JYU DISSERTATIONS 752

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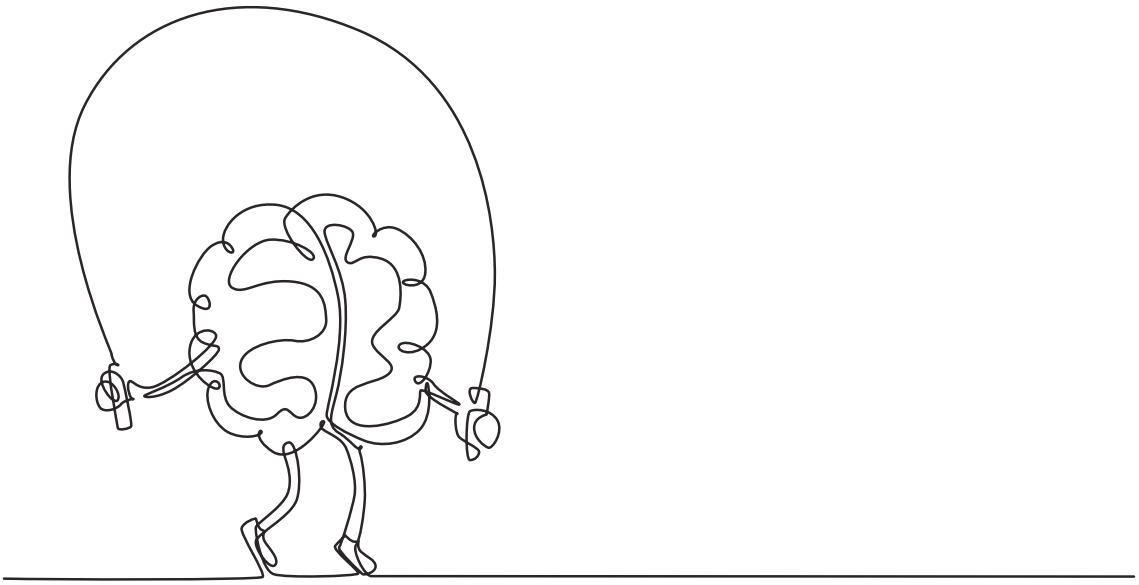
Adaptation of Corticospinal Excitability after Short- and Long-Term Motor Training



UNIVERSITY OF JYVÄSKYLÄ
FACULTY OF SPORT AND
HEALTH SCIENCES

JYU DISSERTATIONS 752

Nijia Hu

Adaptation of Corticospinal Excitability after Short- and Long-Term Motor Training

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ABSTRACT

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The present doctoral thesis aimed to investigate neural adaptation at the supraspinal and spinal level during different proprioception processing-related tasks (translational and rotational ankle perturbation) after short-term motor training, long-term skill and long-term endurance training. Using established translational perturbation protocols, 14 subjects participated in measurements to determine the accuracy and reliability of transcranial magnetic stimulation (TMS) and Hoffmann reflex (H-reflex) in Experiment I. In Experiment II, TMS and H-reflex measurements were used before and after one perturbation training session to examine corticospinal excitability and adaptation during translational perturbation tasks in 14 young adult subjects. To explore neural adaptation from long-term specific motor skill acquisition training, Experiment III investigated neural adaptation mechanisms of 10 skill- and 10 endurance- trained athletes and corticospinal excitability was measured by TMS during ankle rotational perturbation. The results demonstrated good to excellent test-retest reliability in TMS and H-reflex during translational perturbation tasks. Balance control ability for the translational perturbation task was significantly improved after one training session. Potentially decreasing corticospinal excitability, but increasing spinal excitability, suggests that repeated skill training improves motor performance and neural adaptation may transfer from cortical control to more subcortical involvement. After long-term training, skill-trained athletes demonstrated corticospinal excitability plays an important role in voluntary movement and suggests cortical adaptation to a top-down strategy in response to ankle rotational perturbation. For endurance-trained athletes, on the other hand, maintaining intracortical inhibition relates to higher neural modulation at the spinal level in response to ankle rotational perturbation. Therefore, the results of this thesis support that both spinal and supraspinal mechanisms adapt after training. Indeed, the acquisition of motor performance through training resulted in a discernible decrease in cortical influence and an augmentation of spinal influence. These changes align with the strategies employed by endurance trained athletes.

Keywords: motor control, motor skill learning, corticospinal excitability, transcranial magnetic stimulation, H-reflex, athletic training, ankle perturbation

TIIVISTELMÄ (ABSTRACT IN FINNISH)

Hu, Nijia
Kortikospinaalisen herätteen mukautuminen lyhyt- ja pitkäaikaisen motorisen harjoittelun jälkeen
Jyväskylä: Jyväskylän yliopisto, 2024, 98 s.
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Tämän väitöskirjan tarkoituksena oli tutkia lihastyön hermostollisen ohjauksen sopeutumista lyhytaikaiseen taitoharjoitteluun sekä pitkäaikaiseen taito- ja kestävyysharjoitteluun silloin, kun nilkkaniveleen aiheutetaan liikehäiriö. Hermostollista ohjausta tutkittiin sekä kortikaalisella että selkäydintasolla. Ensimmäisessä osatutkimuksessa 14 tutkittavaa osallistui transkraniaalisen magneettistimulaation (TMS) ja H-refleksin toistomittauksiin, joilla pyrittiin selvittämään näiden menetelmien luotettavuutta ja toistettavuutta nilkkanivelen translaatiohäiriön (lineaarinen) aikana. Samat 14 tutkittavaa osallistuivat tutkimuksen toiseen vaiheeseen, jossa edellä mainittuja menetelmiä käytettiin ennen ja jälkeen translationaalista häiriöharjoittelua. Tällä pyrittiin selvittämään kortikospinaalisen radan herkkyyden sopeutumista ja moduloitumista kyseessä olevaan tasapainoharjoitteluun. Tutkimuksen kolmannessa vaiheessa tutkittiin pitkäkestoisen urheiluharjoittelun vaikutuksia hermostolliseen ohjaukseen nilkkanivelen kiertohäiriön aikana kymmenellä taito- ja kymmenellä kestävyysharjoitelleella urheilijalla. Tulokset osoittivat, että TMS:n ja H-refleksin luotettavuus ja toistettavuus vaihtelivat hyvästä erinomaiseen. Tasapainon hallintakyky parani merkittävästi jo yhden tasapainoharjoituskerran jälkeen. Samalla kortikospinaalisen radan herkkyys laski lievästi ja selkäydintason herkkyys kasvoi. Nämä tulokset osoittavat, että lyhytaikainen taitoharjoittelu voi parantaa motorista taitoa, jolloin hermostollinen ohjaus vaikuttaisi siirtyvän kortikaaliselta tasolta enemmän selkäydintasolle. Taitoharjoitelleilla urheilijoilla kortikospinaalisen radan herkkyys oli merkittävästi yhteydessä tahdonalaiseen voimantuottoon kyseessä olevan häiriön aikana. Tällä perusteella kortikaalisen ohjauksen sopeutuminen tapahtuisi taitolajien urheilijoilla ylhäältä–alaspäin suuntautuvan strategian mukaisesti nilkan kiertohäiriössä. Toisaalta intrakortikaalisen inhibition säilyminen kestävyysurheilijoilla viittaisi selkäydintason ohjauksen voimakkaampaan rooliin nilkan kiertohäiriön aiheuttamissa hermostollisissa vasteissa. Tämän väitöskirjan tulosten mukaan fyysinen harjoittelu voi aiheuttaa hermostollista sopeutumista sekä kortikaalisella- että selkäydintasolla, riippuen harjoittelutavasta.

Asiasanat: motorinen kontrolli, motoristen taitojen oppiminen, kortikospinaalinen herkkyys, transkraniaalinen magneettistimulaatio, H-refleksi, urheilijoiden harjoittelu, nilkkanivelen liikehäiriö

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Jyväskylä 12.01.2024
Nijia Hu

ORIGINAL PUBLICATIONS AND AUTHOR CONTRIBUTION

This thesis is based on the following original publications, which will be referred to by their Roman numerals. The thesis also includes unpublished data.

I Hu, N., Avela, J., Kidgell, D., Nevanperä, S., Walker, S., & Piirainen, J. M. (2022). Reliability of transcranial magnetic stimulation and H-reflex measurement during balance perturbation tasks. Frontiers in Physiology, 13. https://doi.org/10.3389/fphys.2022.957650

II Hu, N., Piirainen, J. M., Kidgell, D., Walker, S., & Avela, J. (2023). Corticospinal Adaptation to Short-Term Horizontal Balance Perturbation training. Brain Sciences, 13(8), 1209. https://doi.org/10.3390/brainsci13081209

III Hu, N., Avela, J., Kidgell, D., Piirainen, J. M., & Walker, S. (2022). Modulations of corticospinal excitability following rapid ankle dorsiflexion in skill- and endurance-trained athletes. European Journal of Applied Physiology, 122(9), 2099–2109. https://doi.org/10.1007/s00421-022-04981-9

As the first author of the original publications, considering the comments from the co-authors, the author of the thesis drafted the study questions and designs for the publications, prepared the data collection, performed statistical analysis and took main responsibility of writing the manuscripts. The author was responsible for all experiment design and participated in the data collection in all studies between 2019 and 2023.

ABBREVIATIONS

a120	120 ms after stretch reflex in an active condition
aMT	Active motor threshold
BDNF	Brain-derived neurotrophic factor
CNS	Central nervous system
COP	Centre of pressure
CV	Coefficient of variance
dCOP	Peak-to-peak centre of pressure displacement
EMG	Electromyography
H-reflex	Hoffman reflex
ICC	Intraclass correlation coefficients
ICF	Intracortical facilitation
LLR	Long-latency reflex
LLR_2	The second-long latency reflex
LTD	Long-term depression
LTP	Long-term potentiation
M-wave	Muscle compound action potential
M1	Primary motor cortex
MDC	Minimal detectable change
MEP	Motor evoked potential
MEP_{AVG}	Average motor evoked potential value from 30 trials
MLR	Medium-latency reflex
M_{MAX}	Maximum muscle compound action potential
MVC	Maximal voluntary contraction
N1	A negative potential from electroencephalography
NMDA	N-methyl-d-aspartate
Onset	Onset of the pedal movement
PAS	Paired association stimulation
PS1	The first perturbation session of Experiment II
PS2	The second perturbation session of Experiment II
PS3	The third perturbation session of Experiment II
p120	120 ms after stretch reflex in a passive condition
rMT	Resting motor threshold
S1	The first session of Experiment I
S2	The second session of Experiment I
SD	Standard deviation
SEM	Standard error of measurement
SICI	Short-intracortical inhibition
SLR	Short-latency reflex
SR	Stretch reflex
TMS	Transcranial magnetic stimulation
vCOP	Average centre of pressure velocity
VEGF	Vascular endothelial growth factor

CONTENTS

1 INTRODUCTION

Motor training is a process that involves acquiring information from external sources and performing movement. It is intrinsically linked to neuroplasticity. One significant neural mechanism underlying motor performance improvements observed in motor learning is training-induced corticospinal excitability changes (Bagce et al., 2013; Orban de Xivry et al., 2013). Animal research by Adkins et al. (2006) showed motor skill training leads to long-term potentiation (LTP) and synaptogenesis in the primary motor cortex (M1). Depending on the complexity of motor learning tasks, synaptogenesis requires several repeated training sessions (Carey et al., 2005).

In short-term motor training, new synaptic connections may occur in different brain areas during the skill-learning process (Hikosaka et al., 2002). It has been known that within a single motor skill learning session, there is an increase in corticospinal excitability in the area controlling the corresponding limb (Suzuki et al. 2012). As motor skill becomes acquired, it is believed that the acquired motor skills are stored in the sub-cortical areas, such as brain stem and basal ganglia can be recalled by the cortical-subcortical network when the learned task is repeated (Holland et al., 2015).

Long-term sports training has been found to enhance corticospinal plasticity from motor learning (Hötting & Röder 2013; Singh et al. 2016). Meanwhile, long-term skill training leads to changes in corticospinal excitability and different cortical responsiveness compared to endurance training (Perez et al. 2004). On the other hand, long-term endurance training enhances spinal excitability and improves blood flow, and oxygen delivery through angiogenesis, to brain regions but appears not to directly participate in the modulation of synaptic number or topology (Churchill et al. 2002; Taubert et al. 2015; Chen et al. 2019).

It is well-established that corticospinal excitability during muscle voluntary activation is highly related to motor tasks. Voluntary activation is not only controlled by top (supraspinal level)–down (spinal level) mechanisms, but proprioceptive feedback related to afferent input also plays a crucial role in adjusting corticospinal excitability, particularly when dealing with dynamic

perturbations or unexpected disruptions to the body's equilibrium (Riemann & Lephart, 2002). As such, rotational and translation perturbations are commonly used in research to elicit different reflex functions (Nashner, 1976; Wälchli et al., 2017). For example, when a sudden dorsiflexion perturbation is applied to the ankle, it results in muscle spindle stretching in the triceps surae muscles. Muscle spindle stretching enhances activity in Ia afferents, which are sensory neurons responsible for conveying information from the muscle spindle to the central nervous system (MacKinnon, 2018). This activity, subsequently, affects motor output from supraspinal levels. This mechanism is essential for the coordination and execution of movement. Therefore, this thesis evaluated neural excitability and adaptation from spinal and supraspinal levels after short-term and long-term training in response to two perturbation tasks, i.e., ankle translational perturbation and ankle rotational perturbation tasks.

2 REVIEW OF THE LITERATURE

2.1 Proprioception and motor control

In human motor control, the central nervous system (CNS) receives inputs from three primary subsystems: somatosensory, vestibular, and visual systems (Cree & Weimer, 2003). Among these subsystems, afferent information from the somatosensory system, including proprioception, significantly influences movement control, which is related to maintenance of body posture and coordination (Frontera, 2007). Proprioceptive information is derived from proprioceptors that are present in skin, skeletal muscles, joints, ligaments, and tendons (Lephart et al., 1997). The receptors provide proprioceptive feedback to the CNS, including shape, size, and mass of body segments to regulate muscle tension as well as awareness of the human body's position, movement, and orientation (Delhaye et al., 2018; Grigg, 1994). When muscles stretch, one of the specialized receptors, called muscle spindles, discharge in response to the lengthening of the muscle fibres. Muscle spindles work in conjunction with other proprioceptors, such as Golgi tendon organs and joint receptors (Matthews, 2015). It is well known that the muscle spindle and its afferents are most responsible for the perception of limb movement and position (Matthews & Bagby, 1974; Proske et al., 2000). The role of proprioception in motor control is crucial for the coordination and execution of movements, especially when dealing with dynamic perturbations or unexpected disruptions to the body's equilibrium (Riemann & Lephart, 2002). As shown in Figure 1, during dynamic perturbations, proprioceptive feedback is essential for rapidly detecting and responding to changes in body position, joint angles, and muscle length (MacKinnon, 2018). In the case of sudden perturbations like rotational or translational perturbations, proprioception, vestibular, and the visual system provide sensory information about the orientation of the human body (Shumway-Cook & Woollacott, 2007). Usually, vision and vestibular inputs have a slower processing speed than proprioceptive input (Dietz et al., 1991). Therefore, early postural responses may rely more on proprioceptive input, which enables the CNS to activate

appropriate muscle responses to maintain postural stability and restore balance (Dean, 2013; Frigon et al., 2021). While in the later phase (> 200 ms) of postural control during perturbation, vestibular and vision are more important (Dietz et al., 1991). In sports training, ankle proprioception plays a particularly crucial role in balance control, where the ankle-foot is always the primary point of contact with the ground (Han et al., 2015). It provides essential information for adjusting ankle position and coordinating movements of the entire body. In addition, it can provide successful performance in complex motor tasks associated with human movement and elite sports (Di Giulio et al., 2009; Sasagawa et al., 2009). For example, Han et al. (2015) demonstrated a high correlation between athletes' competition level and ankle proprioception score, which highlights the importance of ankle proprioception for sports success.

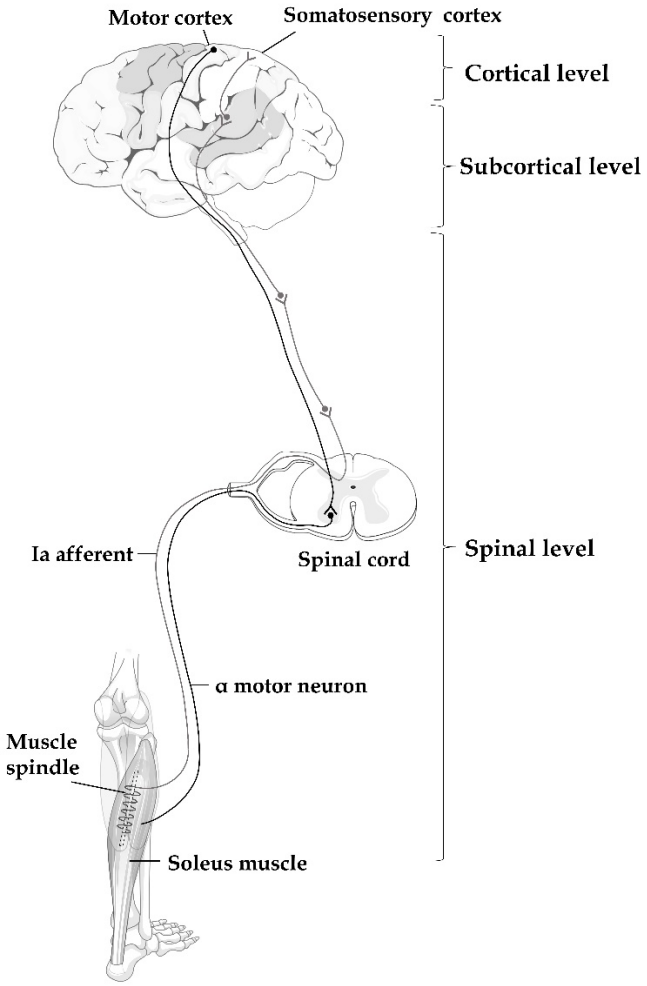

FIGURE 1 Proprioceptive feedback and corticospinal pathways schematic. The diagram of soleus muscle and response loop from Ia afferent to the somatosensory cortex, and M1 to α-motoneuron, whose terminal is at the muscle fibre. The figure is modified from (Proske & Gandevia, 2012).

2.2 Motor control mechanisms in perturbation

2.2.1 Rotational versus translational perturbations

In a rapid ankle perturbation, such as a rotational or translational perturbation, muscle spindles are activated in response to changes in muscle length and velocity (Proske et al., 2000). In a healthy human, when an ankle joint experiences a rotational perturbation, distinct muscle activity responses can be measured by electromyography (EMG). As shown in Figure 2, the main response in resting muscle that occurs approximately 40–50 ms after the stretch is known as the short-latency reflex (SLR), where sensory information from the muscle spindles activates the motoneuron pool directly (Fellows et al., 1993; Lee & Tatton, 1975). Stretching a muscle spindle leads to increased activity in Ia afferents, which are sensory neurons responsible for conveying information from the muscle spindle to CNS. It further evokes the activity of the α-motoneuron that innervates the same muscle. Ia afferents can also excite the motoneurons that innervate synergistic muscles while inhibiting motoneurons that innervate antagonistic muscles (Purves et al., 2001). A classic view is that SLR is mediated by a monosynaptic reflex loop, which is "purely" under spinal control (Fellows et al., 1993; Lee & Tatton, 1975). When giving a perturbation during standing, following the SLR observed from soleus, other reflex responses can be observed in EMG, including the medium-latency reflex (MLR, ~70 ms), the long-latency reflex (LLR, ~90 ms), and even voluntary reaction (Dietz et al., 1984; Latash & Zatsiorsky, 2015; Kurtzer et al., 2010; Schieppati & Nardone, 1997). The MLR is thought to involve II afferent pathways and subcortical processing (Kurtzer, 2015; Taube et al., 2008). Based on the latency time, it has been found that LLR includes contributions from multiple neural pathways (transcortical loops) that are influenced by cortical behaviour (Evarts, 1973; Petersen et al., 1998; Taube et al., 2006). In addition, voluntary reactions engage a more extensive circuitry that includes the premotor cortex and basal ganglia, along with the continued involvement of neural pathways (Kurtzer, 2015). However, the specific neural contributions and functional capabilities of the LLR and voluntary reaction in the lower limb are still not fully understood during rotational perturbation.

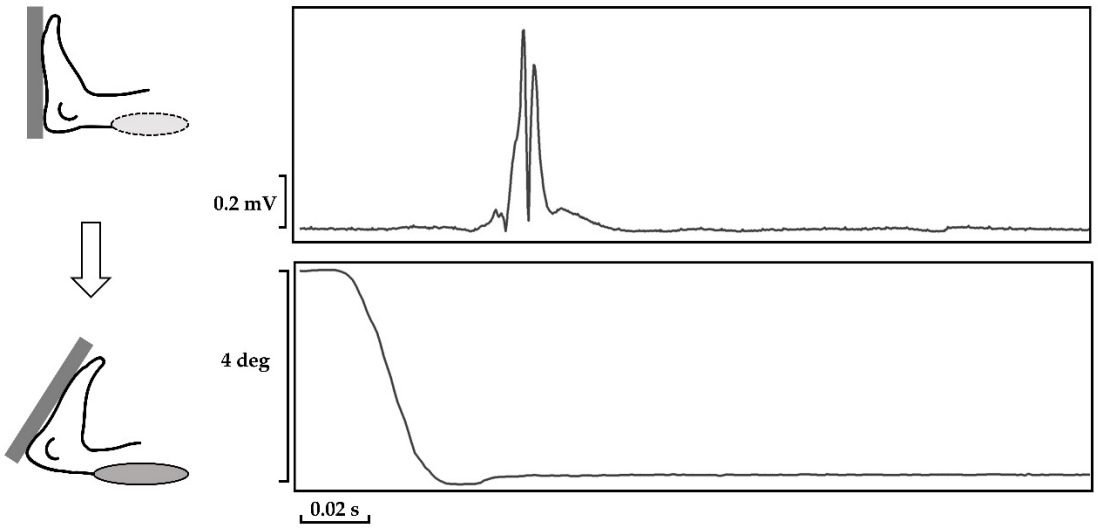

FIGURE 2 A Rotational perturbation example from Experiment III. The upper trace shows the smoothed with a 2 ms window and rectified EMG signal of soleus muscle and short latency reflex occurs ~40 ms after perturbation. The bottom trace shows the rotational signal from the ankle dynamometer.

Translational perturbation is commonly used in motor control research as a balance perturbation task. The balance perturbation task involves sudden external disturbances that disrupt the body's equilibrium, which is commonly applied in research to examine postural feedback responses (Chen et al., 2014). Proprioceptive, somatosensory, and vestibular loops contribute to maintain body balance, which is associated with neural activity in the brainstem, cerebellum, and motor cortex (Horak et al., 1994; Jacobs & Horak, 2007). During these balance perturbation tasks, both feedback control, which occurs in response to sensory feedback, and feedforward control, which refers to anticipation of a voluntary movement are involved in postural control (Dietz et al., 1993).

As mentioned earlier, following ankle movement in translational perturbation tasks, the muscle activity in lower limb muscles (e.g., soleus and tibias anterior) also shows typical patterns. As shown in Figure 3, SLR, MLR, and LLR have been observed as is also the case with the rotational perturbation (Petersen et al., 1998; Piirainen et al., 2013; Taube et al., 2006). However, it is important to note that, even though SLR, MLR, and LLR have been observed in both rotational and translational perturbation tasks, different patterns have been demonstrated between these tasks in a study by Wälchli et al. (2017). Researchers found no clear SLR following the high-velocity translational perturbation, but a prominent LLR was observed, suggesting that higher intercortical activity, which is likely related to anticipation of the movement, is involved in the balance control during high-velocity translational perturbation tasks.

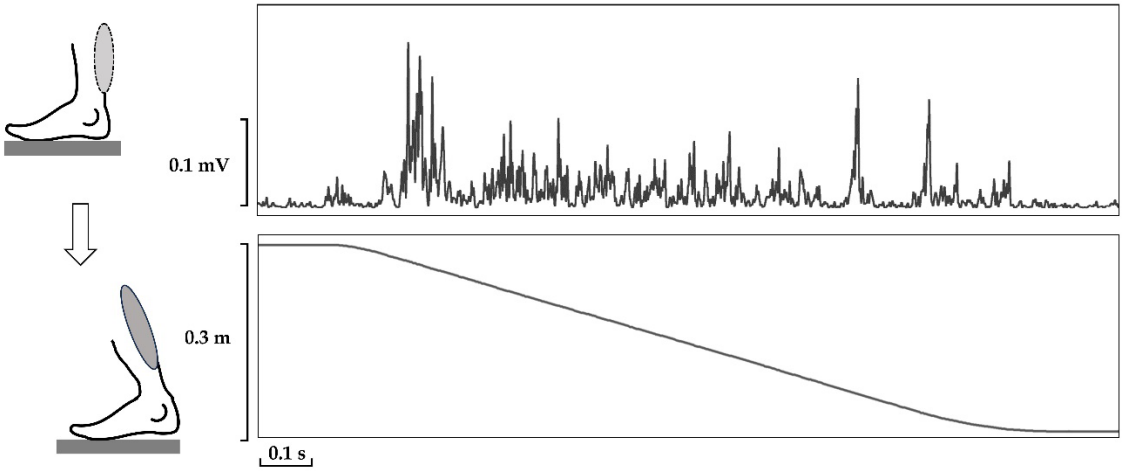

FIGURE 3 A translational perturbation example from Experiment I and II. The upper trace shows the smoothed with a 2 ms window and rectified EMG signal of soleus muscle. Short latency reflex (SLR) was observed ~65 ms after perturbation onset, which should take ankle movement delay into account. medium-latency reflex, long latency reflex, and voluntary activation phases follow SLR. The bottom trace shows perturbation with the balance platform moving backwards by 0.3 m.

The specific contribution and involvement of the corticospinal pathway in the control of lower limb muscles during rapid ankle perturbations remain unclear. Understanding proprioception and its role in motor control is imperative for acquiring a deeper understanding of the neural responses and how they are adjusted across various motor tasks.

2.2.2 Corticospinal excitability

Motor control in humans is a complex process involving interconnected neuromuscular networks. Subcortical neural pathways (e.g., spinal cord, brainstem, and cerebellum) control repetitive and automatic movements, such as daily walking or maintaining a quiet standing position (Waxman, 2020). Notably, decerebrate cats can still generate automatic postural responses to translation, indicating that the circuitry remains intact at the subcortical level (Honeycutt et al., 2009). Conversely, cats with spinal cord transection, which interrupts the connection between the brain and spinal cord, cannot generate direction-specific responses (Macpherson & Fung, 1999). On the other hand, voluntary movement is predominantly controlled by the brain cortex, particularly from the M1, and transmits neural activity via the corticospinal pathway to evoke skeletal muscle contractions (Martin, 2005; Davidoff, 1990). Known as the 'corticospinal pathway', it forms a vital connection between M1 and spinal motoneurons by descending axons.

Corticospinal excitability includes excitability from M1 to spinal motoneurons within the corticospinal pathway (Brouwer & Ashby, 1990; 1992). This pathway is responsible for controlling voluntary movement, and changes in corticospinal excitability can significantly impact motor function (Chen & Hallett,

1999). In human research, corticospinal excitability is typically assessed through indirect methods, including magnetic and electrical stimulation applied to peripheral nerves and/or the motor cortex (Barker et al., 1985; Bestmann & Krakauer, 2015).

2.2.2.1 Transcranial Magnetic Stimulation

Transcranial Magnetic Stimulation (TMS) is a non-invasive technique that can be used to study corticospinal excitability underlying motor control (Wassermann et al., 2008). Bartholow's research in 1874 was the first attempt at TMS stimulation in humans (Bartholow, 1874). Subsequently, Penfield and Jasper conducted systematic electrical stimulation experiments on the human brain during surgery, investigating the creation of the well-known 'homunculus diagram', which represents the motor responses of different body parts (Penfield & Jasper, 1954). As a result, TMS can be used to selectively target specific muscles of the human body by stimulating corresponding areas of the motor homunculus. In practice, a figure-of-eight coil is used to stimulate the M1 region associated with the upper limbs, while a double-cone coil is used to target the leg areas of the brain that are more deeply buried in the interhemispheric fissure. Di Lazzaro and Rothwell's study (2014) showed TMS induces an electrical field in the target tissue to activate axons rather than neuronal cell bodies and, thus induces indirect waves (I-waves). A recent study has proposed a model suggesting that earlier I-waves are primarily driven by synapses located close to the soma, whereas the later ones are structured by inputs of distal synapses (Rusu et al., 2014).

When applying a single supra-threshold intensity TMS on M1, it can elicit muscle twitches in contralateral muscles, which are typically quantified by using EMG in order to record the resulting motor evoked potential (MEP) as shown in Figure 4 (Chen et al., 2008; Rothwell, 1997). The amplitude of MEP is used as an indicator of corticospinal excitability, while the latency of MEP reflects the sum of the time for intracortical processing and neuromuscular transmission (Hallett, 2007; Rossini et al., 1999). Some researchers have used TMS during ankle perturbation to investigate corticospinal excitability and its connection to proprioceptive processing. For example, Taube et al. (2008a) investigated drop jumps, and MEP responses in the soleus muscle were not affected during SLR or MLR but showed facilitation at second-long latency reflex (LLR_2, ~120 ms). This suggests that LLR_2, but not SLR/MLR, is influenced by cortical processes during such tasks. However, it is also important to consider the top-down influence of cognitive processes, such as motor preparation (Bestmann & Duque, 2016; Bonnard et al., 2003). Additionally, MEPs are also influenced by the excitability of the motoneuronal pool (Taylor, 2006). Therefore, when making conclusions about neural excitability from MEP results, assessing the changes occurring at the spinal level is also necessary.

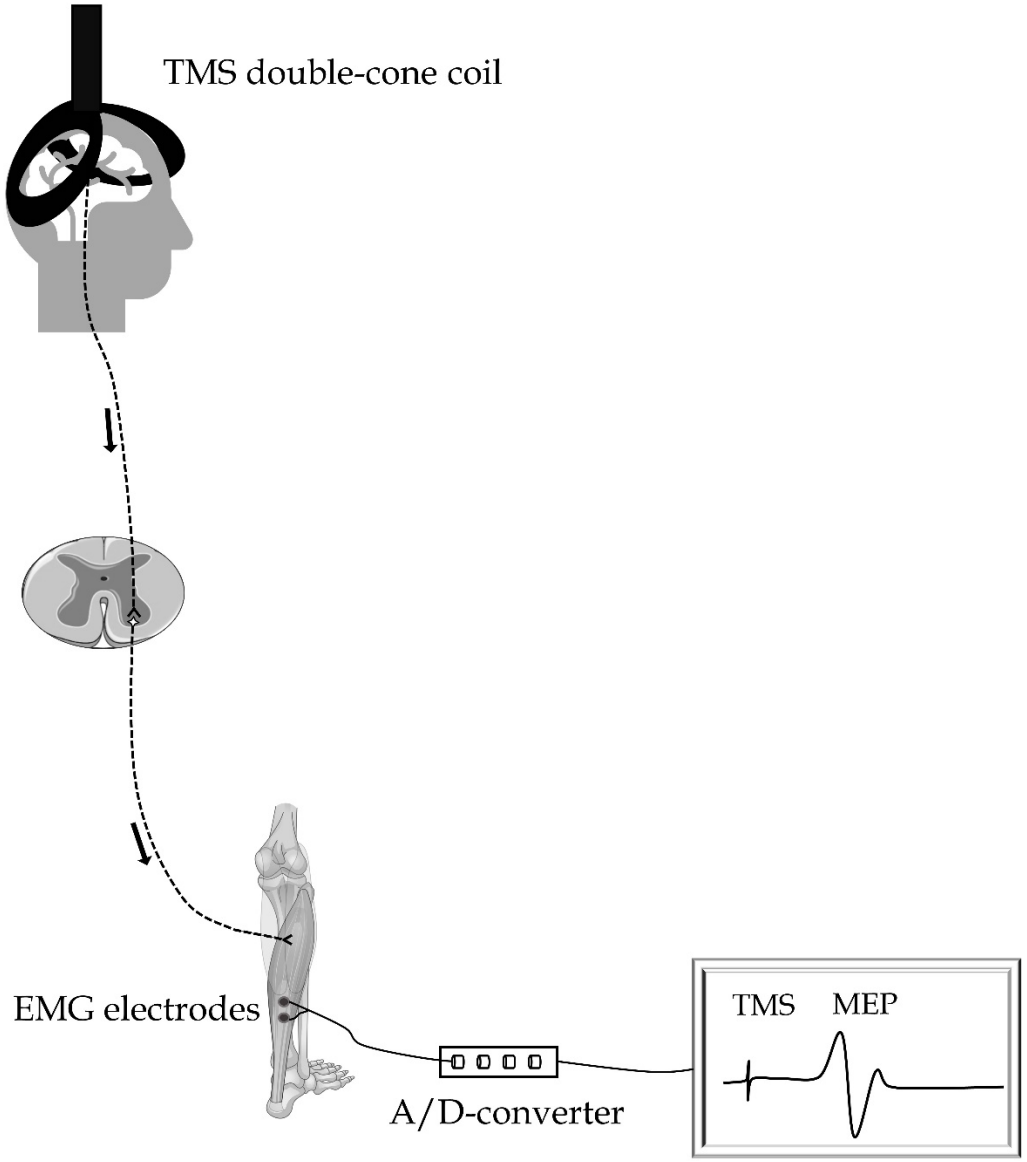

FIGURE 4 Corticospinal excitability schematic. Motor evoked potential (MEP) induced by a single-pulse transcranial magnetic stimulation through the corticospinal pathway is shown with a dashed line and arrow. MEP amplitude is shown in the sampling software in electromyography signals.

2.2.2.2 Hoffmann reflex

To evaluate the excitability and plasticity of a reflex pathway at the spinal level, Hoffmann reflex (H-reflex) is commonly used and elicited peripheral electrical stimulation (Nielsen et al., 1993; Wolpaw & Lee, 1989; Zehr, 2002). The H-reflex was first described by Paul Hoffmann in 1918 as an artificial monosynaptic reflex

of the spinal cord (Hoffmann, 1918). As shown in Figure 5, when a sufficient percutaneous electric stimulation intensity is applied to a peripheral nerve, which contains both afferent and efferent motoneuron axons (e.g., tibia nerve), it activates afferent fibres (Ia sensory) that pass through the motoneuron pool and, thus, activates the efferent (motor) neurons, resulting in the H-reflex. As the stimulation intensity increases, a direct efferent motor response known as Muscle compound action potential (M-wave) also occurs in addition to the H-reflex, which reflects muscle activity associated with the stimulated motoneurons (Capaday, 1997; Zehr, 2002). The H-reflex / M-wave ratio can be used to quantify the percentage of active motoneurons in the motoneuron pool. It is important to note that the H-reflex is elicited by electrical stimulation of somatosensory nerves, but SLR is elicited by lengthening of the muscle. Therefore, the muscle spindle itself is not being stimulated in H-reflex (Palmieri et al., 2004). H-reflex is not solely a monosynaptic reflex originating from group Ia afferents, but it also involves oligosynaptic contributions from Ia and another large-diameter afferent (Misiaszek, 2003).

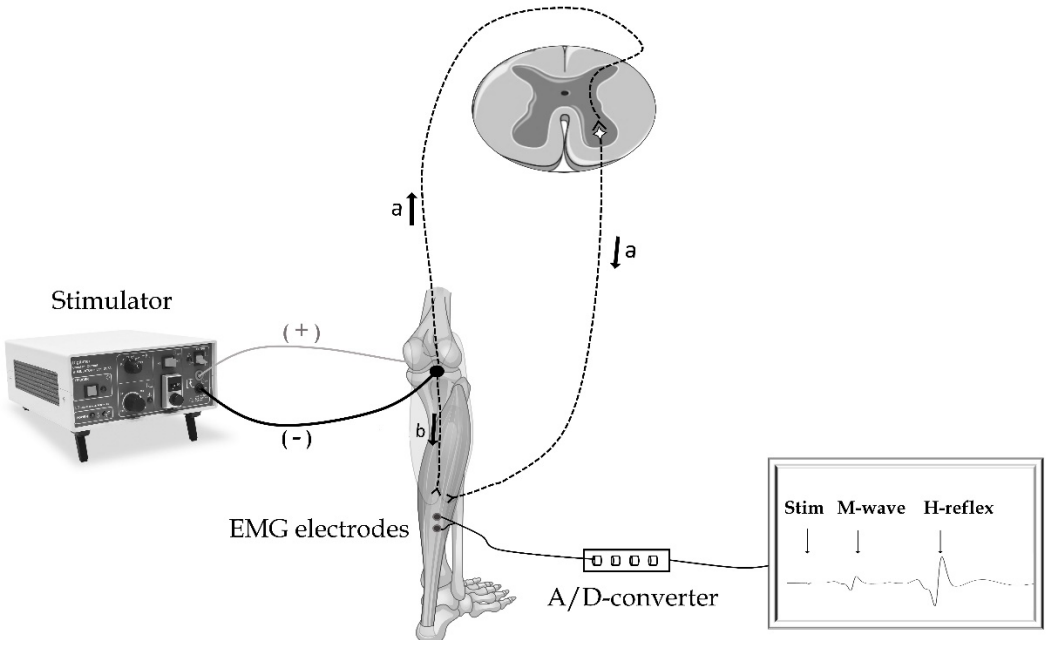

FIGURE 5 H-reflex and M-wave pathway schematic. H-reflex is elicited by the 'a' loop, in which the stimulation activates afferent fibres, and passes through the spinal cord, thus activating the efferent neurons, recorded by electromyography (EMG). When the intensity of stimulation increases, M-wave can be observed before H-reflex in the EMG signal, which passes through the 'b' pathway of stimulated motoneurons.

Many factors have been shown to influence the H-reflex response. Proprioceptive processing significantly influences H-reflex amplitude in various body positions. Studies have shown that the soleus H-reflex amplitude is reduced during standing compared to sitting or lying positions (Angulo-Kinzler et al., 1998;

Mynark & Koceja, 2002). Furthermore, when standing on a foam mat compared to a rigid surface, the H-reflex amplitude is also decreased (Earles et al., 2000). In resting muscles, the soleus H-reflex amplitude decreases during rapid muscle lengthening, which is influenced by the tonic discharge of muscle spindle afferents and transmission within the Ia pathway; but increases during passive muscle shortening, which is influenced by the shortening-induced slack of the muscle spindle (Pinniger et al., 2001). In addition, the H-reflex has been demonstrated to be influenced by presynaptic inhibition (Crone & Nielsen, 1989) and post-synaptic factors (Pierrot-Deseilligny & Burke, 2012). In a study by Taube et al. (2006) using translational perturbation, H-reflex amplitude was greater at LLR compared to SLR. This may be due to a decrease in presynaptic inhibition of Ia afferents. In another study by Piirainen et al. (2013), researchers measured H-reflex between young and elderly participants during transitional perturbation task. Lower H-reflex was observed in elderly in SLR but not LLR, which might be caused by higher presynaptic inhibition. Increased H-reflex amplitude was demonstrated after five weeks of isometric strength training (Lagerquist et al., 2006), which indicated enhanced spinal excitability. It may relate to increasing α-motoneuronal excitability or reducing presynaptic inhibition-related strength development. Supraspinal level modulation was not required in reflex responses, but it may influence the response during different motor tasks. Therefore, interpreting H-reflex measurements requires a thorough understanding of the factors influencing the H-reflex, including neural excitability at the spinal level, proprioceptive processing, and top-down influences from the supraspinal level. Meanwhile, differences between the protocols, for example, H-reflex measurement position, perturbation velocity and direction would also affect the H-reflex results, which should be noted when comparing the studies.

2.3 Motor learning and neural adaptations

Motor control refers to the planning and execution of movements. Since people are interested in improving motor control performance, the process of learning new skills is known as ‘motor learning’ (Muratori et al., 2013). Motor learning refers to the progressive refinement of spatial and temporal accuracy in movements through practice, and it relies on motor control as a foundation that involves the acquisition, retention, and transfer of motor skills (Willingham, 1998). Motor learning is essential for various activities, including daily living tasks and sports training. According to the ‘Dynamic Systems Model’ proposed by Esther Thelen and Linda Smith (1994), motor learning is not a fixed, linear process, but rather a complex, dynamic interaction. Multiple systems interact during motor learning, such as the neuromuscular, perceptual, and environmental systems. In line with this model, motor skills continuously adapt to changes in the environment and the individual's movements (Thelen & Smith, 1994). Likewise, Willingham (1998) presented a neuropsychological theory of

motor skill learning, emphasizing that learning is the foundation of motor control processes, which underscores the idea that the acquisition and refinement of motor skills occur through the motor learning process.

