conventional EEG needle electrodes inserted into the skin 2 cm behind and 5 cm in front of the vertex. The signals were amplified (100 000), filtered (1-500 Hz) and averaged using the electrical stimulation as a trigger (Neuropack Four Mini, MEB-5304K, Nihon Kohden, Tokyo Japan). The intensity of the electrical stimulus was set to motor threshold, where minimal intensity induced a visually observable muscle twitch in the soleus muscle. A total of 200 sweeps were averaged and the occurrence of the first negative peak (P32) was quantified.

4.3.4 Transcranial magnetic stimulation

TMS was delivered using a mono-pulse Magstim 2002 stimulator with a 9-cm double batwing coil inducing a posterior-to-anterior directed current (Magstim, Whitland, UK). The optimal stimulus site for soleus muscle was located on average 1 cm lateral and 1 cm posterior to the vertex on the left hemisphere. A custom made coil holder and rubber straps were used to fix the coil firmly on the head. The position of the coil was marked on a closely fitting cap worn by the subjects. The cap also protected the hair from cold spray (PRF101, Taerosol, Kangasala, Finland), which was used to cool down the coil during the measurements if needed. RMT was defined as the lowest stimulus intensity to elicit a MEP with a peak-to-peak amplitude of 50 μV in three out of five consecutive trials.

4.3.4.1 MEP

Corticospinal excitability was assessed by MEPs with a stimulus intensity of 120% of RMT when one intensity was used (I-III). Ten MEPs were elicited in the resting condition and five in active conditions. During passive measurements the subjects were asked to perform an attention task, which consisted of silently counting backwards from 200. Peak-to-peak amplitudes of the MEPs were determined and averaged.

4.3.4.2 Input-output curve

Stimulus intensity-dependent recruitment of corticospinal projections to the soleus muscle was measured via the MEP IO-curve (I, IV). Ten trials at 90, 100, 110, 120, 130 and 140% of RMT were recorded at rest (no 90% of RMT in IV). The order of the TMS intensities was randomized and the trials were delivered with variable intervals of 7-10 s. Peak-to-peak amplitude for each trial was determined to calculate the mean MEP for each TMS intensity. The slopes of the IO-curves were quantified by linear regression analysis from the steepest part of the IO-curve; between 110 and 140% of RMT in experiment I and between 100 and 120% of RMT in experiment II (Rosenkranz, Williamon & Rothwell 2007). To obtain single MEP values representative of the IO-curve, means of MEP responses of the steepest part were calculated.

4.3.4.3 Silent period

SP refers to a silencing of any ongoing EMG activity after the MEP as a result of TMS being delivered during a voluntary contraction. SP was analyzed in experiment III when MEPs were delivered during contractions at 50% of MVC as recommended by Säisänen et al. (2008), and also during contractions at 20% of MVC. The duration of the SP was determined by visual inspection as the time from MEP offset to the time of reoccurrence of voluntary EMG activity. Each individual trial was analyzed separately and then averaged across trials and subjects.

4.3.5 Measures of spinal excitability

SLSR and its electrically evoked analog, the H-reflex, were used to assess potential excitability changes at the spinal level. The biggest difference between these two reflexes is that the H-reflex is less sensitive to changes in γ-activity because the muscle spindle is bypassed with direct nerve stimulation (Zehr 2002). However, it is unlikely that with the same submaximal force level before and after PAS the spindle sensitivity would change. Different measures of spinal excitability were used in every experiment to obtain the most comprehensive results.

4.3.5.1 H-reflex

In experiment I, the H-reflex recruitment curve of resting soleus muscle was measured. H-reflexes were elicited over intensities of 10-100% of the maximal M-wave intensity (M_{max}) in steps of 10%. Three trials at each of the intensities were recorded in a randomized order. Peak-to-peak amplitude for each trial was determined to calculate the mean H-reflex for each intensity. Mean H-reflex amplitudes were normalized to M_{max}.

In experiment III, M_{max} was measured with supramaximal stimulus intensity, i.e. 150% of the current needed to elicit maximal stimulus response. M_{max} was elicited before all H-reflex measurements. Then 10 submaximal stimuli were applied with a current intensity that evoked peak-to-peak M-wave responses of $20 \pm 5\%$ of M_{max} to quantify the H-reflex. Peak-to-peak amplitude for each trial was determined, averaged and normalized to M_{max}.

In experiment IV, Soleus H-reflex recruitment curve was measured to quantify maximal H-reflex (H_{max}). After the H-reflex threshold intensity was reached, the current was increased in steps of 0.6 mA until the M-wave plateaued. To quantify a reliable M_{max}, supramaximal stimulus intensity (150%) was used. The amplitude of H_{max} was determined from the recruitment curve and normalized to the concomitant M_{max} (Stutzig & Siebert 2015).

4.3.5.2 Short latency stretch reflex

In experiment II, soleus SLSRs were elicited with ten perturbations induced by the ankle dynamometer before setting the coil on the head and after the last TMS measurement to avoid possible coil movements. Subjects were asked to maintain 20% of their maximal plantarflexion force while a 6 deg dorsiflexion perturbation was delivered every 4-7 s with a velocity of 120 deg/s. The onset

of the SLSR was visually determined from ten ensemble-averaged and rectified EMG traces. Root mean square of a 20 ms window from the SLSR onset was calculated. This value was normalized to the root mean square (100 ms window) of the MVC and then used as an indication of the size of SLSR. The background EMG level at 20% of MVC was defined as the root mean square in a window placed from 120 ms to 20 ms prior to the perturbation.

4.3.6 Fatigue test

For the fatigue test (III) subjects were instructed to produce their maximal isometric plantar flexion force and maintain it for 15 seconds (figure 6), during which the force declined towards the end. Verbal encouragement was given throughout the trial. At the end of the fatiguing contraction, neural deficit was estimated using the interpolation twitch technique (ITT) (Merton 1954); a supramaximal (150% of M_{max} intensity) double pulse with 10 ms interval was delivered to the tibial nerve to quantify possible increment in force (superimposed twitch). Immediately following the sustained MVC, the same supramaximal double pulse was delivered to the relaxed muscle to quantify resting twitch. Neural deficit indicates insufficient central drive to the motor neurons and is attributable to central fatigue (Allen, McKenzie & Gandevia 1998).

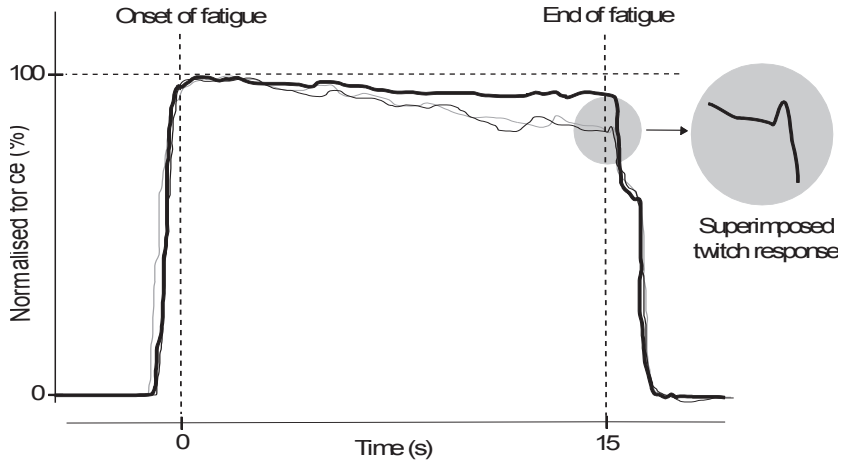

FIGURE 6 Example figure of the fatigue resistance test. Force traces are from one representative subject. The two continuous thin lines demonstrate the test-retest repeatability prior to intervention (fatigue test1 = black line, fatigue retest = gray line). The thicker black line represents the post-intervention test. Force is normalized to maximal voluntary force.

Fatigue resistance was calculated by dividing the average force during the 15 s isometric contraction by the individual MVC. Thus fatigue resistance of 100% corresponds to the theoretical situation where the initial MVC force is maintained for the entire 15 s. Amplitudes of the superimposed twitch and resting twitch were measured and the ratio between them was calculated to estimate neural deficit.

4.4 PAS interventions

4.4.1 Traditional PAS (I, II, III)

PAS consisted of a single electrical stimulation delivered to the tibial nerve at 150% of motor threshold and a single TMS pulse at 120% of RMT. The optimal ISI to induce LTP-like plasticity in the soleus muscle was determined in the first part of experiment I, where four different ISIs were tested. The optimal ISI was SEP plus 18 ms and used from then on in experiments I and II. In experiment III, ISIs to induce LTP- and LTD-like plasticity were selected based on previous experiments. A constant ISI of 50 ms was used for the PAS_{LTP} group (Mrachacz-Kersting et al. 2007; Poon et al. 2008) and a constant ISI of 20 ms was used for the PAS_{LTD} group (Stinear & Hornby 2005; Jayaram & Stinear 2008; Poon et al. 2008). In all PAS protocols, a total of 200 pairs of stimuli were applied at a rate of 0.2 Hz. To optimize the PAS effect, subjects were required to perform an attention task consisting of counting the peripheral stimuli applied to the tibial nerve and to produce a slight plantarflexion force after every 20 stimuli between the sequential stimuli (Stefan, Wycislo & Classen 2004)

4.4.2 Modified PAS (IV)

PAS_{reflex} consisted of a soleus stretch reflex response, which was elicited with a perturbation induced by the ankle dynamometer and a single TMS pulse at 120% of RMT. ISI consisted of afferent conduction time plus 18 ms (I) to account for the central processing time. The afferent conduction time was calculated from the individually determined latencies of SEP plus the difference between the SLSR and H-reflex latencies (to identify the time it takes muscle spindles to be activated and the induced afferent volley to travel to the knee level, where SEP was elicited). The average ISI was 62 ± 6 ms. A total of 200 pairs of stimuli were applied at a rate of 0.2 Hz. To optimize the PAS_{reflex} effect, subjects were required to perform an attention task consisting of counting the perturbations of the ankle dynamometer and to produce a slight plantarflexion force after every 20 perturbations between the sequential stimuli (Stefan, Wycislo & Classen 2004). The intervention for the PAS_{contr} group was similar but without TMS.

4.5 Statistical analysis

Statistical analysis was conducted using IBM SPSS 19.0 (SPSS, Chicago, USA). Normality of variables was tested with Shapiro-Wilk's *W*-tests (II, III, IV). Accordingly, differences between the subject groups for characteristic parameters were tested by unpaired *t*-tests. Averaged soleus MEPs were compared with repeated measures ANOVA (I, II) or with Wilcoxon's signed-rank test (III, IV). To compare MEP changes between the groups, the post-MEPs were normalized

to baseline MEPs and one-way ANOVA (II) or Mann-Whitney *U*-test (III, IV) was performed. For evaluation of IO-curves, repeated measures ANOVA (I) or Wilcoxon's signed-rank test (IV) was used. SPs were analyzed with repeated measures ANOVA. H-reflexes were analyzed using repeated measures ANOVA (I, IV) or Wilcoxon's signed-rank test (III). SLSR responses were analyzed with repeated measures ANOVA. Intraclass correlation coefficient (ICC) was used to measure repeatability.

In experiment III, MVC and fatigue force were compared with two-tailed paired *t*-tests. Fatigue resistance was compared with repeated measures ANOVA and neural deficit with Wilcoxon's signed-rank test. Spearman's coefficient was used to correlate the changes in PAS-induced excitability (at post15; MEPs as percentage of baseline values) and changes in fatigue resistance. In a secondary analysis the abovementioned tests for neural correlates and fatigue resistance were also performed only for the PAS responders (post MEP/pre MEP ratio > 1.00 in the PAS_{LTP} group and < 1.00 in the PAS_{LTD} group). The secondary analysis was done due to the high inter-individual variability of the PAS effects, which have been previously reported (Ridding & Ziemann 2010). In all experiments, the significance level was set at $P < 0.05$. All data are given as mean ± standard deviation (SD).

5 RESULTS

5.1 The optimal PAS intervention for soleus muscle (I)

The average SEP latency after tibial nerve stimulation was 32 ± 2 ms (figure 7). This resulted in four different ISIs: 38 ± 2 ms, 44 ± 2 ms, 50 ± 2 ms and 56 ± 2 ms for the first part of experiment I. The SEP and MEP (30 ± 1 ms) latencies correlated significantly ($r = 0.89$, $P < 0.01$). Following the PAS protocol, resting soleus MEPs only increased significantly (by 88 ± 105%) with an ISI of 50 ± 2 ms (SEP plus 18 ms), which was then used as the optimal ISI to induce LTP-like plasticity. MEPs only decreased significantly (by 31 ± 30%) with an ISI of 38 ± 2 ms (SEP plus 6 ms). There was no significant facilitation in the soleus MEPs in active conditions with any of the ISIs. The effects of different PAS interventions are shown in figure 8A. The morphology of MEPs was examined before and after the optimal PAS to gain more insight into the nature of facilitation (figure 8B). Across the subjects, the main increase of the MEP occurred 2-4 ms after MEP onset while the early part of the MEP remained unchanged.

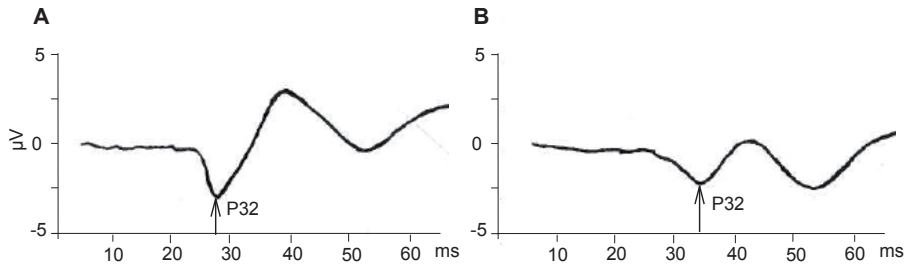

FIGURE 7 SEPs after tibial nerve stimulation for two representative subjects. The first negative peak (P32) occurred at a latency of 28 ms for a 161 cm female subject who was stimulated with a motor threshold of 8 mA (A). The P32 latency was 34 ms for a 176 cm male subject with a motor threshold of 5 mA (B). The traces are an average of 200 sweeps.