The process of motor learning involves the integration of various neural mechanisms (Sanes & Donoghue, 2000). It is currently believed that synaptic plasticity in the form of LTP and long-term depression (LTD) are mechanisms of motor learning (Ziemann et al., 2006). LTP refers to a long-lasting enhancement of synaptic transmission, while LTD refers to the weakening of synaptic transmission, both of which can persist for hours to days after the stimulus (Bliss & Lømo, 1973; Lynch et al., 1977). Motor skill training induced LTP and structural changes at dendritic spines in the M1 has been demonstrated in rats (Kida & Mitsushima, 2018). In human research, non-invasive techniques have been used to provide direct evidence of LTP-like mechanisms related to new motor learning (Rioult-Pedotti et al., 1998). The connection between LTP and LTD in the M1 is continuously modified as a result of appropriate motor patterns and can lead to functional reorganization of motor representation of muscles (Sanes & Donoghue, 2000).

There are also several theories about neural activity contribution to different brain areas during different stages of the motor learning process. According to Kleim et al. (2004), motor map reorganization and synapse formation do not significantly contribute to the early acquisition stage of motor skills but rather represent the consolidation of motor skills during the late stage of training. However, Costa et al. (2004) observed distinct neural processes in different brain structures during both early (fast) and later (slow) learning across multiple sessions. A comprehensive review by Dayan and Cohen (2011) highlighted the functional and structural plasticity across different spatial and temporal scales during motor skill learning. They found that neural activity increased in the dorsolateral prefrontal cortex, the M1, and in the pre-supplementary motor area during the early learning stage, but decreased as learning progressed, while increased activation is observed in the premotor cortex, supplementary motor area, parietal regions, striatum, and the cerebellum during the later learning stage. It has been also reviewed by Hikosaka et al. (2002) that motor learning is a result of multiple neural mechanisms. These mechanisms may contribute to different aspects of learning, such as the acquisition of new movement patterns, the refinement of existing skills, and the transfer of learned skills to different contexts.

In recent years, there has been growing interest in the role of corticospinal plasticity in motor skill learning. Corticospinal plasticity refers to the brain's ability to adapt and change in response to experiences and environmental stimuli, which is essential for motor control and learning (Kantak et al., 2012). At the supraspinal level, neural changes can be generally distinguished into two types that have been highlighted in many studies (Dayan & Cohen, 2011; Papale & Hooks, 2018). The first type involves intracortical serial connections at the cortex level, as demonstrated by Sakai et al. (1998). The second type of neural change involves two sets of loop circuits: cortex-basal ganglia and cortex-cerebellum

circuits. These circuits are part of the corticospinal pathway and play a role in the selection and optimization of movement patterns (Doyon et al., 1997; Jueptner et al., 1997). Therefore, the interaction between distinct cortical and subcortical circuits is crucial for skill acquisition, especially during the early motor learning stage (Doyon et al., 1997; Hikosaka et al., 2002). By understanding the different neural mechanisms that contribute to motor skill learning, researchers can develop more effective interventions that target specific aspects of the learning process and improve overall outcomes.

2.4 Training induced neural adaptation

Training-induced corticospinal adaptation are an important neural mechanism that underlies motor performance improvements observed following motor learning (Bagce et al., 2013; Orban de Xivry et al., 2013). Understanding these changes can help to optimize training protocols for specific training goals and populations. Physical training-induced neural adaptation in both central (cortical) and peripheral (spinal) levels has been explored in many studies (Enoka, 1997; Jensen et al., 2005; Taube et al., 2008). Research has shown that neural adaptation refers to the changes like LTP and synaptogenesis that occur in the brain not only as a result of motor learning but also during the process itself (Cooke & Bliss, 2006; Kleim et al., 2004). Specifically, M1 is engaged during the early stage of motor learning and consolidation (Muellbacher et al., 2001, 2002). Acquired motor skills are stored in the sub-cortical areas, such as basal ganglia and can be recalled by the cortical-subcortical network when the learned task is again repeated (Holland et al., 2015). In general, motor skill learning has been divided into short-term and long-term processes. Even though there is no real consensus on exact definitions of their time course.

2.4.1 Short-term motor skill training

During short-term motor skill training, ranging from several minutes to a few sessions, at least two different fast-acting processes seem to drive motor adaptation. One process retains the information well but responds weakly to errors, while the other responds strongly to errors but has poor retention (Smith et al., 2006). This suggests that the process of the motor learning mechanisms can vary depending on motor task. Zehr (2006) prepared a review of training induced plasticity of afferent reflex pathways. He noted that reflex pathways should be considered when evaluating neural adaptation and should be evoked under the same conditions, as the expression of neural excitability in a given reflex can be different according to the given motor task. Prsa et al. (2011) indicated that motor skill training in laboratory settings (e.g., moving the arm to the required target) is simpler than in 'real-life' situations, where tasks can be more complex and require conscious effort to complete. In other words, simple motor training tasks in the lab may be acquired fast, even after a few trials, and in an unconscious

manner, which is often referred to as 'adaptation'. Perez et al. (2004) found increasing recruitment of motor units during the process of ankle movement-related skill training and the recruitment changes seem to be related to the difficulty of the motor task. Therefore, the complexity of the motor learning tasks may induce different motoneuron recruitment patterns and, thus, differences in corticospinal responses as well.

In short-term training studies, Rosenkranz et al. (2007) demonstrated that as few as five training sessions of rapid thumb abductions induced signs of dormant synaptic connections and even format new synaptic connections. In addition, new synaptic connections may occur in different brain areas during the skill-learning process (Hikosaka et al., 2002). In the study of Li et al. (2001), researchers found that neural adaptation was already observed within a single learning session. At the same time, two different types of memory cells were found by short–and long-term motor skill training respectively. Therefore, corticospinal plasticity may be different in short- and long-term training. Some studies have shown similar results and suggested that already a few minutes of sensorimotor training can induce changes in neural activity in the M1 (Chen & Wise, 1995; Wise et al., 1998). On the other hand, functional magnetic resonance imaging studies have shown that the dorsolateral prefrontal cortex is active during the early learning stage, and parietal areas were activated at a later stage (Floyer-Lea & Matthews, 2005; Sakai et al., 1998). Also, short-term motor learning was associated with decreased activity in the dorsolateral prefrontal, anterior cingulate, posterior parietal, primary motor, and cerebellar cortex, and with increased activity in the right cerebellar dentate nucleus, the left putamen, and left thalamus (Floyer-Lea & Matthews, 2005). This implies that short-term motor skill learning seems to be accompanied primarily with activation in a cortical network specific to the learned movements.

2.4.2 Long-term sports training

Long-term sports training includes repeating movements, and thus motor learning, and has been shown to increase corticospinal excitability (Hötting & Röder, 2013; Singh et al., 2016). Meanwhile, different training categories such as endurance and skill training seem to modify the neural system differently (Schlaffke et al., 2014). MEP-related corticospinal excitability and plasticity changes have been investigated in athletes of different sport types. For example, skill-trained athletes have higher corticospinal plasticity and excitability compared with endurance trained athletes (Kumpulainen et al., 2015).

2.4.2.1 Endurance training

Endurance training is a type of physical exercise that involves sustained physical activity for extended periods (Pate & Branch, 1992). Endurance training can be achieved through various forms of exercise such as running, swimming, cycling, or cross-country skiing. Endurance training aims to increase the capacity of

continuous motor output by repeating the same movement sequence, thus, increasing the efficiency of the movement (Barnes & Kilding, 2015).

Along with the physical benefits, endurance training has also been shown to produce significant neural adaptations and benefits in the brain (Taubert et al., 2015; Winter et al., 2007; Zoladz et al., 2008). A study by Svatkova et al. (2015) used diffusion tensor imaging to investigate white matter integrity in both schizophrenia and healthy controls after cycling training. Increased white matter in both groups was observed after training. Researchers suggested that neural connectivity in the brain was still improved by 'overlearning' even though this motor skill was already acquired. Another study used same approach to investigate the improved microstructural organization of the corpus callosum in response to endurance training in young runners. The results showed increased fractional anisotropy values in several white matter tracts, including the corpus callosum, the superior longitudinal fasciculus, and the corticospinal pathway (Tarumi et al., 2022). This implies that endurance training can lead to improvements in the structural connectivity of the brain. On the other hand, Knaepen et al.'s study (2010) showed endurance training has also been shown to increase the production of neurotrophic factors such as brain-derived neurotrophic factor (BDNF) and vascular endothelial growth factor (VEGF). These factors are involved in promoting the growth and survival of neurons and the formation of new blood vessels, respectively. Increases in these factors have been associated with improvements in cognitive function and corticospinal plasticity (Müller et al., 2020). Endurance training increases cognition and neuroplasticity in several brain regions such as the cerebellum, hippocampus, and cerebral cortex via different mechanisms of global affection, such as altered blood volume in the brain and lactate induces elevation of neural growth factors. However, it does not alter specific motor map organization or synapse number (synaptogenesis), which is produced by motor learning (Taubert et al., 2015; Thomas et al., 2012).

At the spinal level, the excitability of the motoneurons is known to adapt after long-term endurance training (Koceja et al., 2004). Higher H-reflex has been demonstrated to relate to higher neural excitability at the spinal level, which has been discussed previously. A study by Vila-Chã et al. (2012) examined three weeks of endurance and strength training and found a reduction in H-reflex threshold in both while increasing H-reflex amplitude was only observed in the endurance training group. In a study by Ogawa et al. (2009), well-trained swimmers demonstrated higher H-reflex responses following rapid ankle rotation than non-trained individuals (Ogawa et al., 2009). Similar results were later introduced for endurance runners (Ogawa et al., 2012). However, a recent study by Bertschinger et al. (2021) did not support the hypothesis that cyclists have higher H-reflexes compared to recreationally active controls, even though cycling is defined as a typical endurance sport. Contradictory results could be due to methodological differences in assessing the H-reflex or due to training-specific proprioceptive control of the different endurance sports. However,

supraspinal influence should also be considered since it can affect spinal motoneurons' responsiveness to Ia afferent inputs (Gruber et al., 2007).

Overall, long-term endurance training generally results in enhanced spinal excitability and increased blood flow and oxygen delivery through angiogenesis of brain regions. However, there is no clear evidence showing that long-term endurance training directly participates in the modulation of synaptic number or topology, which affects the number or arrangement of synapses in the brain (Chen et al., 2019; Churchill et al., 2002; Taubert et al., 2015).

2.4.2.2 Skill training

Skill training is defined as the acquisition and subsequent refinement of novel movement sequences (Adkins et al., 2006). Skill training such as dancing, martial arts, and gymnastics require a high level of neuromuscular coordination, precision, and fine-tuned movements. According to neuroimaging studies, learning a new sport (e.g., dancing, gymnastics) that triggers motor skill learning processes, produces neural adaptation in the brain that optimizes the neural circuits responsible for controlling a focused task especially when compared to a simple movement, for example grasping and moving small objects (Papale & Hooks, 2018; Ungerleider et al., 2002). During a single motor skill learning session, there is an increase in corticospinal excitability in the area controlling the corresponding limb. During long-term skill training, the size of representation areas of M1 demonstrated adaptative changes depending on the skills (Vaalto et al., 2013). Furthermore, numerous studies have demonstrated that corticospinal excitability increases following long-term training of related muscles (Christiansen et al., 2017; Suzuki et al., 2012).

Long-term skill training involves complex movements that require repeated practice and coordinated control of the neuromuscular system. Through repetitive motor learning, the brain undergoes neural adaptations that optimize the neural circuits responsible for controlling these movements, resulting in increased efficiency of the neural network at both cortical and subcortical levels (Reithler et al., 2010). As the skill-trained athlete practices the same movements repeatedly, the brain undergoes a process of neural plasticity, which involves the formation of new neural connections (Kleim et al., 2004), and the strengthening and/or weakening of existing ones, such as LTP/LTD respectively (Bruel-Jungerman et al., 2007). Also, BDNF and VEGF are released after motor learning, which is related to neural plasticity (Cotman et al., 2007). Neural adaptations that occur in response to skill training are the development of more efficient neural connections in both intracortical and cortical spinal levels. It allows motor control processing to become more efficient at coordinating the movements, reducing the amount of cognitive effort required to execute them (Dayan & Cohen, 2011). A TMS-based experiment showed that skill-trained athletes have a higher capacity for corticospinal plasticity of the test-relevant muscle (soleus) compared to endurance trained athletes (Kumpulainen et al., 2015). Changes in the structure of the brain have also been shown by studies on long-term skill training. For example, a study by Hänggi et al. (2010) found that professional ballet dancers

have increased grey matter volume in brain regions responsible for motor control and coordination, compared to non-dancers. Similarly, Berkowitz and Ansari (2010) found that gymnasts have a greater cortical thickness in brain regions responsible for motor planning and execution compared to non-athletes. Sports training has also been shown to improve cognitive functions such as attention, memory, and decision-making. Chang et al. (2010) reviewed cognition of older adults after Tai Chi training and suggested that cognitive demands of learning complex movements may result in broader cognitive benefits.

While several studies have investigated the neural adaptations that occur in the brain during skill and endurance training, there are still many unanswered questions, such as the mechanism of neural adaptations that occur in response to different training.

2.4.3 Balance training

Postural control involves modification of sensory and motor systems and their adaptation to different tasks and environmental demands (Shumway-Cook & Woollacott, 2007). Many sport activities require good static and/or dynamic postural control ability, and balance training has been shown to benefit sports such as shooting, soccer, and gymnastics (Bekris et al., 2012; Hrysomallis, 2011). For example, balance training suppresses spinal reflex excitability leading to improved balance skills, which are particularly relevant in gymnastics (Hrysomallis, 2011). A recent systematic review introduced that balance training induced spinal reflex excitability changes may also depend on age (Sun et al., 2022). It has been observed that balance training leads to a consistent decrease in the soleus H-reflex amplitude in younger people, while it is less affected or even increased in older individuals (Gruber et al., 2007; Ruffieux et al., 2017; Sun et al., 2022).

On the other hand, during balance perturbation tasks, MEPs have been found to reduce only at LLR, indicating that neural adaptation occurs at the supraspinal rather than the spinal level after balance training (Taube et al., 2008). A study by Mouthon and Taube (2019) demonstrated an increased short-interval intracortical inhibition (SICI) following two-week, including six training sessions of balance training, suggesting enhanced intracortical inhibition. Lauber et al. (2021) showed that SICI initially increased during the early stages of balance training but then returned to baseline as training progressed. Researchers proposed that there may be a high level of cortical drive at the beginning of balance training, and then shifts to a subcortical level once balance control has been acquired (Logan, 1979). This evidence may suggest that long-term balance training induces neural adaptation that transforms from the cortical level to the spinal level. However, the mechanisms underlying balance training-induced corticospinal adaptation seem not to be fully understood, and contradictory results observed in these studies may be attributed to differences in the training duration, balance tasks, and the age of the participants.

3 PURPOSE OF THIS STUDY

Recently, a review by Bestmann and Krakauer (2015) discussed the relationship between motor skill acquisition and modulation of corticospinal excitability. The researchers suggested that there are several possible reasons for the lack of association between the two. Long-term training of some sport discipline usually involves motor skill learning and consequently repeated training. It is well known that motor skill expertise is associated with functional/structural cortical plasticity. Nevertheless, the manner in which this neuroplastic reorganization results in modifications to motor learning processes remain unclear. (Seidel et al., 2017).

Motor control and the underlying process of motor skill learning are closely interconnected with environmental changes and proprioceptive processing. Consequently, this series of studies assessed corticospinal excitability in response to translational and rotational ankle perturbation tasks within the framework of motor skill acquisition. The detailed purposes of the preset studies can be characterized as follows:

The reliability of neither TMS nor H-reflex measurement during high amplitude translational perturbation tasks is known, but such methods are used by researchers to examine differences between groups and/or the effects of interventions. Thus, it is important to determine such reliability to enable full evaluation of the scientific methodology employed within those studies. Therefore, the aim of the first experiment (Experiment I) was to examine the test-retest reliability of MEPs and H-reflex responses as well as corticospinal modulation during a high amplitude translational perturbation task.

Currently, little is known regarding how repeated balance training affects motor performance during translational perturbation tasks, or what the contribution of the spinal and supraspinal mechanisms behind this improvement is. The purpose of the second experiment (Experiment II) was to investigate whether short-term motor learning leads to performance improvement during

ankle translational perturbation. An additional aim was to determine the neural mechanism that might modulate the improvement in balance control ability.

The aim of the third experiment (Experiment III) was to explore the contribution of/and the underlying corticospinal mechanisms mediating motoneuronal responses to rotational perturbation of skill and endurance trained athletes. Both skill and endurance training are known to lead to distinctive neuronal adaption, but there are contentions about how the different types of long-term training affects corticospinal plasticity. Whether muscle stretch influences corticospinal facilitation/inhibition differently in endurance- and skill-trained athletes and, thus, the mechanism(s) behind natural movement remain unknown.

4 METHODS

4.1 Subjects

This dissertation consists of three original manuscripts, each of which is based on a separate experiment (I, II, III). A total of 48 healthy subjects volunteered to participate in these experiments. Descriptive characteristics of the subjects of the three experiments are presented in Table 1. None of the participants had any history of neuromuscular diseases and all participants were informed about the procedures and gave written informed consent. The study was approved by the ethics board of the University of Jyväskylä and was performed in conformity with the declaration of Helsinki. Nonathlete subjects were recruited in Experiment I and II (reference number: 267/13.00.04.00/2021), athletes were recruited in Experiment III (16/7/2019). In Experiment III, the endurance group had trained endurance sports on average for 12 ± 3 years for 11 ± 3 h per week. Three participants practiced cross-country skiing, two long-distance running, three triathlon, and two swimming. The skill group had trained in skill sports on average for 13 ± 3 years for 9 ± 1 h per week. Four participants practiced aerobic gymnastics, three aesthetic group gymnastics, two martial arts, and one dancing.

TABLE 1 Physical characteristics of the study subjects (mean ± standard deviation).

Experi-	Group	N	Sex (M/F)	Age (yr)	Height (cm)	Weight
I	/	14	8/6	35 ± 6	173.5 ± 10.6	71.8 ± 17.0
II	/	14	7/7	33 ± 5	171.3 ± 9.3	72.8 ± 14.2
III	Endurance	10	7/3	25 ± 3	176.3 ± 8.3	69.7 ± 9.5
	Skill	10	1/9	22 ± 3	165.2 ± 8.4	67.0 ± 7.8

Subjects were asked to not participate in exhaustive exercise 24 hours before measurements and not to take any caffeine on the measurement day to avoid interference with the TMS protocol (Turco et al., 2020).

4.2 Experimental design

4.2.1 Pre-study

To investigate stability of the new custom-built helmet for the TMS coil and TMS cable holder system, a pre-study in Experiment I was performed with two subjects (A: 160.0 cm, 45.0 kg; B: 185.0 cm, 96.4 kg). Subjects went through the same translational perturbation setup as in Experiment I. Kinematic data of the TMS coil and the subject were recorded at 150 Hz by a five-camera motion capture system (Vicon Motion System, Oxford, UK). Three markers were placed on the subject's head to build the head coordinate system. Two markers were placed on the coil handle to estimate TMS coil movement since the coil was covered by the helmet, which made it impossible to place any markers on the coil itself (Figure 6).

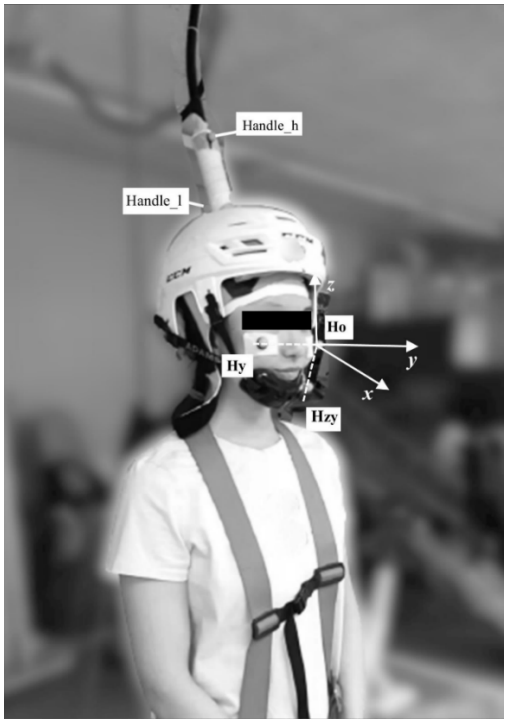

FIGURE 6 Subject with motion capture markers in the pre-study. Three markers were placed on the head: 'Ho' was the origin of the head coordinate system; 'Hy' was utilized to build the y-axis with 'Ho'; 'Hzy' was the point on the zy- plane, which produced x-axis by cross product with y-axis). The z-axis was built by the cross product of x-axis and y-axis. Handle_h was the marker on the higher position of the TMS handle, and Handle_l was the marker placed on the lower position of the TMS handle.

4.2.2 Experiment I

Experiment I was conducted over two sessions with the same tasks repeated and 48 h separated session 1 (S1) and session 2 (S2). In each session, after EMG electrode setup and five minutes cycling warm-up (70 W) on the fitness cycle (Monark, 282E, Varberg, Sweden), 16 translational perturbations as one set without any stimulation were used to collect centre of pressure (COP), muscle activity, and calculate the latency from platform movement to ankle movement onset. Then, subjects were positioned in a custom-built ankle dynamometer (University of Jyväskylä, Finland) to test the isometric maximal voluntary contraction (MVC) of the right leg soleus muscle. The TMS coil was set up and the active motor threshold (aMT) was tested when subjects sat in the ankle dynamometer. With a TMS coil set on the head and held by the custom-built helmet (Figure 7), subjects carefully stood up and moved to the balance platform. MEPs were measured during standing rest, and at four different time points (10 ms, 40 ms, 80 ms, and 140 ms) relative to the onset of ankle movement during translational perturbation in random order. The H-reflex measurements were always performed after TMS due to practical reasons. During standing rest, maximum M-wave (M_{MAX}) and H-reflex/M-wave recruitment curve were measured. In perturbation tasks, H-reflexes were elicited at the same four time points as the MEPs also in random order. The stimulations were delivered during each perturbation, regardless of perturbation direction, but only MEPs and H-reflex during backward perturbations were analysed.

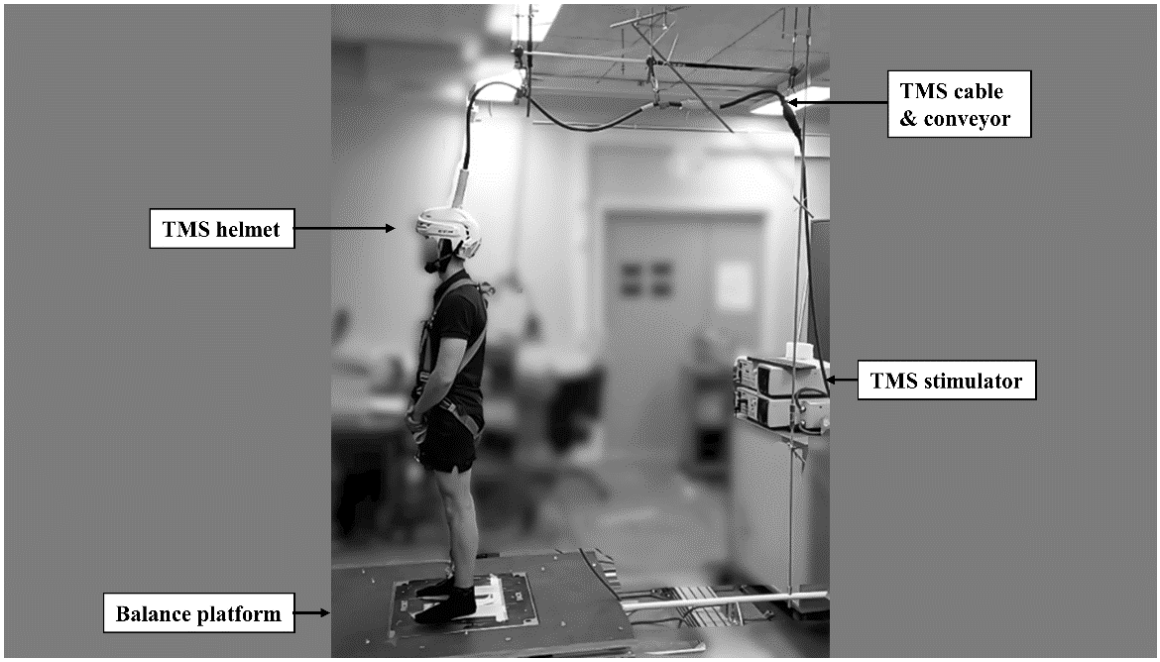

FIGURE 7 Measurement setup in Experiments I and II. The TMS coil's cable was connected to a conveyor on the roof to relieve the weight and moved along with the balance platform during perturbation.

4.2.3 Experiment II

Three perturbation sessions (PS1, PS2, PS3) were conducted with 48 h intervals. The protocol of PS1 and PS3 was similar to Experiment I (Figure 7). TMS and H-reflex measurements were delivered during standing rest and at two time points (40 ms and 140 ms) during translational perturbation tasks. In PS2, 13 perturbation sets, with a total of 208 perturbations were given to subjects with one to two minutes rest between perturbation trials.

4.2.4 Experiment III

In Experiment III, a single-pulse TMS session and a paired-pulse TMS session were conducted. After EMG electrodes were set, subjects were positioned on a custom-built ankle dynamometer (Figure 8, University of Jyväskylä, Finland). Then the M_{MAX} and MVC of the right soleus muscle were measured separately. 10 trials of rotational perturbation were using to measure SLR. After identifying the TMS stimulation hotspot and resting motor threshold (rMT), 10 single-pulse TMS stimuli were administered at 120% of the rMT. In each session, MEPs were measured by TMS in four conditions: at the beginning of the pedal movement (Onset), during SLR, 120 ms after SLR in a passive condition (p120), and 120 ms after SLR while plantar flexing the ankle to 25% of MVC (a120). All single-pulse trials were performed during single-pulse TMS sessions and then, following five days, all paired-pulse trials were performed in paired-pulse TMS sessions.

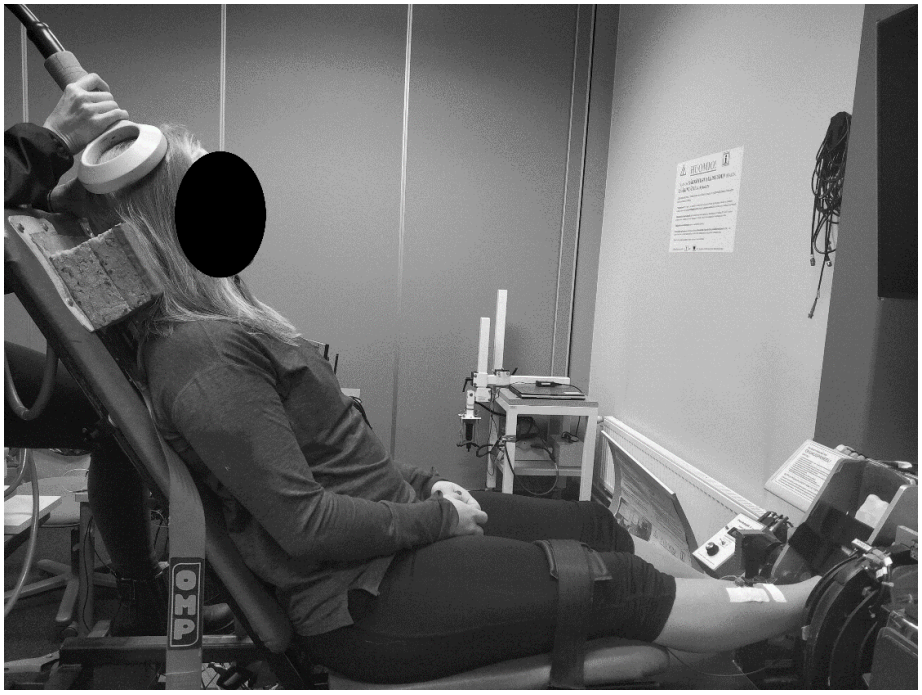

FIGURE 8 Measurement setup and subject position in Experiments III. The subject sat on a custom-built ankle dynamometer during the experiment.

4.3 Measurements

4.3.1 Electromyography

4.3.1.1 EMG setup in Experiments I and II

EMG was measured by bipolar electrodes (Blue Sensor, Ag/AgCl, 28 mm^2, Ambu A/S, Ballerup, Denmark) placed 2 cm below the gastrocnemius on the line of the Achilles tendon for soleus muscle. For the tibialis anterior and gastrocnemius muscles, EMG was placed according to SENIAM guidelines (1999). In the pilot study, discomfort, and strong muscle twitch were reported from some subjects during 140 ms. To reduce potential discomfort and tension caused by high-intensity stimulation, a pseudo-monopolar setup on soleus was used in TMS measurements as has been done previously (Blazevich et al., 2012; Kirk et al., 2019). The pseudo-monopolar setup provides a better representation of the electrical characteristics of the action potentials (Rodriguez-Falces & Place, 2018), resulting in higher MEP amplitude compared to a bipolar arrangement with the same intensity of the stimulus and therefore, a lower intensity could be used during the measurements. In addition, according to practical experience, the shape of the MEP is more consistent with the pseudo-monopolar setup, which is important in dynamic tasks. A disadvantage of this electrode montage is that the signal-to-noise ratio can be compromised, however, this was not the case in the current study. One electrode was placed 2 cm below the gastrocnemius on the line of the Achilles tendon and the reference electrode was placed on the tibia at the same level. For all EMG setups, skin was shaved, carefully abraded with sandpaper, and cleaned with alcohol. Target skin impedance was less than 5 kΩ and if this was not the case, skin preparation was repeated. All EMG data were collected using the Neurolog EMG system (CED Ltd., Cambridge, England), with a gain of 1000. Data were band-passed filtered (15–500 Hz) and further collected using CED 1401 A/D-converter (CED ltd., Cambridge, England) and Spike2 (8.0) software (CED ltd., Cambridge, England) with a sampling rate of 5 kHz.

4.3.1.2 EMG setup in Experiment III

EMG measurements were performed by bipolar electrodes (Blue Sensor N, Ag/AgCl, 28 mm^2, Ambu A/S, Ballerup, Denmark) placed 2 cm below the gastrocnemius on the line of the Achilles tendon for soleus muscle and over the muscle belly for tibialis anterior at 1/3 of the distance between the fibula and medial malleolus. A reference electrode was placed on the ipsilateral medial malleolus. Before electrode placement, skin under the electrodes was shaved, abraded with sandpaper, and cleaned with alcohol to reduce the resistance below 5 kΩ. EMG signals were amplified (1000×) by a preamplifier (NL824; Digitimer, Welwyn Garden City, UK), and then band-pass filtered (10-1000 Hz) by another preamplifier (NL900D/NL820A; Digitimer Ltd., UK). EMG was sampled at 5 kHz and reaction forces were sampled at 1 kHz via a 16-bit AD converter (CED

power 1401, Cambridge Electronics Design Limited, UK). Spike2 software (CED, Cambridge, UK) was used for all online data collection and offline analyses.

4.3.2 Force measurement

4.3.2.1 Force measurements in Experiments I and II

MVC was used to investigate possible muscle fatigue between sessions and to determination of aMT (10% MVC). Subjects were positioned in a custom-built ankle dynamometer (University of Jyväskylä, Jyväskylä, Finland) to assess the MVC with the right foot on the plate at 100° hip angle, 180° knee angle (leg fully extended) and 90° ankle angle. After the positioning procedure, the subject performed 5–7 submaximal plantarflexion trials to practice performance. MVC was performed at least three times at one-minute intervals and the highest force value was considered as the MVC. If the last trial was > 5% greater than the second-best, single additional trials were performed until no further improvement was observed. The typical number of required maximum trials was 3–5. Reaction forces from the dynamometer pedal were measured by a strain gauge transducer with A/D converter and sampled at 1 kHz in Spike2 software.

4.3.2.2 Force measurement in Experiment III

MVC was measured to compare calf muscle force between groups and adjust muscle contraction levels during a120 (25% MVC). Subjects were positioned on a custom-built ankle dynamometer (University of Jyväskylä, Finland) with the hip at 120° and the right knee in a fully extended position of 180°. The right foot ankle was set at 90° and rested on a pedal of the dynamometer. A seat belt restricted movement of the upper body and straps secured the right thigh and foot. Hands were resting and held together during the measurement (Figure 8). The subject contracted the ankle plantar flexors sub-maximally several times for warm-up and then performed three maximal isometric plantarflexion actions with two minutes of rest between trials. The highest force value from the three trials was considered as the MVC. Reaction forces from the dynamometer pedal were measured by a piezoelectric crystal transducer (Kistler Holding, Winterthur, Switzerland) with A/D converter, and sampled at 1 kHz in Spike2 software.

4.3.3 Perturbation tasks

4.3.3.1 Translational perturbation in Experiments I and II

Translational perturbation tasks were completed on a custom-built dynamic balance device (University of Jyväskylä, Finland) modified from Piirainen et al.'s study (2013). The translational perturbation system operated at 0.25 m/s, accelerating at 2.5 m/s^2, over a 0.3 m displacement. During perturbation tasks, 16 perturbations were performed in one set, with eight anterior and eight posterior perturbations in random order which reduced the possible anticipation of the direction of perturbation. The order of perturbation direction was the same

in all sets. Two-min rest periods were given after every perturbation set to minimize possible muscle fatigue (Piirainen et al., 2013).

During translational perturbation tasks, COP values were collected by a force plate embedded inside the balance platform. One strain gauge sensor was placed in each of the four corners of the force plate (BT4 balance platform; HUR Labs, Tampere, Finland). The data was saved using the Coachtech-feedback system (University of Jyväskylä, Finland). Coachtech-feedback monitored COP displacement and only triggered the next perturbation when COP was below ± 5 mm level from the standing baseline for at least 1 s. This approach ensured that the subject was always keeping the initial body position and not anticipating the upcoming perturbation. A fixation point was set on the wall three meters from the subjects at eye level to stabilize the subjects' visual attention during measurements.

4.3.3.2 Rotational perturbation in Experiment III

A motor-driven ankle dynamometer (University of Jyväskylä, Finland) performed ankle rotational perturbation with dorsiflexion (rotational magnitude: 4°, speed: 200 °/s) in Experiment III (Figure 2).

4.3.4 Stretch reflex measurement

4.3.4.1 Short latency response in Experiments I and II

In the pilot study of Experiment I, the time difference between ankle and platform movement was identified by an ankle goniometer (Figure 9: cursor 2) and platform control signal Figure 9: cursor 1). A 17 ms to 33 ms time difference was observed between ankle movement and the platform control signal between different subjects. Therefore, a 25 ms constant delay was defined as the time difference between the platform control signal and the onset of ankle movement. Additionally, the latency of SLR from pilot subjects was calculated by Spike2 software using the average of the waveforms from eight backward perturbations of one perturbation set (Figure 9D). 40 ms after ankle movement was defined as SLR time point (Figure 9: cursor 4).

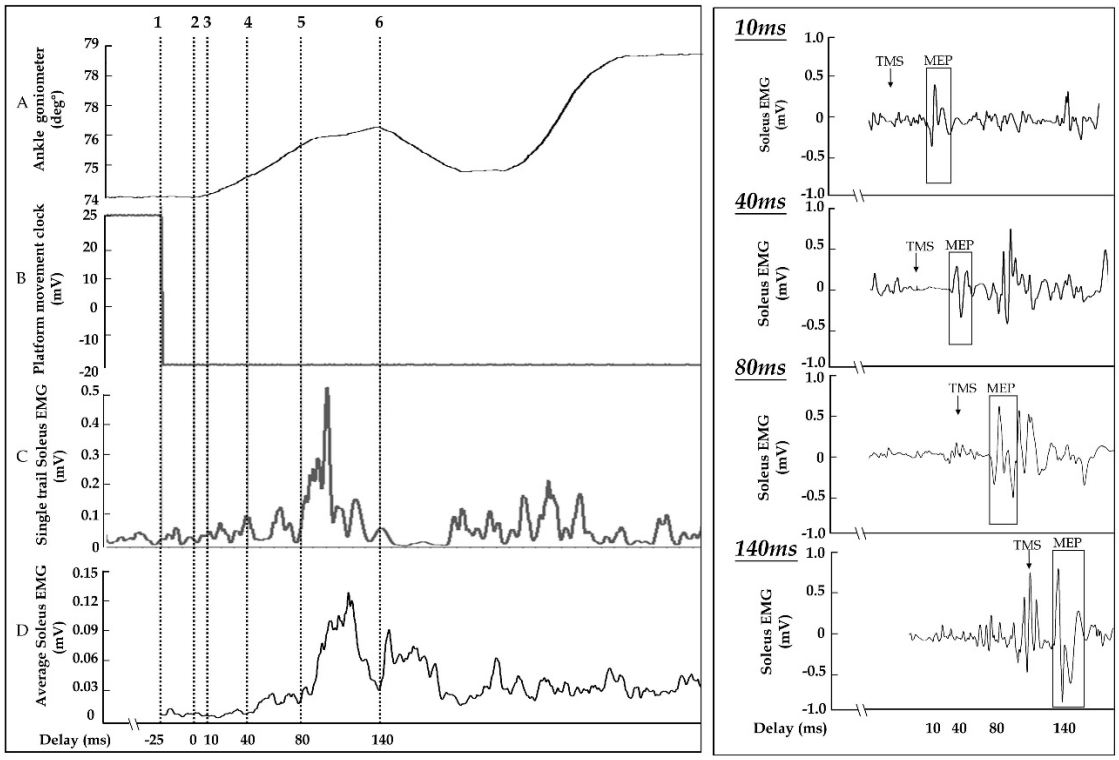

FIGURE 9 The sampling schematic of one posterior perturbation trial. The A channel shows ankle movement from the ankle goniometer (°), and cursor 2 was determined as the onset of ankle movement after perturbation onset. The B channel demonstrates platform movement starting from cursor 1. The C channel shows soleus EMG activities from a single perturbation trial (smoothed with a 2 ms window and rectified). The D channel shows the average EMG activity curve from eight posterior perturbation trials (smoothed with a 2 ms window and rectified) to estimate the delay for stimulation because of the EMG variability between perturbation trials. The right column shows the soleus MEPs from a single trial of perturbation in four time points (10 ms, 40 ms, 80 ms, and 140 ms).

4.3.4.2 Short latency response in Experiment III

The SLR was elicited by rotational perturbation in resting and contracting soleus muscle in experiment III. At the beginning of the experiment, 10 SLR trials were measured during muscle-relaxed condition to calculate the latency of SLR in Spike2 software using the average of the waveforms. The latency of SLR was then defined as the time between the onset of a digital trigger of pedal movement and the start of the ascending EMG signal.

4.3.5 Transcranial magnetic stimulation

4.3.5.1 TMS setup in Experiments I and II

TMS was delivered using a single-pulse Magstim 200² stimulator with a double-cone coil (Magstim, Whitland, UK). A skin-tight (swimming) cap was placed on the head of the subject to increase friction between the coil and the scalp. The optimal TMS stimulus site for the right soleus muscle was located on average 1 cm lateral (left) and 1 cm posterior to the cranial apex. Several stimulations were delivered to determine optimal coil placement and it was then marked on the cap. The aMT was defined as the lowest stimulus intensity to elicit clear MEPs in three out of five stimulations from right ankle plantarflexion with 10% MVC. After the confirmation of aMT, a second swimming cap with a hole in the middle of the vertex (Orca High Visibility Neoprene Swim Cap, Orca, Auckland, New Zealand) was placed over the coil to reduce the gap and relative movement between the coil and head. Then, the custom-made helmet (modified from an ice-hockey helmet; CCM TACK 710 JK-K, CCM Hockey, Montreal, Canada) was attached to the subject's head with a chin strap. Even though the helmet setup was tight, it was ensured that the helmet was as comfortable as possible with no reported discomfort caused to the subject. Then subjects moved to the balance system. The TMS cable was placed on a conveyor adjacent to the safety belt conveyor on the roof and connected with the balance platform by a firm handle, which raised the cable above the subject and moved it in the same direction as the balance platform during perturbations (Figure 7).

In experiment sessions, single-pulse TMS with 110% intensity of aMT was delivered during standing rest and translational perturbation tasks to investigate corticospinal excitability. The 110% intensity of aMT was chosen in order to cause less discomfort than the higher-level stimulations used in the pilot study. During standing rest, 10 trials of TMS were performed to calculate the latency of MEP before perturbation tasks. This allowed precise arrival of the MEP to the soleus muscle to coincide with the desired time points for each participant. In translational perturbation tasks, MEPs were elicited at different time points after ankle movement (i.e., Experiment I: 10 ms, 40 ms, 80 ms, and 140 ms; Experiment II: 40 ms and 140 ms; Figure 9, right column).

4.3.5.2 TMS setup in Experiment III

TMS was delivered using a paired-pulse Magstim 200² stimulator with a double cone coil (Magstim, Whitland, UK). Finding the hotspot of soleus muscle was done the same way as it had been done in Experiments I and II. rMT was defined as the lowest stimulus intensity to elicit clear MEPs in three out of five trials. 10 TMS stimulations with 120% of rMT intensity were delivered to calculate the latency of MEP before perturbation tasks.

In single-pulse TMS session, TMS was delivered during the four tasks. For each task, 10 TMS stimulations with ankle rotational perturbations were given with different intensities (120%, 140%, 150% of rMT) randomly. There were 5–8 s intervals between each TMS stimulation in each trial and 2 min rest between

conditions. In paired-pulse TMS sessions, subjects performed the same rotational perturbation tasks as in single-pulse TMS session paired-pulse TMS sessions. To investigate intracortical facilitation/inhibition, SICI and intracortical facilitation (ICF) were measured during the four conditions. SICI was elicited by paired pulse TMS stimulation with a suprathreshold TMS pulse (120% of rMT) after a subthreshold TMS pulse (80% of rMT) at 3 ms inter-stimulus interval. Similarly, ICF (15 ms inter-stimulus interval) was produced using the same sub- to suprathreshold intensities (Kujirai et al., 1993; Wassermann, 2002; Ziemann et al., 1996).

In both sessions, during passive tasks (Onset, SLR, and p120), participants were asked to perform an attention task, which consisted of counting down from 200 silently. During active task (a120), participants were asked to focus on a line marking 25% MVC level on a screen in front of them and perform plantar flexion to follow the force line throughout the trial.

4.3.6 H-reflex measurement (Experiment I, II)

For H-reflex measurements, subjects stood relaxed during the electrical stimulation set-up. Electrical stimulation was administrated to the tibial nerve in the popliteal fossa. A cathode (1.5 cm × 1.5 cm) was placed over the tibial nerve, and an anode (5 cm × 8 cm) was placed above the patella. Rectangular stimulation pulse (DS7AH, Digitimer Ltd., Hertfordshire, UK) with a duration of 0.2 ms was delivered at 10 s intervals. Once the optimal site of stimulation was established, the site was marked by a marker pen, and an electrode (Blue Sensor, Ag/AgCl, 28 mm^2, Ambu A/S, Ballerup, Denmark) was placed and strapped around the subject's knee with an elastic band. An increasing intensity interval (1-5 mA) was chosen to measure the H-M recruitment curve with at least 30 data points up to the M_{MAX}. The stimulus intensity was adjusted to 5 ± 2% of the M_{MAX}, which was used during perturbation to control H-reflex measurements.

During translational perturbation tasks, H-reflex measurement followed the same protocol as TMS trials. H-reflex was measured during standing rest and different time points in perturbation tasks. The M_{MAX} of soleus muscle was recorded to normalize the muscle response values (MEP, H-reflex, and muscle activity). For H-reflex measurements, a successful trial from one perturbation set was considered when an M-wave response reached 5 ± 2% of the M_{MAX} value. The intensity of electrical stimulation was adjusted during perturbation trials to obtain at least five successful H-reflex trials. In cases where fewer than five successful H-reflex collections were achieved in a set, an extra perturbation set (four backward and four forward) was involved. For each perturbation task, five successful H-reflex were usually completed within one to one normal plus extra set (16-24 perturbations).

4.4 Data analysis and statistical methods

Statistical analyses were conducted using IBM SPSS 20.0 (SPSS, Chicago, USA). Result visualizations were performed using Prism (V9, GraphPad Software, San Diego, California USA). MEPs, H-reflex, and other muscle activity results were normalized by the peak-to-peak value of M_{MAX} and presented as $\%M_{MAX}$ in the results. The significance level was set at $P < 0.05$ and all results were displayed as mean ± standard deviation (SD). In addition, if variables were not normally distributed as tested by Shapiro-Wilk's W tests, variables were processed by log transformation prior to statistical analyses following Nielsen's suggestion (Nielsen, 1996).

4.4.1 Pre-study

Kinematic data were analysed using MATLAB (2019b, The MathWorks, Inc., U.S.). After coordinating transformation from ground coordinate to head coordinate system (H*xyz*, Figure 6), relative offset (maximum displacement) of the coil handle was analysed on *x*-axis, *y*-axis, and *z*-axis respectively to represent coil movement compared with the subject's head movement. In the coordinate system, the *x*-axis was the sagittal axis, the *y*-axis was the frontal axis, and the *z*-axis was the vertical axis.

4.4.2 Experiment I

In standing rest, mean soleus MEPs were determined by peak-to-peak amplitude (in mV) from 10 TMS stimulations. Outliers were identified from the 10 trials (± 2.5 SD of the mean) and removed before analysis (Avenanti et al., 2006). The average MEP latency and duration were calculated in the standing rest condition and then utilized in the translational perturbation condition. MEP was defined as starting when EMG was above the mean +2SD level recorded 100 ms before the TMS trigger and ending when below the mean -2SD level (Hirano et al., 2016). However, this was only used in the standing condition since it was difficult to use these criteria during the perturbation due to increase in EMG. Thus, the MEP amplitude was obtained by calculating the peak-to-peak amplitude within the MEP onset and offset latencies calculated in the standing condition. MEP amplitudes from 7–8 trials when the platform moved backward were selected and averaged after excluding outliers (± 2.5 standard deviations of the mean). H-reflex was determined with peak-to-peak amplitude and averaged from all successful trials (within 3–7% M_{MAX}) in standing rest and translational perturbation tasks.

Test-retest reliability and inter-individual variability of MEPs and H-reflex amplitude between sessions were assessed via intraclass correlation coefficients (ICC) using a two-way mixed effects model with an absolute agreement using the average value from multiple trials. Standard error of measurement (SEM) was estimated as root mean square error ($\sqrt{}$ MSE) from a one-way ANOVA,

which avoids errors associated with ICC calculation. The minimal detectable change (MDC) was calculated as SEM × 1.96 × √2 (Weir, 2005). According to the ICC method guideline (Koo & Li, 2016), ICC was calculated between single stimulation trials, and trial-to-trial coefficient of variance (CV) with homoscedasticity of MEPs and H-reflex amplitudes to determine whether eight MEP/H-reflexes were adequate for calculating the average value. Reliability based on ICCs and 95% CIs were categorized as poor (ICC < 0.5), moderate (0.5 < ICC < 0.75), good (0.75 < ICC < 0.9), or excellent (ICC > 0.9). Bland-Altman plots of MEPs in all conditions were investigated to visualize the agreement between two sessions (Bland & Altman, 1995).

4.4.3 Experiment II

COP in anterior-posterior direction was calculated using the formula:

$$\mathrm{COP}y = \big((\mathrm{Flf} + \mathrm{Frf}) \times 0.26 - (\mathrm{Flr} + \mathrm{Frr}) \times 0.26\big) / (\mathrm{Flf} + \mathrm{Frf} + \mathrm{Frr} + \mathrm{Flr})$$

, where F is the force value from sensors (lf = left front, rf = right front, rr = right rear, lr = left rear), and 0.26 (m) is a sensor distance from the middle line. COP values were analysed in perturbation trials without stimulations. The mean standard deviation of the COP displacement curve was calculated to evaluate the general body sway. As shown in Figure 13, Peak-to-peak COP displacement (dCOP) was analysed in the time window of 1 s before platform movement (Preparation-phase; Pre), during platform movement (Active-phase; Act), and 1 s from the end of platform movement (Recovery-phase; Rec). The COP velocity curve was calculated by differentiating the COP curve by using 20 ms windows, and then the average COP velocity of the velocity curve (vCOP) was analysed in the same time window as dCOP. Both dCOP and vCOP were normalized by individual subject's height × weight (dCOP: mm/(m*kg); vCOP: (mm/s)/(m*kg)) according to the recommendation of Chiari et al. (2002).

In the perturbation tasks without stimulation, the average of all subjects' full wave rectified EMG from soleus, gastrocnemius, and tibialis anterior was analysed 100 ms before the ankle movement to 400 ms. EMG was normalized with maximal EMG during tasks. Furthermore, soleus EMG was analysed using root mean square (RMS) over 20 ms time windows from the perturbation onset (0 ms) to 180 ms after onset. In the perturbation tasks with stimulation, the EMG was calculated using RMS in a 30 ms window before stimulation, which was defined as background EMG.

In order to evaluate neural excitability changes between corticospinal and spinal level MEP / H-reflex ratio was calculated. To explore the relationship between the changes in balance performance and corticospinal correlation from PS1 to PS3, ΔMEP and ΔH-reflex and their correlation with Δ dCOP were analysed by Pearson product-moment correlation. Delta values were calculated using the formula:

Delta value (Δ) = (value of PS3 – value of PS1) / value of PS1 × 100%

All variables of the MEP / H-reflex ratio were processed by log transformation before statistical analyses following Nielsen's suggestion. MVC, M_{MAX}, and H_{MAX} / M_{MAX} values were assessed by paired t-test. Since dCOP and vCOP were analysed by different time windows (i.e., 1 s for Pre and Rec phase, but 1.3 s for the Act phase), between-session differences of dCOP, vCOP, and standard deviation of the COP displacement were examined by paired t-test. To assess adaptation in corticospinal excitability during perturbations, MEPs, H-reflex, Background EMG, and EMG activity were assessed by two-way (2 × 3) repeated-measures ANOVA with the factors SESSION (PS1 and PS3) and TIME (standing rest, 40 ms, 140 ms). When a significant F-value was observed, Mauchly's test was used to evaluate sphericity, and where the assumption was valid F-values were reported with sphericity-assumed degrees of freedom and df error $F_{(\text{sphericity assumed df, df error})}$. Effect sizes for the ANOVA main effects are reported as partial eta squared (η_p^2), where 0.02, 0.13, and 0.26 are considered small, medium, and large, respectively. If significance for TIME was revealed, Bonferroni post hoc analysis was used for pairwise comparisons between levels (i.e., standing rest, 40 ms, 140 ms). Correlations between MEP amplitude, H-reflex amplitude, and EMG activity were analysed by Pearson product moment correlation tests.

4.4.4 Experiment III

In single-pulse TMS session, MEP amplitudes from 120%, 140%, and 150% of rMT trials did not differ between groups. Consequently, the data from all stimulus intensities were averaged and defined as 'MEP_{AVG}', thereby increasing the number of trials per condition to 30. MEP_{AVG} at SLR condition was compared with stretch reflex (SR) values without stimulation to demonstrate the SR / MEP_{AVG} ratio. In PS, the peak-to-peak amplitude of conditioned MEP was compared to the test MEP. SICI and ICF were expressed as a percentage of the test MEP with the following formula:

SICI (or ICF) = (conditioned MEP/ test MEP) × 100%

A higher ICF percentage represents more facilitation, while higher SICI percentage values represent less intracortical inhibition when comparing conditions.

TMS-induced responses were assessed by a two-way repeated measures ANOVA with within-subjects factor of four levels (Onset, SLR, p120, and a120) and between-subjects factor groups of two levels (endurance and skill). When a significant F-value was observed, a post-hoc one-way repeated analysis of variance (ANOVA) with main factor group of four levels (Onset, SLR, p120, and a120) was used. Mauchly's test was used to evaluate sphericity, and where the assumption was valid F-values were reported with sphericity-assumed degrees of freedom and df error $F_{(\text{sphericity assumed df, df error})}$. MEP_{AVG} violated the assumption of sphericity and so F-values were reported along with the Greenhouse-Geisser

adjustments $F_{\text{(Greenhouse-Geisser adjusted df, df error)}}$. Effect sizes for the ANOVA main effects are reported as partial eta squared (η_p^2), where 0.02, 0.13, and 0.26 are considered small, medium, and large, respectively. To assess post-hoc differences between groups, an independent *t*-test was used. Correlations between MEP_{AVG} and stretch reflex, MVC, and MEP_{AVG} were analysed for non-log transformed MEP values and stretch reflex values using Spearman's rank correlation test.

5 RESULTS

5.1 TMS system stability during translational perturbation tasks (Pre-study)

Markers of the TMS handle displacement before (A: *x*-axis, B: *y*-axis, and C: *z*-axis) and after transformation (D: *x*-axis, E: *y*-axis, and F: *z*-axis) are shown in Figure 10. The maximum offset of the Handle_h marker demonstrated 7 ± 2 mm on the *x*-axis, 8 ± 2 mm on the *y*-axis, and 5 ± 1 mm on the *z*-axis. The offset of the Handle_l marker was 5 ± 1 mm on the *x*-axis, 5 ± 2 mm on the *y*-axis, and 4 ± 1 mm on the *z*-axis.

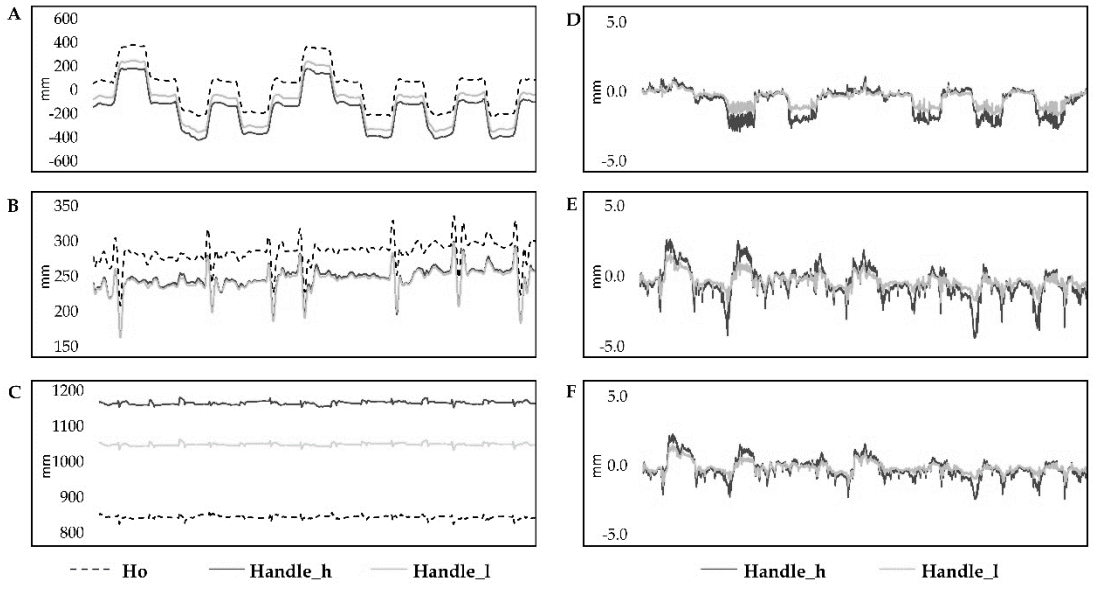

FIGURE 10 Kinematic data from one subject. Average marker's displacement from 14 perturbations is shown with ground coordinate system (left column) and head coordinate system (right column).

5.2 Reliability of using TMS during translational perturbation tasks (Experiment I)

5.2.1 Corticospinal excitability during perturbation tasks

In this study, MEPs were visible in all trials. MEPs demonstrated lower amplitude in standing rest and 10 ms time point of S2 compared to S1. The H-reflex increased in S2 compared to S1 at 10 ms time point (Table 2).

TABLE 2 Normalized MEP and H-reflex value (% maximum M-wave) in both sessions (S1 and S2). Significant differences between time are denoted by '*' ($P < 0.05$).

		S1	S2	$t_{(13)}$	P-value	η_p^2
MEP	Standing rest	7.06 ± 4.62	5.89 ± 3.88	2.217	0.045*	0.592
	10 ms	12.96± 8.14	11.11 ± 7.26	2.211	0.046*	0.591
	40 ms	15.86 ± 9.74	14.36 ± 9.76	1.455	0.169	0.389
	80 ms	16.52 ± 6.65	16.02 ± 6.04	0.561	0.585	0.150
	140 ms	34.27 ± 21.26	30.36 ± 16.84	0.946	0.361	0.253
		S1	S2	$t_{(12)}$	P-value	η_p^2
H-reflex	Standing rest	26.18 ± 10.72	28.87 ± 11.21	-0.720	0.486	-0.200
	10 ms	24.81 ± 8.86	30.96 ± 9.15	-2.460	0.030*	-0.682
	40 ms	27.99 ± 11.22	30.32 ± 11.09	-0.765	0.459	-0.212
	80 ms	38.29 ± 17.61	41.20 ± 14.72	-0.973	0.350	-0.270
	140 ms	42.99 ± 21.46	47.92 ± 17.04	-1.303	0.217	0.362

5.2.2 Within-session test-retest reliability

In S1, the ICC of MEPs showed excellent reliability and narrow 95% CI in standing rest and translational perturbations. In S2, ICC demonstrated good to excellent reliability of all MEPs. Within-session CV% of MEPs showed homoscedasticity when tested by Levene's statistics (Table 3).

TABLE 3 Within-session test-retest reliability (between stimulation trials) of MEPs for both sessions (S1 and S2) are shown in the table with ICC and 95% confidence interval. Coefficient of variance percentage (CV%) is shown as mean ± standard deviation.

	S1	
	ICC [95% CI]	CV%
Standing rest	0.953 [0.906, 0.982]	40.8 ± 14.5
10 ms	0.927 [0.932, 0.993]	39.1 ± 25.8
40 ms	0.964 [0.925, 0.987]	39.0 ± 23.1
80 ms	0.957 [0.909, 0.985]	33.4 ± 17.8
140 ms	0.983 [0.964, 0.994]	20.1 ± 8.0
	S2	
	ICC [95% CI]	CV%
Standing rest	0.934 [0.868, 0.975]	39.4 ± 11.7
10 ms	0.915 [0.924, 0.987]	41.6 ± 17.5
40 ms	0.960 [0.913, 0.986]	38.5 ± 20.7
80 ms	0.854[0.694, 0.948]	35.0 ± 14.5
140 ms	0.976 [0.950, 0.991]	22.7 ± 10.3

Within-session reliability of H-reflex responses showed to be good to excellent in both sessions and narrow 95% CI. Within-session CV% of H-reflex showed homoscedasticity when tested by Levene's statistics (Table 4).

TABLE 4 Within-session test-retest reliability (between stimulation trials) of H-reflex for each session (S1 and S2) are shown in the table with ICC and 95% confidence interval. Coefficient of variance percentage (CV%) is shown as mean ± standard deviation.

	S1	
	ICC [95% CI]	CV%
Standing rest	0.985 [0.968, 0.995]	21.5 ± 9.8
10 ms	0.945 [0.848, 0.989]	27.9 ± 11.8
40 ms	0.945 [0.837, 0.991]	33.1 ± 12.7
80 ms	0.979 [0.905, 0.999]	19.1 ± 11.7
140 ms	0.974 [0.881, 0.999]	24.0 ± 16.7
	S2	
	ICC [95% CI]	CV%
Standing rest	0.986 [0.971, 0.995]	18.6 ± 5.9
10 ms	0.956 [0.725, 1.000]	22.3 ± 8.0
40 ms	0.994 [0.963, 1.000]	22.1 ± 9.8
80 ms	0.874 [0.351, 0.997]	16.9 ± 8.6
140 ms	0.965 [0.843, 0.999]	17.8 ± 8.3

5.2.3 Between-session test-retest reliability

MEPs during standing rest demonstrated excellent test-retest reliability between sessions when considering the 95% CI (Table 5). During perturbation tasks, MEPs also showed excellent reliability (Table 5). From the Bland-Altman plot, the mean bias for MEPs at 10 ms time point (mean bias = 1.85%, 95% CI [-4.09, 7.80]; Figure 11) and 40 ms time point (mean bias = 1.50%, 95% CI [-8.78, 11.79]; Figure 11) were similar. MEPs at 80 ms time point showed the lowest bias (mean bias = 0.50%, 95% CI [-5.76, 6.77]; Figure 11), while MEPs of 140 ms time point demonstrated the highest bias and widest limits of agreement (mean bias = 3.91%, 95% CI [-10.23, 18.04]; Figure 11).

TABLE 5 Between-session test-retest reliability of MEPs (log-transformed data) with ICC and 95% confidence interval. Standard error of measurement (SEM) and minimal detectable change (MDC) is expressed in decimal form, which is the same as the original MEP data.

Time point	ICC [95% CI]	SEM (%M_{MAX})	MDC (%M_{MAX})
Standing rest	0.932 [0.789, 0.978]	0.232	0.644
10 ms	0.935 [0.811, 0.979]	0.210	0.581
40 ms	0.928 [0.797, 0.977]	0.152	0.420
80 ms	0.943 [0.777, 0.982]	0.032	0.088
140 ms	0.947 [0.835, 0.983]	0.084	0.232

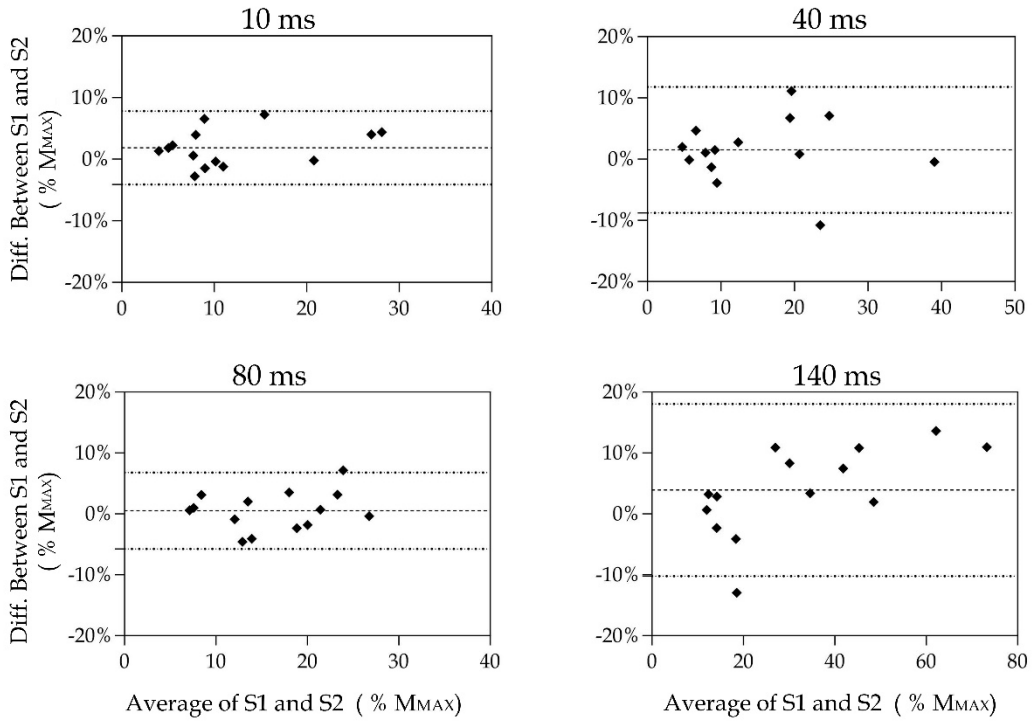

FIGURE 11 Bland-Altman plot for MEPs responses during perturbation tasks between sessions (S1 and S2). Each panel (10 ms, 40 ms, 80 ms, and 140 ms) shows the difference as a function of the average of the two testing sessions with dashed lines indicating the mean bias and 95% confidence intervals indicated by dot lines.

During standing rest, H-reflex demonstrated poor test-retest reliability, but during perturbation tasks, H-reflex showed moderate-to-good reliability. At the 10 ms time point, ICC demonstrated a wider 95% CI compared to the other time points (Table 6). Meanwhile, H-reflex showed the highest bias at the 10 ms time point (mean bias = -6.149%, 95% CI [-23.81, 11.51]; Figure 12). Similar limits of agreement for H-reflex were observed at 40 ms (mean bias = -2.328%, 95% CI [-23.82, 19.17]; Figure 12) and 80 ms time points (mean bias = -2.909%, 95% CI [-24.02, 18.21]; Figure 12). However, H-reflex demonstrated its widest limits of agreement at the 140 ms time point (mean bias = -4.935, 95% CI [-31.68, 21.81]; Figure 12).

TABLE 6 Between-session test-retest reliability of H-reflex (original data) with ICC and 95% confidence interval. Standard error of measurement (SEM) and minimal detectable change (MDC) is expressed in decimal form, which is the same as the original H-reflex data.

Time point	ICC [95% CI]	SEM (%M_{MAX})	MDC (%M_{MAX})
Standing rest	0.475 [-0.771, 0.841]	0.071	0.169
10 ms	0.626 [-0.079, 0.881]	0.158	0.378
40 ms	0.720 [0.086, 0.914]	0.063	0.151
80 ms	0.887 [0.644, 0.965]	0.071	0.169
140 ms	0.865 [0.577, 0.958]	0.126	0.302

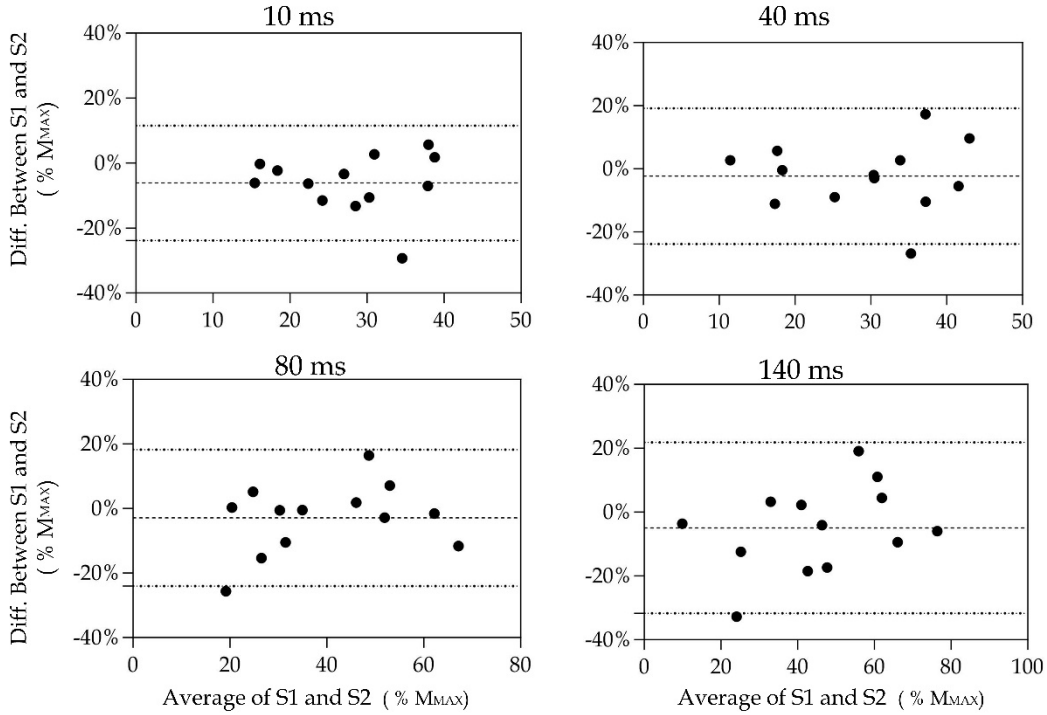

FIGURE 12 Bland-Altman plot for H-reflex during perturbation tasks between sessions (S1 and S2). Each panel (10 ms, 40 ms, 80 ms, and 140 ms) shows the difference as a function of the average of the two testing sessions with dashed lines indicating the mean bias and 95% confidence intervals indicated by dot lines.

5.3 Balance control performance after short-term translational perturbation training (Experiment II)

dCOP (mm/(m*kg)) and vCOP ((mm/s)/(m*kg)) of Pre, Act, and Rec were analysed to explore balance performance in anterior-posterior direction before, during, and after the onset of balance platform movement, respectively (Figure 13). Both dCOP and vCOP at all phases decreased significantly from PS1 to PS3 (Table 7). Standard deviation of the COP displacement demonstrated significant decrease at PS3 compared to PS1 (PS1: 0.16 ± 0.05; PS3: 0.13 ± 0.06, $t_{(13)} = 2.741$, $P = 0.017$).

TABLE 7 COP displacement (mm/(m*kg)) and COP velocity ((mm/s)/(m*kg)) in Pre, Act, and Rec phases are shown with t-value and P-value of the paired *t*-test. Significant differences between time are denoted by '*' ($P < 0.05$) and '**' ($P < 0.001$).

		PS1	PS3	Mean difference	$t_{(13)}$	P-value	Hedge's *g*
dCOP	Pre	0.09 ± 0.04	0.08 ± 0.04	0.01	2.177	0.049*	0.217
	Act	1.14 ± 0.34	1.01 ± 0.27	0.13	2.483	0.027*	0.395
	Rec	0.64 ± 0.21	0.44 ± 0.18	0.20	4.642	< 0.001**	0.995
vCOP	Pre	0.16 ± 0.08	0.13 ± 0.06	0.03	2.951	0.011*	0.429
	Act	1.59 ± 0.57	1.31 ± 0.40	0.28	3.212	0.007*	0.548
	Rec	0.97 ± 0.35	0.66 ± 0.29	0.31	5.405	< 0.001**	0.928

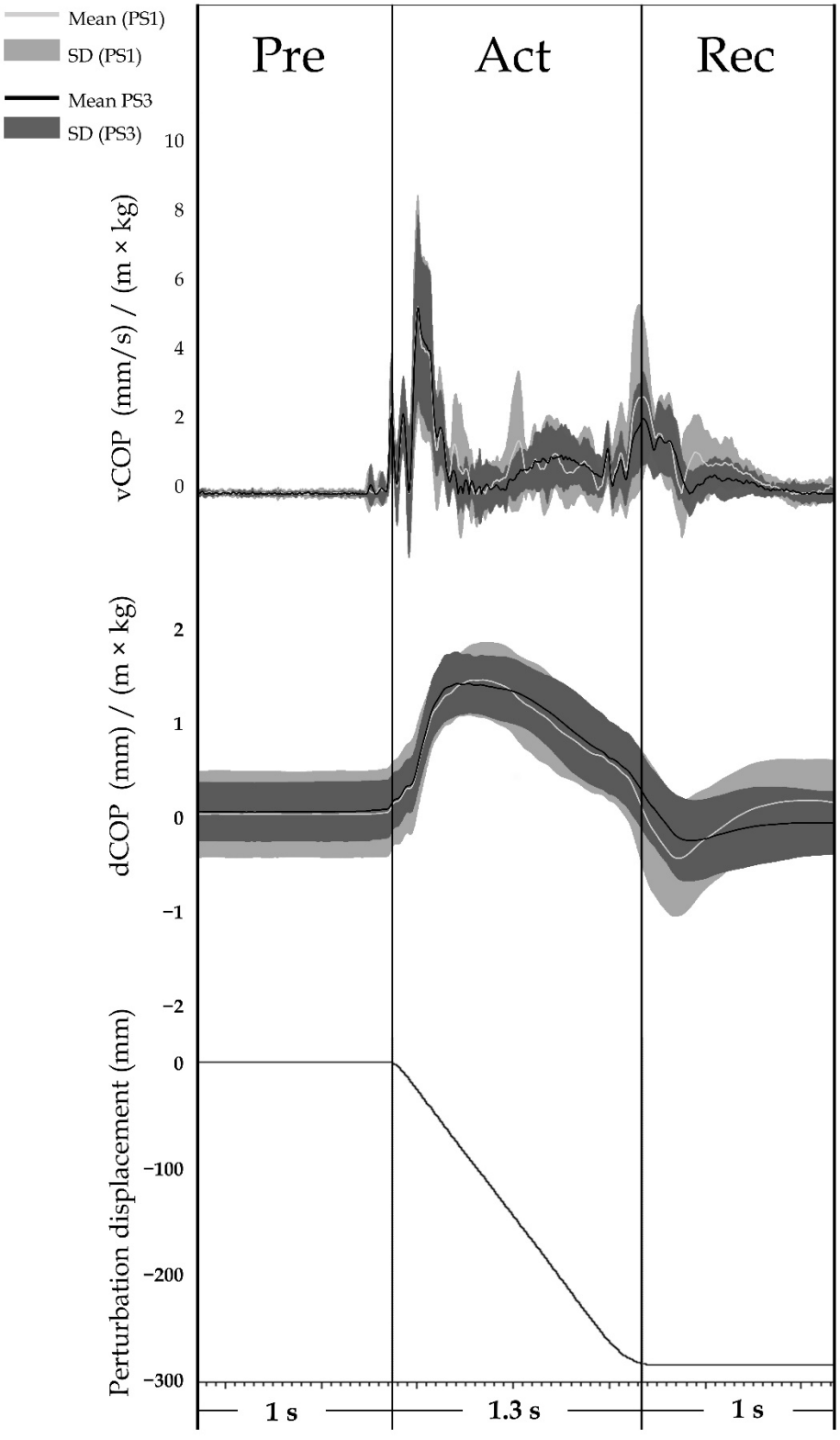

FIGURE 13 Average COP displacement (dCOP) and velocity (vCOP). The signal diagram (bottom part) shows the displacement of the perturbation platform movement (negative value means the platform moved backward).

5.4 Corticospinal modulation after short-term translational perturbation training (Experiment II)

5.4.1 Corticospinal excitability

There was no significant difference demonstrated in M_{MAX}, H_{MAX} / M_{MAX}, or MVC (Table 8).

TABLE 8 Between sessions results of maximum M-wave (M_{MAX}), maximum H-reflex and M-wave ratio (H_{MAX} / M_{MAX}), and maximum voluntary contraction force (MVC).

	PS1	PS3	t(13)	P-value
M_{MAX} (mV)	6.8 ± 1.3	6.6 ± 1.5	0.907	0.381
H_{MAX} / M_{MAX} (%)	48.5 ± 16.2	47.4 ± 18.2	0.279	0.785
MVC (Nm)	1756.9 ± 480.9	1813.7 ± 480.9	-2.070	0.059

A significant main effect for time was observed in MEP ($F_{(1.118, 30.897)} = 39.355$, $P < 0.001$, $\eta_p^2 = 0.602$; Figure 14A), but there was no main effect for session ($F_{(1, 26)} = 0.817$, $P = 0.374$, $\eta_p^2 = 0.031$) or session × time interaction ($F_{(1.118, 30.897)} = 0.267$, $P = 0.650$, $\eta_p^2 = 0.010$). However, significant differences over time were observed from 40 ms to 140 ms ($P < 0.001$). In addition, MEP during the standing rest was lower than 40 ms ($P = 0.012$) and 140 ms ($P < 0.001$). Background EMG values demonstrated significant increase at 140 ms compared to other times (Figure 14B).

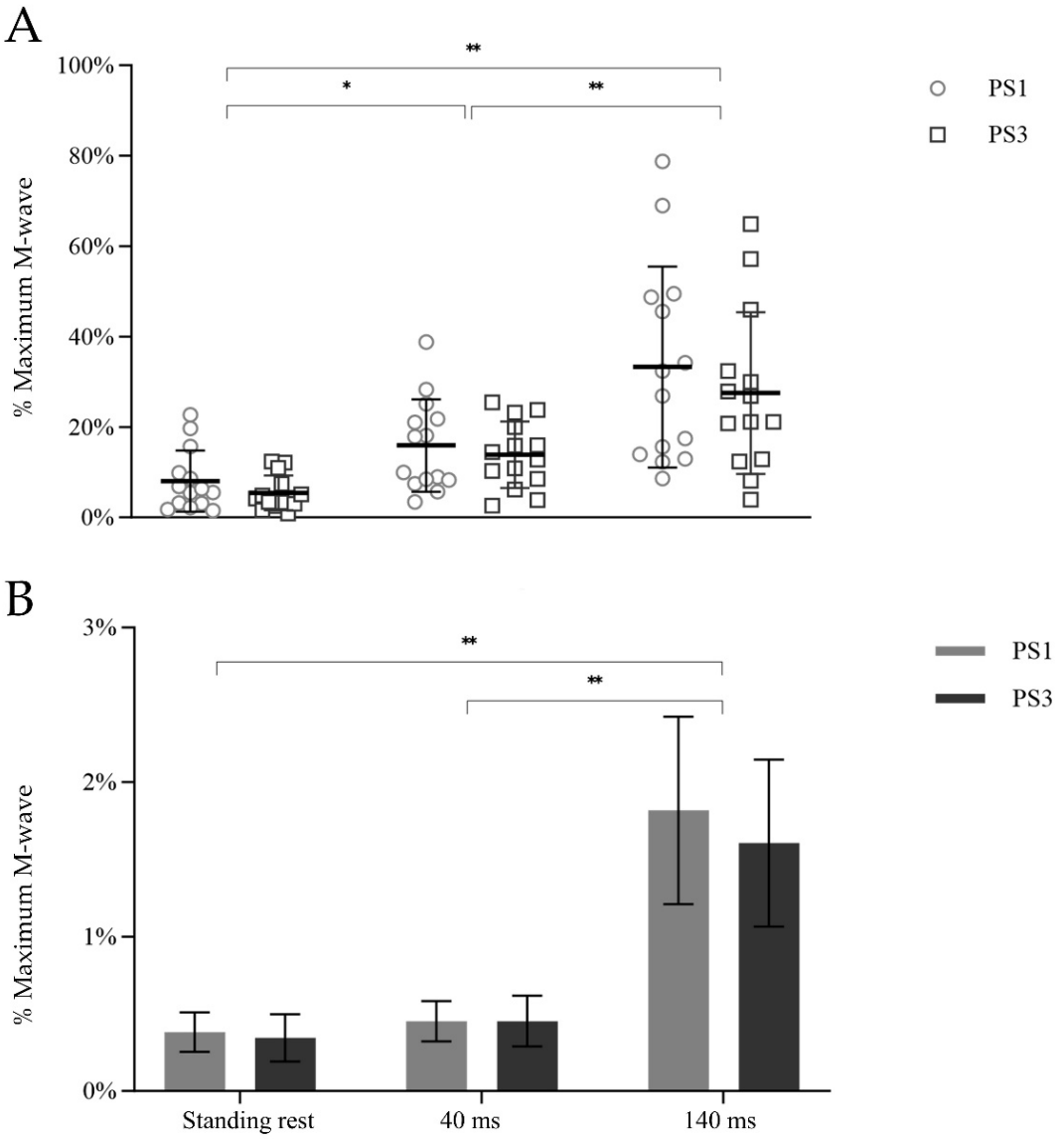

FIGURE 14 A: Motor evoked potential (MEP) and B: background EMG between sessions (PS1 and PS3). Three different times (standing rest, 40 ms, and 140 ms) were shown in the x-axis. Symbols represent the MEP values of individual subjects and bar charts represent the Background EMG activities. Significant differences between time are denoted by '*' ($P < 0.05$) and '**' ($P < 0.001$).

Similarly, no between session difference was shown in H-reflex ($F_{(1,26)} = 0.048$, $P = 0.828$, $\eta_p^2 = 0.002$; Figure 15A) or session × time interaction ($F_{(1.273,33.099)} = 0.638$, $P = 0.466$, $\eta_p^2 = 0.024$). However, a significant main effect over time was observed ($F_{(1.273,33.099)} = 36.269$, $P < 0.001$, $\eta_p^2 = 0.582$). Post hoc tests showed increased H-reflex from 40 ms to 140 ms ($P < 0.001$). In addition, H-reflex during the standing rest was lower than 140 ms ($P < 0.001$). Background EMG values demonstrated an increase at 140 ms compared with the other times ($P < 0.001$; Figure 15B).