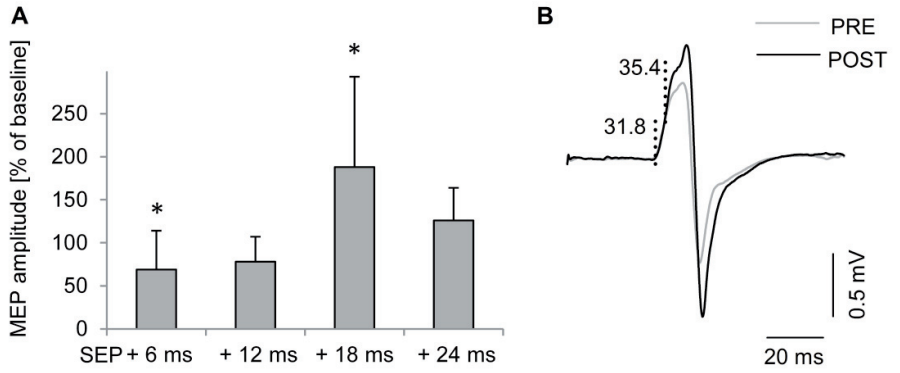

FIGURE 8 The effect of four different PAS interventions on group MEP amplitudes at rest (A). LTD-like plasticity was achieved with an ISI of SEP plus 6 ms and LTP-like plasticity with an ISI of SEP plus 18 ms (* P < 0.05). The effect of the optimal PAS on MEP morphology before (pre) and after (post) the intervention (B). Each MEP is an average of 10 stimuli.

5.1.1 Duration, specificity and site of origin

The effect of the optimal ISI on the IO-curves is shown in figure 9. The IO-curve slope steepened by 59% immediately after and significantly increased by 73% 30 min after PAS. Mean MEP amplitudes increased significantly by 43 ± 44% immediately after and 53 ± 41% 30 min following the PAS intervention. There were no significant changes in the peak-to-peak amplitudes of TA MEPs. The PAS intervention did not affect the H-reflex recruitment curve at any intensity, which can be seen in figure 10.

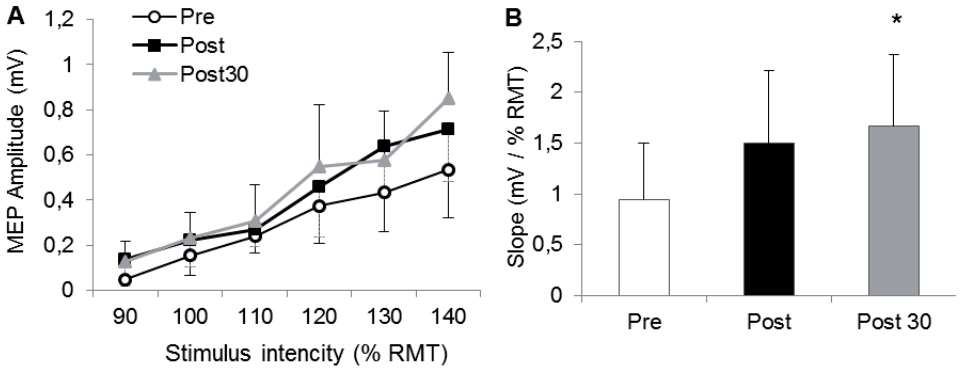

FIGURE 9 Effect of PAS on soleus input-output curve before (pre), immediately after (post) and 30 min after (post30) PAS (A). The slope of the IO-curve was significantly (* P < 0.05) steeper 30 min after PAS (B).

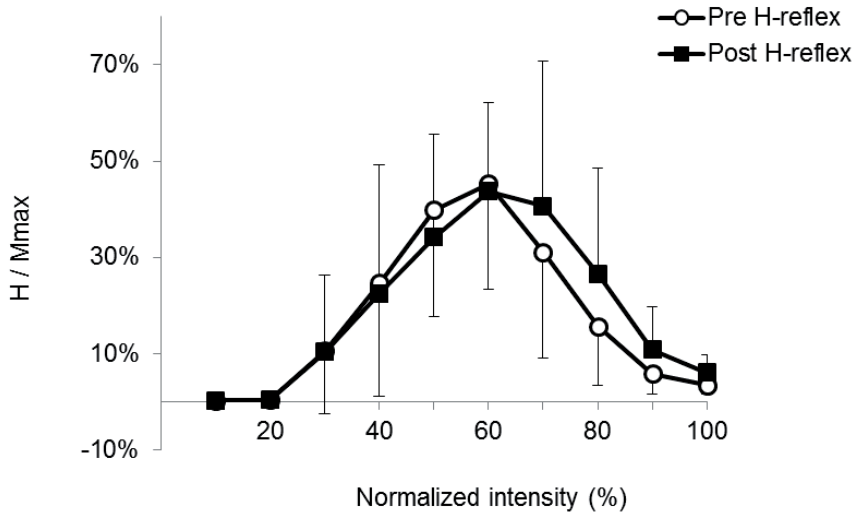

FIGURE 10 Effect of PAS on H-reflex recruitment curves. Mean H-reflex amplitudes are presented as a percentage of maximal M-wave amplitude before (pre) and after (post) PAS. PAS did not affect the H-reflex recruitment curves at any intensity.

5.1.2 Repeatability of the PAS-induced effects

Repeatability of the optimal PAS (SEP plus 18 ms) was tested in two separate sessions. In both repeatability measurements, significant increases in the peak-to-peak amplitudes of the passive soleus MEPs were attained; 46 ± 52% and 36 ± 32%, respectively (figure 11). The ICC value was 0.85. MEPs of the active muscle were neither increased nor repeatable (ICC = 0.18). In addition, the repeatability of RMT and baseline MEPs were evaluated. In the first part of experiment I, the ICC of RMTs between four sessions was 0.89 and ICC of baseline MEPs was 0.82, and in the repeatability experiment ICC of RMTs was 0.94 and ICC of baseline MEPs was 0.95.

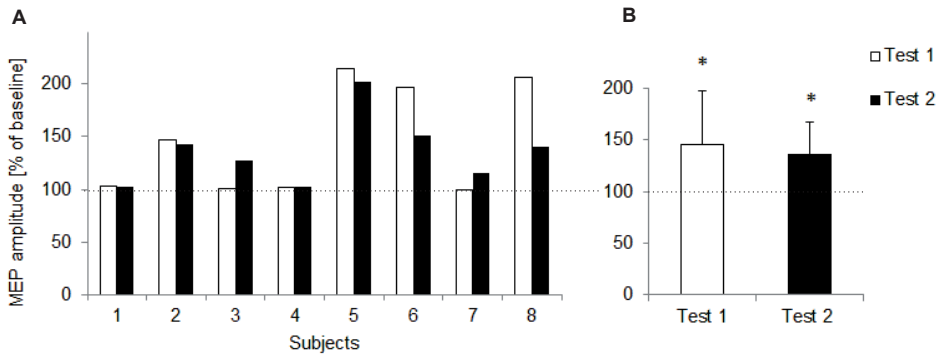

FIGURE 11 Effects of the optimal PAS intervention (SEP plus 18 ms) in two separate testing sessions, the individual results (A) and the group result (B). Significant LTP-like plasticity was achieved in both testing sessions and the ICC value of PAS-induced effects was 0.85 (* $P < 0.05$).

5.2 The effect of training background on motor cortex plasticity tested by PAS (II)

There were no differences between the skill and endurance groups in training years (skill, 14 ± 4 years; endurance, 12 ± 4 years) or MVC (skill, 1250 ± 430 N; endurance, 1150 ± 280 N). There were also no differences between the groups in RMT (skill, 51 ± 10% stimulator output; endurance, 54 ± 4% stimulator output; control, 57 ± 11% stimulator output) or baseline MEPs (skill, 0.47 ± 0.36 mV; endurance, 0.50 ± 0.29 mV; control, 0.36 ± 0.18 mV).

Figure 12 illustrates the main finding with original MEP recordings from one representative skill and one endurance athlete before PAS and 5 min following PAS, as well as the group mean results. A two-way repeated measures ANOVA revealed a significant effect of PAS ($F_{(1,35)}$ = 5.25, P < 0.05) and an interaction of PAS and group ($F_{(2,70)}$ = 9.08, P < 0.01) for the MEPs. Post hoc analysis using a two-tailed paired t-test revealed that MEP amplitude significantly increased by 76 ± 83% only in the skill group (P < 0.01). There was a nonsignificant decrease of 7 ± 35% in the endurance group and an increase of 21 ± 30% in the control group. One-way ANOVA for normalized MEPs revealed a significant effect of group ($F_{(2,35)}$ = 7.68, P < 0.05). Post hoc analysis using Bonferroni test revealed a significant difference between the skill and endurance groups (P < 0.01). The control group did not differ from the other groups.

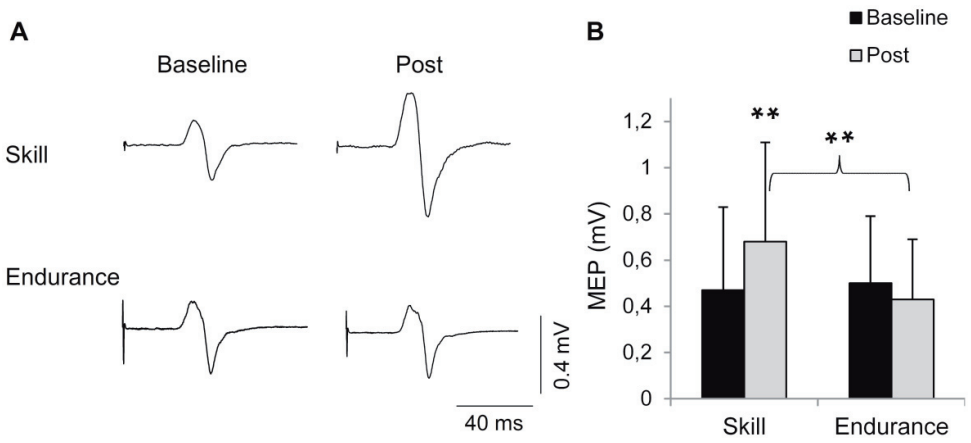

FIGURE 12 MEP traces for one representative skill and one endurance subject before (baseline) and after (post) PAS (A). Each trace is an average of 10 trials. The group MEP amplitudes are presented as percentages of baseline values (B). The average soleus MEP amplitude increased significantly by 76% in the skill group. The ratio of baseline and post MEPs differed significantly between the skill and endurance groups (** P < 0.01).

SLSR responses were not affected by PAS ($F_{(1,28)}$ = 0.317, P > 0.05), group ($F_{(1,28)}$ = 0.33, P > 0.05) or their interaction ($F_{(1,28)}$ = 0.206, P > 0.05). Figure 13 shows SLSR recordings from one representative skill and one endurance athlete before and following PAS. Normalized pre-intervention values were 73 ± 55% in the skill group and 88 ± 53% in the endurance group.

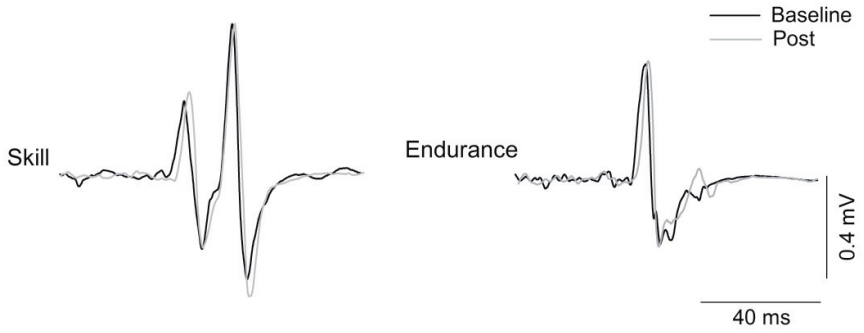

FIGURE 13 Short latency stretch reflex response traces for one representative skill and one endurance subject before (baseline) and after (post) PAS. Each trace is an average of 10 trials. A medium latency stretch reflex can be seen in the trace of the skill athlete. SLSR responses were not affected by PAS.

5.3 Functionality of PAS intervention (III)

5.3.1 Neural correlates

The PAS effect (post MEPs/pre MEPs) was significantly different between PAS_{LTP} and PAS_{LTD} groups at post0 and post15 at rest but not in active conditions. The normalized post-intervention peak-to-peak MEP amplitudes are presented in figure 14A and B. In the PAS_{LTP} group, MEP increased significantly by 73 ± 123% only 15 min after PAS at rest. There were no significant changes in the 20% of MVC condition but MEPs decreased significantly immediately after as well as 15 min after PAS in the 50% of MVC condition. In the PAS_{LTD} group, passive MEP decreased significantly by 27 ± 32% only immediately after PAS. In the 20% of MVC condition, MEP decreased significantly at both measurements after PAS. There were no significant changes in the 50% of MVC condition. The difference between post0 and post15 was never statistically significant.

SPs at a contraction level of 50% of MVC were not affected by time ($F_{(2,56)}$ = 1.54, P > 0.05), group ($F_{(1,28)}$ = 0.87, P > 0.05) or their interaction ($F_{(2,56)}$ = 0.06, P > 0.05). SP values for the PAS_{LTP} group were 75 ± 27 ms before, 78 ± 23 ms immediately after and 79 ± 25 ms 15 min after PAS. In the PAS_{LTD} group, the SP values were 68 ± 19 ms, 72 ± 15 ms and 73 ± 14 ms, respectively.