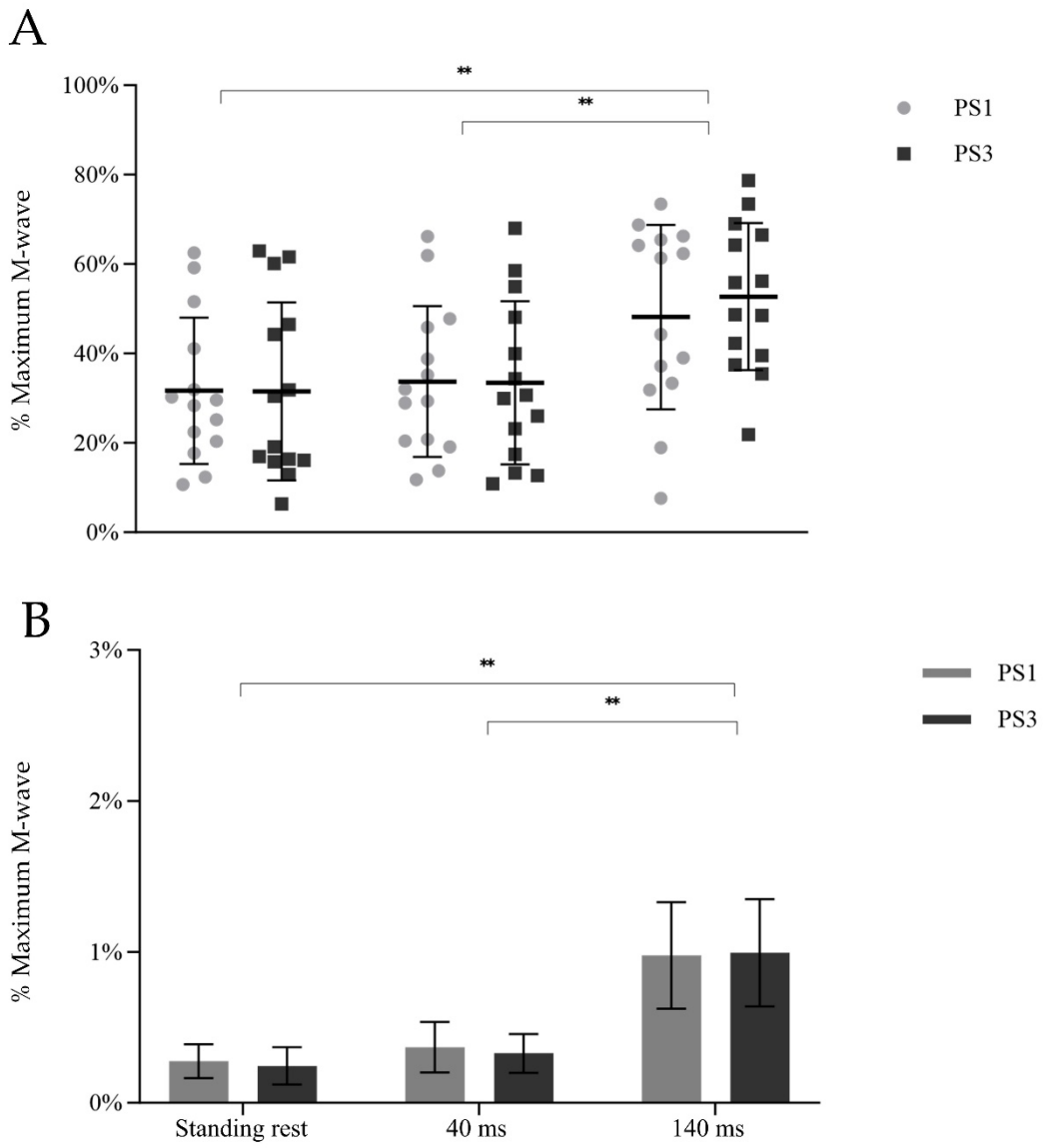

FIGURE 15 A: H-reflex and B: background EMG between sessions (PS1 and PS3). Three different times (standing rest, 40 ms, and 140 ms) were shown in the x-axis, in which symbols represent H-reflex values of individual subjects and bar charts represent the Background EMG activities. Significant differences between time are denoted by '*' ($P < 0.05$) and '**' ($P < 0.001$).

A significant main effect for time was observed in the MEP / H-reflex ratio ($F_{(1.592,\ 41.397)} = 37.174$, $P < 0.001$, $\eta_p^2 = 0.588$; Figure 16), but there was no main effect for session ($F_{(1,\ 26)} = 0.541$, $P = 0.469$, $\eta_p^2 = 0.020$) or session × time interaction ($F_{(1.592,\ 41.397)} = 0.704$, $P = 0.469$, $\eta_p^2 = 0.026$). Post hoc tests demonstrated an enhanced MEP / H- reflex ratio from standing rest to 40 ms ($P < 0.001$) and standing rest to 140 ms ($P < 0.001$).

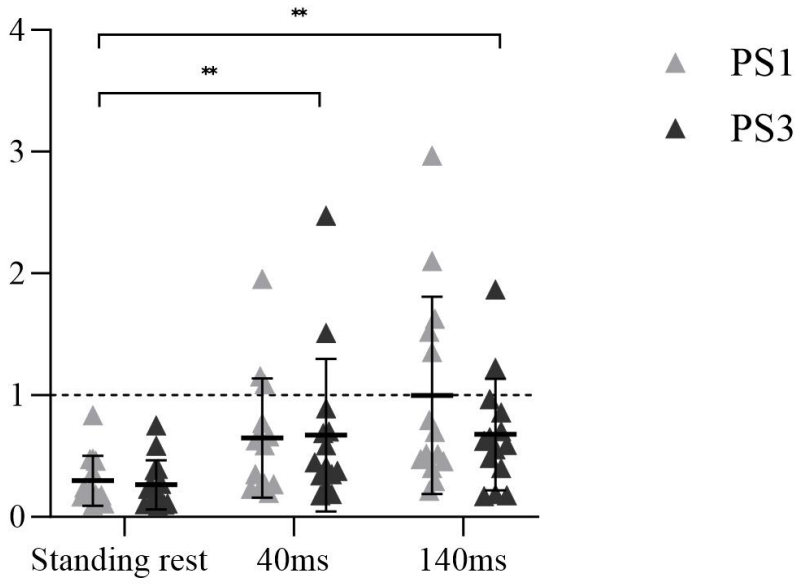

FIGURE 16 MEP / H-reflex ratio. Three different times (standing rest, 40 ms, and 140 ms) were shown in the x-axis. Symbols represent the values of individual subjects, while the mean values with standard deviation bars are also depicted. Statistical significance is denoted by '**' ($P < 0.001$).

5.4.2 Muscle activity during perturbation

Figure 17 shows soleus (A), gastrocnemius (B), and tibialis anterior (C) muscle activity during translational perturbation in PS1 and PS3.

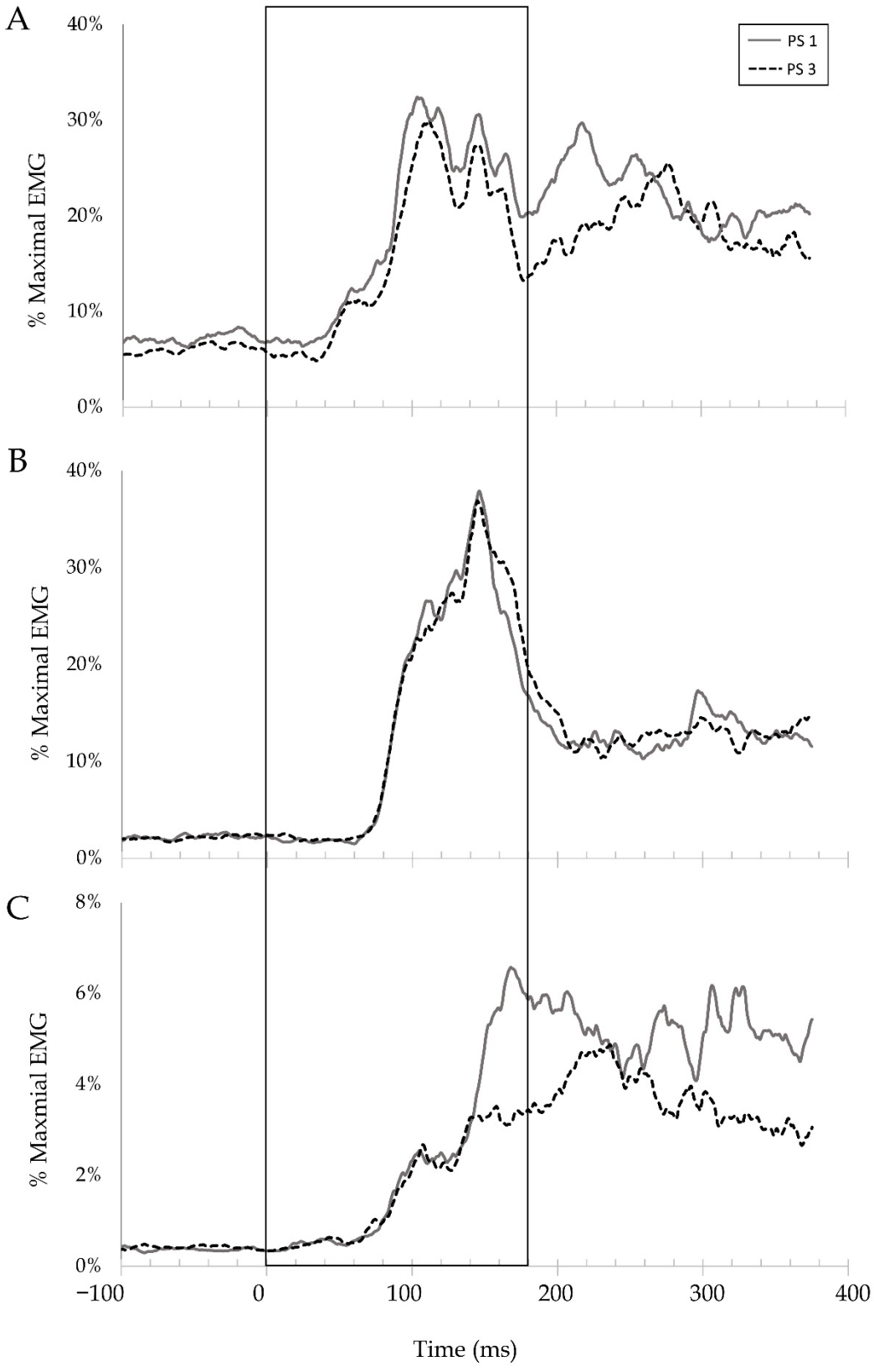

FIGURE 17 Muscle activity during balance perturbation. The average EMG from all subjects in perturbation trials without stimulation (A: soleus; B: gastrocnemius; C: tibialis anterior).

Specifically, RMS of soleus EMG activity did not demonstrate a significant between-session difference in perturbation trials without stimulation. Soleus activity was lower at the 40–60 ms window (PS1: 0.53 ± 0.20%, PS3: 0.47 ± 0.21%) when compared to the 140–160 ms window (PS1: 1.32 ± 0.64%, PS3: 1.27 ± 0.52%; $P < 0.001$; Figure 18), where these windows match the timings of the stimulations.

Since Background EMG was calculated before the stimulations, correlations between EMG activity during no-stimulation trials and MEP and H-reflex amplitudes were also calculated. A positive correlation was observed between EMG activity (40 ms-60 ms) and H-reflex ($r = 0.500$, $P = 0.007$), but not with MEP ($r = 0.161$, $P = 0.412$) at 40 ms. On the contrary, EMG activity showed a positive correlation with MEP ($r = 0.501$, $P = 0.007$), but not H-reflex ($r = 0.241$, $P = 0.218$) at 140 ms.

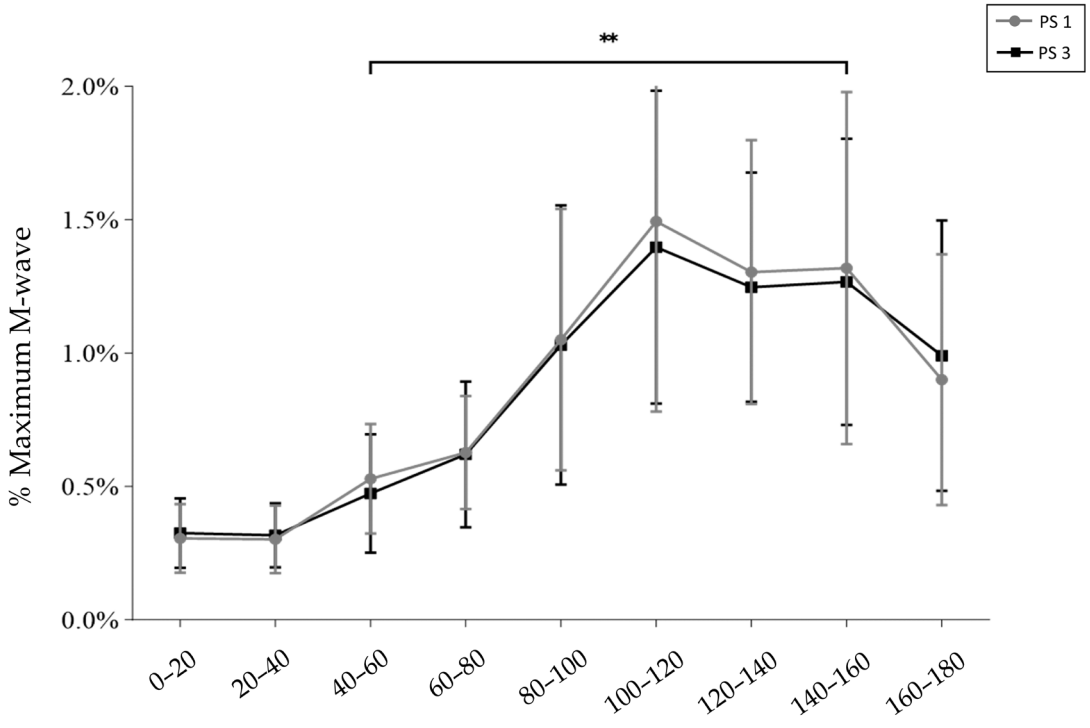

FIGURE 18 Root mean square of soleus EMG during balance perturbation. soleus EMG in every 20 ms window from ankle movement (0 ms) to 180 ms as zoomed in from the dashed square of Figure 17A. Statistical significance is denoted by '**' ($P < 0.001$). Note: There are other significant differences between the time windows which are not marked in the figure since the focus was 40-60 ms and 140-60 ms windows.

5.4.3 Correlations between changes in spinal/corticospinal excitability and balance performance

Figure 19 showed the correlations between changes in the displacement of COP and MEP and H-reflex at 40 ms time points. Δ MEP at 40 ms demonstrated a significant and positive correlation with Δ dCOP in Rec (Figure 19C). However,

Δ H-reflex at 40 ms demonstrated a significant and negative correlation with the Δ dCOP during the Pre (Figure 19D).

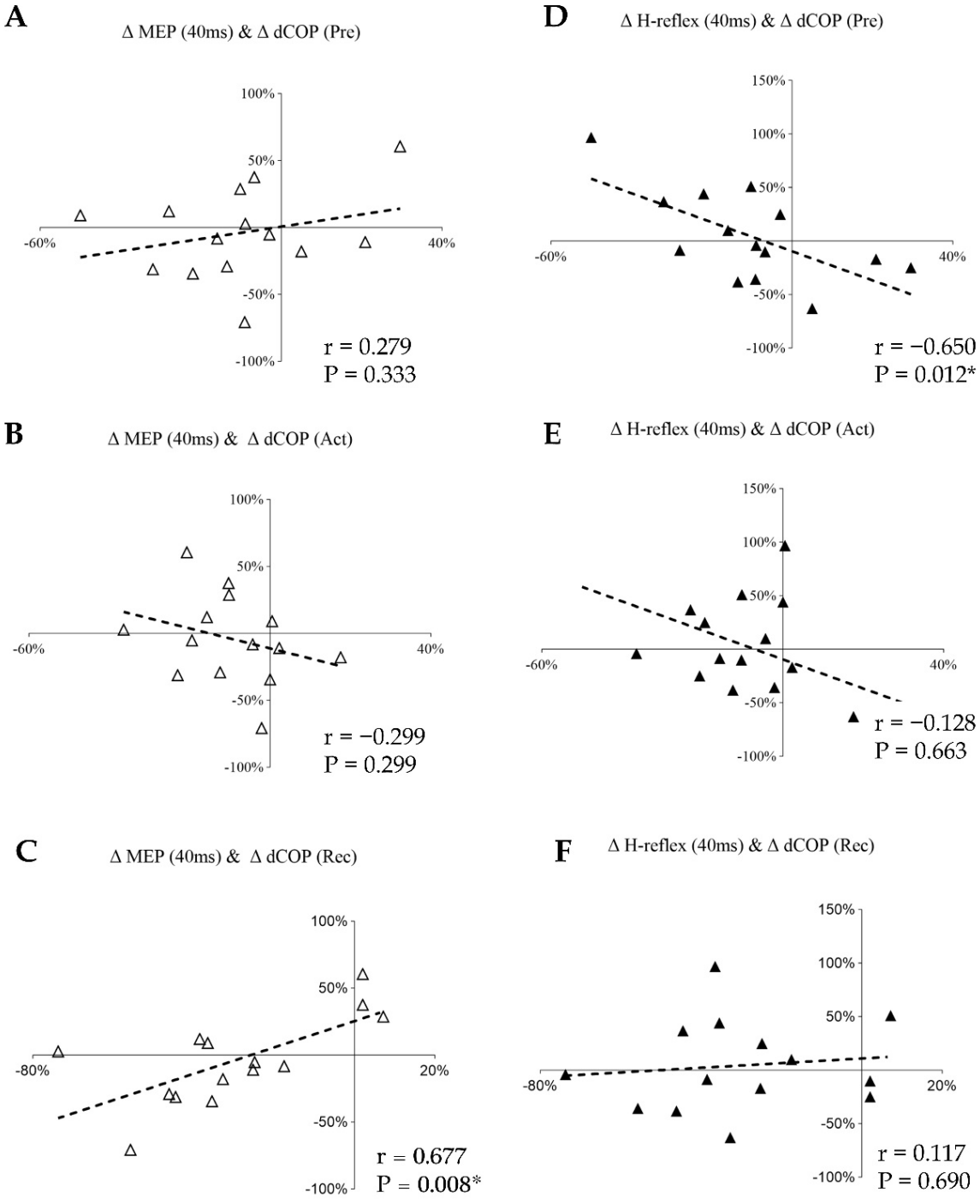

FIGURE 19 Correlation between changes of MEP / H-relfex and COP displacement (dCOP). ' △ ' shows correlation between Δ MEP with Δ dCOP at Pre (A), Act (B), and Rec (C); ' ▲ ' shows correlation between Δ H-reflex with Δ dCOP at Pre (D), Act (E), and Rec (F).

5.5 Corticospinal excitability between skill and endurance training athletes during rotational perturbation tasks (Experiment III)

There were no differences between groups in training years (endurance group: 11 ± 3 years; skill group: 13 ± 3 years), rMT (endurance group: 54 ± 7% stimulator output; skill group: 47 ± 8% stimulator output) or MVC (endurance group: 297 ± 67 Nm; skill group: 227 ± 86 Nm).

5.5.1 Single-pulse MEPs

MEP_{AVG} results demonstrated a significant main effect for condition ($F_{(1.971, 33.51)} = 83.908$, $P < 0.001$, $\eta_p^2 = 0.832$; Figure 20B), but there was no main effect for the group ($F_{(1, 17)} = 0.532$, $P = 0.476$, $\eta_p^2 = 0.030$; Figure 20A) and no group × condition interaction ($F_{(1.971, 33.51)} = 1.88$, $P = 0.169$, $\eta_p^2 = 0.100$). MEP_{AVG} in the endurance group was 1.6 ± 0.8 $\%M_{MAX}$ (Onset), 10.7 ± 9.4 $\%M_{MAX}$ (SLR), 2.1 ± 1.3 $\%M_{MAX}$ (p120), and 10.0 ± 5.0 $\%M_{MAX}$ (a120). MEP_{AVG} in the skill group was 1.7 ± 1.0 $\%M_{MAX}$ (Onset), 14.4 ± 6.4 $\%M_{MAX}$ (SLR), 2.7 ± 1.8 $\%M_{MAX}$ (p120), and 8.9 ± 5.9 $\%M_{MAX}$ (a120).

Significant differences between conditions were observed from Onset to SLR, SLR to p120, p120 to a120, and Onset to a120 for both groups ($P < 0.001$; Figure 20B), In addition, there was a significant difference between Onset and p120 ($P = 0.005$), SLR and a120 ($P = 0.024$) in the skill group only (Figure 20B).

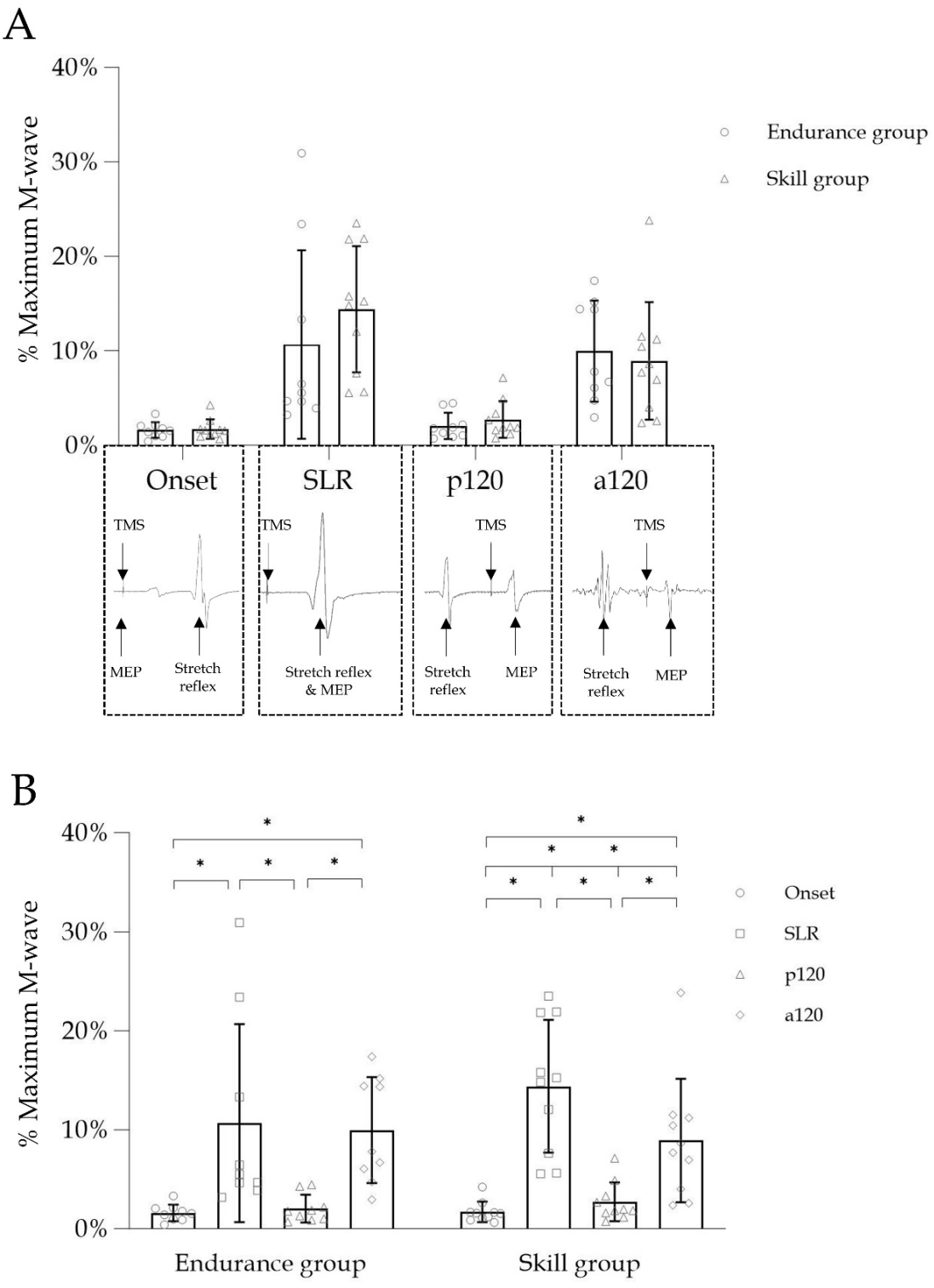

FIGURE 20 Average MEP value (MEPAVG) between-groups (A) and within-groups (B). (A) There was no difference shown in MEPAVG between groups. Individual values are shown with shape symbols ('○' = endurance group, '△' = skill group). The signal of stretch reflex and MEP is from a single TMS trial of one representative participant. (B) In the skill group, there were differences between each condition. Individual values are showed with shape symbols ('○' = Onset, '□' = SLR, '△' = p120, '◇' =a120).

There was a strong correlation between MVC and Onset MEP_{AVG} in the skill group (r = 0.790, P = 0.007, N = 10; Figure 21), but no relationship was observed for the endurance group (r = -0.417, P = 0.265, N = 9).

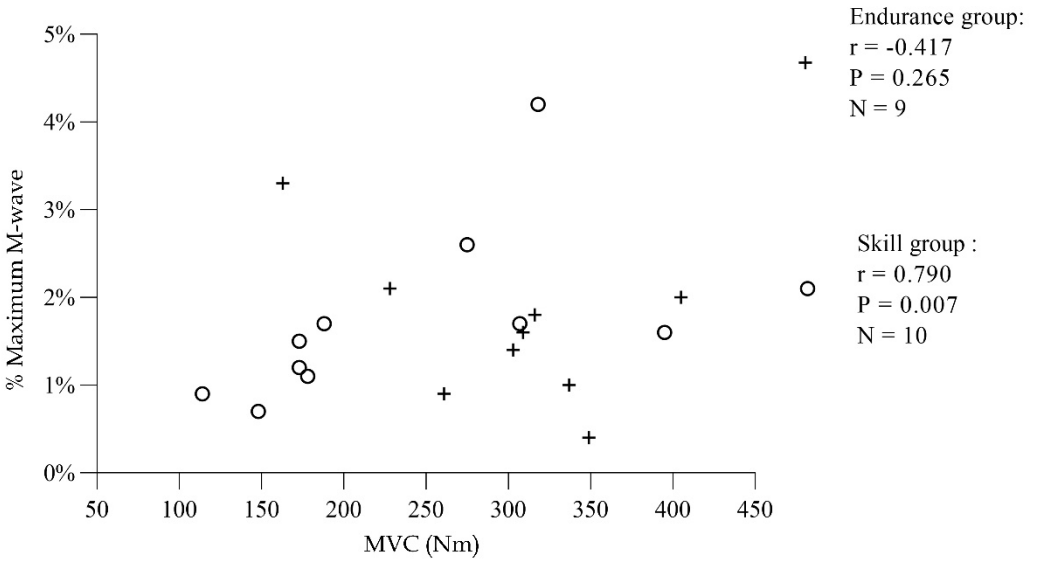

FIGURE 21 Correlation between MVC and average MEP value (MEP_{AVG}). Scatter plot of MVC and MEP_{AVG} of Onset condition in two groups ('+' = endurance group, 'o' = skill group). Data from the skill group (N = 10) showed a positive correlation (P = 0.007). Data from the endurance group (N = 9) did not reach statistical significance (P = 0.265).

SR / MEP_{AVG} ratio revealed that the increase in MEP_{AVG} from Onset to SLR was partly affected by the presence of stretch reflex, and there were no differences between two groups (endurance = 1.8 ± 0.8; skill = 1.3 ± 1.0). However, the correlation of MEP_{AVG} and stretch reflex showed a strong relationship in the endurance group (r = 0.733, P = 0.025, N = 9; Figure 22), but not in the skill group (r = 0.212, P = 0.556, N = 10; Figure 22).

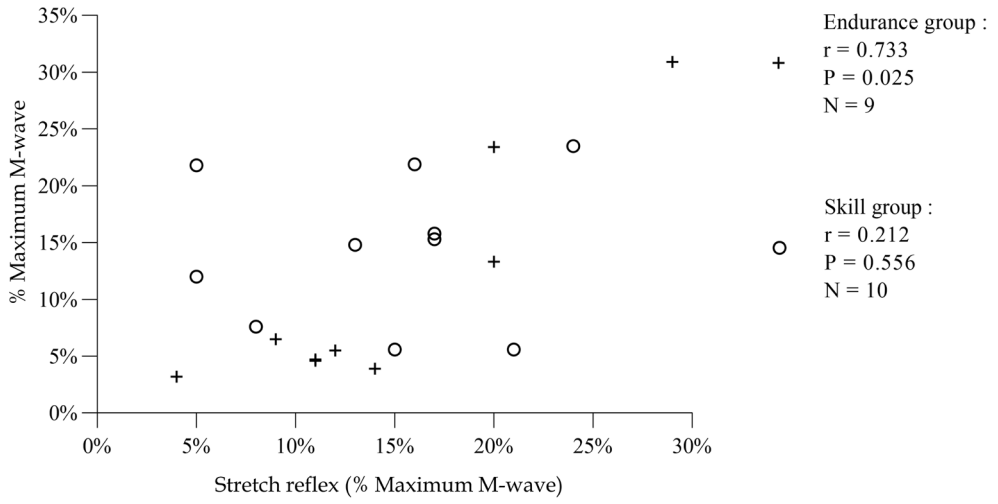

FIGURE 22 Correlation between stretch reflex and average MEP value (MEP_{AVG}). Scatter plot of stretch reflex and MEP_{AVG} in two groups ('+' = endurance group, '○' = skill group). There was a significant positive correlation observed in the endurance group (P = 0.025), but not in the skill group (P = 0.556).

5.5.2 Paired-pulse MEPs

SICI showed a significant main effect for condition ($F_{(3, 42)} = 5.154$, P = 0.004, $\eta_p^2 = 0.269$), but not between groups ($F_{(1, 14)} = 0.409$, P = 0.533, $\eta_p^2 = 0.028$) nor group × condition interaction ($F_{(3, 42)} = 1.074$, P = 0.370, $\eta_p^2 = 0.071$). Post-hoc (Bonferroni) tests for SICI did not reveal significant differences between conditions for each group separately.

ICF showed a significant main effect for condition ($F_{(3, 45)} = 4.64$, P = 0.007, $\eta_p^2 = 0.236$) and for the group ($F_{(1, 15)} = 6.163$, P = 0.025, $\eta_p^2 = 0.291$). There was no group × condition interaction ($F_{(3, 45)} = 0.455$, P = 0.715, $\eta_p^2 = 0.029$). However, one-way ANOVA for ICF did not show significant differences between conditions in either group nor differences between groups observed for any condition by t-test (Table 9).

TABLE 9 Short-intracortical inhibition (SICI) and intracortical facilitation (ICF) at different conditions (mean ± standard deviation).

	SICI		ICF	
	Endurance	Skill group	Endurance	Skill group
Onset	51 ± 21 %	46 ± 28 %	166 ± 90 %	185 ± 51 %
SLR	67 ± 23 %	115 ± 40 %	98 ± 32 %	140 ± 56 %
p120	69 ± 32 %	69 ± 37 %	150 ± 34 %	158 ± 43 %
a120	94 ± 36 %	87 ± 27 %	115 ± 35 %	130 ± 36 %

6 DISCUSSION

6.1 Test-retest reliability of using TMS and H-reflex in ankle translational perturbation tasks.

The TMS setup in translational perturbation tasks (Experiment I and II) was innovative (Figure 7). Since MEP amplitude is sensitive to the position of the stimulation hotspot, it is necessary to test the stability and reliability of this setup at the beginning. Thus, a preliminary motion capture assessment was conducted to examine the relative movement between the subject's head and the TMS coil. In the pre-study, the TMS coil and its handle were considered a rigid body, rotating around the centre point of the head. Two markers on the upper and lower part of the handle were used to estimate the movement of the coil. Because the coil was below the lower marker, the movement of the coil could be considered to be less than the markers range of movement, which was less than 5 mm on the *x*-, *y*-, and *z*-axis. From the study of TMS coil location accuracy with a function-guided navigation system, a 2-5 mm distance around the initially defined hotspot resulted in good accuracy of MEPs, and changes in coil location within a 5 mm distance had no significant effect on MEP amplitude (De Goede et al., 2018). This supports the assertion that the stability of the TMS coil during perturbation tasks provided accurate MEP values in the present study. In recent pilot experiment, the reliability of the same TMS setup was tested using a neuronavigational TMS system and revealed a good match between the two coil tracking methods (unpublished observation).

In Experiment I, paired t-tests were used to test any systematic differences in MEPs and H-reflexes between sessions. The results demonstrated higher H-reflex amplitude in S2 at 10 ms time point (S1: 24.8 $\%M_{MAX}$; S2: 31.0 $\%M_{MAX}$) with lower MEP (S1: 13.0 $\%M_{MAX}$; S2: 11.1 $\%M_{MAX}$). The observed MEP or H-reflex amplitude changes were lower than the between-session MDC, which indicates that the between-session differences may result from the inherent variability of MEPs/H-reflex or noise in the measurements. Therefore, to enhance the reliability of the obtained results and minimize the potential influence of learning

effects, it is recommended that at least two familiarizing perturbation sets should be performed prior to the first measurement session and reduce the number of repeated perturbations in a single session.

TMS measurement demonstrated strong test-retest reliability in both between- and within-session, during standing rest and translational perturbation tasks (ICC > 0.80). The highest test-retest reliability and lowest between-trial variability were observed at 140 ms time point, which is defined as a voluntary activation phase. It indicates that MEPs are more reliable while the contribution of voluntary activation of the muscles is increasing compared with the low voluntary muscle activity at the early response time points during perturbation tasks or muscles at rest. This finding is supported by data from Tallent et al., (2012), in which they showed higher reliability of MEPs in active muscle than passive muscle. Similarly, Darling et al.'s (2006) study has shown that less variance was observed with more muscle activation. On the other hand, sensory inputs (vestibular, vision, proprioception) may influence the excitability of motor units in the corticospinal pathway more during standing rest and early time points after perturbation and, therefore, the variability of MEPs increases (Darling et al., 2006). Because of this typical between-trial variability, which was also shown in Experiment I (within-session CV% = 16.9-46.1) mean MEP values from several individual trials should be examined. Although Goldsworthy et al. (2016) suggested that 20 ~ 30 trials may be optimal for estimating MEPs in the first dorsal interosseous, other TMS studies have shown good reliability with fewer stimulation trials (Bastani & Jaberzadeh, 2012; van Hedel et al., 2007), which indicates that the reliability of MEPs fluctuates in different experimental protocols and it might be muscle-specific (Cavaleri et al., 2017). Eight to ten trials showed excellent reliability (ICC > 0.81) in MEPs of the tibialis anterior muscle of stroke patients (Beaulieu et al., 2017). Lewis et al. (2014) demonstrated good reliability (ICC > 0.80) in soleus muscle in healthy subjects by averaging only six MEPs. According to Cavaleri et al.'s (2017) study, a mean value of ten trials is required to produce consistent condensed reliability, and five trials are the lowest number to achieve excellent within-session reliability. It seems MEPs in lower limb muscles appear to be more reliable than those in upper limb muscles. Therefore, five to ten trials of TMS would be applicable for lower limb muscles research. Based on the results of Experiment I, MEPs of a single subject at every time point were analysed from eight backward perturbation trials, and the average value was calculated (seven to eight trials) after removing outliers. To the best of the author's knowledge, there is only one previous TMS study that has used this method (Hosel & Tremblay, 2021). Since ICC of MEPs demonstrated good-to-excellent within-session (between trials) reliability, calculating average MEP amplitude from eight TMS stimulation trials and removing outlier MEPs beyond 2.5 SD (maximum one outlier in the present results) could be considered sufficient in reducing MEP between-trial variability and producing a reliable TMS procedure in corresponding perturbation tasks.

H-reflex demonstrated better test-retest reliability in translational perturbation tasks than during standing rest; ICC, SEM, and MDC, and within-

session reliability were extremely robust. Similar results that revealed high stability between stimulation trials but lower reliability between the sessions were found in a previous study (Handcock et al., 2001). The possible reasons include more irregular body sway or various lack of attention issues during standing rest compared with more regular body movements and better focus during perturbation tasks. Compared with the standing position, previous studies with subjects who were in a supine or prone position revealed high reliability for the soleus H-reflex (Hopkins et al., 2000; Palmieri et al., 2002), which indicated that the H-reflex reliability may also relate to the body position used in the protocol. Better reliability was shown at 80 ms (ICC = 0.89) and 140 ms (ICC = 0.87) time points than at 10 ms (ICC = 0.63) and 40 ms (ICC = 0.72) time points, even though the reliability in the latter two conditions is still considered acceptable (Portney & Watkins, 2009). The within-session reliability was generally better than between sessions. This observation implies that five successful stimulations are sufficient to be utilized for H-reflex measurements in translational perturbation tasks. Calculating average from eight to ten trials may provide greater reliability between the sessions. However, it should be noted that increasing the number of perturbation trials with stimulation might induce the risk of fatigue and learning effects. It is not surprising that high reliability of H-reflex in soleus muscle was shown between stimulation trials since previous studies from different body positions have also reported similar high-reliability values and suggested that four to five stimulations are needed to obtain reliable results (Al Amer et al., 2020; Hopkins et al., 2000). Although the stimulation intensity and body position were different, results in Experiment I showed important information about reliability of using the H-reflex method in ankle translational perturbation tasks.

6.2 Motor skill performance improves after one session of perturbation training

COP is an important parameter to evaluate balance performance in balance tasks (Zemková, 2011). In a previous study of balance ability between young and older adults, the older subjects showed larger peak COP displacement which implied poorer balance control ability during perturbation (Piirainen et al., 2013). Since the body always sways mostly in the anterior and posterior direction during forward/backward translational perturbation tasks, the anterior-posterior direction of COP movement in Experiments I and II was of interest. In Experiment I, the COP displacement and velocity were analysed only for the backward movement of the platform. COP displacement and velocity did not differ significantly between the two measurement sessions in terms of the Act-phase, indicating the high reliability of COP during the active translational perturbation phase between sessions. However, the velocity of COP during the Pre- and Rec-phases, as well as the maximum COP displacement during Rec-

phase was considerably reduced in S2. The results suggest that there was less body sway in S2 before the perturbation and after the perturbation ended compared to S1.

In Experiment II, a key distinction from Experiment I was that one perturbation training session was placed in between two TMS/H-reflex measurement sessions. An adaptive process was observed as sway reduction in both COP displacement and velocity during perturbation tasks after training. In addition, variability of COP decreased, as shown by the reduction of standard deviation of the COP after training, suggesting that there was less inconsistency in body sway. The study of Tjernström et al. (2002) suggested that previous experience with balance testing helped the subjects to refine their strategies to maintain balance and, thus adaptive motor learning can be induced by challenging postural disturbance training but not by training of fewer perturbations or lower amplitude. It is in line with results of this thesis that one extra perturbation training session can significantly contribute to improved balance control.

During translational perturbation tasks, both feedback control, which occurs in response to sensory feedback, and feedforward control, which refers to anticipation of a voluntary movement, are involved in postural control (Dietz et al., 1993). In the results from Experiment I, balance control ability was already partially learned from S1 to S2, in which the COP decreased in Pre- and Rec- phases in S2. It is well-known that feedforward control is an internal model for accuracy, which does not need a feedback loop and is more related to anticipation and voluntary activation (Kawato, 1999). Therefore, feedforward control may have improved and contributed to the balance control from S1 to S2. On the other hand, feedback control needs information from sensory afferents, which occurs in Act- phase. Results from Experiment II demonstrated that one more training session was needed for the balance performance improvement in the Act- phase. This may imply that feedback control is more involved in the balance control improvement after training. Similar results were demonstrated in the study by Alizadehsaravi et al. (2021) that balance training induced feedback control improvement during perturbation in older adults. In predictable situations (e.g., aiming tasks, same direction balance perturbation), movement control shifts from feedback to feedforward during the skill acquisition, and the learner becomes less dependent on feedback control (Kasuga et al., 2015; Teixeira et al., 2020). However, in unpredictable situations (e.g., random direction balance perturbation), feedforward compensations are less effective (Pavol & Pai, 2002). This may imply that in translational perturbation tasks, motor control from feedback may play a more crucial role than feedforward control.

6.3 Short-term perturbation training induces neural adaptation

6.3.1 Neural modulation during ankle translational perturbation

The time consumption of early cortical somatosensory potentials evoked by tibial electrical stimulation has been demonstrated to be about 40 ms (Fellows et al., 1993). The centre processing time from the somatosensory cortex to the M1 has been measured to be about 18 ms in the representative area of the soleus muscle (Kumpulainen et al., 2012). In Experiment II, we observed that the MEP latency, which demonstrates the neural transformation from the M1 to the soleus muscle, is 35 ms on average. According to the calculating method by Mrachacz-Kersting et al.'s study (2006), M1 contribution to ankle stretch and, thus, activation of the soleus muscle should not arrive earlier than 93 ms (i.e., 93 ms = 40 ms + 18 ms + 35 ms). Therefore, in Experiments I and II, from ankle movement onset to 100 ms (early phase) can be considered to represent subcortical control, and 100 ms (late phase) onwards can be considered as cortical control phase.

MEPs were demonstrated to increase already in 40 ms in Experiment I and II, which is too soon for a cortical response following proprioceptive processing as mentioned previously. It may relate to subjects' anticipation or feedforward control, even though the perturbation system gave random anterior or posterior perturbations. However, it should be noted that MEPs did not increase at the onset of ankle movement compared to the standing rest phase, which, on the other hand, might imply that anticipation-induced increases in corticospinal excitability may not exist. Then again, feedforward control is driven by the internal model for accuracy, which does not need a proprioceptive loop (Kawato, 1999). Therefore, feedforward control, but not likely the anticipation, could explain the increased MEPs without H-reflex changes at a 40 ms time point during perturbation.

In the later perturbation phase, contribution of cortical drive during balance control is known to increase (Taube et al., 2008). In the study of Mierau and colleagues (2015), a negative potential (N1) was recorded by electroencephalography over the centro-parietal cortical area at 100–200 ms after the perturbation onset. N1 is addressed as reflex cortical processing, which is related to perturbation amplitude and postural threat. Meanwhile, the N1 amplitude demonstrated a positive correlation with EMG activity of the gastrocnemius muscle after 100 ms. A similar positive correlation was observed in Experiment II between EMG activity (140–160 ms) and MEP (140 ms phase). It suggests that cortical level neural activity is strongly related to muscle activity during the later phases of translational perturbation.

Since MEP and H-reflex amplitude is highly influenced by background EMG, increased MEP and H-reflex values may be explained by increased motoneuron excitability from 40 ms to 140 ms. On the other hand, this study showed a positive correlation between EMG activity and H-reflex at 40 ms, and between EMG activity and MEP at 140 ms. This implies that the motoneuron excitability relates to H-reflex at the early phase (i.e., SLR), but the reliance of

MEP on motoneuron excitability was more likely at the later phase (i.e., after LLR). Nevertheless, the strength of the correlation was moderate ($r^2 = 0.25$) with other factors contributing most of the variance. Therefore, when a potential decreasing MEP/H-reflex ratio was demonstrated in the results, it can be thought to be because of reduced cortical excitability after one training session. A previous study from Taube et al., (2006) showed a similar conclusion since conditioned H-reflex by MEP decreased after balance training.

6.3.2 Neural adaptation after one session of perturbation training

TMS-induced MEP amplitude demonstrates neural excitability in the whole corticospinal loop. Therefore, the neural adaptation that is explained by MEP changes after perturbation training may occur at cortical and/or subcortical levels.

LTP and its shift to synaptogenesis are important mechanisms for motor skill learning processes (Cooke & Bliss, 2006; Kleim et al., 2004). Depending on the complexity of motor tasks, synaptogenesis needs several repeated training sessions (Carey et al., 2005). Animal studies, such as one by Kleim et al. (2004), have shown that cortical synaptogenesis occurred after ten days of repeated training. In human studies, Rosenkranz et al. (2007), demonstrated evidence of synaptogenesis after five training sessions. In this thesis, non-significant changes in MEPs were demonstrated before and after training in Experiment II. This suggests that the number of training sessions may not have been sufficient to induce morphological changes, resulting in incomplete motor skill learning in some subjects (3 out of 14 subjects showed increased dCOP). However, Shah's study (2008) showed that the information regarding repetitive and learned movements is stored in the basal ganglia, accessible through cortical and subcortical connections when needed. A study by Mouthon and Taube (2019) found that two weeks, including six balance training sessions, led to increased intracortical inhibition, which correlated to improved balance control performance. Additionally, Lauber, et al. (2018) showed that SICI increased during the initial balance training stages but then returned to baseline as training progressed. Researchers also proposed that there may be a high level of cortical drive at the beginning of balance training, and then shifts to a subcortical level once balance control has been acquired (Logan, 1979). Only single-pulse TMS was used in Experiment II of this thesis, which makes it impossible to explore cortical inhibition or facilitation after training. To gain a more comprehensive understanding of intracortical inhibition and facilitation behaviour during balance perturbation tasks, future research should consider using paired-pulse TMS.

It should also be noted that the modulation of automaticity-related neural excitability may occur at the spinal level, according to the modulation of H-reflex to the demands of the task (Adkins et al., 2006). Previous studies have shown decreasing soleus H-reflex amplitude when human body balance is challenged. For example, from lying to standing (Mynark & Koceja, 2002), or from standing to walking (Capaday & Stein, 1986). In this thesis study, reduced dCOP and

vCOP after training indicated that balance control ability was improved after perturbation training. It also suggested that maintaining balance was not as challenging as before, which may indicate why the MEP / H-reflex ratio in the majority of subjects (12 out of 14) was lower after training. A similar finding has been shown in a previous study. Penzer et al. (2015) found decreased MEP slope and increased H-reflex slope of the input-output curve following two times per week in six weeks of balance training in older adults. It suggested an increased efficacy of Ia afferents and a decrease in corticospinal excitability. This is consistent with thesis results that showed increased H-reflex while MEP decreased at 140 ms. However, neural adaptation at the spinal level is related to the duration of training and the age of subjects. For example, four weeks balance training period induced decreasing H-reflex in quiescent muscle (Gruber et al., 2007; Taube et al., 2007), which may be caused by supraspinal influence on presynaptic inhibition of Ia afferent fibres. A recent systematic review demonstrated that the soleus H-reflex consistently decreases in younger people, while it is less affected or increases in older people after balance training (Sun et al., 2022). Therefore, mechanisms of balance training-induced corticospinal adaptation seem to be not fully resolved, and contradictory results in the studies may be related to differences in the duration of training, motor tasks, and the age of the participants.

Automaticity refers to the ability to perform automated tasks with minimal or no interference from demanding secondary tasks (Logan, 1979). Depending on the motor task some studies have linked reduced cortical activity to movement 'automaticity' (Hempel et al., 2004; Jansma et al., 2001). For example, a functional magnetic resonance imaging study by Wu et al. (2004) demonstrated that when the motor task becomes automatic, the related motor network becomes more efficient and cortical areas, such as premotor cortex, cingulate cortex, left caudate nucleus participate less in executing automatic condition. On the other hand, an increase or more complex redistribution of activity in different brain regions has been suggested as well (Poldrack & Gabrieli, 2001). A study on visuomotor control revealed that early performance gains are strongly influenced by prefrontal-caudate interactions, whereas as the task becomes more automatic, later performance gains rely more on subcortical circuit activity (Floyer-Lea & Matthews, 2004). This research found a positive correlation between Δ MEP (40 ms) and Δ dCOP (Rec), but a negative correlation between Δ H-reflex (40 ms) and Δ dCOP (Pre), suggesting that decreased COP displacement may be associated with reduced corticospinal excitability and increased spinal excitability. Taube et al. (2007) also demonstrated a negative correlation between balance performance improvement and changes in TMS-conditioned H-reflex following sensorimotor training, indicating reduced corticospinal input to the soleus muscle, which aligns with current findings. A study by Bakker et al. (2021) found that 30 min balance training improved balance performance, but no neural adaptation, such as altered MEP amplitude or increased SICI as hypothesized, was observed. Researchers speculated that the motor adaptation process after motor learning might be influenced by the subcritical area, potentially within the cerebellum and

basal ganglia (Bakker et al., 2021; Doyon & Benali, 2005). Considering the lack of MEP or H-reflex changes in this thesis study before and after training, and the relationship of Δ MEP and Δ H-reflex with Δ dCOP at the 40 ms time point, it is reasonable to infer that subcortical circuit activity may play an important role in balance control, which induces improved balance performance following perturbation training.

6.4 Neural adaptation responses to rotational perturbation depends on the training background

6.4.1 Neural modulation in the early phase of rotational perturbation between skill- and endurance- trained athletes

Experiment III in this thesis focused on long-term training (i.e., skill- and endurance- trained athletes), and neural adaptation revealed by rotational perturbation. By using rotational perturbation in a sitting position, a more controlled/standardized experimental environment was ensured. SLR here can be considered as a purer stretch reflex than in standing since there is less cortical modulation from the descending pathway. Therefore, MEPs elicited by TMS during SLR and after SLR would demonstrate inherent differential corticospinal and intracortical modulation between groups. During the early phase (SLR) following rotational perturbation, the ratio of stretch reflex amplitude and MEP_{AVG} revealed by TMS has an additive effect on EMG response during rotational perturbation. The ratio in each group (endurance group = 1.8, skill group: = 1.3) showed no between-group differences, indicating that corticospinal excitability was not specifically enhanced in either group during spinal reaction phase. This result matches what has been found previously in that no corticospinal modulation occurred when SLR was elicited in the tibialis anterior or soleus muscles (Petersen et al., 1998; Taube et al., 2006). However, MEP_{AVG} showed a strong positive correlation with stretch reflex in the endurance group only at the SLR time point. This implies that the monosynaptic spinal loop likely contributes more to corticospinal excitability than the supraspinal loop in the fast response phase after ankle rotational perturbation in the endurance group. There were indeed some individuals in the endurance group specifically adapted for high excitability post-stretch. However, the lack of between-group differences may have been due to high within-group variance and low sample size. It is speculated that training-specific sensorimotor control may induce neural adaptation differently. For example, while both long-distance running and swimming are defined as endurance training, there is obviously less rapid ankle perturbations in swimming compared to running. This difference between sports may affect the responsiveness of spinal motoneurons on Ia afferent input. This proposal is in line with a previous study that long-term trained endurance runners demonstrated higher neural excitability at the spinal level (Ogawa et al.,

2012), but on the contrary, low spinal excitability has been observed in superficial cyclists (Bertschinger et al., 2021).

The ankle rotational perturbation-induced stretch reflex is an important part of proprioceptive processing induced by lengthening soleus muscle. As mentioned before, it is generally acknowledged that SLR is provided predominantly by the spinal pathway and that transcortical feedback is involved after SLR (Dietz et al., 1984; Mrachacz-Kersting et al., 2006). However, the outcomes of SICI in Experiment III indicated that there was modulation of cortical inhibitory processing already during SLR in the skill group. It has been known that cortical inhibition is reduced before a voluntary action (Reynolds & Ashby, 1999). However, it is important to emphasize that no voluntary muscular activity occurred before or after SLR had abated in the passive trials of Experiment III. This prompts the consideration of a 'priming' mechanism in the skill group to modulate top-down responses since subjects could anticipate when the rapid perturbation came. This is supported by other research, which demonstrated cortical inhibition decreasing without changes in spinal neuronal excitability during an anticipatory postural task (Chiou et al., 2018). Additionally, a study by Wälchli et al. (2017), using TMS and H-reflex measurement, illustrated that intracortical inhibition is reduced during the preparatory phase when individuals expect a perturbation to happen. These studies suggested cortical inhibition 'release' during anticipating situations, which is in line with the result shown in the skill group in this thesis.

At the SLR time point, increased intracortical inhibition was observed in the endurance group (SICI: 67% of test MEP) but not in the skill group (SICI: 115% of test MEP). In addition, SICI showed inhibition (<100% of test MEP) at all passive conditions (i.e., Onset, SLR, and p120) in the endurance group, which implies that intracortical inhibition was maintained during ankle rotational perturbation. This finding seems to contradict a previous study by Singh et al. (2014), which showed that aerobic training increased cortical facilitation and decreased inhibition at rest. However, it is essential to note that the increased intracortical inhibition in the endurance group was specifically observed during SLR. It is possible that the Ia afferent input modulated SICI amplitude in this case. When considering the correlation found between stretch reflex and MEPs in the endurance group, the findings suggested that endurance athletes might rely more on spinal mechanisms and have lower cortical reliance. According to Mouthon and Taube (2019), the role of enhanced intracortical inhibition is believed to avoid unnecessary coactivation during motor control tasks. Since endurance training includes long-term repetitive movements reflective of motor technique learning (Adkins et al., 2006), maintaining cortical inhibition and reduced corticospinal excitability may benefit reducing unnecessary coactivation and lead to more efficient movements. On the other hand, the skill group demonstrated more variability in SICI during the entire process of ankle rotational perturbation, which included 'facilitation' at the SLR time point and inhibition at other time points. It implied a wider range of modulatory capacity at the cortical level in the skill group, which possibly related to their ability to

learn complex motor tasks and new motor skills. Previous research has shown that ability to modulate SICI is crucial for behavioural function, and limiting the ability of SICI modulation is related to behavioural function declines (e.g., balance performance) by aging (Heise et al., 2014; Papegaaij et al., 2016). Additionally, a greater capacity for inhibitory modulation can be essential for learning complex motor tasks (Taube et al., 2020). In the present study, higher variability of SICI during passive ankle perturbation suggests a wider modulatory capacity range in the skill group and, thus, it may relate to long-term new motor skill learning and complex motor skill training.

6.4.2 Neural modulation in the late phase of rotational perturbation between skill- and endurance- trained athletes

In Experiment III, increased MEPs at p120 compared to Onset were observed only in the skill group. The p120 time point took place 120 ms after SLR, which can be considered a voluntary activation phase and is close to the LLR_2 phase reported by Taube et al. (2008). The increased MEPs at LLR_2 indicated modulation of corticospinal excitability while reduced H-reflex at the same time suggested that this modulation was cortical. At the p120 time point, the ascending time of MEP is sufficient to allow different pathways, including cortical and spinal, to contribute to its facilitation and inhibition. Greater MEPs in the skill group compared to the endurance group suggest that they have greater or more long-lasting corticospinal excitability than endurance-trained athletes after ankle rotational perturbation, even in a passive condition.

Less intracortical inhibition at SLR may at least partly explain the enhanced MEP at p120 in the skill group. Hence, reducing inhibition supports the assumption of 'releasing the break before impending movement' (Floeter & Rothwell, 1999). Since there was no muscle activity at the p120 condition, but MEP was enhanced in the skill group, 'priming' for voluntary contraction seems to arise in the skill group but not in the endurance group. The possible explanation might be that for skill-trained athletes, there are more voluntary movement changes in training and competition, which need to be controlled by the motor cortex, cerebella, or somatosensory association cortex (Kurtzer, 2015; Suminski et al., 2007). In support of this contention, a strong positive correlation between MVC and resting MEPs was observed in the skill group only. MVC force is dependent on recruitment of motor units and the force-producing capacity of muscle fibres. A higher MEP value is related to higher excitability of motor cortical output cells and motoneurons during voluntary contraction (Taylor et al., 2002). Therefore, for skill-trained athletes, corticospinal excitability plays an important part in voluntary movement and suggests cortical adaptation to a top-down strategy in response to ankle rotational perturbation.

Higher cortical facilitation in skill athletes was found with ICF by paired pulse TMS. The average ICF value from all conditions was higher in the skill than in the endurance group, and a significant main effect for group was observed. Nevertheless, there were no (pairwise) statistical between-group differences at any condition, which may dilute confidence in making such inferences. The

cortical mechanisms of ICF are not fully clear, especially in the lower limb. It is generally thought to be mediated by glutamatergic interneurons and activation of the N-methyl-d-aspartate (NMDA) receptor (Kujirai et al., 1993; McDonnell et al., 2006). Increased ICF is either a result of decreased GABAergic inhibition or a separate increase in glutamatergic facilitation. Since the GABAergic inhibition (SICI) demonstrated no difference at Onset, p120, or a120, it may be that potentially higher ICF in skill athletes was due to glutamatergic facilitation. Although many controversies exist, the review by Park et al. (2014) showed that glutamatergic facilitation, which is believed to be triggered by the synaptic activation of the NMDA receptor, is one of the important molecular mechanisms for LTP. Therefore, a greater ICF found in the skill group would support Kumpulainen et al.'s (2015) findings that skill athletes have higher corticospinal plasticity than endurance athletes.

6.5 Motor learning mechanisms of short- and long-term training.

One important suggestion regarding the mechanisms of motor learning-induced cortical plasticity is related to LTP at the cortical level, which involves modifying synaptic connections (Rioult-Pedotti et al., 2000). LTP is a prerequisite for synaptogenesis, and studies have demonstrated that skill training leads to synaptogenesis in the motor cortex (Adkins et al., 2006). To induce LTP-like plasticity, researchers have used paired association stimulation (PAS) with TMS of the corresponding area in the M1. The amount of PAS-induced LTP-like plasticity increase depends on the number of active synapses. Therefore, a PAS intervention has been used as a measure of corticospinal plasticity (Di Lazzaro et al., 2009). In a study by Kumpulainen et al. (2015), PAS induced increased MEP in skill athletes, but not in endurance athletes or untrained adults, revealing higher corticospinal plasticity and greater synaptogenesis at the cortical level of skill-trained athletes. Previous findings suggest that skill training enhances the adaptability of corticospinal plasticity. Therefore, skill-trained athletes may rely more on cortical sources for voluntary movement, as observed in the present study during the p120 and a120 conditions in Experiment III.

On the other hand, Experiment II focused on repeated translational perturbation training and demonstrated a potential reduction in cortical control at the 100 ms time point after SLR. It indicates that repeated training may result in a greater reliance on spinal sources after motor acquisition. Just like endurance training, which involves repetitive stretch-shortening cycle actions, may lead to a decrease in muscle stiffness and Ia afferent presynaptic inhibition (Avela & Komi, 1998). During passive and active ankle perturbation, such as stance and swing phase in running, rapid modulation through spinal processes plays a vital role in maintaining balance during body oscillations, as cortical processes would be too slow to respond effectively (Tahayori & Koceja, 2012). Consistently, higher stretch reflex responses observed in endurance runners highlight enhanced modulation of spinal excitability (Ogawa et al., 2012). This is corroborated by a

study performed using H-reflex measurement, which found that well-trained swimmers demonstrated greater spinal excitability with increased H-reflex compared to non-trained individuals (Ogawa et al., 2009). The findings from Experiments II and III partially supported the hypothesis that repeated training and long-term endurance training prioritize spinal responses to ankle perturbation. This suggests that the repetitive training leads to adaptation in which the spinal control mechanisms overdrive cortical involvement in response to perturbations at the ankle joint. On the other hand, anticipation induced neural excitability at the cortical level was observed in the early phase of perturbation, which is known as the 'spinal drive' phase. This implies that a top-down strategy or feedforward control can occur very early during motor tasks in skill-trained athletes when responding to ankle perturbation tasks.

Some study limitations should be considered in this thesis.

The pre-study on the stability of the helmet system only included two subjects, which did not provide any statistical results. On the other hand, both subjects showed relatively small movement of the coil, which is in line with the literature (De Goede et al., 2018). The small sample size in Experiment I is another limitation. In addition, since MEPs (pseudo-monopolar) and background EMG (bipolar) were measured with different EMG electrode arrangements MEP normalization by background EMG is complicated. Therefore, the discussion about corticospinal modulation and changes in background EMG were made separately.

In Experiment II, large variability was observed in both MEP and H-reflex amplitudes when responding to translational perturbation tasks. This variability may have led to the lack of significant differences between sessions. Therefore, more subjects in future studies would be ideal. Despite utilizing a random perturbation order and COP monitoring to reduce anticipation and body sway before perturbation onset, it was very difficult to rule out all anticipation effects. Due to a similar order and time interval between perturbations of each set, it is still possible that subjects could have learned some movement patterns, which might be one of the reasons for increasing MEP amplitude at the 40 ms time point.

In Experiment III, only 20 participants were recruited and divided into two groups. Therefore, this sample size may not have been sufficient to determine between-group differences considering the large variabilities of MEP amplitudes when responding to rapid ankle dorsiflexion. Although conducting sample size estimation *a priori* for novel measurements is challenging, the convention within the field is that a typical sample size would be approximately 15 per group (e.g., Kumpulainen et al., 2015; Wälchli et al., 2017). Consequently, it is conceivable that only the clearest differences in corticospinal plasticity between skill- and endurance- athletes may have reached the level of statistical significance in this study. This may explain, for example, significant main effects for ICF but no significant differences were observed when post-hoc tests were performed to ascertain specific differences between groups or between conditions. Moreover, it is possible that different loading patterns may induce differences in corticospinal plasticity between sports. For example, the two swimmers included

in the present study may have added variance to the results given that their sport does not include stretch-shortening cycle actions in the triceps surae muscles through repetitive ground contact as in running. It is recommended that sport and training characteristics should be considered when recruiting athletes as participants if the research involves corticospinal responses during motor tasks.

7 PRIMARY FINDINGS AND CONCLUSIONS

The stability of the new TMS setup used in a translational perturbation task was verified in the pre-study experiment. Both MEPs and H-reflex demonstrated acceptable reliability between two measurement sessions based on ICCs and have good-to-excellent test-retest reliability between stimulation trials. During the perturbation phase, no differences between the sessions were observed in MEPs, H-reflex responses, COP displacement, or COP velocity during translational perturbations indicating good reliability of the test methods.

In repeated perturbation training, balance control ability improved after only one perturbation training session, which was evidenced by decreased COP displacement and velocity during translational perturbation in Experiment II. Increased MEPs were observed in SLR time points (40 ms after ankle movement), which suggests that feedforward control plays an important role in balance control during translational perturbations. However, no significant neural adaptation was demonstrated at the supraspinal or spinal level before and after training sessions. Nonetheless, the correlation between ΔCOP and ΔMEP/ΔH-reflex may imply potential alterations in corticospinal excitability that parallel with balance performance improvement. These findings suggest that when using repeated motor tasks in the experimental designs, motor learning, and neural adaptation even after a few sessions should be considered. Furthermore, neural adaptation might occur at a subcortical level, which may be related to repeated short-term translational perturbation training. In general, enhancing balance control could potentially prevent injuries related to falls in various populations, ranging from athletes to elderly individuals. This study showed that short-term repeated balance training significantly improved balance performance. Neural adaptation might occur at a subcortical level, which is important for fast response in the changing environment (also in line with findings from endurance athletes in this study). Therefore, repeated training may benefit fast response for certain population groups. For example, training using forward translational perturbations before winter for older adults may replicate potential slipping and effectively improve their balance performance, such as preventing falls, on icy

surfaces during winter. Nevertheless, future research is required in the elderly population to validate findings similar to those observed in the present study.

With long-term sports training, skill- and endurance-trained athletes demonstrated a similar pattern of corticospinal modulation, as evidenced by MEP_{AVG} following ankle rotational perturbation. However, the endurance group showed maintained SICI, while the skill group demonstrated removal of SICI during the SLR time point (~40 ms after perturbation onset). Furthermore, in skill-trained athletes, corticospinal excitability (MEP_{AVG}) was enhanced at 120 ms after muscle stretch, suggesting a "priming" of corticospinal excitability during the voluntary activation phase. Skill-trained athletes demonstrated a positive relationship between MEP amplitude and MVC, emphasizing the significance of corticospinal excitability for voluntary action which is particularly important for skill athletes. On the other hand, in endurance-trained athletes, a positive correlation emerged between stretch reflex and MEP amplitude at SLR. This suggests that the spinal loop response assumes a more important role in the fast response phase after rapid ankle rotational perturbation in endurance athletes. Meanwhile, considering SICI variability during rotational perturbation in the endurance group, maintained cortical inhibition may potentially reduce inefficient motor response to muscular stretch.

YHTEENVETO (SUMMARY IN FINNISH)

Esitutkimuksessa selvitettiin aivojen magneettistimulaation (TMS) luotettavuutta ja toistettavuutta tilanteessa, jossa tasapainoon aiheutettiin translationaalinen häiriö. Lihaksen sähköinen vaste TMS-stimuloinnille (MEP) sekä H-refleksivaste sähköiselle stimuloinnille osoittivat hyvää luotettavuutta kahden mittauskerran välillä. Mittausten sisäinen luotettavuus oli molempien vasteiden osalta hyvästä erinomaiseen. Häiriövaiheen aikana mittauskertojen välisiä eroja MEP- ja H-refleksivasteissa ja painekeskipisteen (COP) siirtymässä tai sen nopeudessa ei havaittu, mikä osoittaa testimenetelmien hyvää luotettavuutta.

Toistuvassa tasapainohäiriöharjoittelussa tasapainonhallintakyky parani jo yhden harjoituskerran jälkeen, mikä näkyi COP:n siirtymän ja nopeuden vähenemisenä translaatiohäiriön aikana. Lyhyen latenssin venytysrefleksivaiheen (SLR) aikapisteessä (40 ms nilkan liikkeen jälkeen) havaittiin MEP:n kasvua, mikä viittaa siihen, että myötäkytkentä (feedforward) ohjauksella on tärkeä rooli tasapainon hallinnassa translaatiohäiriön aikana. Selkäytimen tai sen yläpuolisen tason hermostollisessa ohjauksessa ei kuitenkaan havaittu merkittävää sopeutumista harjoittelujakson jälkeen. COP:n ja MEP/H-refleksi suhteen muutosten välinen tilastollisesti merkitsevä korrelaatio voi kuitenkin viitata mahdolliseen muutokseen kortikospinaalisen radan herkkyydessä, joka on samansuuntainen tasapainosuorituskyvyn paranemisen kanssa. Nämä havainnot viittaavat siihen, että tutkimuksissa olisi jatkossa syytä ottaa huomioon motorinen oppiminen ja hermostollinen sopeutuminen, joiden vaikutusmekanismit voivat käynnistyä jo muutaman suorituskerran jälkeen, erityisesti silloin kun koejärjestelyssä käytetään toistuvia motorisia tehtäviä. Lisäksi lyhytaikaisen ja toistuvan translaatiohäiriöharjoittelun hermostollinen sopeutuminen saattaa tapahtua motorista aivokuorta alemmalla tasolla.

Tutkimus osoitti, että pitkäaikaista taito- ja kestävyysharjoittelua harrastaneilla urheilijoilla kortikospinaalisen radan herkkyyden modulaatiomalli (keskiarvoinen MEP muutos) nilkan kiertohäiriön aikana oli samankaltainen. Kestävyysryhmässä lyhyen intervallin intrakortikaalinen inhibitio (SICI) kuitenkin säilyi, kun taas taitoryhmässä vastaava inhibitio poistui SLR-aikapisteen aikana. Lisäksi taitoa harjoittelevilla urheilijoilla kortikospinaalisen radan herkkyys (keskiarvoinen MEP) lisääntyi 120 ms kiertohäiriön alun jälkeen, mikä viittaa kortikospinaalisen radan herkkyyden pohjustamiseen tahdonalaisen aktivointivaiheen aikana. Taitolajiurheilijoilla MEP:n ja tahdonalaisen maksimaalisen isometrisen voimantuoton välillä oli tilastollisesti merkitsevä positiivinen korrelaatio. Tämä korostaa kortikospinaalisen radan herkkyyden merkitystä tahdonalaiselle toiminnalle, mikä näyttäisi olevan erityisen tärkeää taitolajiurheilijoille. Toisaalta kestävyysurheilijoilla ilmeni tilastollisesti merkitsevä positiivinen korrelaatio venytysrefleksin ja MEP-amplitudin välillä SLR-aikapisteessä kiertohäiriön alun jälkeen. Tämä viittaa siihen, että selkäytimen monosynaptisella vasteella on tärkeämpi rooli nopeassa vastevaiheessa nilkan nopean kiertohäiriön jälkeen erityisesti kestävyysurheilijoilla. Samalla, kun otetaan huomioon kestävyysryhmän SICI:n vaihtelevuus kiertohäiriön aikana, säilytetty intrakortikaalinen inhibitio

voi mahdollisesti vähentää tehotonta motorista vastetta kiertohäiriöön eli lihasvenytykseen.

REFERENCE

Adkins, D. L., Boychuk, J., Remple, M. S., & Kleim, J. A. (2006). Motor training induces experience-specific patterns of plasticity across motor cortex and spinal cord. *Journal of Applied Physiology*, 101(6), 1776–1782. https://doi.org/10.1152/japplphysiol.00515.2006

Al Amer, H. S., Sabbahi, M. A., & Olson, S. L. (2020). Reliability of the soleus H-reflex in different sitting postures. *Medical Sciences*, 8(4), 48. https://doi.org/10.3390/medsci8040048

Alizadehsaravi, L., Bruijn, S. M., & van Dieën Jh. (2021). Balance training improves feedback control of perturbed balance in older adults. BioRxiv. 2021-03. https://doi.org/10.1101/2021.03.31.437824

Angulo-Kinzler, R. M., Mynark, R. G., & Koceja, D. M. (1998). Soleus H-Reflex Gain in Elderly and Young Adults: Modulation Due To Body Position. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences*, 53A(2), M120–M125. https://doi.org/10.1093/gerona/53a.2.m120

Avela, J., & Komi, P. V. (1998). Interaction between muscle stiffness and stretch reflex sensitivity after long-term stretch-shortening cycle exercise. *Muscle & Nerve*, *21*(9), 1224–1227. https://doi.org/10.1002/(sici)1097-4598(199809)21:9

Avenanti, A., Paluello, I. M., Bufalari, I., & Aglioti, S. M. (2006). Stimulus-driven modulation of motor-evoked potentials during observation of others' pain. *NeuroImage*, 32(1), 316–324. https://doi.org/10.1016/j.neuroimage.2006.03.010

Bagce, H. F., Saleh, S., Adamovich, S. V., Krakauer, J. W., & Tunik, E. (2013). Corticospinal excitability is enhanced after visuomotor adaptation and depends on learning rather than performance or error. *Journal of Neurophysiology*, *109*(4), 1097–1106. https://doi.org/10.1152/jn.00304.2012

Bakker, L. B. M., Nandi, T., Lamoth, C. J. C., & Hortobágyi, T. (2021). Task specificity and neural adaptations after balance learning in young adults. *Human Movement Science*, 78, 102833. https://doi.org/10.1016/j.humov.2021.102833

Barker, A. T., Jalinous, R., & Freeston, I. L. (1985). Non-invasive magnetic stimulation of human motor cortex. *The Lancet*, 325(8437), 1106–1107. https://doi.org/10.1016/s0140-6736(85)92413-4

Barnes, K. R., & Kilding, A. E. (2015). Running economy: measurement, norms, and determining factors. *Sports Medicine–Open*, 1(1),1-15. https://doi.org/10.1186/s40798-015-0007-y

Bartholow, R. (1874). Experiments on the functions of the human brain. *British medical journal*, 1(700), 727–727. https://doi.org/10.1136/bmj.1.700.727-a

Bastani, A., & Jaberzadeh, S. (2012). A higher number of TMS-elicited MEP from a combined hotspot improves intra- and inter-session reliability of the upper limb Muscles in Healthy Individuals. *PLoS ONE,* 7(10), e47582. https://doi.org/10.1371/journal.pone.0047582

Beaulieu, L.-D., Massé-Alarie, H., Ribot-Ciscar, E., & Schneider, C. (2017). Reliability of lower limb transcranial magnetic stimulation outcomes in the ipsi- and contralesional hemispheres of adults with chronic stroke. *Clinical Neurophysiology*, 128(7), 1290–1298. https://doi.org/10.1016/j.clinph.2017.04.021

Bekris, E., Georgios, K., Konstantinos, A., Gissis, I., Papadopoulos, C., & Aristomenis, S. (2012). Proprioception and balance training can improve amateur soccer players' technical skills. *Journal of Physical Education and Sport*, *12*, 81–89.

Berkowitz, A., & Ansari, D. (2010). Expertise-related deactivation of the right temporoparietal junction during musical improvisation. *NeuroImage*, *49*, 712–719. https://doi.org/10.1016/j.neuroimage.2009.08.042

Bertschinger, R., Giboin, L., & Gruber, M. (2021). Endurance Trained Athletes Do Not per se Have Higher Hoffmann Reflexes Than Recreationally Active Controls. *Frontiers in Physiology*, *12*. https://doi.org/10.3389/fphys.2021.736067

Bestmann, S., & Duque, J. (2016). Transcranial Magnetic Stimulation: Decomposing the Processes Underlying Action Preparation. *The Neuroscientist*, *22*(4), 392–405. https://doi.org/10.1177/1073858415592594

Bestmann, S., & Krakauer, J. W. (2015). The uses and interpretations of the motor-evoked potential for understanding behaviour. *Experimental Brain Research*, *233*(3), 679–689. https://doi.org/10.1007/s00221-014-4183-7

Bland, J. M., & Altman, D. G. (1995). Comparing methods of measurement: why plotting difference against standard method is misleading. *The Lancet*, *346*(8982), 1085–1087. https://doi.org/10.1016/s0140-6736(95)91748-9

Blazevich, A. J., Kay, A. D., Waugh, C., Fath, F., Miller, S., & Cannavan, D. (2012). Plantarflexor stretch training increases reciprocal inhibition measured during voluntary dorsiflexion. *Journal of Neurophysiology*, *107*(1), 250–256. https://doi.org/10.1152/jn.00407.2011

Bliss, T. V., & Lømo, T. (1973). Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. *The Journal of Physiology*, *232*(2), 331–356. https://doi.org/10.1113/jphysiol.1973.sp010273

Bonnard, M., Camus, M., de Graaf, J., & Pailhous, J. (2003). Direct Evidence for a Binding between Cognitive and Motor Functions in Humans: A TMS Study. *Journal of Cognitive Neuroscience*, *15*(8), 1207–1216. https://doi.org/10.1162/089892903322598157

Brouwer, B., & Ashby, P. (1990). Corticospinal projections to upper and lower limb spinal motoneurons in man. *Electroencephalography and Clinical Neurophysiology*, *76*(6), 509–519. https://doi.org/10.1016/0013-4694(90)90002-2

Brouwer, B., & Ashby, P. (1992). Corticospinal projections to lower limb motoneurons in man. *Experimental Brain Research*, *89*(3), 649–654. https://doi.org/10.1007/BF00229889

Bruel-Jungerman, E., Davis, S., & Laroche, S. (2007). Brain plasticity mechanisms and memory: A party of four. *The Neuroscientist*, *13*(5), 492–505. https://doi.org/10.1177/1073858407302725

Capaday, C. (1997). Neurophysiological methods for studies of the motor system in freely moving human subjects. *Journal of Neuroscience Methods*, *74*(2), 201–218. https://doi.org/10.1016/s0165-0270(97)02250-4

Capaday, C., & Stein, R. B. (1986). Amplitude modulation of the soleus H-reflex in the human during walking and standing. *Journal of Neuroscience*, *6*(5), 1308–1313. https://doi.org/10.1523/JNEUROSCI.06-05-01308.1986

Carey, J. R., Bhatt, E., & Nagpal, A. (2005). Neuroplasticity promoted by task complexity. *Exercise and Sport Sciences Reviews*, *33*(1), 24–31. https://pubmed.ncbi.nlm.nih.gov/15640717

Cavaleri, R., Schabrun, S. M., & Chipchase, L. S. (2017). The number of stimuli required to reliably assess corticomotor excitability and primary motor cortical representations using transcranial magnetic stimulation (TMS): a systematic review and meta-analysis. *Systematic Reviews*, *6*(1). https://doi.org/10.1186/s13643-017-0440-8

Chang, Y.-K., Nien, Y.-H., Tsai, C.-L., & Etnier, J. L. (2010). Physical Activity and Cognition in Older Adults: The Potential of Tai Chi Chuan. *Journal of Aging and Physical Activity*, *18*(4), 451–472. https://doi.org/10.1123/japa.18.4.451

Chen, C.-L., Lou, S.-Z., Wu, H.-W., Wu, S.-K., Yeung, K.-T., & Su, F.-C. (2014). Effects of the type and direction of support surface perturbation on postural responses. *Journal of NeuroEngineering and Rehabilitation*, *11*(1), 50. https://doi.org/10.1186/1743-0003-11-50

Chen, K., Ge, R., Cheng, Y., & Dai, Y. (2019). Three-week treadmill training changes the electrophysiological properties of spinal interneurons in the mice. *Experimental Brain Research*, *237*(11), 2925–2938. https://doi.org/10.1007/s00221-019-05647-3

Chen, L. L., & Wise, S. P. (1995). Neuronal activity in the supplementary eye field during acquisition of conditional oculomotor associations. *Journal of Neurophysiology*, *73*(3), 1101–1121. https://doi.org/10.1152/jn.1995.73.3.1101

Chen, R., Cros, D., Curra, A., Di Lazzaro, V., Lefaucheur, J.-P., Magistris, M. R., Mills, K., Rösler, K. M., Triggs, W. J., Ugawa, Y., & Ziemann, U. (2008). The clinical diagnostic utility of transcranial magnetic stimulation: Report of an IFCN committee. *Clinical Neurophysiology*, *119*(3), 504–532. https://doi.org/10.1016/j.clinph.2007.10.014

Chen, R., & Hallett, M. (1999). The time course of changes in motor cortex excitability associated with voluntary movement. *Canadian Journal of Neurological Sciences*, *26*(3), 163–169. https://doi.org/10.1017/s0317167100000196

Chiari, L., Rocchi, L., & Cappello, A. (2002). Stabilometric parameters are affected by anthropometry and foot placement. *Clinical Biomechanics*, *17*(9), 666–677. https://doi.org/10.1016/S0268-0033(02)00107-9

Chiou, S., Hurry, M., Reed, T., Quek, J. X., & Strutton, P. H. (2018). Cortical contributions to anticipatory postural adjustments in the trunk. *The Journal of Physiology*, *596*(7), 1295–1306. https://doi.org/10.1113/jp275312

Christiansen, L., Larsen, M. N., Grey, M. J., Nielsen, J. B., & Lundbye-Jensen, J. (2017). Long-term progressive motor skill training enhances corticospinal excitability for the ipsilateral hemisphere and motor performance of the untrained hand. *European Journal of Neuroscience*, *45*(12), 1490–1500. https://doi.org/10.1111/ejn.13409

Churchill, J. D., Galvez, R., Colcombe, S. J., Swain, R. A., Kramer, A. F., & Greenough, W. T. (2002). Exercise, experience and the aging brain. *Neurobiology of Aging*, *23*(5), 941–955. https://doi.org/10.1016/s0197-4580(02)00028-3

Cooke, S. F. (2006). Plasticity in the human central nervous system. *Brain*, *129*(7), 1659–1673. https://doi.org/10.1093/brain/awl082

Costa, R. M., Cohen, D., & Nicolelis, M. a. L. (2004). Differential corticostriatal plasticity during ffast and slow motor skill learning in mice. *Current Biology*, *14*(13), 1124–1134. https://doi.org/10.1016/j.cub.2004.06.053

Cotman, C. W., Berchtold, N. C., & Christie, L.-A. (2007). Exercise builds brain health: Key roles of growth factor cascades and inflammation. *Trends in Neurosciences*, *30*(9), 464–472. https://doi.org/10.1016/j.tins.2007.06.011

Cree, B. A., & Weimer, L. H. (2003). Sensory System, Overview. In *Elsevier eBooks* (pp. 234–241). https://doi.org/10.1016/b0-12-226870-9/00897-2

Crone, C., & Nielsen, J. (1989). Methodological implications of the post activation depression of the soleus H-reflex in man. *Experimental Brain Research*, *78*(1), 28–32. https://doi.org/10.1007/BF00230683

Darling, W. G., Wolf, S. L., & Butler, A. (2006). Variability of motor potentials evoked by transcranial magnetic stimulation depends on muscle activation. *Experimental Brain Research*, *174*(2), 376–385. https://doi.org/10.1007/s00221-006-0468-9

Dayan, E., & Cohen, L. G. (2011). Neuroplasticity subserving motor skill learning. *Neuron*, *72*(3), 443–454. https://doi.org/10.1016/j.neuron.2011.10.008

De Goede, A. A., Ter Braack, E. M., & Van Putten, M. J. (2018). Accurate coil positioning is important for single and paired pulse TMS on the subject level. *Brain Topography*, *31*(6), 917–930. https://doi.org/10.1007/s10548-018-0655-6

Dean, J. C. (2013). Proprioceptive feedback and preferred patterns of human movement. *Exercise and Sport Sciences Reviews*, *41*(1), 36–43. https://doi.org/10.1097/jes.0b013e3182724bb0

Delhaye, B. P., Long, K. H., & Bensmaia, S. J. (2018). Neural basis of touch and proprioception in primate cortex. *Comprehensive Physiology*, *8*(4), 1575–1602. https://doi.org/10.1002/cphy.c170033

Di Giulio, I., Maganaris, C. N., Baltzopoulos, V., & Loram, I. D. (2009). The proprioceptive and agonist roles of gastrocnemius, soleus and tibialis anterior muscles in maintaining human upright posture. *The Journal of*

Physiology, *587*(10), 2399–2416. https://doi.org/10.1113/jphysiol.2009.168690
Di Lazzaro, V., & Rothwell, J. C. (2014). Corticospinal activity evoked and modulated by non-invasive stimulation of the intact human motor cortex. *The Journal of Physiology*, *592*(19), 4115–4128. https://doi.org/10.1113/jphysiol.2014.274316
Dietz, V., Quintern, J., & Berger, W. (1984). Cerebral evoked potentials associated with the compensatory reactions following stance and gait perturbation. *Neuroscience Letters*, *50*(1–3), 181–186. https://doi.org/10.1016/0304-3940(84)90483-x
Dietz, V., Trippel, M., & Horstmann, G. A. (1991). Significance of proprioceptive and vestibulo–spinal reflexes in the control of stance and gait. *Advances in Psychology* (pp. 37–52). https://doi.org/10.1016/s0166-4115(08)60737-2
Dietz, V., Trippel, M., Ibrahim, I. K., & Berger, W. (1993). Human stance on a sinusoidally translating platform: Balance control by feedforward and feedback mechanisms. *Experimental Brain Research*, *93*(2), 352–362. https://doi.org/10.1007/BF00228405
Doyon, J., & Benali, H. (2005). Reorganization and plasticity in the adult brain during learning of motor skills. *Current Opinion in Neurobiology*, *15*(2), 161–167. https://doi.org/10.1016/j.conb.2005.03.004
Doyon, J., Gaudreau, D., Laforce, R., Castonguay, M., Bédard, P. J., Bédard, F., & Bouchard, J. P. (1997). Role of the striatum, cerebellum, and frontal lobes in the learning of a visuomotor sequence. *Brain and Cognition*, *34*(2), 218–245. https://doi.org/10.1006/brcg.1997.0899
Earles, D. R., Koceja, D. M., & Shively, C. W. (2000). Environmental changes in soleus H-reflex excitability in young and elderly subjects. *International Journal of Neuroscience*, *105*(1–4), 1–13. https://doi.org/10.3109/00207450009003261
Enoka, R. M. (1997). Neural adaptations with chronic physical activity. *Journal of Biomechanics*, *30*(5), 447–455. https://doi.org/10.1016/s0021-9290(96)00170-4
Evarts, E. V. (1973). Motor cortex reflexes associated with learned movement. *Science*, *179*(4072), 501–503. https://doi.org/10.1126/science.179.4072.501
Fellows, S. J., Dömges, F., Töpper, R., Thilmann, A. F., & Noth, J. (1993). Changes in the short- and long-latency stretch reflex components of the triceps surae muscle during ischaemia in man. *The Journal of Physiology*, *472*(1), 737–748. https://doi.org/10.1113/jphysiol.1993.sp019970
Floeter, M. K., & Rothwell, J. C. (1999). Releasing the brakes before pressing the gas pedal. *Neurology*, *53*(4), 664. https://doi.org/10.1212/wnl.53.4.664
Floyer-Lea, A., & Matthews, P. M. (2004). Changing brain networks for visuomotor control with increased movement automaticity. *Journal of Neurophysiology*, *92*(4), 2405–2412. https://doi.org/10.1152/jn.01092.2003
Floyer-Lea, A., & Matthews, P. M. (2005). Distinguishable brain activation networks for Short- and Long-Term Motor Skill learning. *Journal of Neurophysiology*, *94*(1), 512–518. https://doi.org/10.1152/jn.00717.2004

Frigon, A., Akay, T., & Prilutsky, B. I. (2021). Control of mammalian locomotion by somatosensory feedback. *Comprehensive Physiology*, 2877–2947. https://doi.org/10.1002/cphy.c210020

Frontera, W. R. (2007). Clinical sports medicine: medical management and rehabilitation. *Elsevier Health Sciences*.