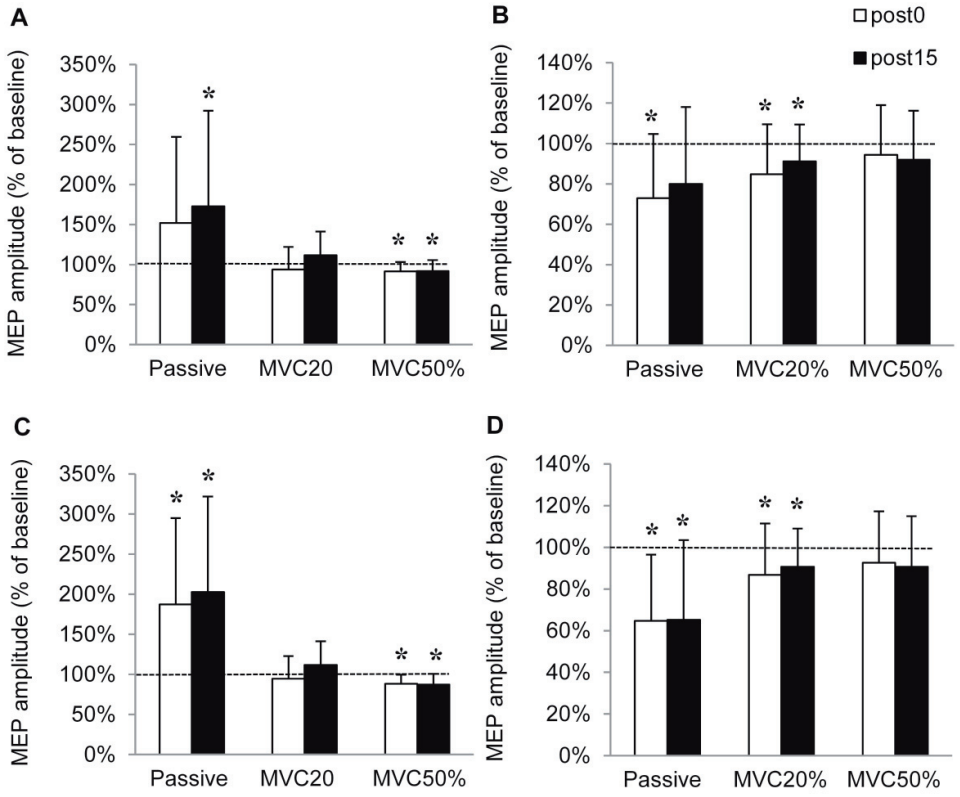

FIGURE 14 Effect of PAS on MEP responses immediately after (post0) and 15 min after (post15) the intervention. Mean post-intervention MEP amplitudes (normalized to baseline) in the PAS_{LTP} group (A) and in the PAS_{LTD} group (B). The same results are presented for responders only in the PAS_{LTP} group, 11 subjects (C) and in the PAS_{LTD} group, 12 subjects (D), $^*P < 0.05$.

Correspondingly, there were no changes in SP duration at 20% of MVC. The coefficient of variation was significantly higher, 0.25 at 20% of MVC compared to 0.16 at 50% of MVC. Neither M_{max} amplitude nor H/M_{max} ratio changed significantly throughout the protocol.

5.3.2 Fatigue resistance

In both groups the 15 s fatigue test induced significant force reduction prior to PAS; in the PAS_{LTP} group MVC was 1380 ± 420 N and average fatigue force was significantly less, 1230 ± 340 N ($P < 0.001$). In the PAS_{LTD} group, MVC force was 1190 ± 320 N and average fatigue force was significantly less, 1110 ± 280 N ($P < 0.01$). Corresponding fatigue resistance values were 90 ± 8% in the PAS_{LTP} group and 93 ± 7% in the PAS_{LTD} group. Contrary to our hypothesis, fatigue resistance was not affected by time ($F_{(1,28)} = 0.11$, $P > 0.05$), group ($F_{(1,28)} = 0.59$, $P > 0.05$) or their interaction ($F_{(1,28)} = 0.86$, $P > 0.05$). After the PAS interventions, fatigue re-

sistance was 92 ± 1% in the PAS_{LTP} group and 92 ± 7% in the PAS_{LTD} group. However, when the groups were combined, the change in excitability (post15 MEPs as a percentage of baseline values) correlated significantly with the change in fatigue resistance (N = 30, R = 0.40, $P < 0.05$), which can be seen in figure 15.

In the PAS_{LTP} group, neural deficit was 1.6 ± 2.4% prior to PAS and 0.9 ± 4.4% following PAS. In the PAS_{LTD} group, neural deficit was 6.5 ± 13.3% prior to PAS and 5.9 ± 11.2% following PAS. Neural deficit decreased non-significantly by 44 ± 79% in PAS_{LTP} and by 10 ± 30% in PAS_{LTD}. Test-retest comparison (N=10) revealed excellent repeatability of the fatigue resistance test ($P < 0.001$).

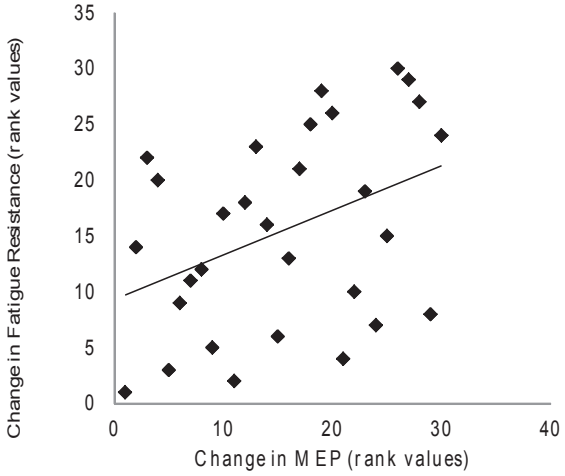

FIGURE 15 Relationship between excitability and fatigue resistance. Spearman's coefficient was used to correlate PAS-induced changes in excitability, presented on the horizontal axis, with changes in fatigue resistance, presented on the vertical axis (N = 30, R = 0.40, $P < 0.05$).

5.3.3 Secondary analysis

The secondary analysis with only responders is shown in figure 14B and C. Passive MEPs increased significantly in both measurements after PAS in the PAS_{LTP} group (11 subjects) and decreased significantly in both measurements in the PAS_{LTD} group (12 subjects). Otherwise the significances in MEP and SP results did not differ from the whole group analysis. However, two-way repeated measures ANOVA of the fatigue resistance test revealed a significant interaction of time and group ($F_{(1,21)}$ = 4.8, $P < 0.05$), which can be seen in figure 16. Post hoc analysis using two-tailed paired t-tests showed a non-significant improvement in fatigue resistance by 3.1 ± 8.4% in the PAS_{LTP} group and a reduction of 3.0 ± 7.5% in the PAS_{LTD} group. Otherwise the significances in force results did not differ from the whole group analysis.

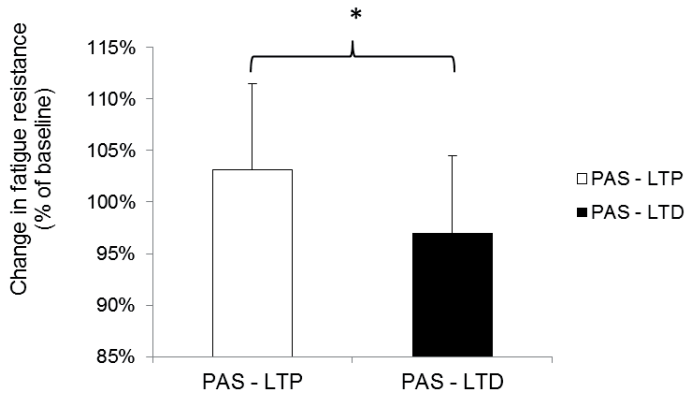

FIGURE 16 Change in fatigue resistance among responders in the PAS_{LTP} group (11 subjects) and in the PAS_{LTD} group (12 subjects). There was a significant interaction of time and group but no significant change in fatigue resistance within the groups of responders (*$P < 0.05$).

5.4 Modified PAS intervention; PAS_{reflex} (IV)

There were no differences between the groups in age (PAS_{reflex}, 27 ± 6 years; PAS_{cont}, 28 ± 4 years) or RMT (PAS_{reflex}, 55 ± 10% stimulator output; PAS_{contr}, 54 ± 7% stimulator output).

The soleus IO-curves for both groups are shown in figure 17A and B. In the PAS_{reflex} group, there was no change in the slope of the IO-curve immediately after the intervention, but it increased significantly 30 min after. There was also a significant difference between the post0 and post30 measurements. The slope of the IO-curve increased in 5 out of 14 subjects immediately after and in 10 out of 14 subjects 30 min after PAS. In the PAS_{contr} group the slopes of the IO-

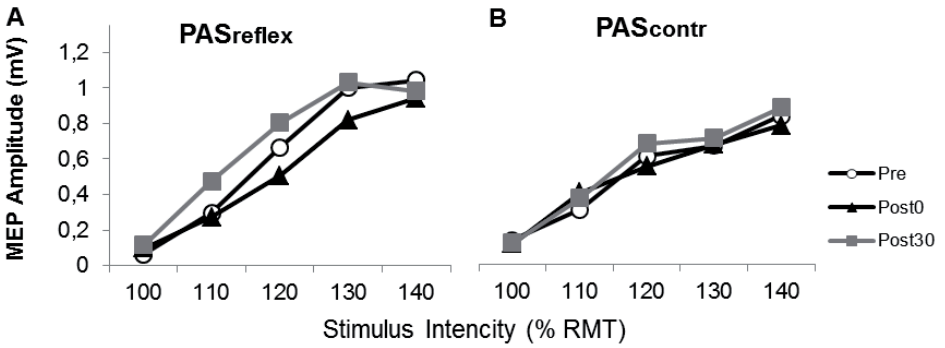

FIGURE 17 Effect of PAS_{reflex} (A) and PAS_{contr} (B) on MEP IO-curves before, immediately after and 30 min after interventions. Group MEP amplitude data for IO-curves are from 14 subjects in the PAS_{reflex} group and nine subjects in the PAS_{contr} group.

curves did not change. However, the change in the slope of the IO-curve (post values as a percentage of baseline values) was not different between the groups at post0 or at post30.

The mean slope values and mean MEP amplitudes for both groups are shown in figure 18. The Mean MEPs increased significantly 30 min after the intervention in the PAS_{reflex} group and there was also a significant difference between the post0 and post30 measurements. There were no changes in MEPs in the PAS_{contr} group. Again the change in the MEPs at post0 and post30 (post values as a percentage of baseline values) was not different between the groups.

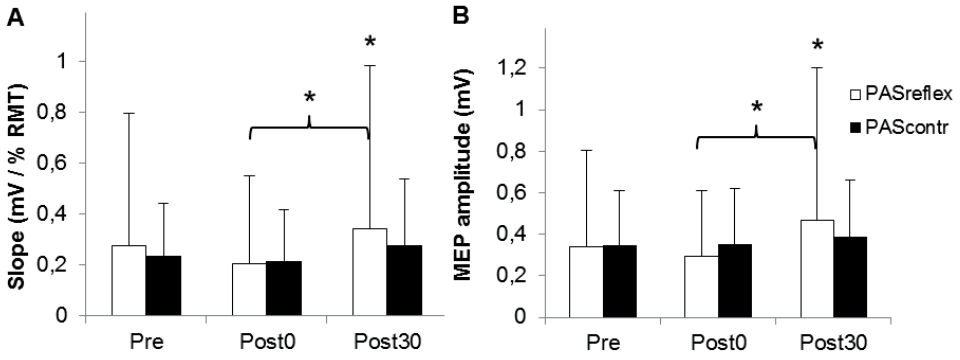

FIGURE 18 Effect of PAS_{reflex} and PAS_{contr} on the slope of the MEP IO-curve (A) and mean MEP responses (B). The slopes of the IO-curves and mean MEP amplitudes behaved similarly; there was a significant increase at post30 and a significant difference between post0 and post30 measurements in the PAS_{reflex} group. There were no changes in slopes or mean MEPs in the PAS_{contr} group (* $P < 0.05$).

M_{max} and H_{max}/M_{max} ratio were not affected by time, group, or their interaction. H_{max}/M_{max} ratios for the PAS_{reflex} group were 0.72 ± 0.21% before, 0.74 ± 0.21% immediately after and 0.70 ± 0.25% 30 min after the intervention. H_{max}/M_{max} ratios for the PAS_{contr} group were 0.71 ± 0.26%, 0.66 ± 0.17% and 0.66 ± 0.26%, respectively.

6 DISCUSSION

The purpose of this thesis was to investigate the plasticity of the soleus area of the motor cortex using PAS interventions. This was the first study to show PAS-induced LTP/LTD-like effects in the antigravity soleus muscle. The current results indicated that the optimal ISI for PAS to repeatedly induce LTP-like plasticity in the soleus area of the motor cortex was a latency of SEP plus 18 ms. With the optimal PAS protocol, skill trained athletes showed significantly higher motor cortex plasticity compared to endurance trained athletes. On average, fatigue resistance did not change following the PAS interventions and consequently, the functionality of PAS was not evident. Although PAS_{reflex} did not decrease the variability of PAS-induced effects, it produced similar results compared to traditional PAS protocols. Due to the nature of the study, methodological considerations and limitations have been incorporated into the discussion.

6.1 Characteristics of the optimal PAS intervention when the soleus muscle is targeted (I)

Significant LTP-like plasticity was attained with an ISI consisting of the latency of the P32 component of the SEP plus 18 ms ($\approx$ 50 ms). With this optimal PAS intervention the soleus MEP amplitude increased by 88 ± 105%. The PAS effect was long-lasting, input-specific and supraspinal in origin. The ICC to test the repeatability of the PAS intervention with the optimal ISI was 0.85. As hypothesized, the excitability of cortical projections to the soleus muscle was repeatedly increased after PAS with an optimal ISI of SEP plus 18 ms.

6.1.1 Optimal ISI to induce LTP-like plasticity

The optimal ISI of PAS targeting soleus muscle was longer than the optimal ISI of PAS targeting hand muscles (SEP plus 5 ms $\approx$ 25 ms). This may in part be

due to the longer time required for the afferent volley generated by the electrical stimulus to reach the sensory cortex, as reflected by the longer SEP latencies. However, in that case, one would expect the ISI to be made up of the P32 component plus 5 ms. Surprisingly, at least 18 ms had to be added to induce a significant LTP-like effect, whereas a significant LTD-effect was induced with the addition of only 6 ms. This may indicate that a longer processing time of afferent signals arising from lower limb muscles is required. Earlier studies by Nielsen at al. (1997) and Petersen et al. (1998) have estimated central processing times of 10-15 ms for the tibialis anterior muscle, which is close to the 18 ms processing time in the current experiment.