Goldsworthy, M. R., Hordacre, B., & Ridding, M. C. (2016). Minimum number of trials required for within- and between-session reliability of TMS measures of corticospinal excitability. *Neuroscience*, *320*, 205–209. https://doi.org/10.1016/j.neuroscience.2016.02.012

Grigg, P. (1994). Peripheral neural mechanisms in proprioception. *Journal of Sport Rehabilitation*, *3*(1), 2–17. https://doi.org/10.1123/jsr.3.1.2

Gruber, M., Taube, W., Gollhofer, A., Beck, S., Amtage, F., & Schubert, M. (2007). Training-specific adaptations of H- and stretch reflexes in human soleus muscle. *Journal of Motor Behavior*, 39(1), 68–78. https://doi.org/10.3200/JMBR.39.1.68-78

Hallett, M. (2007). Transcranial magnetic stimulation: a primer. *Neuron*, *55*(2), 187–199. https://doi.org/10.1016/j.neuron.2007.06.026

Han, J., Anson, J., Waddington, G., Adams, R., & Liu, Y. (2015). The role of ankle proprioception for balance control in relation to sports performance and injury. *BioMed Research International*, *2015*, 1–8. https://doi.org/10.1155/2015/842804

Handcock, P. J., Williams, L. R., & Sullivan, S. J. (2001). The reliability of H-reflex recordings in standing subjects. *Electromyogr Clin Neurophysiol*, *41*(1), 9–15. https://pubmed.ncbi.nlm.nih.gov/11234570

Hänggi, J., Koeneke, S., Bezzola, L., & Jäncke, L. (2010). Structural neuroplasticity in the sensorimotor network of professional female ballet dancers. *Human Brain Mapping*, *31*(8), 1196–1206. https://doi.org/10.1002/hbm.20928

Heise, K.-F., Niehoff, M., Feldheim, J., Liuzzi, G., Gerloff, C., & Hummel, F. (2014). Differential behavioral and physiological effects of anodal transcranial direct current stimulation in healthy adults of younger and older age. *Frontiers in Aging Neuroscience*, *6*, 146. https://doi.org/10.3389/fnagi.2014.00146

Hempel, A., Giesel, F. L., Caraballo, N. M. G., Amann, M., Meyer, H., Wüstenberg, T., Essig, M., & Schröder, J. (2004). Plasticity of cortical activation related to working memory during training. *American Journal of Psychiatry*, *161*(4), 745–747. https://doi.org/10.1176/appi.ajp.161.4.745

Hermens, H., Freriks, B., Merletti, R., Hägg, G., Stegeman, D. F., Blok, J. H., Hägg, G., & Blok, W. J. (1999). SENIAM 8: European recommendations for surface electromyography.

Hikosaka, O., Nakamura, K., Sakai, K., & Nakahara, H. (2002). Central mechanisms of motor skill learning. *Current Opinion in Neurobiology*, *12*(2), 217–222. https://doi.org/10.1016/s0959-4388(02)00307-0.

Hirano, M., Kubota, S., Koizume, Y., Tanaka, S., & Funase, K. (2016). Different effects of implicit and explicit motor sequence learning on latency of

motor evoked potential evoked by transcranial magnetic stimulation on the primary motor cortex. *Frontiers in Human Neuroscience*, *10*, 671. https://doi.org/10.3389/fnhum.2016.00671

Hoffmann, P. (1918). Uber die beziehungen der sehnenreflexe zur willkurichen bewegung und zum tonus. *Z Biol*, *68*, 351–370.

Holland, L., Murphy, B., Passmore, S., & Yielder, P. (2015). Time course of corticospinal excitability changes following a novel motor training task. *Neuroscience Letters*, *591*, 81–85. https://doi.org/10.1016/j.neulet.2015.02.022

Honeycutt, C. F., Gottschall, J. S., & Nichols, T. R. (2009). Electromyographic responses from the hindlimb muscles of the decerebrate cat to horizontal support surface perturbations. *Journal of Neurophysiology*, *101*(6), 2751–2761. https://doi.org/10.1152/jn.91040.2008

Hopkins, J. T., Ingersoll, C. D., Cordova, M. L., & Edwards, J. E. (2000). Intrasession and intersession reliability of the soleus H-reflex in supine and standing positions. *Electroencephalography and Clinical Neurophysiology*, *40*(2), 89–94. https://pubmed.ncbi.nlm.nih.gov/10746184

Horak, F. B., Shupert, C. L., Dietz, V., & Horstmann, G. A. (1994). Vestibular and somatosensory contributions to responses to head and body displacements in stance. *Experimental Brain Research*, *100*(1). https://doi.org/10.1007/bf00227282

Hosel, K., & Tremblay, F. (2021). Facilitation of motor evoked potentials in response to a modified 30 Hz intermittent Theta-Burst stimulation protocol in healthy adults. *Brain Sciences*, *11*(12), 1640. https://doi.org/10.3390/brainsci11121640

Hötting, K., & Röder, B. (2013). Beneficial effects of physical exercise on neuroplasticity and cognition. *Neuroscience & Biobehavioral Reviews*, *37*(9), 2243–2257. https://doi.org/10.1016/j.neubiorev.2013.04.005

Hrysomallis, C. (2011). Balance ability and athletic performance. *Sports Medicine*, *41*(3), 221–232. https://doi.org/10.2165/11538560-000000000-00000

Jacobs, J., & Horak, F. B. (2007). Cortical control of postural responses. *Journal of Neural Transmission*, *114*(10), 1339–1348. https://doi.org/10.1007/s00702-007-0657-0

Jansma, J., Ramsey, N. F., Slagter, H. A., & Kahn, R. S. (2001). Functional anatomical correlates of controlled and automatic processing. *Journal of Cognitive Neuroscience*, *13*(6), 730–743. https://doi.org/10.1162/08989290152541403

Lundbye-Jensen, J., Marstrand, P. C. D., & Nielsen, J. B. (2005). Motor skill training and strength training are associated with different plastic changes in the central nervous system. *Journal of Applied Physiology*, *99*(4), 1558–1568. https://doi.org/10.1152/japplphysiol.01408.2004

Jueptner, M., Frith, C. D., Brooks, D. J., Frackowiak, R. S. J., & Passingham, R. E. (1997). Anatomy of motor learning. II. Subcortical structures and learning

by trial and error. *Journal of Neurophysiology*, *77*(3), 1325–1337. https://doi.org/10.1152/jn.1997.77.3.1325
Kantak, S., Stinear, J. W., Buch, E. R., & Cohen, L. G. (2011). Rewiring the brain. *Neurorehabilitation and Neural Repair*, *26*(3), 282–292. https://doi.org/10.1177/1545968311420845
Kasuga, S., Telgen, S., Ushiba, J., Nozaki, D., & Diedrichsen, J. (2015). Learning feedback and feedforward control in a mirror-reversed visual environment. *Journal of Neurophysiology*, *114*(4), 2187–2193. https://doi.org/10.1152/jn.00096.2015
Kawato, M. (1999). Internal models for motor control and trajectory planning. *Current Opinion in Neurobiology*, *9*(6), 718–727. https://doi.org/10.1016/s0959-4388(99)00028-8
Kida, H., & Mitsushima, D. (2018). Mechanisms of motor learning mediated by synaptic plasticity in rat primary motor cortex. *Neuroscience Research*, *128*, 14–18. https://doi.org/10.1016/j.neures.2017.09.008
Kirk, B., Trajano, G. S., Pulverenti, T. S., Rowe, G., & Blazevich, A. J. (2019). Neuromuscular factors contributing to reductions in muscle force after repeated, High-Intensity muscular efforts. *Frontiers in Physiology*, *10*. https://doi.org/10.3389/fphys.2019.00783
Kleim, J. A., Hogg, T. M., VandenBerg, P. M., Cooper, N. R., Bruneau, R., & Remple, M. S. (2004). Cortical synaptogenesis and motor map reorganization occur during late, but not early, phase of motor skill learning. *The Journal of Neuroscience*, *24*(3), 628–633. https://doi.org/10.1523/jneurosci.3440-03.2004
Knaepen, K., Goekint, M., Heyman, E. M., & Meeusen, R. (2010). Neuroplasticity—exercise-induced response of peripheral brain-derived neurotrophic factor. *Sports Medicine*, *40*(9), 765–801. https://doi.org/10.2165/11534530-000000000-00000
Koceja, D. M., Davison, E., & Robertson, C. T. (2004). Neuromuscular characteristics of endurance- and power-trained athletes. *Research Quarterly for Exercise and Sport*, *75*(1), 23–30. https://doi.org/10.1080/02701367.2004.10609130
Koo, T. K., & Li, M. Y. (2016). A Guideline of Selecting and Reporting Intraclass Correlation Coefficients for Reliability Research. *Journal of chiropractic medicine*, 15(2), 155–163. https://doi.org/10.1016/j.jcm.2016.02.012
Kumpulainen, S., Avela, J., Grüber, M., Bergmann, J., Voigt, M., Linnamo, V., & Mrachacz-Kersting, N. (2014). Differential modulation of motor cortex plasticity in skill- and endurance-trained athletes. *European Journal of Applied Physiology*, *115*(5), 1107–1115. https://doi.org/10.1007/s00421-014-3092-6
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*, *221*(3), 241–249. https://doi.org/10.1007/s00221-012-3165-x

Kurtzer, I. (2015). Long-latency reflexes account for limb biomechanics through several supraspinal pathways. *Frontiers in Integrative Neuroscience*, *8*. https://doi.org/10.3389/fnint.2014.00099

Kurtzer, I., Pruszynski, J. A., & Scott, S. H. (2010). Long-latency and voluntary responses to an arm displacement can be rapidly attenuated by perturbation offset. *Journal of Neurophysiology*, *103*(6), 3195–3204. https://doi.org/10.1152/jn.01139.2009

Lagerquist, O., Zehr, E. P., & Docherty, D. (2006). Increased spinal reflex excitability is not associated with neural plasticity underlying the cross-education effect. *Journal of Applied Physiology*, *100*(1), 83–90. https://doi.org/10.1152/japplphysiol.00533.2005

Latash, M. L., & Zatsiorsky, V. M. (2015). Biomechanics and motor control: defining central concepts.

Lauber, B., Gollhofer, A., & Taube, W. (2018). Differences in motor cortical control of the soleus and tibialis. *The Journal of Experimental Biology*. https://doi.org/10.1242/jeb.174680

Lauber, B., Gollhofer, A., & Taube, W. (2021). What to train first: Balance or explosive strength? Impact on performance and intracortical inhibition. *Scandinavian Journal of Medicine & Science in Sports*, *31*(6), 1301–1312. https://doi.org/10.1111/sms.13939

Di Lazzaro, V., Dileone, M., Pilato, F., Profice, P., Oliviero, A., Mazzone, P., Insola, A., Capone, F., Ranieri, F., & Tonali, P. (2009). Associative motor cortex plasticity: direct evidence in humans. *Cerebral Cortex*, *19*(10), 2326–2330. https://doi.org/10.1093/cercor/bhn255

Lee, R., & Tatton, W. G. (1975). Motor responses to sudden limb displacements in primates with specific CNS lesions and in human patients with motor system disorders. *Canadian Journal of Neurological Sciences*, *2*(3), 285–293. https://doi.org/10.1017/s0317167100020382

Lephart, S. M., Pincivero, D. M., Giraido, J. L., & Fu, F. H. (1997). The role of proprioception in the management and rehabilitation of athletic injuries. *The American Journal of Sports Medicine*, *25*(1), 130–137. https://doi.org/10.1177/036354659702500126

Li, C.-S. R., Padoa-Schioppa, C., & Bizzi, E. (2001). Neuronal correlates of motor performance and motor learning in the primary motor cortex of monkeys adapting to an external force field. *Neuron*, *30*(2), 593–607. https://doi.org/10.1016/S0896-6273(01)00301-4

Logan, G. D. (1979). On the use of a concurrent memory load to measure attention and automaticity. *Journal of Experimental Psychology: Human Perception and Performance*, *5*, 189–207. https://doi.org/10.1037/0096-1523.5.2.189

Lynch, G. S., Dunwiddie, T., & Gribkoff, V. (1977). Heterosynaptic depression: A postsynaptic correlate of long-term potentiation. *Nature*, *266*(5604), 737–739. http://dx.doi.org/10.1038/266737a0

MacKinnon, C. D. (2018). Sensorimotor anatomy of gait, balance, and falls. *Handbook of Clinical Neurology*, *159*, 3–26.

Macpherson, J. M., & Fung, J. (1999). Weight support and balance during perturbed stance in the chronic spinal cat. *Journal of Neurophysiology*, *82*(6), 3066–3081. https://doi.org/10.1152/jn.1999.82.6.3066

Martin, J. H. (2005). The corticospinal system: From development to motor control. *The Neuroscientist*, *11*(2), 161–173. https://doi.org/10.1177/1073858404270843

Matthews, P. B. C. (2015). Where anatomy led, physiology followed: a survey of our developing understanding of the muscle spindle, what it does and how it works. *Journal of Anatomy*, *227*(2), 104–114. https://doi.org/10.1111/joa.12345

Matthews, P. B. C., & Bagby, R. M. (1974). Mammalian muscle receptors and their central actions. *Medicine & Science in Sports & Exercise*, *6*(2). https://doi.org/10.1249/00005768-197400620-00005

McDonnell, M. N., Orekhov, Y., & Ziemann, U. (2006). The role of GABAb receptors in intracortical inhibition in the human motor cortex. *Experimental Brain Research*, *173*(1), 86–93. https://doi.org/10.1007/s00221-006-0365-2

Mierau, A., Hülsdünker, T., & Strüder, H. K. (2015). Changes in cortical activity associated with adaptive behavior during repeated balance perturbation of unpredictable timing. *Frontiers in Behavioral Neuroscience*, *9*. https://doi.org/10.3389/fnbeh.2015.00272

Misiaszek, J. E. (2003). The H-reflex as a tool in neurophysiology: Its limitations and uses in understanding nervous system function. *Muscle & Nerve*, *28*(2), 144–160. https://doi.org/10.1002/mus.10372

Mouthon, A., & Taube, W. (2019). Intracortical Inhibition Increases during Postural Task Execution in Response to Balance Training. *Neuroscience*, *401*, 35–42. https://doi.org/10.1016/j.neuroscience.2019.01.007

Mrachacz-Kersting, N., Grey, M. J., & Sinkjaer, T. (2006). Evidence for a supraspinal contribution to the human quadriceps long-latency stretch reflex. *Experimental Brain Research*, *168*(4), 529–540. https://doi.org/10.1007/s00221-005-0120-0

Muellbacher, W., Ziemann, U., Boroojerdi, B., Cohen, L., & Hallett, M. (2001). Role of the human motor cortex in rapid motor learning. *Experimental Brain Research*, *136*(4), 431–438. https://doi.org/10.1007/s002210000614

Muellbacher, W., Ziemann, U., Wissel, J., Dang, N., Kofler, M., Facchini, S., Boroojerdi, B., Poewe, W., & Hallett, M. (2002). Early consolidation in human primary motor cortex. *Nature*, *415*(6872). https://doi.org/10.1038/nature712

Müller, P., Duderstadt, Y., Leßmann, V., & Müller, N. G. (2020). Lactate and BDNF: key mediators of exercise induced neuroplasticity? *Journal of Clinical Medicine*, *9*(4), 1136. https://doi.org/10.3390/jcm9041136

Muratori, L. M., Lamberg, E. M., Quinn, L., & Duff, S. V. (2013). Applying principles of motor learning and control to upper extremity rehabilitation. *Journal of Hand Therapy : Official Journal of the American Society of Hand Therapists*, *26*(2), 94–103. https://doi.org/10.1016/j.jht.2012.12.007

Mynark, R. G., & Koceja, D. M. (2002). Down training of the elderly soleus H-reflex with the use of a spinally induced balance perturbation. *Journal of Applied Physiology*, *93*(1), 127–133. https://doi.org/10.1152/japplphysiol.00007.2001

Nashner, L. M. (1976). Adapting reflexes controlling the human posture. Experimental *Brain Research*, 26(1), 59-72. https://doi.org/10.1007/BF00235249

Nielsen, J., Crone, C., & Hultborn, H. (1993). H-reflexes are smaller in dancers from the royal danish ballet than in well-trained athletes. European Journal of *Applied Physiology and Occupational Physiology*, *66*(2), 116–121. https://doi.org/10.1007/BF01427051

Nielsen, J. F. (1996). Logarithmic distribution of amplitudes of compound muscle action potentials evoked by transcranial magnetic stimulation. *Journal of Clinical Neurophysiology*, *13*(5), 423–434. https://doi.org/10.1097/00004691-199609000-00005

Ogawa, T., Kawashima, N., Suzuki, S., & Nakazawa, K. (2012). Different modulation pattern of spinal stretch reflex excitability in highly trained endurance runners. *European Journal of Applied Physiology*, *112*(10), 3641–3648. https://doi.org/10.1007/s00421-012-2351-7

Ogawa, T., Kim, G., Sekiguchi, H., Akai, M., Suzuki, S., & Nakazawa, K. (2008). Enhanced stretch reflex excitability of the soleus muscle in experienced swimmers. *European Journal of Applied Physiology*, *105*(2), 199–205. https://doi.org/10.1007/s00421-008-0890-8

Orban de Xivry, J.-J., Ahmadi-Pajouh, M. A., Harran, M. D., Salimpour, Y., & Shadmehr, R. (2013). Changes in corticospinal excitability during reach adaptation in force fields. *Journal of Neurophysiology*, *109*(1), 124–136. https://doi.org/10.1152/jn.00785.2012

Palmieri, R. M., Hoffman, M. A., & Ingersoll, C. D. (2002). Intersession Reliability for H-reflex measurements arising form the soleus, peroneal, and tibialis anterior musculature. *International Journal of Neuroscience*, *112*(7). https://doi.org/10.1080/00207450290025851

Palmieri, R. M., Ingersoll, C. D., & Hoffman, M. A. (2004). The Hoffmann reflex: methodologic considerations and applications for use in sports medicine and athletic training research. *Journal of Athletic Training*, *39*(3), 268–277.

Papale, A. E., & Hooks, B. M. (2018). Circuit changes in motor cortex during motor skill learning. *Neuroscience*, *368*, 283–297. https://doi.org/10.1016/j.neuroscience.2017.09.010

Papegaaij, S., Baudry, S., Négyesi, J., Taube, W., & Hortobágyi, T. (2016). Intracortical inhibition in the soleus muscle is reduced during the control of upright standing in both young and old adults. *European Journal of Applied Physiology*, *116*(5). https://doi.org/10.1007/s00421-016-3354-6

Park, P., Volianskis, A., Sanderson, T. M., Bortolotto, Z. A., Jane, D. E., Zhuo, M., Kaang, B., & Collingridge, G. L. (2014). NMDA receptor-dependent long-term potentiation comprises a family of temporally overlapping forms of synaptic plasticity that are induced by different patterns of

stimulation. *Philosophical Transactions of the Royal Society B*, *369*(1633), 20130131. https://doi.org/10.1098/rstb.2013.0131
Pate, R. R., & Branch, J. D. (1992). Training for endurance sport. *Medicine and Science in Sports and Exercise*, *24*(9), 340-343. https://doi.org/10.1249/00005768-199209001-00007
Pavol, M. J., & Pai, Y.-C. (2002). Feedforward adaptations are used to compensate for a potential loss of balance. *Experimental Brain Research*, *145*(4). https://doi.org/10.1007/s00221-002-1143-4
Penfield, W., & Jasper, H. (1954). Epilepsy and the functional anatomy of the human brain. *Southern Medical Journal*, 47(7), 704. https://doi.org/10.1097/00007611-195407000-00024
Penzer, F., Duchateau, J., & Baudry, S. (2015). Effects of short-term training combining strength and balance exercises on maximal strength and upright standing steadiness in elderly adults. *Experimental Gerontology*, *61*, 38–46. https://doi.org/10.1016/j.exger.2014.11.013
Perez, M. A., Lungholt, B. K. S., Nyborg, K., & Nielsen, J. B. (2004). Motor skill training induces changes in the excitability of the leg cortical area in healthy humans. *Experimental Brain Research*, *159*(2), 197–205. https://doi.org/10.1007/s00221-004-1947-5
Petersen, N., Christensen, L. O. D., Morita, H., Sinkjær, T., & Nielsen, J. B. (1998). Evidence that a transcortical pathway contributes to stretch reflexes in the tibialis anterior muscle in man. *The Journal of Physiology*, *512*(1), 267–276. https://doi.org/10.1111/j.1469-7793.1998.267bf.x
Pierrot-Deseilligny, E., & Burke, D. (2012). The circuitry of the human spinal cord: Spinal and corticospinal mechanisms of movement. Cambridge University Press. http://dx.doi.org/10.1017/CBO9781139026727
Piirainen, J. M., Linnamo, V., Cronin, N. J., & Avela, J. (2013). Age-related neuromuscular function and dynamic balance control during slow and fast balance perturbations. *Journal of Neurophysiology*, *110*(11), 2557–2562. https://doi.org/10.1152/jn.00476.2013
Pinniger, G. J., Nordlund, M., Steele, J. R., & Cresswell, A. B. (2001). H-reflex modulation during passive lengthening and shortening of the human triceps surae. *The Journal of Physiology*, *534*(3), 913–923. https://doi.org/10.1111/j.1469-7793.2001.00913.x
Poldrack, R. A., & Gabrieli, J. D. E. (2001). Characterizing the neural mechanisms of skill learning and repetition priming. *Brain*, *124*(1), 67–82. https://doi.org/10.1093/brain/124.1.67
Portney, L. G., & Watkins, M. P. (2002). Foundations of clinical research: applications to practice. *Survey of Ophthalmology*, *47*(6), 598. https://doi.org/10.1016/s0039-6257(02)00362-4
Proske, U., & Gandevia, S. C. (2012). The proprioceptive senses: their roles in signaling body shape, body position and movement, and muscle force. *Physiological Reviews*, 92(4), 1651–1697. https://doi.org/10.1152/physrev.00048.2011

Proske, U., Wise, A. K., & Gregory, J. E. (2000). The role of muscle receptors in the detection of movements. *Progress in Neurobiology*, *60*(1), 85–96. https://doi.org/10.1016/S0301-0082(99)00022-2

Prsa, M., & Thier, P. (2011). The role of the cerebellum in saccadic adaptation as a window into neural mechanisms of motor learning. *European Journal of Neuroscience*, *33*(11), 2114–2128. https://doi.org/10.1111/j.1460-9568.2011.07693.x

Purves, D., Augustine, G. J., Fitzpatrick, D., Katz, L. C., LaMantia, A.-S., McNamara, J. O., & Williams, S. M. (2001). The spinal cord circuitry underlying muscle stretch reflexes. *Neuroscience. 2nd edition*. Sinauer Associates. https://www.ncbi.nlm.nih.gov/books/NBK10809/

Reithler, J., Van Mier, H., & Goebel, R. (2010). Continuous motor sequence learning: Cortical efficiency gains accompanied by striatal functional reorganization. *NeuroImage*, *52*, 263–276. https://doi.org/10.1016/j.neuroimage.2010.03.073

Reynolds, C., & Ashby, P. (1999). Inhibition in the human motor cortex is reduced just before a voluntary contraction. *Neurology*, *53*(4). https://doi.org/10.1212/wnl.53.4.730

Riemann, B. L., & Lephart, S. M. (2002). The sensorimotor system, Part II: The role of proprioception in motor ccontrol and functional joint stability. *Journal of Athletic Training*, *37*(1), 80–84.

Rioult-Pedotti, M.-S., Friedman, D., & Donoghue, J. P. (2000). Learning-induced LTP in neocortex. *Science*, *290*(5491), 533–536. https://doi.org/10.1126/science.290.5491.533

Rioult-Pedotti, M.-S., Friedman, D., Hess, G., & Donoghue, J. P. (1998). Strengthening of horizontal cortical connections following skill learning. *Nature Neuroscience*, *1*(3). https://doi.org/10.1038/678

Robert A. Davidoff, M. D. (1990). The pyramidal tract. *Neurology*, *40*(2), 332–332. https://doi.org/10.1212/WNL.40.2.332

Rodriguez-Falces, J., & Place, N. (2018). End-of-fiber signals strongly influence the first and second phases of the M wave in the vastus lateralis: implications for the study of muscle excitability. *Frontiers in Physiology*, *9*. https://doi.org/10.3389/fphys.2018.00162

Rosenkranz, K., Kacar, A., & Rothwell, J. (2007). Differential modulation of motor cortical plasticity and excitability in early and late phases of human motor learning. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *27*, 12058–12066. https://doi.org/10.1523/JNEUROSCI.2663-07.2007

Rossini, P. M., Rossi, S., Pasqualetti, P., & Tecchio, F. (1999). Corticospinal excitability modulation to hand muscles during movement imagery. *Cerebral Cortex*, *9*(2), 161–167. https://doi.org/10.1093/cercor/9.2.161

Rothwell, J. C. (1997). Techniques and mechanisms of action of transcranial stimulation of the human motor cortex. *Journal of Neuroscience Methods*, *74*(2), 113–122. https://doi.org/10.1016/S0165-0270(97)02242-5

Ruffieux, J., Mouthon, A., Keller, M., Wälchli, M., & Taube, W. (2017). Behavioral and neural adaptations in response to five weeks of balance training in older adults: A randomized controlled trial. *Journal of Negative Results in BioMedicine*, *16*(1), 11. https://doi.org/10.1186/s12952-017-0076-1

Rusu, C. V., Murakami, M., Ziemann, U., & Triesch, J. (2014). A model of TMS-induced I-waves in motor cortex. *Brain Stimulation*, *7*(3), 401–414. https://doi.org/10.1016/j.brs.2014.02.009

Sakai, K., Hikosaka, O., Miyauchi, S., Takino, R., Sasaki, Y., & Pütz, B. (1998). Transition of brain activation from frontal to parietal areas in visuomotor sequence learning. *The Journal of Neuroscience*, *18*(5), 1827–1840. https://doi.org/10.1523/jneurosci.18-05-01827.1998

Sanes, J. N., & Donoghue, J. P. (2000). Plasticity and primary motor cortex. *Annual Review of Neuroscience*, *23*, 393–415. https://doi.org/10.1146/annurev.neuro.23.1.393

Sasagawa, S., Ushiyama, J., Masani, K., Kouzaki, M., & Kanehisa, H. (2009). Balance control under different passive contributions of the ankle extensors: Quiet standing on inclined surfaces. *Experimental Brain Research*, *196*(4), 537–544. https://doi.org/10.1007/s00221-009-1876-4

Schieppati, M., & Nardone, A. (1997). Medium-latency stretch reflexes of foot and leg muscles analysed by cooling the lower limb in standing humans. *The Journal of Physiology*, *503*(3), 691–698. https://doi.org/10.1111/j.1469-7793.1997.691bg.x

Schlaffke, L., Lissek, S., Lenz, M., Brüne, M., Juckel, G., Hinrichs, T., Platen, P., Tegenthoff, M., & Schmidt-Wilcke, T. (2014). Sports and brain morphology–A voxel-based morphometry study with endurance athletes and martial artists. *Neuroscience*, *259*, 35–42. https://doi.org/10.1016/j.neuroscience.2013.11.046

Seidel, O., Carius, D., Kenville, R., & Ragert, P. (2017). Motor learning in a complex balance task and associated neuroplasticity: a comparison between endurance athletes and nonathletes. *Journal of Neurophysiology*, *118*(3), 1849–1860. https://doi.org/10.1152/jn.00419.2017

Shah, A. (2008). Biologically-based functional mechanisms of motor skill acquisition. University of Massachusetts Amherst. https://scholarworks.umass.edu/dissertations/AAI3337030

Shumway-Cook, A., & Woollacott, M. H. (2006). Motor control: translating research into clinical practice. http://lib.ugent.be/en/catalog/rug01:002051786

Singh, A. M., Duncan, R. E., Neva, J. L., & Staines, W. R. (2014). Aerobic exercise modulates intracortical inhibition and facilitation in a nonexercised upper limb muscle. *BMC Sports Science, Medicine and Rehabilitation*, *6*(1), 23. https://doi.org/10.1186/2052-1847-6-23

Singh, A. M., Neva, J. L., & Staines, W. R. (2016). Aerobic exercise enhances neural correlates of motor skill learning. *Behavioural Brain Research*, *301*, 19–26. https://doi.org/10.1016/j.bbr.2015.12.020

Smith, M. A., Ghazizadeh, A., & Shadmehr, R. (2006). Interacting adaptive processes with different timescales underlie short-term motor learning. *PLOS Biology*, *4*(6), e179. https://doi.org/10.1371/journal.pbio.0040179

Suminski, A. J., Rao, S. M., Mosier, K. M., & Scheidt, R. A. (2007). Neural and electromyographic correlates of wrist posture Control. *Journal of Neurophysiology*, *97*(2). https://doi.org/10.1152/jn.01160.2006

Sun, Y., Hurd, C. L., Barnes, M. M., & Yang, J. F. (2022). Neural plasticity in spinal and corticospinal pathways induced by balance training in neurologically intact adults: a systematic review. *Frontiers in Human Neuroscience*, *16*, 921490. https://doi.org/10.3389/fnhum.2022.921490

Suzuki, M., Kirimoto, H., Onishi, H., Yamada, S., Tamaki, H., Maruyama, A., & Yamamoto, J. (2012). Reciprocal changes in input–output curves of motor evoked potentials while learning motor skills. *Brain Research*, *1473*, 114–123. https://doi.org/10.1016/j.brainres.2012.07.043

Svatkova, A., Mandl, R. C. W., Scheewe, T. W., Cahn, W., Kahn, R. S., & Hulshoff Pol, H. E. (2015). Physical exercise keeps the brain connected: biking increases white matter integrity in patients with schizophrenia and healthy controls. *Schizophrenia Bulletin*, *41*(4), 869–878. https://doi.org/10.1093/schbul/sbv033

Tahayori, B., & Koceja, D. (2012). Activity-dependent plasticity of spinal circuits in the developing and mature spinal cord. *Neural Plasticity*, *2012*, 964843. https://doi.org/10.1155/2012/964843

Tallent, J., Goodall, S., Hortobágyi, T., St Clair Gibson, A., French, D. N., & Howatson, G. (2012). Repeatability of corticospinal and spinal measures during lengthening and shortening contractions in the human tibialis anterior muscle. *PLoS One*, *7*(4). https://doi.org/10.1371/journal.pone.0035930

Tarumi, T., Fukuie, M., Yamabe, T., Kimura, R., Zhu, D. C., Ohyama-Byun, K., Maeda, S., & Sugawara, J. (2022). Microstructural organization of the corpus callosum in young endurance athletes: a global tractography study. *Frontiers in Neuroscience*, *16*, 1042426. https://doi.org/10.3389/fnins.2022.1042426

Taube, W., Gollhofer, A., & Lauber, B. (2020). Training-, muscle- and task-specific up- and downregulation of cortical inhibitory processes. *European Journal of Neuroscience*, *51*(6). https://doi.org/10.1111/ejn.14538

Taube, W., Grüber, M., Beck, S., Faist, M., Gollhofer, A., & Schubert, M. (2007). Cortical and spinal adaptations induced by balance training: correlation between stance stability and corticospinal activation. *Acta Physiologica*, *189*(4), 347–358. https://doi.org/10.1111/j.1748-1716.2007.01665.x

Taube, W., Grüber, M., & Gollhofer, A. (2008). Spinal and supraspinal adaptations associated with balance training and their functional relevance. *Acta Physiologica*, *193*(2), 101–116. https://doi.org/10.1111/j.1748-1716.2008.01850.x

Taube, W., Leukel, C., Schubert, M., Gruber, M., Rantalainen, T., & Gollhofer, A. (2008). Differential modulation of spinal and corticospinal excitability

during drop jumps. *Journal of Neurophysiology*, *99*(3), 1243–1252. https://doi.org/10.1152/jn.01118.2007
Taube, W., Leukel, C., Schubert, M., Gruber, M., Rantalainen, T., & Gollhofer, A. (2008). Differential modulation of spinal and corticospinal excitability during drop jumps. *Journal of Neurophysiology*, *99*(3), 1243–1252. https://doi.org/10.1152/jn.01118.2007
Taube, W., Schubert, M., Grüber, M., Beck, S., Faist, M., & Gollhofer, A. (2006). Direct corticospinal pathways contribute to neuromuscular control of perturbed stance. *Journal of Applied Physiology*, *101*(2), 420–429. https://doi.org/10.1152/japplphysiol.01447.2005
Taubert, M., Villringer, A., & Lehmann, N. (2015). Endurance exercise as an "endogenous" neuro-enhancement strategy to facilitate motor learning. *Frontiers in Human Neuroscience*, *9*(692). https://doi.org/10.3389/fnhum.2015.00692
Taylor, J. L. (2006). Stimulation at the cervicomedullary junction in human subjects. *Journal of Electromyography and Kinesiology*, *16*(3), 215–223. https://doi.org/10.1016/j.jelekin.2005.07.001
Taylor, J. L., Petersen, N., Butler, J. E., & Gandevia, S. C. (2002). corticospinal transmission after voluntary contractions. In *Springer eBooks* (435–441). https://doi.org/10.1007/978-1-4615-0713-0_49
Teixeira, L. A., Maia Azzi, N., de Oliveira, J. Á., Ribeiro de Souza, C., da Silva Rezende, L., & Boari Coelho, D. (2020). Automatic postural responses are scaled from the association between online feedback and feedforward control. *European Journal of Neuroscience*, *51*(10), 2023–2032. https://doi.org/10.1111/ejn.14625
Smith, L. B., & Thelen, E. (1994). A dynamic systems approach to the development of cognition and action. *The MIT Press eBooks*. https://doi.org/10.7551/mitpress/2524.001.0001
Thomas, A. L. R., Dennis, A., Bandettini, P. A., & Johansen-Berg, H. (2012). The effects of aerobic activity on brain structure. *Frontiers in Psychology*, *3*. https://doi.org/10.3389/fpsyg.2012.00086
Turco, C. V., Arsalan, S. O., & Nelson, A. J. (2020). The influence of recreational substance use in TMS research. *Brain Sciences*, *10*(10). https://doi.org/10.3390/brainsci10100751
Ungerleider, L. G., Doyon, J., & Karni, A. (2002). Imaging brain plasticity during motor skill learning. *Neurobiology of Learning and Memory*, *78*(3). https://doi.org/10.1006/nlme.2002.4091
Vaalto, S., Julkunen, P., Säisänen, L., Könönen, M., Määttä, S., & Karhu, J. (2013). Long-term plasticity may be manifested as reduction or expansion of cortical representations of actively used muscles in motor skill specialists. *NeuroReport*, *24*(11). https://doi.org/10.1097/WNR.0b013e3283628636
van Hedel, H. J., Murer, C., Dietz, V., & Curt, A. (2007). The amplitude of lower leg motor evoked potentials is a reliable measure when controlled for

torque and motor task. *Journal of Neurology*, *254*(8). https://doi.org/10.1007/s00415-006-0493-4
Vila-Chã, C., Falla, D., Correia, M. V., & Farina, D. (2012). Changes in H reflex and V wave following short-term endurance and strength training. *Journal of Applied Physiology*, *112*(1), 54–63. https://doi.org/10.1152/japplphysiol.00802.2011
Wälchli, M., Tokuno, C. D., Ruffieux, J., Keller, M., & Taube, W. (2017). Preparatory cortical and spinal settings to counteract anticipated and non-anticipated Perturbations. *Neuroscience*, *365*, 12–22. https://doi.org/10.1016/j.neuroscience.2017.09.032
Wassermann, E., Epstein, C., Ziemann, U., Walsh, V., Paus, T., & Lisanby, S. (2008). The Oxford handbook of transcranial stimulation. *Oxford University Press eBooks*. https://doi.org/10.1093/oxfordhb/9780198568926.001.0001
Wassermann, E. M. (2002). Variation in the response to transcranial magnetic brain stimulation in the general population. *Clinical Neurophysiology*, *113*(7). https://doi.org/10.1016/S1388-2457(02)00144-X
Waxman, S. G. (2020). Clinical neuroanatomy (twenty-ninth edition). McGraw Hill Medical. https://neurology.mhmedical.com/book.aspx?bookid=2850
Weir J. P. (2005). Quantifying test-retest reliability using the intraclass correlation coefficient and the SEM. *Journal of strength and conditioning research*, 19(1), 231–240. https://doi.org/10.1519/15184.1
Willingham, D. B. (1998). A neuropsychological theory of motor skill learning. *Psychological Review*, *105*, 558–584. https://doi.org/10.1037/0033-295X.105.3.558
Winter, B., Breitenstein, C., Mooren, F. C., Voelker, K., Fobker, M., Lechtermann, A., Krueger, K., Fromme, A., Korsukewitz, C., Floel, A., & Knecht, S. (2007). High impact running improves learning. *Neurobiology of Learning and Memory*, *87*(4), 597–609. https://doi.org/10.1016/j.nlm.2006.11.003
Wise, S. P., Moody, S. L., Blomstrom, K. J., & Mitz, A. R. (1998). Changes in motor cortical activity during visuomotor adaptation. *Experimental Brain Research*, *121*(3), 285–299. https://doi.org/10.1007/s002210050462
Wolpaw, J. R., & Lee, C. L. (1989). Memory traces in primate spinal cord produced by operant conditioning of H-reflex. *Journal of Neurophysiology*, *61*(3), 563–572. https://doi.org/10.1152/jn.1989.61.3.563
Wu, T., Kansaku, K., & Hallett, M. (2004). How self-initiated memorized movements become automatic: a functional MRI study. *Journal of Neurophysiology*, *91*(4), 1690–1698. https://doi.org/10.1152/jn.01052.2003
Zehr, E. P. (2002). Considerations for use of the Hoffmann reflex in exercise studies. *European Journal of Applied Physiology*, *86*(6), 455–468. https://doi.org/10.1007/s00421-002-0577-5
Zehr, E. P. (2006). Training-induced adaptive plasticity in human somatosensory reflex pathways. *Journal of Applied Physiology (1985)*, *101*(6). https://doi.org/10.1152/japplphysiol.00540.2006

Zemková, E. (2011). Assessment of balance in sport: science and reality.*Serbian Journal of Sports Sciences*, (4).