The SEP latencies reported here correspond to the latencies reported in previous studies (Vas, Cracco & Cracco 1981; Brooke et al. 1997; Hauck et al. 2006). An unproven assumption is that the afferent signals reach the motor cortex via the somatosensory area. If the afferent volley traverses other areas to reach the motor cortex, the longer central processing delay may be explained. In addition, different TMS propagation in the motor cortex of the leg area has been proposed in some earlier studies (Prior & Stinear 2006; Nielsen, Petersen & Ballegaard 1995), which could then necessitate a longer ISI. However, other previous studies have shown that TMS activates the hand and leg areas in a similar manner (Terao et al. 2000; Di Lazzaro et al. 2001). TMS elicits a series of descending corticospinal volleys- I-waves- at 1.5 ms intervals. The I3-waves in particular are altered following PAS of the hand area (Kujirai et al. 2006; Di Lazzaro et al. 2009), whereas the PAS effects on I-waves from the leg motor area have not been described. Changes in MEP morphology after PAS (figure 8) in the current study seem to be similar to changes obtained from the hand area (Kujirai et al. 2006). Kujirai et al. (2006) showed that the increase in MEPs occurred 2-3 ms after the onset latency, suggesting that the later I-waves were affected. They also showed that the latency of MEPs induced by anterior-posterior current, which tends to recruit later arriving I3-waves, is 2-3 ms longer.

The PAS results are consistent with previous studies. Mrachacz-Kersting et al. (2007) measured tibialis anterior muscle and also achieved the largest LTP-like plasticity with ISIs of 50-55 ms and LTD-like plasticity with an ISI of 40 ms. Roy et al. (2007) showed LTP-like effects with an ISI of 35 ms in the passive tibialis anterior muscle, which contrasts to the present results. However, they had different protocol parameters, which might affect the results, as stated in the literature review. In addition, when comparing tibialis anterior and soleus muscles, muscle specificity cannot be ignored. Poon et al. (2008) and Roy and Gorassini (2008) studied the effect of a peripheral tibial nerve conditioning stimulus (at 150% MT) on the soleus MEP. Electrical stimulation was timed to arrive at the motor cortex either prior to or following TMS. At ISIs ranging from 50 to 60 ms, a significant increase in the conditioned soleus MEPs was observed, while ISIs of 35 – 40 ms showed a significant decrease in the conditioned soleus MEPs (table 1). Comparable ISIs were used in the PAS interventions in this study and they produced similar changes in unconditioned MEPs. The results of this study differ from PAS studies done during walking. Jayaram et al. (2007),

Prior and Stinear et al. (2006) and Stinear and Hornby et al. (2005) measured the effect of PAS during walking and achieved LTP-like plasticity with ISIs of 35 - 40 ms in the tibialis anterior muscle. However, these studies are not comparable since different conditions in the muscle can affect the length of the SEP latency as well as the central processing time (Duysens et al. 1990; Brooke et al. 1997).

No significant changes were seen in the MEPs of active muscle in this study, which is in line with previous studies (Stefan et al. 2000; Roy, Norton & Gorassini 2007; Poon et al. 2008). The interpretation of the effect of PAS on active muscles is more complicated, as can be seen in the study of Lu et al. (2009), where a decrease of the movement-related cortical potentials (MRCP) in EEG-recordings was reported after an LTP-like PAS protocol. Accordingly, performing the same movement pattern- simple thumb abduction- generated a decreased MRCP negativity after the PAS intervention. MRCP reflects executive aspects of the forthcoming motor action, and decreased MRCP negativity denotes weaker volitional motor output, which may indicate that the LTP-like effect results in decreased or unchanged MEPs in active muscle. However, the current active MEP results should be interpreted with some caution since only five responses were recorded. Nonetheless, a low number of TMS stimuli (three to five) have frequently been used in experiments in active conditions (Priori et al. 1993; Taube et al. 2008; Gruber et al. 2009), and it has also been shown that relative variability of single MEPs is lower during a maintained contraction than in passive muscle (Darling, Wolf & Butler 2006).

6.1.2 Duration, specificity and site of origin

Several criteria have been established for LTP/LTD-like plasticity in the human cortex, such as rapid onset, associativity, duration, specificity, and NMDA-receptor dependence. Previous PAS studies in the upper limbs have confirmed that all of these criteria are fulfilled (Stefan et al. 2000; Stefan et al. 2002; Wolters et al. 2003). Four of these were evident in the present study. The effects of PAS evolved rapidly (< 20 min), were strongly dependent on the order of the two successive stimuli, persisted long after the stimulation (> 30 min) and were muscle specific. MEP amplitudes of the soleus IO-curves were significantly affected by time, intensity and their interaction. Since soleus H-reflexes did not change significantly, the excitability change was likely to be supraspinal in origin, which has also been shown in previous PAS studies with F-waves, electrical brainstem stimulation (Stefan et al. 2000), H-reflexes (Mrachacz-Kersting et al. 2007) and implanted electrode recordings from the cervical epidural space (Di Lazzaro et al. 2009). Although not conclusive, these findings provide evidence that LTP/LTD-like plasticity is also evident in the leg motor area following PAS intervention.

6.1.3 Repeatability

A number of factors have been suggested to influence PAS-induced plasticity. Previous studies report wide variability of PAS-induced effects between sub-

jects and also between sessions in the same subject (Fratello et al. 2006; Sale, Ridding & Nordstrom 2007). Even with a stable group LTP-like effect (1.46 and 1.34) between two sessions, large inter-individual variability was also observed in the present results with SD values of ± 0.52 and ± 0.32, which can be seen in figure 8. Inter-individual variability has been reported in several studies (Fratello et al. 2006; Sale, Ridding & Nordstrom 2007; Stefan, Wycislo & Classen 2004; Stefan et al. 2006; Wolters et al. 2003) and it has been associated with differences in brain anatomy, genes, age and training background (Ridding & Ziemann 2010). The important new observation in the present study is that individual responses to PAS targeting soleus muscle were remarkably consistent (ICC= 0.85) compared to those of previous studies (Fratello et al. 2006; Sale, Ridding & Nordstrom 2007). Intra-individual variability can be reduced by careful planning of the experimental design. It is likely that controlling the three plasticity determinants- the attention level of the subject, history of recent neuronal activity and the time of day- may have positively affected the current results. This study confirms the efficiency of PAS in causing plastic changes in the cortical projections to the soleus muscle at rest, and shows repeatability of this effect within subjects and in the group as a whole.

6.2 The effect of training background on motor cortex plasticity tested by PAS (II)

Significant LTP-like plasticity was attained in the skill group, while there were no significant changes in the endurance or control groups following PAS. As hypothesized, the change in excitability (baseline MEP/post MEP) was significantly different between the skill and endurance groups. Sport background was the main factor separating the skill and endurance groups, and it is therefore suggested that the different PAS-induced effects arise from exercise-specific adaptations in the corticomotoneuronal system. The findings of experiment II suggest that long-term skill training induced preferable adaptations in the task-related areas of the motor cortex because increased plasticity is known to enhance motor learning.

6.2.1 Cortical factors

The significantly greater plasticity induced by skill training is in line with what has been reported previously for professional musicians (Rosenkranz, Williamon & Rothwell 2007). PAS-induced plasticity was significantly larger in musicians compared to non-musicians when PAS was applied to task-related hand muscles. There is convincing evidence from animal and human studies that skill training increases the amount of neurotrophic factors, synaptogenesis and map reorganization within the motor cortex, which drive the acquisition and performance of skilled movements (Monfils, Plautz & Kleim 2005). These coordinated neuronal changes might also have led to higher motor cortex plas-

ticity in the skill group in the present study since larger representation areas of tibialis anterior muscles have been observed in figure skaters compared to controls (Vaalto et al. 2013). It has been demonstrated that these neuronal changes are not simply due to increased use of target muscles (Plautz, Milliken & Nudo 2000; Kleim et al. 2004). Endurance training has been shown to increase angiogenesis in the motor cortex accompanied by increased neurotrophic factors (Kleim, Cooper & VandenBerg 2002; Vaynman & Gomez-Pinilla 2005; Erickson et al. 2012). Synaptogenesis is one separating factor between skill and endurance training adaptations, and can partly explain the different motor cortex plasticity changes in the present study. The increased number of synapses caused by skill training facilitates a stronger PAS effect since excitability changes occur in synapses.

However, other factors are also likely to contribute to insignificant plasticity in the task-related muscles of the endurance group because Cirillo et al. (2009) found heightened plasticity in the task-unrelated, abductor pollicis brevis muscle in endurance-trained subjects compared to physically inactive subjects. Endurance-trained rats showed improved cognitive learning, but not motor learning, when the motor learning task was task-specific to running (Wikgren et al. 2012). It seems that the lower motor cortex plasticity induced by endurance training might be restricted to task-related lower limb muscles, although the reason for this remains unknown. It should be noted that during the time course of long-term training, the cortical activation decreases and possibly shifts from cortical towards subcortical motor regions as movements become more automatic (Wu, Kansaku & Hallett 2004; Floyer-Lea & Matthews 2004; Picard, Matsuzaka & Strick 2013; Debarnot et al. 2014). It seems that extensive practice over a long period of time leads to less of the neural network being activated for movement control, and this reduction in use may also lead to lower plasticity of the particular brain area.

MEP amplitude increase did not reach statistical significance in the non-active control group, which is in line with the study of Cirillo et al. (2009), where sedentary subjects were not facilitated after PAS intervention. In experiment I, where the subjects represented a wide range of sports backgrounds, eight subjects were sufficient to show significant changes after an identical PAS intervention. Even the change in excitability (baseline MEP/post MEP) of the control group did not differ significantly from the sport groups; this is a crucial finding, in that the training adaptations of skill and endurance groups go in opposite directions from the control group.

Task-specific changes in MEPs of lower limb muscles have previously been reported in a study by Beck et al. (2007), where four weeks of balance training resulted in decreased MEP amplitude, and ballistic strength training increased MEP recruitment in tibialis anterior muscle. Balance training involves fast automatic adjustments during stabilization of stance while ballistic strength training consists of self-initiated voluntary movements. Similar to this study, Beck et al. (2007) found no differences in spinal excitability between the training groups either before or after the intervention. These results for balance and bal-

listic strength training were further extended by Schubert et al. (2008), who found a significant interaction of task and training; motor cortical influence was reduced during the trained task but increased during a non-trained motor task, which supports the previously mentioned findings about reduced cortical activity during the time course of training (Wu, Kansaku & Hallett 2004; Floyer-Lea & Matthews 2004; Picard, Matsuzaka & Strick 2013; Debarnot et al. 2014).

6.2.2 Subcortical factors

It is noteworthy that possible training adaptations in supraspinal locomotor centers like the cerebellum and brainstem are largely unknown. An animal study by Klintsova et al. (2004) showed different expression patterns of neurotrophic factors in the cerebellum between skill- and endurance-trained rats. BDNF increased in expression across the first training week in the cerebellar molecular layer in both groups while it remained elevated after 14 days of training in the skill group but not in the endurance group. The cerebellum is known to affect PAS-induced plasticity (Hamada et al. 2012), thus training adaptations in the cerebellum might also have affected the current results.

SLSR remained unchanged after the PAS intervention in both groups suggesting a lack of changes at the spinal level. In this study, SLSR of the skill group was not significantly different from the endurance group. Previous studies by Perot et al. (1991) and Vila-Cha et al. (2012) showed increased H-reflex responses along with increased endurance capacity following endurance training. The suggested mechanism was increased motor neuron excitability and decreased presynaptic inhibition of Ia terminals. In addition, an animal study reported increased BDNF expression in the spinal cord of rats following 28 days of treadmill walking (Macias et al. 2007). Contrary to our results, Nielsen et al. (1993) reported lower H-reflex excitability in a group of ballet dancers compared with endurance trained athletes. The suggested mechanism was increased chronic co-contraction of lower limb muscles during ballet training, leading to increased pre-synaptic inhibition. The reason for inconsistent results might be that SLSRs are known to be less susceptible to presynaptic Ia inhibition than H-reflexes (Morita et al. 1998). Studies of skill training have mainly focused on cortical areas, so spinal cord adaptations are largely unknown (Adkins et al. 2006).

6.2.3 Factors to consider

As a fixed TMS intensity (120% RMT) was used throughout experiment II to assess possible changes within the corticospinal tract, it cannot be excluded that other intensities could have resulted in increased plasticity in the endurance group. However, the soleus muscle is a unique muscle consisting mainly of slow twitch fibers, which are recruited progressively from rest to a contraction strength close to 95% of MVC (Oya, Riek & Cresswell 2009). Thus the recruitment of motor units should continue well beyond the intensity of 120% RMT in both subject groups and allow further increases in soleus MEP amplitudes.

Conversely, higher intensities may have led to an even wider difference in the plastic changes in the motor cortex, since endurance training increases the type 1, low-threshold myosin heavy chain proportion in skeletal muscle (Ahtiainen et al. 2009). In addition, there were no significant differences in MEP amplitudes at an intensity of 120% RMT before PAS between the groups, suggesting that this is unlikely to be a major contributor to the results.

A number of factors are known to influence PAS-induced plasticity, including history of synaptic activity, age, attention to the procedure, time of day of the experiments, gender, genetics and regular exercise (Ridding & Ziemann 2010). Most of these factors were matched between the skill and endurance groups except for gender, genetics and the type of regular exercise. There is some evidence that females are somewhat more responsive to non-invasive brain stimulation (Ridding & Ziemann 2010). However, there were only 2 less males in the skill group and when only including females in the analysis, the result remains the same. There was still a significant change in soleus MEP amplitudes in the 12 skilled female subjects and no significant change in the 10 endurance trained females. It is not known whether these skill and endurance trained athletes shared different BDNF-polymorphisms, which are known to have a different influence on PAS-induced plasticity. However, it is known from numerous studies that endurance trained subjects typically have enhanced plasticity in task-unrelated brain areas (Kramer & Erickson 2007; Cirillo et al. 2009; Erickson et al. 2009; Thomas et al. 2012), suggesting that it is very unlikely that the endurance athletes in the current study would carry unfavorable BDNF-polymorphisms.