Ziemann, U., Ilić, T. V., & Jung, P. (2006). Long-term potentiation (LTP)-like plasticity and learning in human motor cortex – investigations with transcranial magnetic stimulation (TMS). In C. Barber, S. Tsuji, S. Tobimatsu, T. Uozumi, N. Akamatsu, & A. Eisen , *Functional Neuroscience: Evoked Potentials and Related Techniques* (Vol. 59, pp. 19–25). Elsevier. https://doi.org/10.1016/S1567-424X(09)70007-8

Ziemann, U., Rothwell, J., & Ridding, M. C. (1996). Interaction between intracortical inhibition and facilitation in human motor cortex. *The Journal of Physiology*, *496*(3), 873–881. https://doi.org/10.1113/jphysiol.1996.sp021734

Zoladz, J. A., Pilc, A., Majerczak, J., Grandys, M., Zapart-Bukowska, J., & Duda, K. (2008). Endurance training increases plasma brain-derived neurotrophic factor concentration in young healthy men. *Journal of physiology and pharmacology : an official journal of the Polish Physiological Society*, 59 Suppl 7, 119–132.

ORIGINAL PAPERS

I

RELIABILITY OF TRANSCRANIAL MAGNETIC STIMULATION AND H-REFLEX MEASUREMENT DURING BALANCE PERTURBATION TASKS

by

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Reliability of transcranial magnetic stimulation and H-reflex measurement during balance perturbation tasks

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Following ankle movement, posterior balance perturbation evokes short- (SLR ~30–50 ms), medium- (MLR ~50–60 ms), and long-latency responses (LLR ~70–90 ms) in soleus muscle before voluntary muscle contraction. Transcranial magnetic stimulation (TMS) and Hoffmann-reflex (H-reflex) measurements can provide insight into the contributions of corticospinal and spinal mechanisms to each response. Motor evoked potential (MEP) and H-reflex responses have shown good reliability in some dynamic muscle contraction tasks. However, it is still unclear how reliable these methods are in dynamic balance perturbation and corticospinal modulation during long amplitude balance perturbation tasks. 14 subjects completed two test sessions in this study to evaluate the reliability of MEPs, H-reflex, and corticospinal modulation during balance perturbation. In each session, the balance perturbation system operated at 0.25 m/s, accelerating at 2.5 m/s^2 over 0.3 m displacement. MEPs and H-reflexes were elicited in the right leg soleus muscle at four delays after ankle movement (10 ms, 40 ms, 80 ms, and 140 ms), respectively. Test-retest reliability of MEP and H-reflex amplitudes were assessed *via* intraclass correlation coefficients (ICC) both between- and within-session. Between-session test-retest reliability for MEPs was excellent (ICC = 0.928–0.947), while H-reflex demonstrated moderate-to-good reliability (ICC = 0.626–0.887). Within-session reliability for both MEPs and H-reflex was excellent (ICC = 0.927–0.983). TMS and H-reflex measurements were reliable at different delays after perturbation between- and within-sessions, which indicated that these methods can be used to measure corticospinal excitability during balance perturbation.

KEYWORDS

dynamic balance control, voluntary activation, motor evoked potential, intraclass correlation coefficients, corticospinal modulation

Introduction

Human standing balance control is defined as maintaining the stability limits between the center of mass and base of support (Maki and Mcilroy, 1997). In dynamic balance tasks, the human center of mass is led to more challenging conditions, in which the somatosensory system plays a more crucial role in selecting an appropriate muscle response for maintaining balance (Horak et al., 1990). When a sudden and unexpected posterior perturbation occurs, the movement at the ankle joint leads to muscle stretch within the shank, which evokes complex reflexes with short- (SLR ~30–50 ms after ankle plantarflexion), medium- (MLR ~50–60 ms), and long-latency responses (LLR ~70–90 ms) (Taube et al., 2006; Latash and Zatsiorsky, 2015). SLR has been demonstrated to be elicited by a pure monosynaptic response at the spinal level, while LLR is influenced more by the transcortical loop, which has been suggested to include supraspinal level involvement since it has enough time to exert its influence (Taube et al., 2008). As perturbation amplitude increases, there is greater time for body sway that predicts more voluntary activation involved to maintain body balance. Stronger calf muscle voluntary contraction ability is related to better balance control that is observed more in young people who use an 'ankle strategy' than in e.g. older adults who more often use a 'hip strategy' to maintain balance during perturbation (Horak et al., 1992). It has been suggested that both supraspinal and spinal level mechanisms may be at play during a balance perturbation task. However, corticospinal and spinal excitability modulation of responses and voluntary activation during the balance perturbation task is still not clear with higher amplitude perturbation and such studies have been limited.

Transcranial magnetic stimulation (TMS) and Hoffmann's reflex (H-reflex) measurements are commonly used to induce involuntary responses and study the role of corticospinal and spinal excitability as well as their modulation during various tasks (Pinniger et al., 2001; Trimble and Koceja, 2001; Knikou, 2008). In TMS measurements, a significant practical challenge faced by researchers is the stabilization of the TMS coil during the experiment, which may be more precise by using a TMS navigation system particularly in static conditions. For now, only a small number of studies have used TMS in anterior and posterior balance perturbation, and the maximum perturbation amplitude is 15 cm (Taube et al., 2007; Wälchli et al., 2017; Fujio et al., 2019). A higher amplitude balance perturbation may lead to larger and faster body swaying, which may result in unexpected movement of the TMS coil. Thus, the stability of the TMS coil is critical during TMS experiments, especially in the absence of a neuronavigation system (Chipchase et al., 2012). Stabilization of the TMS coil should be carefully considered when examining dynamic balance tasks. Further, the motor evoked potential (MEP) elicited by TMS is very sensitive to changes in the environment outside of the body (i.e., environment noise) and inside (i.e., awareness switch) (Chipchase et al., 2012). Therefore, testing reliability and variability of MEPs are also crucial within this setting. Many studies have observed acceptable reliability of using TMS in static and dynamic conditions, such as in relaxed muscle, knee contraction, and squat tasks (Tallent et al., 2012; Proessl et al., 2021). However, better reliability has been observed in static tasks (i.e., isometric knee extensions) compared with dynamic tasks (i.e., squats) (Proessl et al., 2021), which suggests that complex tasks with extra technical and physiological noise are more variable when using TMS.

H-reflex measurement has been used to assess spinal (motoneuron pool) excitability (Táboríková and Sax, 1968). Good reliability has been observed in many studies (Hopkins et al., 2000; Hayes et al., 2009), for example, when measuring H-reflex at rest, excellent test-retest reliability was observed in soleus and tibialis anterior muscles (ICC >0.9) (Palmieri et al., 2002; Hayes et al., 2009). Good reliability was also shown in ankle plantarflexion and dorsiflexion positions during isometric contraction and walking in soleus muscle (Chen et al., 2010; Simonsen and Dyhre-Poulsen, 2011). However, increased variability in reliability values was observed in different sitting postures (e.g., erect sitting, slumped sitting, and slouched sitting), while the overall reliability of H-reflex was still good (ICC >0.8) (Al Amer et al., 2020). In summation, H-reflex has been demonstrated to have good reliability during various tasks, but not yet during dynamic balance perturbation trials.

Currently, the reliability of neither TMS nor H-reflex measurement during high amplitude balance perturbation tasks is known, but such methods are used by researchers to examine differences between groups and/or the effect of interventions (Taube et al., 2007; Fujio et al., 2019). Thus, it is important to determine such reliability to enable full evaluation of the scientific methodology employed within those studies. Also, in a previous study, the TMS coil was held by the halo vest on the subject's shoulder (Taube et al., 2006), but the vest may affect the natural body movement during balance perturbation. In our system holding the TMS coil, the entire coil is connected with the platform, which helps the TMS coil move with the balance platform during perturbation. Therefore, the aim of the present study was to examine the test-retest reliability of MEPs and H-reflex responses as well as corticospinal modulation during a high amplitude balance perturbation task.

Methods

Subjects

Fourteen voluntary subjects participated in the study (8 males, 6 females, age: 35 ± 6 years, height: 173.5 ± 10.6 cm, weight: 71.8 ± 17.0 kg, and BMI: 25.0 ± 4.7). None of the subjects had any history of neuromuscular or orthopedic diseases and all

subjects were informed about the procedures and gave written informed consent. Subjects were fully introduced to the protocol and they had the opportunity to withdraw from the study at will in any phase. The study was approved by the ethics board of the University (diari number: 1267/13.00.04.00/2021) and the study was performed in conformity with the declaration of Helsinki (2013).

Experimental design

Tests were conducted over two sessions with the same tasks repeated and 46 ± 7 h separated Session 1 (S1) and Session 2 (S2). In each session, after electromyography (EMG) electrode setup and 5 min cycling warm-up (70W) on the fitness cycle (Monark, 282E, Varberg, Sweden), 16 balance perturbations without any stimulation were used to collect center-of-pressure (COP) and EMG activity data. Then, subjects were positioned in a custom-built ankle dynamometer (University of Jyväskylä, Jyväskylä, Finland) to test the isometric maximal voluntary contraction (IMVC) of the right leg. The TMS coil was set up and the active motor threshold (aMT) was tested when subjects sat in the ankle dynamometer. With a TMS coil set on the head and held by the custom-built helmet (Figure 1), subjects carefully stood up and moved to the balance platform. In the balance perturbation task with stimulation, MEPs were evoked at four different delays after the onset of ankle movement during balance perturbation in random order. The H-reflex measurements were always performed after TMS due to practical reasons. H-reflexes were elicited at the same four delays as the MEPs also in random order. The stimulations were delivered during each balance perturbation, regardless of perturbation direction, but only MEPs and H-reflex during backward perturbations were analyzed.

Pre-study design

A pre-study experiment was performed with two subjects with different height and weight before the main experiment to investigate the stability of the custom-built TMS coil helmet and TMS cable holder system. Kinematic data of the TMS coil and the head of the subject were recorded at 150 Hz by a five-camera motion capture system (Vicon Motion System, Oxford, United Kingdom). Three markers were placed on the subject's head to build the head coordinate system. Two markers were placed on the coil handle to estimate TMS coil movement since the coil was totally covered by the helmet, which made it impossible to place any markers on the coil itself. Kinematic data were analyzed using MATLAB (2019b). After coordinate transformation from ground coordinate to head coordinate system (see Figure 1), relative offset (maximum displacement) of the coil handle was analyzed to represent coil movement compared with the subject's head movement. The *x*-axis was the sagittal axis, the *y*-axis was the frontal axis, and the *z*-axis was the vertical axis.

Electromyography

EMG was measured by bipolar electrodes (Blue Sensor, Ag/AgCl, 28 mm^2, Ambu A/S, Ballerup, Denmark) placed 2 cm below the gastrocnemius on the line of the Achilles tendon for soleus muscle (SOL) and tibialis anterior (TA) and gastrocnemius (GM) muscles according to SENIAM guidelines (Hermens, 1999). As part of TMS measurement, we used the pseudo-monopolar setup to collect the MEPs considering potential discomfort and intension of subjects caused by high intensity stimulation during balance perturbation, especially during 140 ms (voluntary activation phase). The pseudo-monopolar setup allowed MEPs of higher amplitude to be recorded compared with bipolar connection, which in turn also decreased the intensity of the stimulus needed to evoke a detectable MEP (Kirk et al., 2019). According to our practical experience, the shape of the MEP is more consistent with the pseudo-monopolar setup, which is important for the dynamic

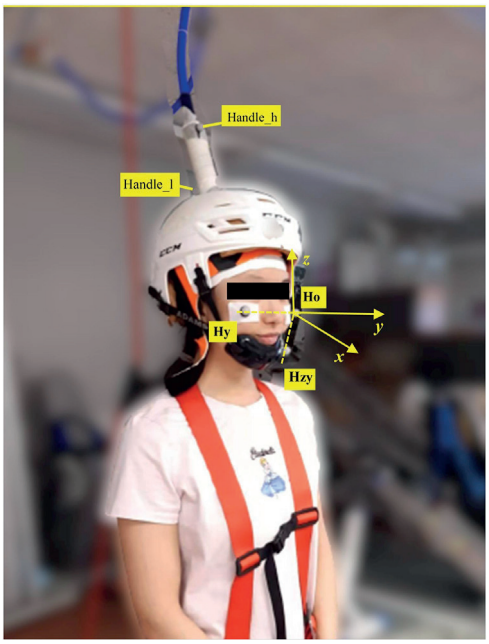

FIGURE 1
Motion capture markers were placed as shown in the picture. Three markers were placed on the head, i.e., 'Ho' was the origin of the head coordinate system; 'Hy' was utilized to build y-axis with 'Ho'; 'Hzy' was the point on the zy-plane, which produced *x*-axis by cross product with *y*-axis). *z*-axiz was built by cross product of *x*-axis and *y*-axis. Handle_h was the marker on the higher position of the TMS handle, and Handle_l was the marker placed on the lower position of the TMS handle.

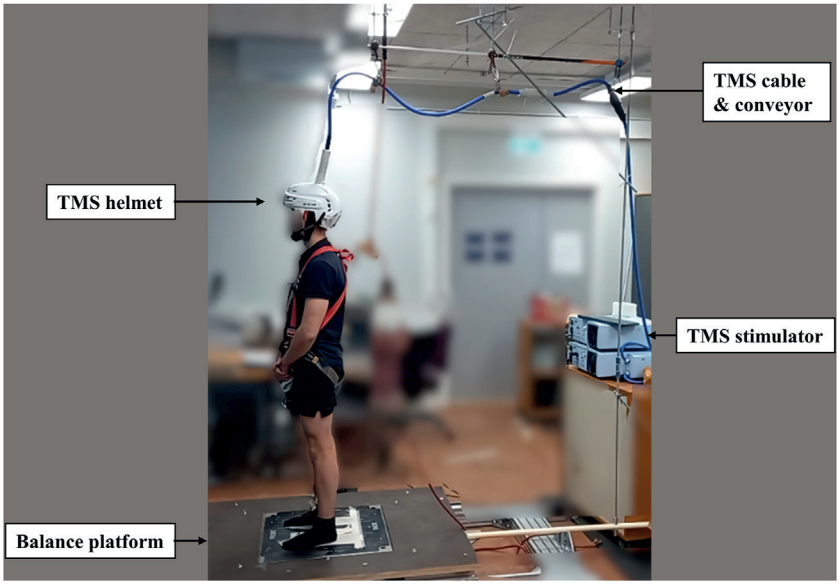

FIGURE 2
The figure shows the modified helmet to stabilize the TMS coil. The TMS coil's cable was connected with a conveyor on the roof to relieve the weight and moved along with the balance platform during perturbation.

tasks. A disadvantage of this electrode montage is that the signal-to-noise ratio can be compromised. However, this was not a problem in the current setup. One electrode was placed 2 cm below the gastrocnemius on the line of the Achilles tendon and the reference electrode was placed on the tibia at the same level. The skin was shaved, carefully abraded with sandpaper, and cleaned with alcohol. Skin target impedance was less than 5 kΩ and if this was not the case, skin preparation was repeated. All EMG data were collected using the Neurolog EMG system (CED ltd., Cambridge, England), with a gain of 1000. Data were band-passed (15–500 Hz) filtered and further collected using CED 1401 A/D-converter (CED ltd., Cambridge, England) and Spike 2 (8.0) software (CED ltd., Cambridge, England) with a sampling rate of 5 kHz.

Isometric maximal voluntary contraction

Isometric maximal voluntary contraction (IMVC) was used to investigate possible muscle fatigue between sessions and to measure aMT. After EMG setup and a 5 min warm-up, subjects were positioned in a custom-built ankle dynamometer (University of Jyväskylä, Jyväskylä, Finland) to test the IMVC with the right foot on the plate at 100° hip angle, 180° knee angle (leg fully extended) and 90° ankle angle. After the positioning procedure, the subject contracted 5 - 7 submaximal plantarflexion trials to practice the performance. IMVC was performed at least three times at one-minute intervals and the highest force value was considered as the IMVC. If the last trial was >5% higher than the second-best, single additional trials were performed until no further improvement was observed. The typical number of required maximum trials was 3–5. Reaction forces from the dynamometer pedal were measured and maximum IMVC amplitude was analyzed by a strain gauge transducer sampled at 1 kHz in Spike2 software.

TMS and H-reflex measurement setup

TMS was delivered using a single-pulse Magstim 200^2 stimulator with a double cone coil (Magstim, Whitland, United Kingdom). A skin-tight (swimming) cap was placed on the head of the subject to increase friction between the coil and the scalp. The optimal TMS stimulus site for the right soleus muscle was located on average 1 cm lateral (left) and 1 cm posterior to the cranial apex. Several stimulations were delivered to determine optimal coil placement and it was then marked by a marker pen on the cap. The aMT was defined as the lowest stimulus intensity to elicit clear MEPs in three out of five stimulation from right ankle plantarflexion with 10% IMVC. After the confirmation of aMT, a second swimming cap with a hole in the middle of the vertex (Orca High Visibility Neoprene Swim Cap, Orca, Auckland, New Zealand) was placed over the coil to reduce the gap and relative movement between the coil and head. Then, the custom-made helmet (modified from an ice-hockey helmet; CCM TACK 710 JK-K, CCM Hockey, Montreal, Canada) was attached to the subject's head with a chin

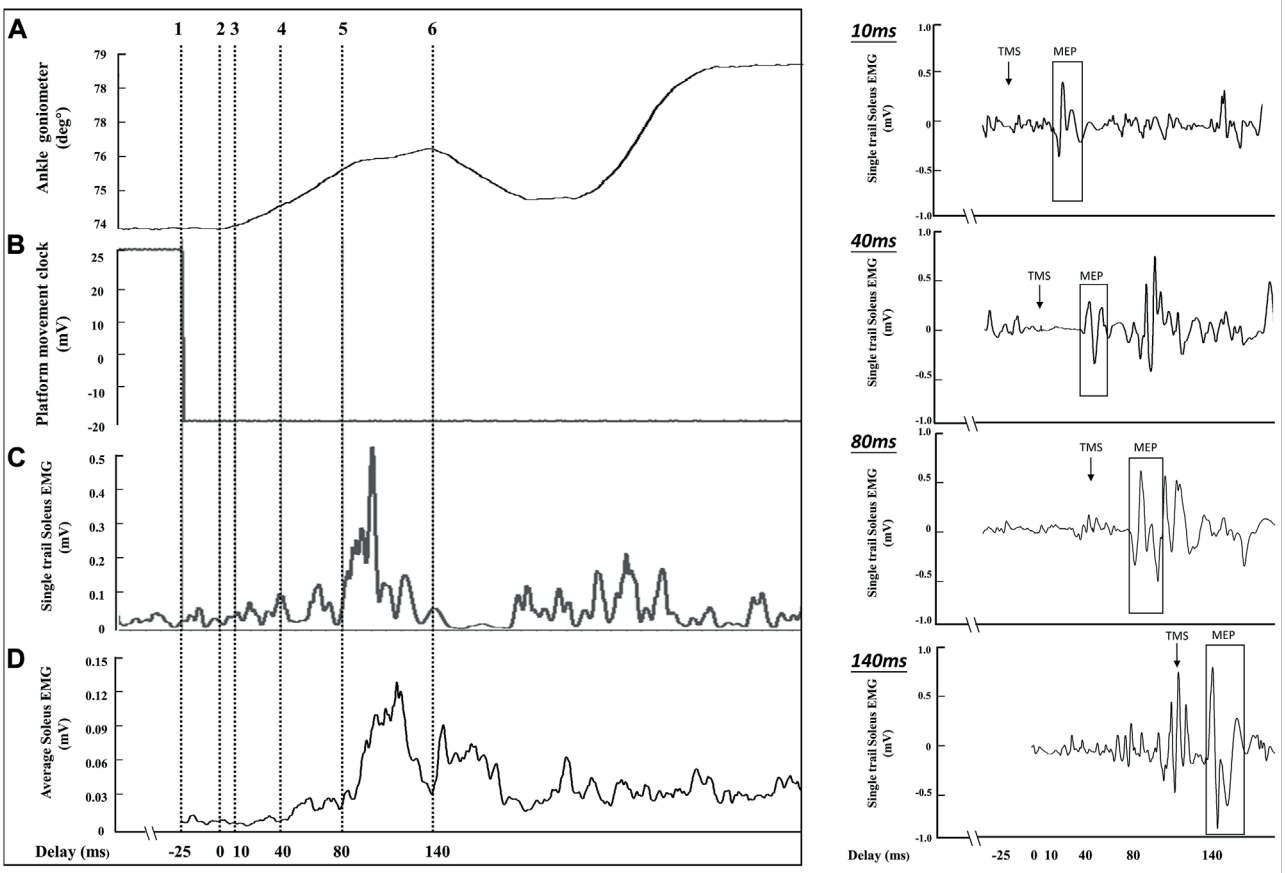

FIGURE 3
The sampling schematic of one balance perturbation phase. The **(A)** channel shows ankle movement from the ankle goniometer (°), and cursor 2 was determined as the onset of ankle movement after balance perturbation. The **(B)** channel demonstrates platform movement starting from cursor 1. The **(C)** channel shows soleus EMG activities from a single perturbation trial (smoothed with a 2 ms window and rectified). The **(D)** channel shows the average EMG activity curve from 8 posterior perturbation trials (smoothed with a 2 ms window and rectified) to estimate the delay for stimulation because of the EMG variability between perturbation trials. The right part of the figure shows the soleus MEPs from a single trial of perturbation in four delays (10 ms, 40 ms, 80 ms, and 140 ms).

strap. Even though the helmet setup was tight, it was ensured that the helmet was as comfortable as possible with no reported pain caused to the subject. Then subjects moved to the balance system. The TMS cable was placed on a conveyor adjacent to the safety belt conveyor on the roof and connected with the balance platform by a firm handle, which raised the cable above the subject and moved it in the same phase and direction as the balance platform during perturbation (Figure 2). Single-pulse TMS with 110% intensity of aMT was delivered during standing rest and balance perturbation tasks to investigate corticospinal excitability.

For H-reflex measurements, subjects stood relaxed during the electrical stimulation set-up. Electrical stimulation was administrated to the tibial nerve in the popliteal fossa. A cathode (1.5 cm × 1.5 cm) was placed over the tibial nerve, and an anode (5 cm × 8 cm) was placed above the patella. Rectangular stimulation pulse (DS7AH, Digitimer Ltd., Hertfordshire, United Kingdom) with a duration of 0.2 ms was delivered at 10 s intervals. Once the optimal site of stimulation was established, the site was marked by a marker pen, and an electrode (Blue Sensor, Ag/AgCl, 28 mm^2, Ambu A/S, Ballerup, Denmark) was placed and strapped around the subject's knee with an elastic band. An increasing intensity interval (1–5 mA) was chosen to measure the H-M recruitment curve with at least 30 data points up to the maximal M-wave. The stimulus intensity was adjusted to 5% (±2%) of the maximum M-wave, which was used during balance perturbation to control H-reflex measurements.

Dynamic balance perturbations with TMS and H-reflex

Balance perturbation tasks utilized a custom-built dynamic balance device (University of Jyväskylä, Jyväskylä, Finland)

modified from Piirainen et al.'s study (2013). The balance perturbation system operated at 0.25 m/s, accelerating at 2.5 m/s^2 over a 0.3 m displacement. During balance perturbation tasks, 16 balance perturbations were delivered in anterior (plate moved forward) and posterior (plate moved backward) directions in random order with 6–12 s intervals. A fixation point was set on the wall 3 m from the subjects at eye level to stabilize the subjects' visual attention during measurements.

During balance perturbation tasks, the COP displacement and velocity in anterior and posterior (AP) directions were collected by custom designed balance platform, with one strain gauge sensor in each of the four corners of the force plate (BT4 balance platform; HUR Labs, Tampere, Finland) and saved and analyzed using the Coachtech-feedback system (University of Jyväskylä, Jyväskylä, Finland). COP in anterior-posterior direction was calculated using the formula COPy = ((Frr + Frf) × 0.26 − (Frr + Flr) × 0.26)/(Flf + Frf + Frr + Flr), where lf = left front, rf = right front, rr = right rear, lr = left rear and 0.26 m is sensor distances from middle line.

In the pilot study, the time difference between ankle movement identified by the ankle goniometer (Figure 3A: cursor 2) and platform control signal (Figure 3B: cursor 1) was analyzed. A 17 ms–33 ms time difference was observed between ankle movement (cursor 1) and the platform control signal (cursor 2). Therefore, a 25 ms constant delay was defined as the time difference between the platform control signal and the onset of ankle movement. During the balance perturbation task, MEPs and H-reflexes were elicited at four delays after the platform control signal: 35 ms, 65 ms, 105 ms, and 165 ms. Delays of MEPs and H-reflex's in this study were represented as 10 ms, 40 ms, 80 ms, and 140 ms, using the onset of the ankle movement as the delay timepoint (see Figure 3: cursor 3, 4, 5, and 6). Delays were designed to represent the onset of ankle movement, SLR, LLR, and the voluntary activation phase. Using the same protocol as TMS trials, H-reflex was measured in standing rest and the same four delays during balance perturbation. The maximum compound action potential (M-max) of soleus muscle with was recorded in order to normalize the muscle response values (MEP, H-reflex, and voluntary EMG activity).

In the dynamic balance perturbation tasks with stimulation, 16 perturbations were performed in one set of trials, with 8 anterior and 8 posterior perturbations in random order, which ensured subjects were not able to anticipate the direction of perturbation. Two-min rest periods were given after every perturbation set to minimize possible muscle fatigue (Piirainen et al., 2013). During H-reflex balance perturbation trials, a successful trial was defined as an M-wave response of 5% (±2%) M-max value. The intensity of electrical stimulation was adjusted during perturbation trials to obtain at least five successful trials. If less than five successful backward trials in a normal 16-trial perturbation set were achieved, an extra 8-trial balance perturbation set, four backward and four forward, with random order was performed. For each perturbation task, five successful trials were usually completed within 16–24 perturbations (16-trials + 8-trials), followed by 2 minutes of rest.

Data and statistical analysis

The COP velocity curve was calculated by differentiating the COP curve by using 20 ms windows. Trials were performed at 6–12 s intervals and triggered when COP was at least 1 s within ±5 mm level from zero level. With this approach, the subject was always standing straight without any anticipation for the upcoming perturbation. Peak COP displacement and the average COP velocity were analyzed in the time window of 1 s before platform movement (Preparation-phase; Pre), during platform movement (Active-phase; Act), and 1 s from the end of platform movement (Recovery-phase; Rec).

EMG activity from balance perturbation was collected from the balance perturbation set without stimulation, which was calculated by the root-mean-square (RMS) with a 20 ms window for SOL, TA, and GM during the perturbation from ankle movement (0 ms) to 160 ms. RMS over a 100 ms window was applied before plate movement. All EMG activity data were normalized by maximum RMS with a 20 ms window during balance perturbation and presented %MaxSOL, %MaxTA, and % MaxGM in the results (Piirainen et al., 2013). Background EMG with stimulation trials was analyzed by RMS with a 30 ms window before stimulation and normalized by Mmax of monopolar (MEPs) and bipolar (H-reflex), respectively.

In standing rest, mean soleus MEPs were determined with peak-to-peak amplitude (in mV) from 10 TMS stimulations. Outliers were identified from the ten trials (±2.5 SD of the mean) and removed before analysis (Avenanti et al., 2006). The average MEP latency and duration were calculated in the standing rest condition and then utilized in the balance perturbation condition. The MEP was defined as starting when EMG was above the mean + 2SD level recorded 100 ms before the TMS trigger and ending when below the mean - 2SD level (Hirano et al., 2016). However, this was only used in the standing condition since it was difficult to use these criteria during the perturbation due to increase in EMG. Thus, the MEP amplitude was obtained by calculating the peak-to-peak amplitude within the MEP onset and offset latencies calculated in the standing condition. Selecting MEP amplitudes from 7 - 8 trials when the platform moved backward and averaged after excluding outliers (±2.5 standard deviation of the mean). All MEPs were normalized by the peak-to-peak value of maximum M-wave and presented as %M-max in the results. H-reflex was determined with peak-to-peak amplitude and averaged from all successful trials (within 3%–7% M-max) in standing rest and balance perturbation tasks. H-reflex was normalized by

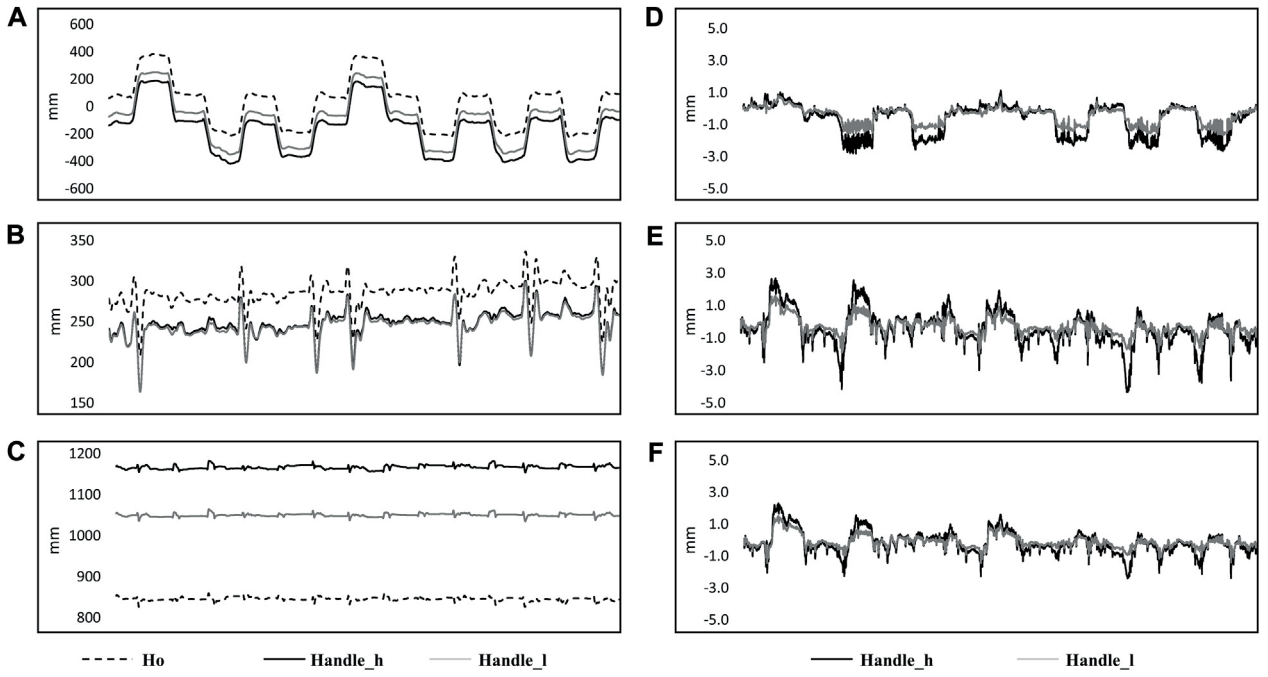

FIGURE 4
Kinematic data from a single subject in 14 perturbations are shown in the figure. Original data in the ground coordinate system are shown on the left side [**(A)** x-axis; **(B)** y-axis; **(C)** z-axis] with markers on the subject's head (Ho: dashed line), higher position of the handle (Handle_h: black solid line) and lower position (Handle_l: gray solid line). Coordinate transformed data from markers on the higher position (Handle_l: black solid line) and lower position of the handle (Handle_h: grey solid line) are shown on the right side, which represented the movement of the markers related to the coordinate system of the head [**(D)**: x-axis; **(E)** y-axis; **(F)** z-axis].

the peak-to-peak amplitude of the maximum M-wave and presented as % M-max in the results.

Statistical analyses were conducted using IBM SPSS 20.0 (SPSS, Chicago, United States). Result visualizations were performed using Prism (V9, GraphPad Software, San Diego, California United States). All variables of MEPs and RMS of EMG activity were processed by log transformation prior to statistical analyses following Nielsen's suggestion (Nielsen, 1996) since the original data was not normally distributed, which resulted in data being normally distributed as assessed by Shapiro-Wilk's W tests. Between-session differences for IMVC, TMS intensity of aMT, maximum COP displacement and average COP velocity were examined by paired t-test.

To assess modulation in corticospinal excitability during balance perturbations, MEPs, H-reflex, EMG activity without stimulation, and background EMG before stimulation data were assessed by two-way (2 × 4) repeated-measures ANOVA with the factors SESSION (S1 and S2) and DELAY (10 ms, 40 ms, 80 ms, and 140 ms). When a significant F-value was observed, Mauchly's test was used to evaluate sphericity, and where the assumption was valid F-values were reported with sphericity-assumed degrees of freedom and df error [i.e., $F_{(\text{sphericity assumed df, df error})}$]. Effect sizes for the ANOVA main effects are reported as partial eta squared (η_p^2), where 0.02, 0.13, and 0.26 are considered small, medium, and large, respectively. If significance for DELAY was revealed, Bonferroni post-hoc analysis was used for pairwise comparisons between levels (0 ms, 40 ms, 80 ms, and 140 ms). The significance level was set at $p < 0.05$ and all results were displayed as Mean ± SD.

For the research question of test-retest reliability, a paired t-test was used to test the reliability of log transformed MEP and H-reflex amplitudes between sessions at each delay separately. Test-retest reliability and inter-individual variability of MEPs and H-reflex amplitude between sessions were assessed *via* intraclass correlation coefficients (ICC) using a two-way mixed effects model with an absolute agreement using the average value from multiple trials. Standard error of measurement (SEM) was estimated as root mean square error ($\sqrt{MSE}$) from a one-way ANOVA, which avoids errors associated with ICC calculation. The minimal detectable change (MDC) was calculated as SEM × 1.96 × $\sqrt{2}$ (Weir, 2005). According to the ICC method guideline (Koo and Li, 2016), ICC was calculated between single stimulation trials, and trial-to-trial coefficient of variance (CV) with homoscedasticity of MEPs and H-reflex amplitudes to determine whether eight MEP/H-reflexes were adequate for calculating the average value. Reliability based on ICCs and 95% CIs were categorized as poor (ICC <0.5), moderate (0.5 < ICC <0.75), good (0.75 < ICC <0.9),

TABLE 1 Between-session test-retest reliability of MEPs (log-transformed data) and H-reflex (original data) with ICC and 95% confidence intervals. In H-reflex, SEM/MDC is expressed in decimal form, which is the same as the original H-reflex data.

	MEPs			H-reflex		
	ICC [95%CI]	SEM	MDC	ICC [95%CI]	SEM(%Mmax)	MDC (%Mmax)
Standing rest	0.932 [0.789, 0.978]	0.232	0.644	0.475 [−0.771, 0.841]	0.071	0.169
10 ms delay	0.935 [0.811, 0.979]	0.210	0.581	0.626 [−0.079, 0.881]	0.158	0.378
40 ms delay	0.928 [0.797, 0.977]	0.152	0.420	0.720 [0.086, 0.914]	0.063	0.151
80 ms delay	0.943 [0.777, 0.982]	0.032	0.088	0.887 [0.644, 0.965]	0.071	0.169
140 ms delay	0.947 [0.835, 0.983]	0.084	0.232	0.865 [0.577, 0.958]	0.126	0.302

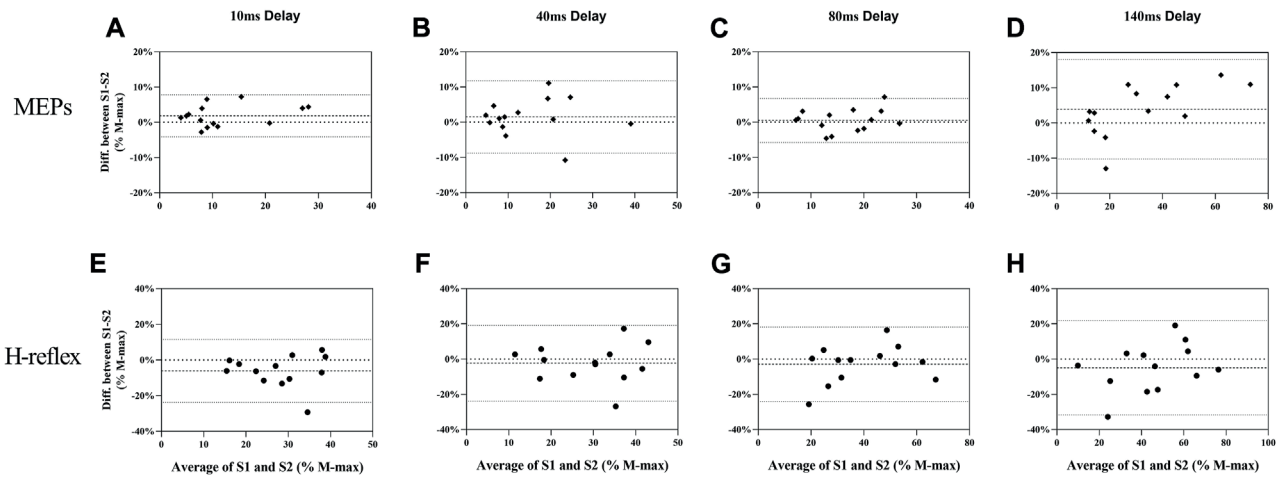

FIGURE 5
Bland-Altman plot for MEPs **(A–D)** and H-reflex **(E–H)** responses in balance perturbation between S1 and S2. Each panel (10 ms, 40 ms, 80 ms, and 140 ms) shows the difference as a function of the average of the two testing sessions with dashed lines indicating the mean bias and 95% confidence intervals indicated by dot lines.

or excellent (ICC >0.9). Bland-Altman plots of MEPs in all conditions were investigated to visualize the agreement between two sessions (Bland and Altman, 1995).

Results

Motion capture results from pre-study

Markers of the TMS handle displacement before (A: *x*-axis, B: *y*-axis, and C: *z*-axis) and after transformation (D: *x*-axis, E: *y*-axis, and F: *z*-axis) are shown in Figure 4. The maximum offset of the marker on the higher position of the TMS handle demonstrated 7 ± 2 mm in the *x*-axis, 8 ± 2 mm in the *y*-axis, and 5 ± 1 mm in the *z*-axis. The offset of the marker on the lower position of the TMS handle was 5 ± 1 mm in the *x*-axis, 5 ± 2 mm in the *y*-axis, and 4 ± 1 mm in the *z*-axis.

Between-session test-retest reliability

The peak-to-peak amplitude of MEPs and H-reflexes varied from 0.87 ± 0.61 to 2.51 ± 1.47 mV and from 1.54 ± 0.64 to 3.20 ± 1.68 mV, respectively. In addition, MEPs were visible in 100% of the trials. By paired-t test, MEPs demonstrate lower amplitude in rest standing [$t_{(13)} = 2.217$, $p = 0.045$, $\eta^2 = 0.592$] and 10 ms delay ($t_{(13)} = 2.211$, $p = 0.046$, $\eta^2 = 0.591$) in the perturbation task of S2. No significant difference was demonstrated for MEP amplitude in other delays of the perturbation tasks [40 ms: $t_{(13)} = 1.455$, $p = 0.169$, $\eta^2 = 0.389$; 80 ms: $t_{(13)} = 0.561$, $p = 0.585$, $\eta^2 = 0.150$;

TABLE 2 Within-session test-retest reliability (between stimulation trials) of MEPs for S1 and S2 are shown in the table with ICC and 95% confidence interval. CV% was shown as mean ± sd.

	S1		S2	
	ICC [95%CI]	CV%	ICC [95%CI]	CV%
Standing rest	0.953 [0.906, 0.982]	40.8 ± 14.5	0.934 [0.868, 0.975]	39.4 ± 11.7
10 ms delay	0.927 [0.932, 0.993]	39.1 ± 25.8	0.915 [0.924, 0.987]	41.6 ± 17.5
40 ms delay	0.964 [0.925, 0.987]	39.0 ± 23.1	0.960 [0.913, 0.986]	38.5 ± 20.7
80 ms delay	0.957 [0.909, 0.985]	33.4 ± 17.8	0.854 [0.694, 0.948]	35.0 ± 14.5
140 ms delay	0.983 [0.964, 0.994]	20.1 ± 8.0	0.976 [0.950, 0.991]	22.7 ± 10.3

140 ms: $t_{(13)} = 0.946$, $p = 0.361$, $\eta^2 = 0.253$]. The H-reflex increased in S2 compared to S1 at 10 ms delay [$t_{(12)} = -2.460$, $p = 0.03$, $\eta^2 = -0.682$], but there were no differences in the other conditions [standing rest: $t_{(12)} = -0.720$, $p = 0.486$, $\eta^2 = -0.200$; 40 ms: $t_{(12)} = -0.765$, $p = 0.459$, $\eta^2 = -0.212$; 80 ms: $t_{(12)} = -0.973$, $p = 0.350$, $\eta^2 = -0.270$; 140 ms: $t_{(12)} = -1.303$, $p = 0.217$, $\eta^2 = -0.362$].