Taking all of these arguments together, it seems reasonable to assume that a large proportion of the differential modulation of motor cortex plasticity is due to the different training backgrounds of the two groups.

6.3 Functionality of PAS intervention (III)

In experiment III, PAS induced associative plasticity changes in the cortical projections to the resting soleus. Contrary to the hypothesis, fatigue resistance during a 15 second sustained maximal isometric contraction did not change following PAS interventions. Thus, functionality of PAS interventions was not evident with the current experimental design. However, PAS-induced excitability changes correlated significantly with changes in fatigue resistance, indicating that subjects whose excitability increased also demonstrated improved fatigue resistance and vice versa. In addition, among responders, fatigue resistance showed a significant interaction of time and group. This suggests that PAS might have slightly affected central fatigue during short maximal contractions.

6.3.1 Neural correlates

The results of the present study indicate that associative modulation of excitability to the cortical projections of soleus was achieved after PAS_{LTP} and PAS_{LTD} interventions with constant ISIs. Accordingly, PAS_{LTP} induced enhancement of synaptic transmission whereas PAS_{LTD} induced weakening of synaptic transmission. There was no significant increase in MEP size at post0 after PAS_{LTP}, but there was a significant increase at post15. A similar trend has been seen in previous studies that have shown an increasing PAS_{LTP} effect over time (Prior & Stinear 2006; Stefan et al. 2000). On the contrary, MEP size decreased significantly at post0 but not at post15 after PAS_{LTD}, which is in line with a previous study by Di Lazzaro et al. (2011), where a significant effect was achieved immediately after but not 30 min after PAS_{LTD}. MEPs were not increased in the active conditions following PAS_{LTP}; on the contrary, MEPs were significantly decreased in the 50% of MVC condition after PAS_{LTP}. This is an interesting result since, to our knowledge, PAS-induced effects have not been measured at such high contraction levels, and a significant decrease in MEP size after PAS_{LTP} has not been reported before. However, there are studies showing no change in MEPs during slight contractions (Stefan et al. 2000; Stefan, Wycislo & Classen 2004). As stated in experiment I, the interpretation of the effect of PAS on active muscles is more complicated, and PAS_{LTP} might decrease the level of effort needed to produce force, resulting in decreased or unchanged MEPs in active muscle.

The most stable and informative SP is measured at a contraction level of 50% of MVC (Säisänen et al. 2008). It has been shown that the initial part of the SP is influenced by spinal circuitries, whereas the latter part to a higher extent represents the state of cortical inhibitory interneurons, which are mediated by $GABA_B$ receptors (Ziemann 2004). SP remained unchanged in the current study, which is in line with the study of Di Lazzaro et al. (2011) that reported unchanged SPs after PAS_{LTP} or PAS_{LTD} when MEPs were elicited during contraction at 50% of MVC. H/M_{max} ratios remained unchanged after the interventions, suggesting a lack of changes at the spinal level, which has also been shown in previous PAS studies (Stefan et al. 2000; Wolters et al. 2003).

6.3.2 Functional implications

A 15 s sustained maximal isometric contraction induced significant force loss on all occasions but fatigue resistance did not change after the interventions. This is in line with a previous study by Milanovic et al. (2011), which showed no changes in a fatigue test involving sustained isometric contractions at 50% of MVC after $PAS_{LTP.}$ However, a significant relationship was found between the change in excitability of the cortical projections and change in fatigue resistance in the present study, which was not reported in the study by Milanovic et al. (2011). One possible reason for this difference could be that they used the duration of submaximal contraction as an indication of fatigue, which may involve a greater peripheral component of fatigue compared to the present protocol

(Lentz & Nielsen 2002). In addition, endurance time and changes in maximal capacity to produce force might provide information about different processes induced by the exercise (Vøllestad 1997). Thus, endurance time may not directly correlate with motor cortex excitability. Benwell et al. (2006) found a significant reduction in the rate of force loss during a ten second MVC of hand muscles after increasing corticomotor excitability using a spike-timing dependent repetitive TMS intervention.

In the current study the lack of change in fatigue resistance at the group level may be due to considerable inter-individual variability in PAS-induced plasticity within the groups. The use of constant ISIs might be one reason for the relatively high inter-individual variability (SD = ± 108% and 123%) observed in this study. Nevertheless, the present values are comparable to the variability (SD = ± 105% and 52%) and number of responders (12 responders from 16 subjects) obtained in experiment I, where the ISI was optimized to the individual SEP latency. Since an earlier study by Mrachacz-kersting et al. (2007) found significant LTP during a 10 ms time window with constant ISIs of 45-55 ms, the constant ISI of 50 ms used here should be sufficient to induce LTP in most subjects. The optimal ISI to induce LTD in the leg area has not been studied but an ISI of 20 ms is mostly used. Stinear and Hornby (2005) reported a similar number of LTD responders to the present study; 12 responders from 14 subjects. Previous studies have also reported wide variability of PAS-induced effects between subjects, which has been associated with differences in brain anatomy, genes, and training background (Ridding & Ziemann 2010). Because of differences in brain anatomy, TMS can preferentially activate different neuronal circuits in different subjects (Sakai et al. 1997), causing variability in the PAS-induced effects. The genetic polymorphisms of neurotrophins can influence the induction of plasticity (Ridding & Ziemann 2010). However, there is only one BDNF-polymorphism that has been shown to limit PAS-induced motor cortex plasticity (Cheeran et al. 2008). Furthermore, physical activity level has been found to affect motor cortex plasticity (Cirillo et al. 2009).

When only responders were included in the analysis, there was a significant interaction of time and group in fatigue resistance. Nonetheless, the change in fatigue resistance was not significant within the groups. This suggests that PAS affects fatigue resistance among PAS responders and supports the result of a significant correlation. ITT was conducted in order to reveal possible changes in neural deficit induced by PAS_{LTP} or PAS_{LTD} at the end of fatiguing contractions. No significant differences were found, suggesting that there were no differences in central fatigue after the interventions. However, since we found a significant correlation between changes in MEP and fatigue resistance, small changes in central fatigue may have occurred within the variation of ITT. Low sensitivity of the ITT method has been reported at maximal contraction intensities (Taylor 2009).

6.3.3 Candidate mechanisms behind PAS and fatigue

Intracortical neural circuits through which the effects of PAS and fatigue emerge have been investigated with single and paired-pulse TMS in several previous studies (Carson & Kennedy 2013; Gruet et al. 2013; Taylor & Gandevia 2001). However, as different studies have used different interventions and target muscles, the results are difficult to interpret. Prolongation of SP has been observed after various fatiguing exercises including sustained MVC of soleus muscle (Iguchi & Shields 2012; McKay et al. 1996). Since prolongation of SP is smaller after cervicomedullary stimulation-induced MEPs, additional inhibition at the cortical level has been suggested to occur (Levenez et al. 2008; Taylor et al. 1996). In addition, Hilty et al. (2011) showed that central projections of group III-IV muscle afferents may facilitate a fatigue-induced increase in SP. Therefore, it seems that $GABA_B$-mediated intracortical inhibitory circuits have a role in the development of central fatigue. Since SP duration remained unchanged after PAS interventions in this study, it seems that, at least to some extent, fatigue and PAS affect different cortical interneurons. This could explain why we found no significant effect of PAS on fatigue resistance. However, among PAS responders, the PAS-induced LTP might have compensated for fatigue-induced inhibition in the motor cortex and thus affected fatigue resistance. In addition, PAS-induced LTP/LTD-like plasticity has been shown to be accompanied by other neuronal circuits in the cerebral cortex (Carson & Kennedy 2013), which might have contributed to the observed significant correlation. For example, LAI, which is also $GABA_B$-mediated, is decreased following PAS_{LTP} (Meunier et al. 2012; Russmann et al. 2009) and might increase motor drive to the exercising muscle during fatiguing tasks. LAI reflects the activity of somatosensory inputs, and is obtained when the interval between peripheral afferent stimulation and subsequent TMS is in the region of 200 ms (Carson & Kennedy 2013).

6.4 Modified PAS intervention; PAS_{reflex} (IV)

On average the soleus IO-curve slope did not change immediately after but increased significantly 30 min after PAS_{reflex}, where there were ten responders out of 14 subjects. Mean MEP amplitudes behaved correspondingly; significantly increasing at post30 and significantly different between the post0 and post30 measurements. There were no changes in slopes or mean MEPs in the PAS_{contr} group. H_{max}/M_{max} ratio did not change in either of the groups. Thus, although the asynchronous natural stretch reflex volley did not decrease the variability of PAS-induced effects as hypothesized, it produced similar results compared to traditional PAS protocols.

6.4.1 The effect of PAS_{reflex}

Similarly to experiment I, the slope of the soleus IO-curve was significantly increased only at post30. However, there was a significant increase in the mean MEPs immediately after the traditional PAS intervention as applied in experiment I, and no difference in the slopes of the IO-curves between post0 and post30 measurements (P = 0.68), while mean MEPs and the slopes of the IO-curve were significantly different between post0 and post30 after PAS_{reflex}. It is likely that the natural stretch reflex combined with TMS induced more multifactorial effects in the motor cortex, for which there may be several possible reasons. PAS_{reflex} could have produced a mixture of effects including a short lasting suppressive effect and a longer lasting excitatory effect. One explanation is that the longer duration of afferent feedback arising from the imposed stretch reflex, when combined with TMS, led to short-term synaptic depression, for example due to depletion of neurotransmitter vesicles, which has been observed shortly after neuronal stimulation (Abrahamsson, Gustafsson & Hanse 2005). Since neurotransmitter vesicles can recover within one minute (Armbruster & Ryan 2011), TMS pulses may have been sufficient to maintain the depletion at post0.

Another possibility is that the afferent volley induced by the stretch reflex caused complex interactions between different neuronal populations within the motor cortex. The optimal ISI to induce LTP-like plasticity in the soleus area of the motor cortex with the traditional PAS intervention was shown to be the afferent conduction time plus 18 ms to account for the central processing delay (I). The central processing time of 18 ms and the afferent conduction time of 44 ms estimated in this study correspond to values of 15 ms and 47 ms obtained by Petersen et al. (1998), where the SEP was evoked by the stretch reflex. However, as type II afferents are more likely to be activated by the stretch reflex than by electrical stimulation (Cussons, Hulliger & Matthews 1977; Burke, Gandevia & McKeon 1983; Simonetta-Moreau et al. 1999), it cannot be ruled out that the afferent volley elicited by slower type II afferents arrived at the motor cortex after the TMS stimulus. Consequently, while the first volley arising from type I afferents might have induced LTP-like plasticity, the later volley from type II afferents might have induced LTD-like plasticity at different sets of synapses. Since not all muscle spindles have type II afferents, the longer lasting LTP-like plasticity could be expected. Thus, the optimal ISI for the PAS_{reflex} intervention might be different. In addition, cutaneous afferents may contribute to the current results since they have been shown to be activated by stretch reflex (Burke, Gandevia & McKeon 1983). In humans type I afferents and cutaneous afferents might have same conduction velocity (Macefield, Gandevia & Burke 1989). However, in the absence of invasive neuronal investigations, any hypothesis about the precise nature of PAS_{reflex}-induced effects remains speculative.

There were no changes in any of the parameters in the PAS_{contr} group, suggesting that the stretch reflex alone did not affect excitability. However, the after-effects were not significantly different between the groups. H_{max}/M_{max} ratios remained unchanged after the interventions, suggesting a lack of changes

at the spinal level, which has also been shown in previous PAS studies (Stefan et al. 2000; Wolters et al. 2003).

6.4.2 Other modified PAS interventions

The traditional PAS intervention has been modified by Thabit et al. (2012), who replaced the artificial stimuli with a natural physiological activation by pairing movement with TMS. Thus, when the MRCP preceded TMS, increases in MEP amplitudes were obtained immediately after and 15 minutes after the intervention but not 30 minutes after. Mrachacz-Kersting et al. (2012) combined electrical stimulation with motor imagery and found significant increases in motor cortical output immediately after the intervention. Sowman et al. (2014) paired auditory stimuli with TMS and demonstrated enhancement of motor cortex excitability immediately after and 15 min after the intervention. Contrary to the present results, these studies showed significant plasticity right after the cessation of the interventions, though the number of responders was not reported. McNickle and Carson (2015) paired short trains of peripheral afferent stimulation with burst of high frequency transcranial alternating current stimulation and compared the effect of two different modes of afferent stimulation on corticospinal excitability. They found that afferent stimulation by muscle tendon vibration induced more reliable elevations of excitability than afferent stimulation by electrical stimulation but the two conditions did not differ significantly. Both, type I and II afferents have been shown to respond to muscle vibration (Burke et al. 1976) while also higher sensitivity of Ia-afferents has been reported (Roll, Vedel & Ribot 1989).

7 MAIN FINDINGS AND CONCLUSIONS

The findings of this thesis will help to understand the behavioural and neural signals that drive plasticity of the lower limb area of the motor cortex. Thus, the results will have important practical significance for guiding the development of novel therapeutic interventions for the treatment of various brain disorders and also for the development of optimal motor learning interventions in school and sport settings. Specific findings and conclusions of the four experiments are as follows:

1) This was the first study to show PAS-induced LTP-like effects in the antigravity soleus muscle. The optimal ISI to induce LTP-like plasticity in the cortical projections to the soleus muscle was SEP latency plus 18 ms. The PAS effect was long-lasting, input-specific and supraspinal in origin. It is important for future paired stimulation protocols to establish the optimal ISI based on individual SEP latencies (P32 + 18 ms) as it differed remarkably from the optimal ISI established for the hand area (N20 + 5 ms). The good level of repeatability of the PAS effects supports the possibility of its use for rehabilitation purposes and for studying motor control adaptation.

2) PAS intervention revealed differential modulation of motor cortex plasticity in skill- and endurance-trained athletes. Significant LTP-like plasticity was demonstrated only in skill-trained athletes. This finding suggests that in the long-term, versatile skill training might induce preferable adaptations in the task-related areas of the motor cortex and thus might enhance rehabilitation and motor learning of muscle groups involved in the exercise.

3) On average fatigue resistance did not change after PAS_{LTP} or PAS_{LTD}. However, changes in MEP peak-to-peak amplitudes after the PAS interventions correlated with changes in fatigue resistance, and among responders fatigue resistance showed a significant interaction of

time and group. This suggests that PAS might have slightly affected central fatigue during short maximal contractions. Therefore, PAS might have implications for improving performance in rehabilitation settings.

4) The effect of PAS_{reflex} was interesting since the change in excitability was different immediately after and 30 min after the intervention. Most likely, there were several phenomena taking place in the motor cortex or elsewhere in subcortical structures. In addition, ten responders from 14 subjects at post30 exhibited a significant increase in the slope of the IO-curve. Therefore, although the natural stretch reflex volley did not decrease the variability of PAS effects, it did produce similar results compared to traditional PAS protocols.

YHTEENVETO (FINNISH SUMMARY)

Aivot muovautuvat läpi elämän ajatusten, tekojen ja kokemusten mukaan ja tätä aivojen kykyä muovautua kutsutaan plastisuudeksi. Ymmärrys aivojen plastisuudesta ja siihen vaikuttavista tekijöistä on erityisen tärkeää opettajille, valmentajille ja kuntouttajille, jotka ovat kiinnostuneita oppimisesta ja uudelleen oppimisesta vammautumisen jälkeen. Mitä plastisempi liikeaivokuori on, sitä paremmin ihminen oppii uusia liikeratoja ja kuntoutuu vammautumisen jälkeen. Liikeaivokuoren jalkojen alueen plastisuudesta ei tiedetä vielä paljoakaan, vaikka sillä on merkittävä rooli ihmisen liikkumisessa ja siten motorisessa oppimisessa.

Erilaisia keinotekoisia aivostimulaatioprotokollia on kehitetty tuottamaan aivoihin plastisuutta, joka kestää minuuteista tunteihin. Parillinen assosiatiivinen stimulaatio (PAS) keksittiin vuosisadan vaihteessa neurologi Hebb:n teoriaan pohjautuen, jonka mukaan kahden hermon lähes yhtäaikainen aktivoituminen johtaa samojen hermojen synapsisen yhteyden vahvistumiseen. PAS:ssa sensorisen hermoradan sähköstimulus ja motorisen aivokuoren magneettistimulus kohtaavat lähes yhtä aikaa aivokuorella ja näitä parillisia stimuluksia annetaan yleensä 200 kertaa. Riippuen stimulusten välisestä aikaerosta synapsista yhteyttä voidaan joko herkistää tai heikentää. PAS:a voidaan käyttää sekä liikeaivokuoren tutkimiseen että kuntoutukseen.

Optimaalinen PAS interventio on löydetty liikeaivokuoren käsien alueelle, mutta samalla kaavalla toteutettu interventio jalkojen alueelle ei ole toiminut. Tämän väitöskirjatutkimuksen tarkoituksena oli määrittää optimaalinen PAS interventio leveän kantalihaksen alueelle liikeaivokuorella. Näin kehitetyn PAS:n avulla tutkittiin seuraavaksi erilaisen liikuntataustojen vaikutuksia motorisen aivokuoren plastisuuteen. PAS:n toiminnallisuutta tutkittiin kolmannessa osatutkimuksessa 15 sekunnin maksimaalisen voimaväsytystehtävän avulla. Neljännen osatutkimuksen tarkoitus oli modifioida PAS interventiota korvaamalla sähköstimulus luonnollisella venytysrefleksi vasteella ja näin mahdollisesti vähentää PAS-tulosten vaihtelevuutta. Optimaalinen PAS interventio jaloille erosi käsille käytetyistä interventiosta sillä kahden stimuluksen välinen aikaero oli huomattavasti pidempi (somatosensorisen herätepotentiaalin latenssi + 18 ms). Kehitetyllä PAS protokollalla havaittiin että taitourheilijoilla oli merkittävästi plastisempi jalkojen alueen liikeaivokuori verrattuna kestävyysurheilijoihin. Tämä johtuu mahdolliseksi erilaisista harjoittelun aiheuttamista adaptaatioista liikeaivokuorella. Väsymysmäärä ei muuttunut merkittävästi PAS:n jälkeen, joten toiminnallisuutta ei näytetty toteen kolmannessa tutkimuksessa. Kuitenkin PAS:n jälkeiset muutokset väsymyksessä ja aivokuoren herkkyydessä korreloivat merkittävästi keskenään ja näin ollen on mahdollista että PAS:lla on vaikutuksia samoihin mekanismeihin kuin sentraalisella väsymyksellä. PAS tulosten vaihtelevuus ei pienentynyt modifioidun PAS interventionavulla.

Kaiken kaikkiaan nämä tulokset antavat tärkeää tietoa motorisen kontrollin adaptaatio mekanismeista ja liikeaivokuoren muovautuvuuteen vaikuttavis-

ta tekijöistä. Tämän pohjalta voidaan määrittää parempia motorisen oppimisen ohjelmia esimerkiksi koululiikuntaan, urheiluvalmennukseen ja aivokuntouttamiseen. Tuloksilla on tärkeä käytännön merkitys oppimisen kannalta lapsille, urheilijoille, vanhuksille ja potilaille.

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ORIGINAL PAPERS

I

THE OPTIMAL INTERSTIMULUS INTERVAL AND REPEATABILITY OF PAIRED ASSOCIATIVE STIMULATION WHEN THE SOLEUS MUSCLE IS TARGETED

by

Kumpulainen Susanne, Mrachacz-Kersting Natalie, Peltonen Jussi, Voigt Michael & Avela Janne, 2012

Experimental Brain Research Sep;221(3):241-9

II

DIFFERENTIAL MODULATION OF MOTOR CORTEX PLASTICITY IN SKILL- AND ENDURANCE-TRAINED ATHLETES

by

Kumpulainen Susanne, Avela Janne, Gruber Markus, Bergmann Julian, Voigt Michael, Linnamo Vesa & Mrachacz-Kersting Natalie, 2015.

European Journal of Applied Physiology May;115(5):1107-15.

III

THE EFFECT OF PAIRED ASSOCIATIVE STIMULATION ON FATIGUE RESISTANCE

by

Kumpulainen Susanne, Peltonen Jussi, Gruber Markus, Cresswell Andrew, Peurala Sinikka, Linnamo Vesa & Avela Janne, 2015.

Neuroscience Research Jun;95:59-65

Neuroscience Research 95 (2015) 59–65

Contents lists available at ScienceDirect

Neuroscience Research

journal homepage: www.elsevier.com/locate/neures

The effect of paired associative stimulation on fatigue resistance

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ARTICLE INFO

Article history:
Received 22 November 2014
Received in revised form 22 January 2015
Accepted 29 January 2015
Available online 7 February 2015

Keywords:
Fatigue
Central fatigue
Motor cortex
Transcranial magnetic stimulation
Paired associative stimulation

ABSTRACT

Paired associative stimulation (PAS) is a non-invasive stimulation method developed to induce bidirectional changes in the excitability of the cortical projections to the target muscles. However, very few studies have shown an association between changes in motor evoked potentials (MEP) after PAS and behavioral changes in healthy subjects. In the present study we hypothesized that the functional relevance of PAS can be seen during fatiguing exercise, since there is always a central contribution to the development of fatigue. Transcranial magnetic stimulation was applied over the motor cortex to measure changes in the MEPs of the soleus muscle before and after PAS. Furthermore, fatigue resistance was tested during 15 s sustained maximal isometric contractions before and after PAS. On average, fatigue resistance did not change after PAS, however the change in excitability correlated significantly with the change in fatigue resistance. *Discussion*: Functionality of PAS intervention was not demonstrated in this study. However, the observed relationship between excitability and fatigue resistance suggests that PAS might have affected central fatigue during short maximal contractions.

1. Introduction

Paired associative stimulation (PAS) is a non-invasive method developed to induce bidirectional changes in the excitability of the cortical projections to the target muscles. PAS combines electrical stimulation of a peripheral somatosensory nerve with transcranial magnetic stimulation (TMS) over the contralateral motor cortex. Depending on the interstimulus interval (ISI), PAS can produce either long-term potentiation (LTP) – or long-term depression (LTD) – like plasticity in the target synapse (Kumpulainen et al., 2012; Stefan et al., 2000; Wolters et al., 2003), showing properties such as rapid onset, associativity, duration, specificity, and NMDA-receptor dependence (Ziemann et al., 2008). Thus, spike-timing dependent plasticity is considered the most likely mechanism behind PAS (Stefan et al., 2000; Wolters et al., 2003). PAS and motor training have been shown to share common neural mechanisms, which suggests that PAS can be used as a test for functionally relevant neuronal circuits within the motor cortex (Jung and Ziemann, 2009; Stefan et al., 2006; Ziemann et al., 2004). However, very few studies have shown functionality of PAS-induced excitability changes in healthy subjects (Frantseva et al., 2008; Jung and Ziemann, 2009; Rajji et al., 2011).

Functional relevance of PAS can probably be seen during fatiguing exercises since it is well known that both central and peripheral factors contribute to the development of fatigue (Gandevia, 2001). Fatigue can be defined as any exercise-induced reduction in the ability of a muscle to generate maximal force or power (Gandevia, 2001). Central fatigue refers to processes proximal to the neuromuscular junction and peripheral fatigue to processes at or distal to it (Gandevia, 2001). The relative contribution of the central and peripheral components depends on the intensity and duration of the fatiguing exercise. Short maximal sustained contractions have been shown to have a substantial central contribution to the development of fatigue (Gandevia et al., 1996; Hunter et al., 2006, 2008; Lentz and Nielsen, 2002; Szubski et al., 2007; Taylor et al., 1996, 1999). Central fatigue has been defined as a progressive reduction in the voluntary activation of a muscle during exercise and it can originate at both spinal and supraspinal levels (Gandevia, 2001). Previous studies suggest that central fatigue at least partially originates from inadequate cortical drive to the motor neurons (Gandevia, 2001; Hunter et al., 2006, 2008).

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http://dx.doi.org/10.1016/j.neures.2015.01.015
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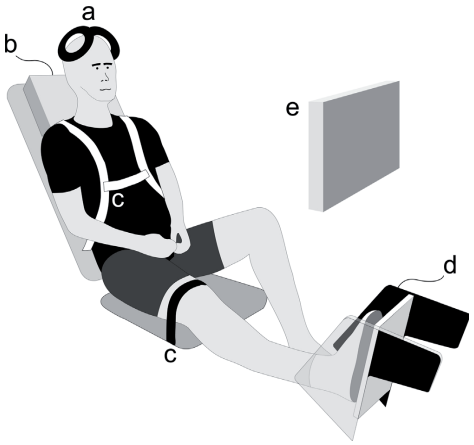

Fig. 1. Schematic of the measurement setup in the ankle dynamometer. (a) A stimulating coil was placed and secured over the left hemisphere and (b) subject's neck was comfortably supported by a head rest. (c) Body movement was restricted with seat belts and a knee strap. (d) Right foot was installed to a force pedal and (e) the force was displayed on a computer screen in front of the subject.

Because PAS can be used to systematically alter the responsiveness of neurons in the primary motor cortex, the current study was designed to investigate the effect of two different PAS interventions on fatigue resistance during 15 s sustained maximal isometric contractions. The PAS interventions targeted the soleus muscle (SOL) as this is an important antigravity muscle during standing and a major contributor to force production during the impact phase of walking and running (Ishikawa et al., 2005). It was hypothesized that after PAS_{LTP} fatigue resistance would increase whereas after PAS_{LTD} fatigue resistance would decrease.

2. Materials and methods

2.1. Ethical approval and subjects

Thirty healthy subjects volunteered for the study and were divided into two groups: PAS induced LTP-like plasticity group (PAS_{LTP}; 9 females and 6 males, 25 ± 4 years, 62 ± 10 kg, 168 ± 11 cm) and LTD-like plasticity group (PAS_{LTD}; 9 females and 6 males, 25 ± 4 years, 63 ± 7 kg, 168 ± 7 cm). Subjects were blinded to the PAS intervention they were undergoing and thus the PAS_{LTP} and PAS_{LTD} were considered as each other's control. None of the subjects had any history of neuromuscular or orthopedic diseases and all subjects were naïve to the experiments. Before testing, subjects were informed about the procedures and gave written consent. The study was approved by the ethics board from the University of Jyväskylä and was performed in conformity with the latest revision of the declaration of Helsinki.

2.2. Experimental design

Participants were positioned on a custom built ankle dynamometer (University of Jyväskylä, Finland) with the hip at 110°, the knee in an extended position at 180°, the ankle at 90° and the right foot resting on a pedal (Fig. 1). A seat belt restricted movement of the upper body and straps secured the right leg and foot. Hands were resting in the lap during all measurements. Prior to the measurements, the participants performed three maximal isometric plantar flexions with a 3 min rest interval between trials. The highest force value was considered as the maximal voluntary contraction (MVC). The experimental protocol is shown in Fig. 2; the upper panel represents the main protocol and the lower panel the additional procedures for sub-groups. The main protocol included transcranial magnetic stimulation (TMS) to measure changes in the motor evoked potentials (MEPs) of SOL before (pre) and twice after the PAS intervention; immediately after (post0) and 15 min after (post15) PAS. The fatigue resistance tests were performed before (pre) and after all TMS measurements (post15). To test for changes at the spinal level, SOL Hoffman reflexes (H-reflexes) were elicited in a subgroup at the pre, post0 and post15 measurements. Fatigue, MEP and PAS procedures were conducted so that fatigue would not affect MEPs or PAS intervention. To avoid possible fatigue effects there was at least 40 min between the last fatiguing contraction and the PAS intervention in the pre measurements. In the post measurements, fatigue resistance was measured after all the MEPs were recorded but within 25 min of the PAS protocol, because LTP/LTD effects have been shown to last for a minimum of 30 min (Kumpulainen et al., 2012; Mrachacz-Kersting et al., 2007; Stefan et al., 2000; Wolters et al., 2003).

2.3. Recordings

For electromyographic (EMG) measurements, a pseudomonopolar electrode placement protocol was used where one surface electrode of a pair (Unilect, Ag/AgCl, Unomedical Ltd., Redditch, UK) was placed on the right SOL and the other over a bony surface of the tibia. A ground electrode was placed over the lateral malleolus (Hoffman et al., 2009). The pseudomonopolar setup allowed MEPs of higher amplitude to be recorded, which in turn also decreased the intensity of the stimulus needed to evoke a detectable MEP. Prior to electrode placement, the skin was shaved, abraded and cleaned with alcohol to reduce resistance below 5 kΩ. EMG signals were amplified (100×), band-pass filtered (10–1000 Hz) and sampled at 5 kHz (Neural Systems NL 900D and NL 844, Digitimer Ltd., Hertfordshire, UK). EMG data and reaction forces from the pedal were collected with a computer via 16-bit AD converter (CED power 1401, Cambridge Electronics Design Limited, UK) and stored for later analysis.

2.4. Procedures

A rectangular current pulse with a duration of 1 ms was delivered to the common tibial nerve using a constant-current stimulator (DS7AH, Digitimer Ltd., Hertfordshire, UK) for the PAS protocol in addition to evoking H-reflexes and maximal M-waves (M_{max}). A circular cathode with a pickup area of 77 mm^2 (Unilect short-term ECG Electrodes, Ag/AgCl, Unomedical Ltd., UK) was placed over the tibial nerve on the popliteal fossa and an oval shaped (5.08 cm/10.16 cm) anode (V-trodes neurostimulation electrodes, Mattler Electronics Corp., USA) was placed above the patella. Motor threshold (MT) was defined as the minimal intensity that induced a visually identifiable muscle twitch in SOL. To quantify reliable M_{max}, supramaximal stimulus intensity was used, being 150% of the current needed to elicit maximal stimulus response.

For the fatigue test subjects were instructed to produce their maximal isometric plantar flexion force and maintain it for 15 s (Fig. 3), during which the force declined toward the end. Verbal encouragement was given throughout the trial. At the end of the fatiguing contraction, neural deficit was estimated using the interpolation twitch technique (ITT); a supramaximal (M_{max} intensity) double pulse with 10 ms interval was delivered to the tibial nerve to quantify possible increment in force (superimposed twitch).

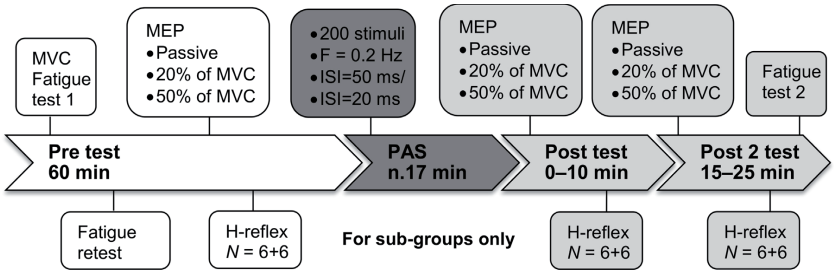

Fig. 2. The experimental protocol. Timeline's upper panel represents the main protocol and lower panel additional procedures for sub-groups only. TMS, transcranial magnetic stimulation; MVC, maximal voluntary contraction; ISI, inter-stimulus interval; PAS, paired associated stimulation.

Immediately following the sustained MVC, the same supramaximal double pulse was delivered to the relaxed muscle to quantify resting twitch. Neural deficit indicates insufficient central drive to the motor neurons and is attributable to central fatigue. Test–retest repeatability was determined by repeating the fatigue resistance test prior to intervention in ten subjects. There was a mandatory break of 10 min between the test and the retest to reduce the possibility of any fatigue effect. It has been shown in previous studies that 10 s sustained maximal force is recovered within 5 min (Benwell et al., 2006).

TMS was delivered using a mono-pulse Magstim 2002 stimulator with a 9 cm double batwing coil (Magstim, Whitland, UK). The optimal stimulus site for SOL was usually located 1 cm lateral and 1 cm posterior to the vertex. A custom-made coil holder and rubber straps were used to fix the coil firmly to the head. The position of the coil was marked on a closely fitted cap worn by the subjects. The cap also protected the hair and head from cold spray (PRF101, Taerosol, Kangasala, Finland), which was used to prevent the coil from overheating. Resting motor threshold (RMT) was defined as the lowest stimulus intensity required to elicit a MEP with a peak-to-peak amplitude of 50 μV in three out of five consecutive trials. Stimulus intensity was then set to 120% of RMT and this intensity was used throughout the experiment. TMS was delivered ten times to passive muscle and five times to active muscle at 20% of MVC and 50% of MVC. It has been previously demonstrated that MEP responses increase progressively from rest to a contraction strength of 80% of MVC in the SOL (Oya et al., 2008). As the force–time curve was displayed on the screen in front of the subjects, they were able to reach their target level with an accuracy of ±2% during the active conditions.

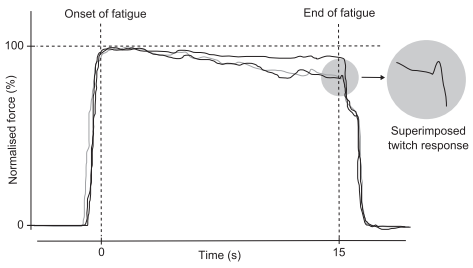

Fig. 3. Example figure of the fatigue resistance test. Force traces are from one representative subject. Two united thin lines demonstrate the test-retest repeatability prior to intervention (fatigue test 1 = black line, fatigue retest = gray line). The thicker black line represents the post-intervention test. Force (*F*) is normalized to maximal voluntary force.

H-reflexes were measured in sub-groups of six subjects from both PAS_{LTP} and PAS_{LTD} groups during rest. M_{max} was elicited before all H-reflex measurements. Then 10 submaximal stimuli were applied with a current intensity that evoked peak-to-peak M-wave responses of 20 ± 5% of M_{max} to quantify the H-reflex.

PAS consisted of a single electrical stimulation delivered to the tibialis nerve at 150% of MT and a single TMS pulse at 120% of RMT. ISIs to induce LTP- and LTD-like plasticity were selected based on previous experiments. A constant ISI of 50 ms was used for the PAS_{LTP} group (Kumpulainen et al., 2012; Mrachacz-Kersting et al., 2007; Poon et al., 2008) and constant ISI of 20 ms was used for the PAS_{LTD} group (Jayaram and Stinear, 2008; Poon et al., 2008; Stinear and Hornby, 2005). A total of 200 pairs of stimuli were applied at a rate of 0.2 Hz. To optimize the PAS effect, subjects were asked to produce 5% of MVC plantar flexion with their right leg during the PAS protocol (Mrachacz-Kersting et al., 2007).

2.5. Data analysis and statistics

Commercially available software (Spike2, CED, Cambridge, England) was used for all offline analyses. Fatigue resistance was calculated by dividing the average force during the 15 s isometric contraction by the individual MVC. Thus fatigue resistance of 100% corresponds to the theoretical situation where the initial MVC force is maintained for the entire 15 s. Amplitudes of the superimposed twitch and resting twitch were measured and the ratio between them was calculated to estimate neural deficit. To determine the efficacy of PAS, peak-to-peak MEP amplitudes were measured from SOL and averaged. Spinal efficacy was determined by taking H-reflex and M_{max} peak-to-peak amplitudes and calculating the ratio of these two measures (H/M_{max}). Cortical silent period (SP) was analyzed when MEPs were delivered during contractions at 50% of MVC as recommended by Säisänen et al. (2008) and also during contractions at 20% of MVC. SP refers to a silencing of any ongoing EMG activity after the MEP as a result of TMS being delivered during a voluntary contraction. The duration of the SP was determined by visual inspection as the time from MEP offset to the time of reoccurrence of voluntary EMG activity. Each individual trial was analyzed separately and then averaged across trials and subjects.

Normality of variables was tested with Shapiro–Wilk's *W*-tests. Accordingly, SOL MEPs, neural deficit and H/M_{max} were compared using Wilcoxon's signed-rank test separately for both groups. Differences between groups for PAS effect (post MEPs as percentage of baseline values) were tested with Mann–Whitney *U*-tests. MVC and fatigue force were compared with two-tailed paired *t* test. Fatigue resistance was compared with a two-way repeated measures ANOVA with within factor time of two levels

(pre PAS/post PAS) and between-subjects factor group of two levels (PAS_{LTP}/PAS_{LTD}). SPs were analyzed with a two-way repeated measures ANOVA with within factor time of three levels (pre PAS/post0 PAS/post15) and between-subjects factor group of two levels (PAS_{LTP}/PAS_{LTD}). Spearman's coefficient was used to correlate the changes in PAS-induced excitability (at post15; MEPs as percentage of baseline values) and changes in fatigue resistance. The inter-class correlation coefficient (ICC) was calculated for fatigue resistance test-retest repeatability. In a secondary analysis the above-mentioned tests for neural correlates and fatigue resistance were also performed only for the PAS responders (post MEP/pre MEP ratio >1.00 in the PAS_{LTP} group and <1.00 in the PAS_{LTD} group). The secondary analysis was done due to the high inter-individual variability of the PAS effects, which have been previously reported (Ridding and Ziemann, 2010). The significance level was set at $P < 0.05$. All data are given as mean ± standard deviation (SD).

3. Results

3.1. Change in excitability

Fig. 4 shows original resting MEP recordings for one representative subject in the PAS_{LTP} and PAS_{LTD} groups before, immediately after and 15 min after PAS. The PAS effect (post MEPs/pre MEPs) was significantly different between groups at post0 and post15 at rest ($P < 0.05$) but not in active conditions ($P > 0.05$). The normalized post-intervention peak-to-peak MEP amplitudes are presented in Fig. 5A and B. In the PAS_{LTP} group, MEP increased by 51 ± 108% at post0 ($P > 0.05$) and significantly by 73 ± 123% ($P < 0.05$) at post15 at rest. There were no significant changes in the 20% of MVC condition but MEPs decreased significantly at both post0 by 9 ± 12% ($P < 0.05$) and post15 by 8 ± 14% ($P < 0.05$) in the 50% of MVC

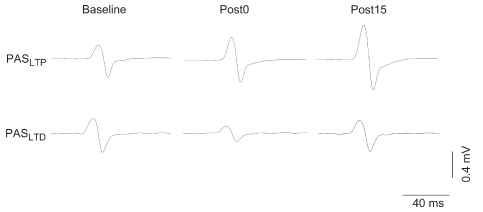

Fig. 4. Resting MEP traces. Traces are from one representative subject in the PAS_{LTP} and PAS_{LTD} groups before and after the interventions. Each trace is an average of 10 trials.

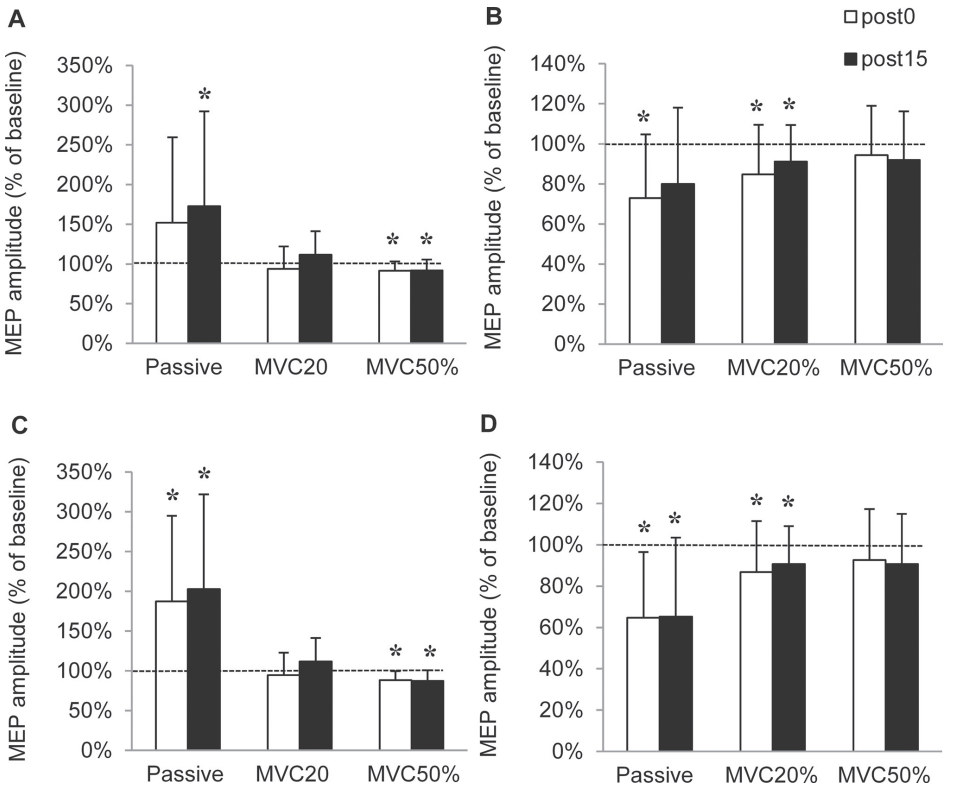

Fig. 5. Effect of PAS on MEP responses. Mean post-intervention MEP amplitudes (normalized to baseline) (A) in the PAS_{LTP} group (15 subjects) and (B) in the PAS_{LTD} group (15 subjects). The same results are presented also for responders only (C) in the PAS_{LTP} group (11 subjects) and (D) in the PAS_{LTD} group (12 subjects), *$P < 0.05$.

condition. In the PAS_{LTD} group, passive MEP decreased significantly at post0 by 27 ± 32% ($P < 0.05$) but not at post15, where MEP decreased by 20 ± 38% ($P > 0.05$). MEP decreased significantly by 15 ± 25% ($P < 0.05$) at post0 and by 9 ± 18% ($P < 0.05$) in the 20% of MVC condition. There were no significant changes in the 50% condition. The difference between the post0 and post15 was never statistically significant. SPs at contraction level of 50% of MVC were not affected by time ($F_{(2,56)} = 1.54$, $P > 0.05$) group ($F_{(1,28)} = 0.87$, $P > 0.05$) nor their interaction ($F_{(2,56)} = 0.06$, $P > 0.05$). SP values for the PAS_{LTP} group were 75 ± 27 ms at pre, 78 ± 23 ms at post0 and 79 ± 25 ms at post15, and for the PAS_{LTD} group 68 ± 19 ms, 72 ± 15 ms and 73 ± 14 ms, respectively. Correspondingly, there were no changes in SP duration at 20% of MVC; time ($F_{(2,56)} = 1.83$, $P > 0.05$) group ($F_{(1,28)} = 1, 66$, $P > 0.05$) nor their interaction ($F_{(2,56)} = 0.19$, $P > 0.05$). The coefficient of variation was significantly higher, 0.25 at 20% of MVC compared to 0.16 at 50% of MVC ($P < 0.05$). Neither M_{max} amplitude nor H/M_{max} ratio changed significantly throughout the protocol ($P > 0.05$). M_{max} values for the PAS_{LTP} group were 19 ± 3 mV at pre, 18 ± 3 mV at post0 and 18 ± 3 mV at post15, and for the PAS_{LTD} group 19 ± 3 mV, 19 ± 3 mV and 19 ± 3 mV, respectively. H/M_{max} ratios for the PAS_{LTP} group were 0.60 ± 0.07 at pre, 0.58 ± 0.12 at post0 and 0.60 ± 0.09 at post15, and for the PAS_{LTD} group 0.49 ± 0.24, 0.47 ± 0.22 and 0.51 ± 0.24, respectively. The secondary analysis with only responders is shown in Fig. 5C and B; MEPs increased significantly at both post0 and post15 ($P < 0.05$) in the PAS_{LTP} group (11 subjects) and decreased significantly at both post0 and post15 ($P < 0.05$) in the PAS_{LTD} group (12 subjects). Otherwise the significances in MEP and SP results did not differ from the whole group analysis.

3.2. Fatigue resistance

In both groups the 15 s fatigue test induced significant force reduction prior to PAS; in the PAS_{LTP} group MVC was 1380 ± 420 N and average fatigue force was significantly less, 1230 ± 340 N ($P < 0.001$). In the PAS_{LTD} group, MVC force was 1190 ± 320 N and average fatigue force was significantly less, 1110 ± 280 N ($P < 0.01$). Corresponding fatigue resistance values were 90 ± 8% in the PAS_{LTP} group and 93 ± 7% in the PAS_{LTD} group. Contrary to our hypothesis, fatigue resistance was not affected by time ($F_{(1,28)} = 0.11$, $P > 0.05$) group ($F_{(1,28)} = 0.59$, $P > 0.05$) nor their interaction ($F_{(1,28)} = 0.86$, $P > 0.05$). After the PAS interventions, fatigue resistance was 92 ± 1% in the PAS_{LTP} group and 92 ± 7% in the PAS_{LTD} group. However, when the groups were combined, the change in excitability (post15 MEPs as a percentage of baseline values) correlated significantly with the change in fatigue resistance ($N = 30$, $R = 0.40$, $P < 0.05$), which can be seen in Fig. 6. In the PAS_{LTP} group, neural deficit was 1.6 ± 2.4% prior to PAS and 0.9 ± 4.4% following PAS. In the PAS_{LTD} group, neural deficit was 6.5 ± 13.3% prior to PAS and 5.9 ± 11.2% following PAS. Neural deficit decreased non-significantly ($P > 0.05$) by 44 ± 79% in PAS_{LTP} and by 10 ± 30% in PAS_{LTD}. Test–retest comparison ($N = 10$) revealed excellent repeatability of the fatigue resistance test ($P < 0.001$). When only responders (PAS_{LTP} group = 11 subjects and PAS_{LTD} group = 12 subjects) were included in the secondary analysis, two-way repeated measures of ANOVA revealed a significant interaction of time and group ($F_{(1,21)} = 4.8$, $P < 0.05$). Post hoc analysis using two-tailed paired *t*-test showed a non-significant improvement in fatigue resistance by 3.1 ± 8.4% in the PAS_{LTP} group and a reduction of 3.0 ± 7.5% in the PAS_{LTD} group. Otherwise the significances in force results did not differ from the whole group analysis.

4. Discussion

The aim of this study was to investigate the functional relevance of PAS by determining the effect of two different PAS intervention

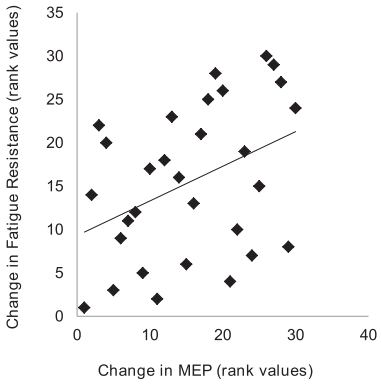

Fig. 6. Relationship between excitability and fatigue resistance. Spearman's coefficient was used to correlate PAS-induced changes in excitability, presented on the horizontal axis, with changes in fatigue resistance, presented on the vertical axis ($N = 30$, $R = 0.40$, $P < 0.05$).

on fatigue resistance during 15 s sustained maximal isometric contraction. On average fatigue resistance did not change after PAS_{LTP} or PAS_{LTD}, however the changes in MEP peak-to-peak amplitudes after PAS correlated with the changes in fatigue resistance. Subjects whose MEP size increased also demonstrated improved fatigue resistance and vice versa.

4.1. Neural correlates – changes in cortico-spinal and spinal excitabilities after PAS

The results of the present study indicate that associative modulation of excitability to the cortical projections of SOL was achieved after PAS_{LTP} and PAS_{LTD} interventions with constant ISIs. Accordingly, PAS_{LTP} induced enhancement of synaptic transmission whereas PAS_{LTD} induced weakening of synaptic transmission. There was no significant increase in MEP size at post0 after PAS_{LTP}, but a significant increase at post15. A similar trend has been seen in previous studies that have shown an increasing PAS_{LTP} effect over time (Kumpulainen et al., 2012; Prior and Stinear, 2006; Stefan et al., 2000). On the contrary, MEP size decreased significantly at post0 but not at post15 after PAS_{LTD}, which is in line with a previous study by Di Lazzaro et al. (2011) where a significant effect was achieved immediately after but not 30 min after PAS_{LTD}. MEPs were not increased in the active conditions following PAS_{LTP}; on the contrary, MEPs were significantly decreased in the 50% of MVC condition after PAS_{LTP}. This is an interesting result since, to our knowledge, PAS-induced effects have not been measured at such high contraction levels, and a significant decrease in MEP size after PAS_{LTP} has not been reported before. However, there are studies showing no change in MEPs during slight contractions (Kumpulainen et al., 2012; Stefan et al., 2000, 2004). The interpretation of the effect of PAS on active muscles is more complicated as can be seen in the study of Lu et al. (2009) where a decrease of the movement-related cortical potentials (MRCP) in electroencephalography recordings was reported after an LTP-like PAS-protocol. Accordingly, performing the same movement pattern, simple thumb abduction, generated a decreased MRCP negativity after the PAS intervention. MRCP reflects executive aspects of the forthcoming motor action and decreased MRCP negativity indicates weaker volitional motor output. This may indicate that the LTP-like effect decreases the level of effort needed to

produce force, resulting in decreased or unchanged MEPs in active muscle.

The most stable and informative SP is measured at a contraction level of 50% of MVC (Säisänen et al., 2008). It has been shown that the initial part of the SP is influenced by spinal circuitries, whereas the latter part to a higher extent represents the state of cortical inhibitory interneurons, which are mediated by $GABA^b$ receptors (Ziemann, 2004). SP remained unchanged in the current study, which is in line with the study by Di Lazzaro et al. (2011) that reported unchanged SPs after PAS_{LTP} or PAS_{LTD} when MEPs were elicited during contraction at 50% of MVC. H/M_{max} ratios remained unchanged after the interventions, suggesting a lack of changes at the spinal level, which has also been shown in previous PAS studies (Kumpulainen et al., 2012; Stefan et al., 2000; Wolters et al., 2003).

4.2. Functional implications – changes in fatigue resistance after PAS

The 15 s sustained maximal isometric contraction induced significant force loss on all occasions but fatigue resistance did not change after interventions. This is in line with a previous study by Milanović et al. (2011) which showed no changes in a fatigue test involving sustained isometric contraction at 50% of MVC after PAS_{LTP}. However, a significant relationship was found between the change in excitability of the cortical projections and change in fatigue resistance in the present study, which was not reported in the study by Milanović et al. (2011). One possible reason for this difference could be that they used the duration of submaximal contraction as an indication of fatigue, which may involve a greater peripheral component of fatigue compared to the present protocol (Lentz and Nielsen, 2002). It has been demonstrated that endurance time and changes in maximal capacity to generate force provide information about different processes induced by the exercise (Vøllestad, 1997). Thus, endurance time may not directly correlate with motor cortex excitability. Benwell et al. (2006) found a significant reduction in the rate of force loss during a 10-s MVC of hand muscles after increasing corticomotor excitability using a spike timing-dependent repetitive TMS intervention. In the current study the lack of changes in fatigue resistance at the group level may be due to considerable inter-individual variability in the PAS-induced plasticity changes within the groups. The chosen constant ISIs might be one reason for relatively high inter-individual variability (SD = ±108% and 123%) observed in this study. Nevertheless, the present values are comparable to the variability (SD = ±105% and 52%) and number of responders (12 responders from 16 subjects) obtained in a previous study (Kumpulainen et al., 2012), where the ISI was optimized to the individual somatosensory-evoked potential and was 50 ± 2 ms on average. Since an earlier study by Mrachacz-Kersting et al. (2007) found significant LTP during a 10 ms time window with constant ISIs of 45–55 ms, the constant ISI of 50 ms used here should be sufficient to induce LTP in most subjects. The optimal ISI to induce LTD in the leg area has not been studied but an ISI of 20 ms is mostly used. Stinear and Hornby (2005) reported the number of LTD responders and it was quite similar to ours; 12 responders from 14 subjects. Also previous studies have reported wide variability of PAS-induced effects between subjects, which has been associated with differences in brain anatomy, genes, and training background (Ridding and Ziemann, 2010). Because of the dissimilarities in brain anatomy, TMS can preferentially activate different neuronal circuits in different subjects (Sakai et al., 1997) causing variability in the PAS-induced effects. The genetic polymorphisms of neurotrophins can influence the induction of plasticity (Ridding and Ziemann, 2010). However, there is only one brain-derived neurotrophic factor – polymorphism, which has been shown to limit PAS-induced motor cortex plasticity (Cheeran et al., 2008). Furthermore, it has been found that skill athletes have higher motor cortex plasticity compared to endurance trained athletes (Kumpulainen et al., 2014). When only responders were included in the analysis, there was significant interaction of time and group in fatigue resistance. Still, the change in fatigue resistance was not significant within the groups. This suggests that PAS affects fatigue resistance among PAS responders and supports the result of significant correlation. ITT was conducted in order to reveal possible changes in neural deficit induced by PAS_{LTP} or PAS_{LTD} at the end of fatiguing contractions. No significant differences were found, suggesting that there were no differences in central fatigue after the interventions. However, since we found a significant correlation between changes in MEP and fatigue resistance, small changes in central fatigue may have occurred within the variation of ITT. Low sensitivity of the ITT method has been reported at maximal contraction intensities (Taylor, 2009).

4.3. Candidate mechanisms behind PAS and fatigue

It has been proposed that PAS-induced LTP occurs within the motor cortex (Di Lazzaro et al., 2009; Stefan et al., 2000), where the somatosensory stimulus can arrive via the dorsal column-medial leminiscal route or via a longer pathway involving the cerebellum (Strigaro et al., 2014). Intracortical neural circuits through which the effects of PAS and fatigue emerge have been investigated with single and paired pulse TMS in several previous studies (Carson and Kennedy, 2013; Gruet et al., 2013; Taylor and Gandevia, 2001). Paired pulse TMS is a tool to examine facilitatory and inhibitory circuits in the cerebral cortex. However, as different studies have used different interventions and target muscles, the results are difficult to interpret. Prolongation of SP has been observed after various fatiguing exercises including sustained MVC of soleus muscle (Iguchi and Shields, 2012; McKay et al., 1996). Since prolongation of SP is less after cervicomedullary stimulation-induced MEP, additional inhibition at the cortical level has been suggested (Levenez et al., 2008; Taylor et al., 1996). In addition, Hilty et al. (2011) showed that central projections of group III–IV muscle afferents may facilitate a fatigue-induced increase in SP. Therefore, it seems that $GABA^b$-mediated intracortical inhibitory circuits have a role in the development of central fatigue. Since SP duration remained unchanged after PAS interventions in this study, it seems that, at least partly, fatigue and PAS affect different cortical interneurons. This corresponds to the current results showing no significant effect of PAS on fatigue resistance. However, among PAS responders the PAS-induced LTP might have compensated for fatigue-induced inhibition in the motor cortex and thus affected fatigue resistance. In addition, PAS induced LTP/LTD-like plasticity has been shown to be accompanied with other neuronal circuits in the cerebral cortex (Carson and Kennedy, 2013), which might have contributed to the observed significant correlation. For example, long afferent inhibition (LAI), which is also $GABA^b$-mediated, is decreased following PAS_{LTP} (Meunier et al., 2012; Russmann et al., 2009) and might increase motor drive to the exercising muscle during fatiguing tasks. LAI reflects the activity of somatosensory inputs, and is obtained when the interval between peripheral afferent stimulation and subsequent TMS is in the region of 200 ms.

5. Conclusions

In the present study, PAS induced associative plasticity changes in the cortical projections to the resting SOL. On average, fatigue resistance during a 15-s sustained maximal isometric contraction did not change following PAS interventions. Thus, functionality of PAS interventions was not evident with the current experimental design. However, among responders fatigue resistance showed

significant interaction of time and group and PAS-induced excitability changes correlated significantly with changes in fatigue resistance. This suggests that PAS might have slightly affected central fatigue during short maximal contractions. Therefore, PAS might have implications for improving performance in rehabilitation settings.

Acknowledgments

The authors are very grateful to the subjects who participated in the study and to the laboratory staff from the Neuromuscular Research Center (Department of Biology of Physical Activity) of the University of Jyväskylä, Finland, for their valuable contributions to this project.

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IV

REPEATED PAIRING OF STRETCH REFLEX AND TRANSCRANIAL MAGNETIC STIMULATION TO INDUCE MOTOR CORTEX PLASTICITY CHANGES

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Submitted for publication