MEPs during standing rest demonstrated excellent test-retest reliability between sessions (ICC = 0.932; Table 1) when considering the 95% CIs. During balance perturbation tasks, MEPs also showed excellent reliability (ICC = 0.928–0.947; Table 1). From the Bland-Altman plot, the mean bias for MEPs at 10 ms delay (Figure 5A, mean bias = 1.85%, 95%CI [−4.09%, 7.80%]) and 40 ms delay (Figure 5B, mean bias = 1.50%, 95%CI [−8.78%, 11.79%]) were similar. MEPs at 80 ms delay showed the lowest bias (Figure 5C, mean bias = 0.50%, 95%CI [−5.76%, 6.77%]), while MEPs of 140 ms delay demonstrated the highest bias and widest limits of agreement (Figure 5D, mean bias = 3.91%, 95%CI [−10.23%, 18.04%]).

During standing rest, H-reflex demonstrated poor test-retest reliability (ICC = 0.475; Table 1). During balance perturbation tasks, H-reflex showed moderate-to-good reliability (ICC = 0.626–0.887). At 10 ms delay, ICC demonstrated a wider 95% CI [−0.079, 0.881] compared to the other delays. Meanwhile, H-reflex showed highest bias at the 10 ms delay (Figure 5E, mean bias = −6.149%, 95%CI [−23.81%, 11.51%]). Similar limits of agreement for H-reflex were observed at 40 ms (Figure 5F, mean bias = −2.328%, 95%CI [−23.82%, 19.17%]) and 80 ms delays (Figure 5G, mean bias = −2.909%, 95%CI [−24.02%, 18.21%]). However, H-reflex demonstrated its widest limits of agreement at the 140 ms delay (Figure 5H, mean bias = −4.935, 95%CI [−31.68%, 21.81%]).

Within-session test-retest reliability

In S1, ICC of MEPs showed excellent reliability and narrow 95% CI in standing rest and balance perturbations (ICC = 0.927–0.983). In S2, ICC demonstrated good to excellent reliability of all MEPs (ICC = 0.854–0.976). Within-session CV% of MEPs ranged from 20.1% to 41.6% in both sessions and showed homoscedasticity when tested by Levene's statistics (Table 2).

Within-session reliability of H-reflex responses showed to be good to excellent in both sessions (ICC = 0.874–0.994), and narrow 95% CI. Within-session CV% of H-reflex was 16.9–33.1% in both sessions and Levene's test indicated homoscedasticity (Table 3).

COP in balance perturbation

COP displacement and velocity of Pre-, Act-, and Rec-phases were analyzed to explore the balance performance in AP

TABLE 3 Within-session test-retest reliability (between stimulation trials) of H-reflex for S1 and S2 are shown in the table with ICC and 95% confidence interval. CV% was shown as mean ± sd.

	S1		S2	
	ICC [95%CI]	CV%	ICC [95%CI]	CV%
Standing rest	0.985 [0.968, 0.995]	21.5 ± 9.8	0.986 [0.971, 0.995]	18.6 ± 5.9
10 ms delay	0.945 [0.848, 0.989]	27.9 ± 11.8	0.956 [0.725, 1.000]	22.3 ± 8.0
40 ms delay	0.945 [0.837, 0.991]	33.1 ± 12.7	0.994 [0.963, 1.000]	22.1 ± 9.8
80 ms delay	0.979 [0.905, 0.999]	19.1 ± 11.7	0.874 [0.351, 0.997]	16.9 ± 8.6
140 ms delay	0.974 [0.881, 0.999]	24.0 ± 16.7	0.965 [0.843, 0.999]	17.8 ± 8.3

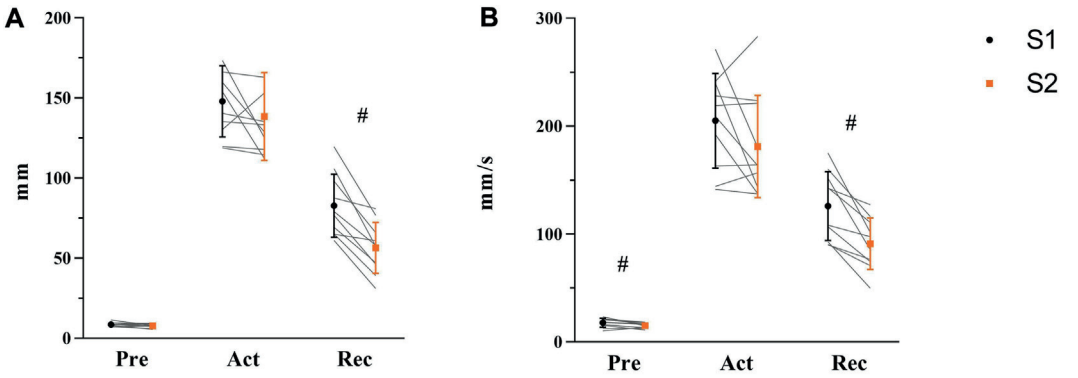

FIGURE 6
Maximum COP displacement **(A)** and average COP velocity **(B)** in Pre-, Act-, and Rec-phases were shown respectively with mean, standard deviation, and individual data (light gray line) in the figure (S1: black symbol; S2: orange symbol, N = 10). Significant differences were marked by '#' between sessions ($p < 0.05$).

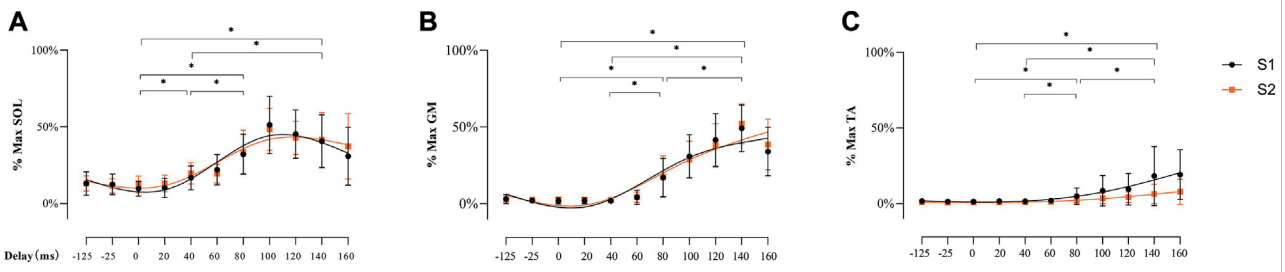

FIGURE 7
EMG activity of SOL **(A)**, GM **(B)**, and TA **(C)** during balance perturbation respectively Significant differences between delays were marked by '*' ($p < 0.05$). EMG activity was demonstrated with mean and standard deviation. The line was processed by smoothing the spline of data knots for S1 (black) and S2 (orange).

direction before, during, and after of balance platform moving, respectively (see Figure 6). Before perturbation (Pre), paired t-test results indicated no change in maximum COP displacement from S1 to S2 [$t_{(9)} = 1.665$, $p = 0.132$, $\eta^2 = 0.235$]. However, velocity was lower in S2 (15 ± 2 mm/s) than S1 (18 ± 4 mm/s) [$t_{(9)} = 2.817$, $p = 0.020$, $\eta^2 = 0.469$]. During perturbation (Act), there was no difference shown either in COP displacement [$t_{(9)} = 1.247$, $p = 0.244$, $\eta^2 = 0.147$] or velocity ($t_{(9)} = 1.650$, $p = 0.133$, $\eta^2 = 0.232$). After perturbation (Rec), significant differences between S1 and S2 were demonstrated from both COP displacement [S1: 83 ± 20 mm; S2: 56 ± 16 mm, $t_{(9)} = 5.962$, $p < 0.001$, $\eta^2 = 0.798$] and velocity [S1: 126 ± 32 mm/s; S2: 91 ± 24 mm/s, $t_{(9)} = 5.043$, $p = 0.001$, $\eta^2 = 0.739$].

EMG activity during balance perturbation

There was no main effect of soleus muscle EMG activity for SESSION [$F_{(1, 26)} = 0.128$, $p = 0.723$, $\eta_p^2 = 0.005$], but a significant main effect was demonstrated for DELAY [Figures 7A,F $_{(3.326, 60.485)} = 65.839$, $p < 0.001$, $\eta_p^2 = 0.718$]. At the delays studied, post-hoc analyses showed lower EMG activity at 10 ms delay than 40 ms, 80 ms, and 140 ms (all $p < 0.001$). At 40 ms delay, EMG activity was lower than 80 ms and 140 ms respectively (both $p < 0.001$), but there was no difference between 80 ms and 140 ms ($p = 0.706$).

Similarly, there was no main effect observed in gastrocnemius medial muscle EMG activity for SESSION [$F_{(1, 26)} = 1.513$, $p = 0.230$, $\eta_p^2 = 0.055$], but a significant main effect was observed for DELAY [Figures 7B,F $_{(2.077, 54.009)} = 219.095$, $p < 0.001$, $\eta_p^2 = 0.894$]. Specifically, post-hoc analysis showed lower EMG activity at 10 ms delay than 80 ms, and 140 ms (both $p < 0.001$), and EMG activity at 40 ms delay was lower compare with 80 ms and 140 ms (both $p < 0.001$). Significantly lower EMG activity was also observed at 80 ms than 140 ms ($p < 0.001$).

The EMG activity of tibialis anterior muscle demonstrated no main effect for SESSION ($F_{(1, 26)} = 3.488$, $p = 0.073$, $\eta_p^2 = 0.118$), but significant main effect for DELAY [Figures 7C,F $_{(2.194,}$

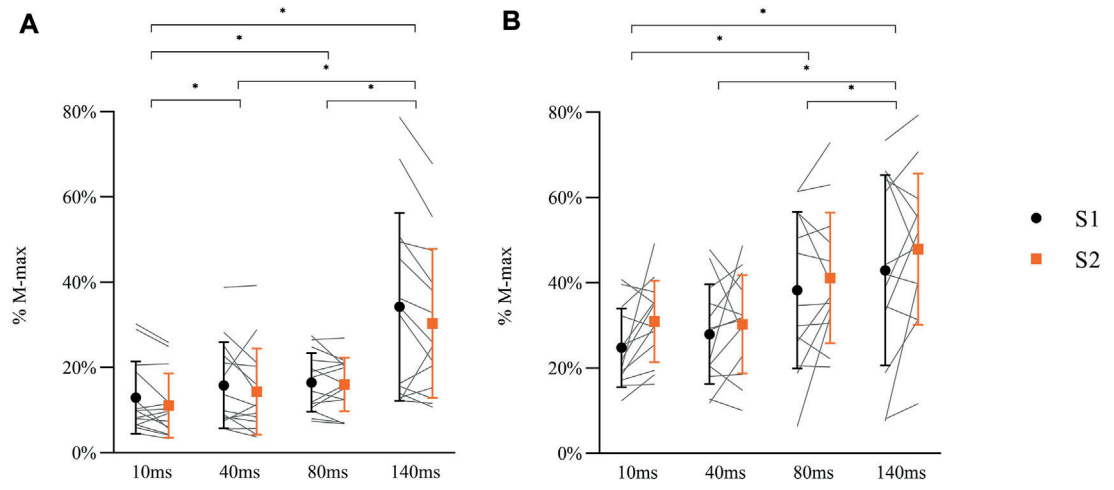

FIGURE 8
Peak-to-peak amplitude of MEPs (**A**: N = 14) and H-reflex responses (**B**: N = 13) in balance perturbation with delays (10 ms, 40 ms, 80 ms, 140 ms) from two sessions (S1: black symbol, S2: orange symbol). Data shown are mean, standard deviation, and individual data (light gray line). The significant differences are shown between delays by '*' ($p < 0.05$), but there were no between-session differences in MEPs or H-reflex.

$_{57.045)}$ = 122.897, $p < 0.001$, $\eta_p^2 = 0.825$]. Specifically, post-hoc analyses showed lower EMG activity at 10 ms delay than 80 ms, and 140 ms (both $p < 0.001$). EMG activity at 40 ms was lower compared with 80 ms and 140 ms (both $p < 0.001$), and significantly lower EMG activity was observed at 80 ms than 140 ms ($p < 0.001$).

The background EMG before TMS did not differ between sessions [$F_{(1, 26)} = 0.317$, $p = 0.578$, $\eta_p^2 = 0.12$], but significant increases were observed between 140 ms delay (1.72%) with other delays (10 ms: 0.53%, $p < 0.001$; 40 ms: 0.50%, $p < 0.001$; 80 ms: 0.58%, $p < 0.001$). A significant difference was also found between 40 ms and 80 ms delays ($p = 0.014$). Background EMG before electrical stimulation has shown similar results. No difference between sessions ($F_{(1, 24)} = 0.383$, $p = 0.542$, $\eta_p^2 = 0.016$). Compared to other delays (10 ms: 0.44%, $p < 0.0001$, 40 ms: 0.48%, $p < 0.0001$, 80 ms: 0.46%, $p < 0.0001$), background EMG at 140 ms delay was significantly higher (1.02%).

Corticospinal excitability during balance perturbation

There was no difference observed in IMVC (S1: 1814.6 ± 499 Nm, S2: 1871.9 ± 522 Nm, $p = 0.894$) or TMS intensity of aMT (S1: 35% ± 4%, S2: 35% ± 4%, $p = 0.769$) between sessions.

A significant main effect for DELAY in MEPs during balance perturbation was observed [Figures 8A,$F_{(3, 78)} = 56.764$, $p < 0.001$, $\eta_p^2 = 0.686$], while no changes were shown between sessions [$F_{(1, 26)} = 0.033$, $p = 0.858$, $\eta_p^2 = 0.001$]. Post-hoc analyses demonstrated significant lower MEPs at 10 ms compared with other delays (40 ms: $p = 0.009$; 80 ms: $p = 0.001$; 140 ms: $p < 0.001$). MEPs at 140 ms delay were higher than 40 ms and 80 ms delays (both $p < 0.001$), but no differences were observed between 40 ms delay and 80 ms delay ($p = 0.249$) (Figure 8A).

A main effect for DELAY was observed in H-reflex [Figures 8B,$F_{(1.594, 38.249)} = 19.366$, $p < 0.001$, $\eta_p^2 = 0.447$], while no differences between sessions were observed [$F_{(1, 24)} = 0.692$, $p = 0.414$, $\eta_p^2 = 0.028$]. Post-hoc analyses demonstrated that H-reflex at 10 ms was lower than 80 ms ($p = 0.001$) and 140 ms ($p < 0.001$) delays. Lower H-reflex was also shown at 40 ms delay compared to 80 ms and 140 ms (80 ms: $p = 0.001$, 140 ms: $p = 0.001$). There was no difference between 80 ms and 140 ms delay ($p = 0.172$) (Figure 8B).

Discussion

In the present study, we investigated the reliability of corticospinal (MEPs) and spinal excitability (H-reflex) during balance perturbation, using variances estimated from a two-session test-retest paradigm. At the beginning of the balance perturbation phase (10 ms delay), MEPs and H-reflexes demonstrated a significant difference between sessions assessed by paired t-test. ICC demonstrated good-to-excellent test-retest reliability in the TMS measurements, which was generally better than that of the H-reflex measurements. Within each session, both measurements showed excellent reliability, although variability was also shown between trials. No differences between the sessions were observed in MEPs, H-reflex responses, COP displacement, or COP velocity during

balance perturbations indicating good reliability of the test methods. Neither EMG activity without stimulation nor background activity before stimulation demonstrated changes between sessions, indicating constant muscle activity between measurement sessions.

Test-retest reliability of the experiment method

In the pre-study, the coil and its handle were considered as a rigid body, which rotated around the head as the center. Two markers on the upper and lower part of the handle were used to estimate the movement of the coil. Because the coil was below the lower marker, the movement of the coil could be considered to be less than the markers on the handle, which was less than 5 mm in the *x*-, *y*-, and *z*-axis. From the study of TMS coil location accuracy with a function-guided navigation system, 2 mm–5 mm distance around the initially defined hotspot resulted in good accuracy of MEPs, and changes in coil location within 5 mm distance had no significant effect on MEP amplitude (De Goede et al., 2018). This supports our assertion that the stability of the TMS coil during balance perturbation trials provided accurate MEP values in the present study.

Paired t-tests were used to test any systematic differences in MEPs and H-reflexes between sessions in this study. According to paired t-test results, 10 ms delay of S2 resulted in higher H-reflex amplitude (S1: 24.8% Mmax; S2: 31.0% Mmax) with lower MEP (S1: 13.0% Mmax; S2: 11.1% Mmax). The observed MEP or H-reflex amplitude changes were lower than the between-session MDC, which indicates that the between session differences may result from the variability of MEPs/H-reflex or noise in the measurements. Therefore, data should be interpreted carefully because systemic error may occur in some conditions. It would be recommended that at least two familiarizing perturbation sets should be performed before the first measurement session to reduce possible learning effects.

TMS measurement demonstrated strong test-retest reliability, both between- and within-session during standing rest and balance perturbation tasks (ICC >0.80). The highest test-retest reliability and lowest between-trial variability were observed at 140 ms delay, which is defined as a voluntary activation phase in the present study. It indicates that MEPs are more reliable while the contribution of voluntary activation of the muscles is increasing compared with the low voluntary muscle activity at the early response phases after balance perturbation or muscles at rest. This finding is supported by Tallent et al.'s (2012) study, in which they showed higher reliability of MEPs in active muscle than resting muscle. In Darling et al.'s (2006) study, less variance was also observed with more muscle activation. Sensory inputs (vestibular, vision, proprioception) may influence the excitability of motor units in the corticospinal pathway more at standing rest and early phases after perturbation and, therefore, the variability of MEPs increases (Darling et al., 2006). Another reason, such as intersession intervals (>72 h), would reduce the TMS measurement reliability (Luc et al., 2014; Cavaleri et al., 2017). There may be a reason for the good between-session (<53 h) MEPs reliability in this study. Examine the mean MEP value from several individual trials because of typical between-trial variability, which was also shown in this study (within-session CV% = 16.9%–46.1%). Although Goldsworthy et al. (2016) suggested that 20–30 trials may be optimal for estimating MEPs in the first dorsal interosseous by TMS, other TMS studies have also shown good reliability with fewer stimulation trials (Van Hedel et al., 2007; Bastani and Jaberzadeh, 2012), which indicates that the reliability of MEPs fluctuates in different experimental protocols and it might be muscle specific (Cavaleri et al., 2017). MEPs in lower limb muscles, on the other hand, appear to be more reliable than those in upper limb muscles. For example, eight to ten trials of MEPs showed excellent reliability (ICC >0.81) in the tibias anterior muscle of stroke patients (Beaulieu et al., 2017). In addition, Lewis et al.'s (2014) demonstrated good reliability (ICC >0.80) in soleus muscle in healthy subjects by averaging only six MEPs. According to Cavaleri et al.'s (2017) study, a mean value of ten trials is required to produce consistent condensed reliability, and five trials are the lowest number to achieve excellent within-session reliability. In the present study, MEPs of a single subject at every delay were analyzed from 8 backward balance perturbation trials and the average value was calculated (7 – 8 trials) after removing outliers. To the best of our knowledge, there is only one previous TMS study that has used this method (Hosel and Tremblay, 2021). Since ICC of MEPs demonstrated good-to-excellent within-session (between trials) reliability, calculating average MEP amplitude from 8 TMS stimulation trials and removing outlier MEPs beyond 2.5 SD (maximum one outlier in the present results) could be considered as sufficient in reducing MEP between-trial variability and producing a reliable TMS procedure in corresponding balance perturbation tasks.

H-reflex demonstrated better test-retest reliability in balance perturbation task than at standing rest; ICC, SEM, and MDC, and within-session reliability were extremely robust. Similar results that revealed high stability between stimulation trials but lower reliability between the sessions were found in a previous study (Handcock et al., 2001). The possible reasons include more irregular body sway or various lack of attention issues during standing rest compared with more regular body movements and better focus during balance perturbation. Compared with the standing position, previous studies with subjects who were in supine or prone position revealed high reliability for the soleus H-reflex (Hopkins et al., 2000; Palmieri et al., 2002), which indicated that the H-reflex reliability may relate to the body position used in the protocol. Better reliability was shown at

80 ms (ICC = 0.89) and 140 ms (ICC = 0.87) delays than at 10 ms (ICC = 0.63) and 40 ms (ICC = 0.72) delays, even though the reliability in the latter two conditions are still acceptable (Portney and Watkins, 2009). The within-session reliability was generally better than between sessions in the present study. This observation implies that five successful stimulations, i.e., at the range of 3%–7% M-max, is sufficient to be utilized for H-reflex measurements in balance perturbation tasks. The average of a larger number of trials (8–10) may provide greater reliability between the sessions. However, it should be noted that in this kind of protocol, the number of perturbation trials will increase with increasing stimulation responses, which might increase the risk of fatigue. It is not surprising that high reliability of H-reflex in soleus muscle was shown between stimulation trials, since previous studies from different body positions have also reported similar high reliability values, and, thus suggested that 4 to 5 stimulations are needed to obtain reliable results (Hopkins et al., 2000; Al Amer et al., 2020). Although the stimulation intensity and body position were different in this study, the present study adds important information about reliability of using the H-reflex method in dynamic balance perturbation tasks.

Corticospinal modulation in balance perturbation

During balance perturbation tasks, COP is an important parameter to evaluate balance performance (Zemková, 2011). In a previous study of balance ability between young and older adults, the older subjects showed larger peak COP displacement which implied poor balance control ability during perturbation (Piirainen et al., 2013). In the present study, we were more interested in the AP direction of the body sway, thus the COP displacement and velocity were analyzed only to backward movement of the platform. COP displacement and velocity did not differ significantly between the two measurement sessions in terms of Act-phase, indicating high reliability of COP during the active balance perturbation phase between sessions. However, the velocity of COP during Pre- and Rec-phases, as well as the maximum COP displacement during Rec-phase was considerably reduced in S2. The results suggest that there was less body sway before perturbation began and after perturbation ended in S2, which may indicate effects of learning. Nevertheless, these changes were not observed during the Act-phase when stimulations were delivered.

As we already know, a rapid ankle joint perturbation (dorsiflexion) can lead to a relatively stereotypical pattern response around 40 ms in the soleus muscle, which is addressed as the 'SLR'. When H-reflex was produced at this time, it showed facilitation in a previous drop jump study, which was explained by enhanced Ia-afferent transmission (Taube et al., 2008). However, H-reflex responses in the present results did not show any difference between the 10 ms delay and 40 ms delay, which was similar to the case of Piirainen et al.'s study (Piirainen et al., 2013). It may relate to the different ankle movement patterns between balance perturbation (translation) and drop jump (rotation). As demonstrated by Wälchli et al.'s study, it involved higher speed perturbations (0.74 m/s), in which SLR decreased while the LLR increased, inferring a top-down control from supraspinal sources (Wälchli et al., 2017). MEP amplitudes were slightly enhanced at 40 ms with EMG activity of the soleus muscle increasing from the onset of ankle movement (no stimulation trials) but background EMG before stimulation did not change. Meanwhile, gastrocnemius and tibialis anterior muscles have not been active implying no co-contraction of the agonist-antagonist muscle groups at this delay (see Figure 7). As a result of the present findings, it seems that cortical control contributes to the initial phase following perturbation. However, there is no literature demonstrated that the transcortical loop triggers the early phase after perturbation. Therefore, the enhanced MEP may relate to extra caution during perturbation tasks.

H-reflexes were found to be enhanced from SLR to LLR during balance perturbation in Taube et al.'s previous study (Taube et al., 2006), suggesting that the LLR is part of the transcortical loop in the soleus muscle. The present results did not show a significant difference between 40 ms (SLR) to 80 ms (LLR) delay but between 10 ms and 80 ms/140 ms delay, which is not entirely consistent, but not in conflict with Taube's study, since both studies indicate an increase in H-reflex during balance perturbation at the later phase. It is also important to consider the random direction of perturbation in this study, as well as different speeds and displacements of the balance platform movement. Therefore, direct comparison is not possible. There was a significant increase in background EMG levels (before stimulation) at 140 ms delay. Increased voluntary muscle contractions result in increased MEP and H-reflex values (Škarabot et al., 2019), which may explain the increased MEPs and H-reflexes in the muscle voluntary contraction phase during perturbation tasks.

Limitations

Some study limitations should be considered when interpreting the current findings. We did not use a neuronavigation TMS system in the present study. However, it is very difficult to utilize such a system in the dynamic task and with the helmet used in the current experiment. The helmet and the coil conveyor made it possible to stabilize the coil during the experiment and eliminate the tension of the cable during perturbations. The pre-study on the stability of the helmet system only included two subjects, which did not provide any statistical results. On the other hand, both subjects showed relatively small movement of the coil, which is in line with the literature (De Goede et al., 2018). The small sample size in this study is another

limitation and since MEPs (pseudo-monopolar) and background EMG (bipolar) used different arrangement normalizing MEPs by background EMG is complicated. Therefore, we were only able to discuss the corticospinal modulation and changes in background EMG separately.

Conclusion

In conclusion, this study investigated the reliability of TMS and H-reflex measurements during different phases of a sliding-platform balance perturbation task. The TMS coil stability was verified in the pre-study experiment with kinematic data. Both MEPs and H-reflex demonstrated acceptable reliability between two measurement sessions based on ICCs and have good-to-excellent test-retest reliability between stimulation trials. However, careful placement/stability of the coil and control of the M-wave during dynamic balance perturbation trials must be ensured to obtain such reliable data. MEPs increased in the early phase (SLR) implying that the corticospinal loop may play a role in overcoming balance perturbation at an earlier delay than previously thought.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the ethics board of the University (diari number: 1267/13.00.04.00/2021) and the study was performed in conformity with the declaration of Helsinki (2013). The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

NH, JA, and JP contributed to conception and design of the study. SN contributed with participants' recruitment, communication and data collection. NH, JP, SW, and DK performed the statistical analysis. NH analyzed the data and wrote the first draft of the manuscript. NH, JA, DK, SN, SW, and JP contributed to manuscript revision, read, and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

Al Amer, H. S., Sabbahi, M. A., and Olson, S. L. (2020). Reliability of the soleus H-reflex in different sitting postures. *Med. Sci.* 8, 48. doi:10.3390/medsci8040048

Avenanti, A., Paluello, I. M., Bufalari, I., and Aglioti, S. M. (2006). Stimulus-driven modulation of motor-evoked potentials during observation of others' pain. *NeuroImage* 32, 316–324. doi:10.1016/j.neuroimage.2006.03.010

Bastani, A., and Jaberzadeh, S. (2012). A higher number of TMS-elicited MEP from a combined hotspot improves intra- and inter-session reliability of the upper limb muscles in healthy individuals. *PLoS One* 7, e47582. doi:10.1371/journal.pone.0047582

Beaulieu, L. D., Massé-Alarie, H., Ribot-Ciscar, E., and Schneider, C. (2017). Reliability of lower limb transcranial magnetic stimulation outcomes in the ipsi- and contralesional hemispheres of adults with chronic stroke. *Clin. Neurophysiol.* 128, 1290–1298. doi:10.1016/j.clinph.2017.04.021

Bland, J. M., and Altman, D. G. (1995). Comparing methods of measurement: Why plotting difference against standard method is misleading. *Lancet* 346, 1085–1087. doi:10.1016/s0140-6736(95)91748-9

Cavaleri, R., Schabrun, S. M., and Chipchase, L. S. (2017). The number of stimuli required to reliably assess corticomotor excitability and primary motor cortical representations using transcranial magnetic stimulation (TMS): A systematic review and meta-analysis. *Syst. Rev.* 6, 48. doi:10.1186/s13643-017-0440-8

Chen, Y. S., Zhou, S., Cartwright, C., Crowley, Z., Baglin, R., and Wang, F. (2010). Test-retest reliability of the soleus H-reflex is affected by joint positions and muscle force levels. *J. Electromyogr. Kinesiol.* 20, 980–987. doi:10.1016/j.jelekin.2009.11.003

Chipchase, L., Schabrun, S., Cohen, L., Hodges, P., Ridding, M., Rothwell, J., et al. (2012). A checklist for assessing the methodological quality of studies using transcranial magnetic stimulation to study the motor system: An international

consensus study. *Clin. Neurophysiol.* 123, 1698–1704. doi:10.1016/j.clinph.2012.05.003

Darling, W. G., Wolf, S. L., and Butler, A. J. (2006). Variability of motor potentials evoked by transcranial magnetic stimulation depends on muscle activation. *Exp. Brain Res.* 174, 376–385. doi:10.1007/s00221-006-0468-9

De Goede, A. A., Ter Braack, E. M., and Van Putten, M. (2018). Accurate coil positioning is important for single and paired pulse TMS on the subject level. *Brain Topogr.* 31, 917–930. doi:10.1007/s10548-018-0655-6

Fujio, K., Obata, H., Kawashima, N., and Nakazawa, K. (2019). Presetting of the corticospinal excitability in the tibialis anterior muscle in relation to prediction of the magnitude and direction of postural perturbations. *Front. Hum. Neurosci.* 13, 4. doi:10.3389/fnhum.2019.00004

Goldsworthy, M. R., Hordacre, B., and Ridding, M. C. (2016). Minimum number of trials required for within- and between-session reliability of TMS measures of corticospinal excitability. *Neuroscience* 320, 205–209. doi:10.1016/j.neuroscience.2016.02.012

Handcock, P. J., Williams, L. R., and Sullivan, S. J. (2001). The reliability of H-reflex recordings in standing subjects. *Electromyogr. Clin. Neurophysiol.* 41, 9–15.

Hayes, B. T., Hicks-Little, C. A., Harter, R. A., Widrick, J. J., and Hoffman, M. A. (2009). Intersession reliability of Hoffmann reflex gain and presynaptic inhibition in the human soleus muscle. *Arch. Phys. Med. Rehabil.* 90, 2131–2134. doi:10.1016/j.apmr.2009.07.023

Hermens, H. J. M. R. F. B. (1999). *SENIAM: European recommendations for surface electromyography*. Enschede, Netherlands: Roessingh Research and Development.

Hirano, M., Kubota, S., Koizume, Y., Tanaka, S., and Funase, K. (2016). Different effects of implicit and explicit motor sequence learning on latency of motor evoked potential evoked by transcranial magnetic stimulation on the primary motor cortex. *Front. Hum. Neurosci.* 10, 671. doi:10.3389/fnhum.2016.00671

Hopkins, J. T., Ingersoll, C. D., Cordova, M. L., and Edwards, J. E. (2000). Intrasession and intersession reliability of the soleus H-reflex in supine and standing positions. *Electromyogr. Clin. Neurophysiol.* 40, 89–94.

Horak, F. B., Nutt, J. G., and Nashner, L. M. (1992). Postural inflexibility in parkinsonian subjects. *J. Neurol. Sci.* 111, 46–58. doi:10.1016/0022-510x(92)90111-w

Horak, F., Nashner, L., and Diener, H. (1990). Postural strategies associated with somatosensory and vestibular loss. *Exp. Brain Res.* 82, 167–177. doi:10.1007/BF00230848

Hosel, K., and Tremblay, F. (2021). Facilitation of motor evoked potentials in response to a modified 30 Hz intermittent theta-burst stimulation protocol in healthy adults. *Brain Sci.* 11 (12), 1640. doi:10.3390/brainsci11121640

Kirk, B. J. C., Trajano, G. S., Pulverenti, T. S., Rowe, G., and Blazevich, A. J. (2019). Neuromuscular factors contributing to reductions in muscle force after repeated, high-intensity muscular efforts. *Front. Physiol.* 10, 783. doi:10.3389/fphys.2019.00783

Knikou, M. (2008). The H-reflex as a probe: Pathways and pitfalls. *J. Neurosci. Methods* 171, 1–12. doi:10.1016/j.jneumeth.2008.02.012

Koo, T. K., and Li, M. Y. (2016). A guideline of selecting and reporting intraclass correlation coefficients for reliability research. *J. Chiropr. Med.* 15, 155–163. doi:10.1016/j.jcm.2016.02.012

Latash, M. L., and Zatsiorsky, V. (2015). *Biomechanics and motor control: Defining central concepts*. Department fo Kinesiology, The Pennsylvania State University, PA, United States: Academic Press.

Lewis, G. N., Signal, N., and Taylor, D. (2014). Reliability of lower limb motor evoked potentials in stroke and healthy populations: How many responses are needed? *Clin. Neurophysiol.* 125, 748–754. doi:10.1016/j.clinph.2013.07.029

Luc, B. A., Lepley, A. S., Tevald, M. A., Gribble, P. A., White, D. B., and Pietrosimone, B. G. (2014). Reliability of corticomotor excitability in leg and thigh musculature at 14 and 28 days. *J. Sport Rehabil.* 23, 330–338. doi:10.1123/jsr.2013-0069

Maki, B. E., and Mcilroy, W. E. (1997). The role of limb movements in maintaining upright stance: The "Change-in-support" strategy. *Phys. Ther.* 77, 488–507. doi:10.1093/ptj/77.5.488

MATLAB (2019b). Natick, Massachusetts: The MathWorks Inc.

Nielsen, J. F. (1996). Logarithmic distribution of amplitudes of compound muscle action potentials evoked by transcranial magnetic stimulation. *J. Clin. Neurophysiol.* 13, 423–434. doi:10.1097/00004691-199609000-00005

Palmieri, R. M., Hoffman, M. A., and Ingersoll, C. D. (2002). Intersession reliability for H-reflex measurements arising from the soleus, peroneal, and tibialis anterior musculature. *Int. J. Neurosci.* 112, 841–850. doi:10.1080/00207450290025851

Piirainen, J. M., Linnamo, V., Cronin, N. J., and Avela, J. (2013). Age-related neuromuscular function and dynamic balance control during slow and fast balance perturbations. *J. Neurophysiol.* 110, 2557–2562. doi:10.1152/jn.00476.2013

Pinniger, G. J., Nordlund, M., Steele, J. R., and Cresswell, A. G. (2001). H-Reflex modulation during passive lengthening and shortening of the human triceps surae. *J. Physiol.* 534, 913–923. doi:10.1111/j.1469-7793.2001.00913.x

Portney, L. G., and Watkins, M. P. (2009). *Foundations of clinical research: Applications to practice*. Saddle River, NJ: Pearson/Prentice Hall Upper.

Proessl, F., Beckner, M. E., Sinnott, A. M., Eagle, S. R., Lagoy, A. D., Conkright, W. R., et al. (2021). Reliability of corticospinal excitability estimates for the vastus lateralis: Practical considerations for lower limb TMS task selection. *Brain Res.* 1761, 147395. doi:10.1016/j.brainres.2021.147395

Simonsen, E. B., and Dyhre-Poulsen, P. (2011). Test–retest reliability of the soleus H-reflex excitability measured during human walking. *Hum. Mov. Sci.* 30, 333–340. doi:10.1016/j.humov.2010.02.009

Škarabot, J., Ansdell, P., Brownstein, C. G., Hicks, K. M., Howatson, G., Goodall, S., et al. (2019). Reduced corticospinal responses in older compared with younger adults during submaximal isometric, shortening, and lengthening contractions. *J. Appl. Physiol.* 126, 1015–1031. doi:10.1152/japplphysiol.00987.2018

Táboríková, H., and Sax, D. S. (1968). Motoneurone pool and the H-reflex. *J. Neurol. Neurosurg. Psychiatry* 31, 354–361. doi:10.1136/jnnp.31.4.354

Tallent, J., Goodall, S., Hortobágyi, T., St Clair Gibson, M., Gollhofer, A., French, D. N., and Howatson, G. (2012). Repeatability of Corticospinal and Spinal Measures during Lengthening and Shortening Contractions in the Human Tibialis Anterior Muscle. *PLoS One* 7, e35930.

Taube, W., Gruber, M., Beck, S., Faist, M., Gollhofer, A., and Schubert, M. (2007). Cortical and spinal adaptations induced by balance training: Correlation between stance stability and corticospinal activation. *Acta Physiol.* 189, 347–358. doi:10.1111/j.1748-1716.2007.01665.x

Taube, W., Leukel, C., Schubert, M., Gruber, M., Rantalainen, T., and Gollhofer, A. (2008). Differential modulation of spinal and corticospinal excitability during drop jumps. *J. Neurophysiol.* 99, 1243–1252. doi:10.1152/jn.01118.2007

Taube, W., Schubert, M., Gruber, M., Beck, S., Faist, M., and Gollhofer, A. (2006). Direct corticospinal pathways contribute to neuromuscular control of perturbed stance. *J. Appl. Physiol.* 101, 420–429. doi:10.1152/japplphysiol.01447.2005

Trimble, M. H., and Koceja, D. M. (2001). Effect of a reduced base of support in standing and balance training on the soleus H-reflex. *Int. J. Neurosci.* 106, 1–20. doi:10.3109/00207450109149734

Van Hedel, H. J., Murer, C., Dietz, V., and Curt, A. (2007). The amplitude of lower leg motor evoked potentials is a reliable measure when controlled for torque and motor task. *J. Neurol.* 254, 1089–1098. doi:10.1007/s00415-006-0493-4

Wälchli, M., Tokuno, C. D., Ruffieux, J., Keller, M., and Taube, W. (2017). Preparatory cortical and spinal settings to counteract anticipated and non-anticipated perturbations. *Neuroscience* 365, 12–22. doi:10.1016/j.neuroscience.2017.09.032

Weir, J. P. (2005). Quantifying test-retest reliability using the intraclass correlation coefficient and the SEM. *J. Strength Cond. Res.* 19, 231–240. doi:10.1519/15184.1

Zemková, E. (2011). *Assessment of balance in sport: Science and reality*. Serbian Journal of Sports Sciences.

II

CORTICOSPINAL ADAPTATION TO SHORT-TERM HORIZONTAL BALANCE PERTURBATION TRAINING

by

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Corticospinal Adaptation to Short-Term Horizontal Balance Perturbation Training

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Abstract: Sensorimotor training and strength training can improve balance control. Currently, little is known about how repeated balance perturbation training affects balance performance and its neural mechanisms. This study investigated corticospinal adaptation assessed by transcranial magnetic stimulation (TMS) and Hoffman-reflex (H-reflex) measurements during balance perturbation induced by perturbation training. Fourteen subjects completed three perturbation sessions (PS1, PS2, and PS3). The perturbation system operated at 0.25 m/s, accelerating at 2.5 m/s^2 over a 0.3 m displacement in anterior and posterior directions. Subjects were trained by over 200 perturbations in PS2. In PS1 and PS3, TMS and electrical stimulation elicited motor evoked potentials (MEP) and H-reflexes in the right leg soleus muscle, at standing rest and two time points (40 ms and 140 ms) after perturbation. Body sway was assessed using the displacement and velocity of the center of pressure (COP), which showed a decrease in PS3. No significant changes were observed in MEP or H-reflex between sessions. Nevertheless, Δ MEP at 40 ms demonstrated a positive correlation with Δ COP, while Δ H-reflex at 40 ms demonstrated a negative correlation with Δ COP. Balance perturbation training led to less body sway and a potential increase in spinal-level involvement, indicating that movement automaticity may be suggested after perturbation training.

Keywords: balance control; motor learning; transcranial magnetic stimulation; H-reflex; automaticity

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1. Introduction

Balance control is a fundamental motor skill that requires rapid adaptation to a dynamically changing environment (e.g., balance perturbation tasks) [1]. Especially in dynamic tasks, balance control involves proprioceptive, somatosensory, and vestibular loops, which are related to neuronal activity in the brain stem, cerebellum, and motor cortex [2,3]. It has been determined that inherent muscle activity and the action of reflex loops contribute to maintaining standing balance during balance perturbation tasks [4,5]. Muscle activity recorded by electromyography (EMG) in lower limb muscles, such as the soleus and tibialis anterior, exhibits a stereotypical pattern referred to as short-latency response (SLR), medium-latency response (MLR), and long-latency response (LLR) following ankle movement subsequent to the disturbance of a standing position [6,7]. Although there have been different opinions regarding latencies and the mechanisms of these responses, SLR is generally considered a monosynaptic reflex and can enhance muscle stiffness [8,9]. Alternatively, MLR is more likely due to group-II afferent involvement [10] and is likely subcortical in origin, while LLR is associated with transcortical loops [11,12].

Several studies have examined how sensorimotor training [12], explosive strength training [13], and cognitive training [14] can improve balance control. In this regard, neural adaptation at both spinal and supraspinal levels has been demonstrated following

training [12–18]. The Hoffman reflex (H-reflex) is a commonly used method to evaluate neural excitability at the spinal level. Correia et al. [19] reviewed that there were only a few studies that had used H-reflex in fast movement tasks. Even though H-reflex changes (i.e., spinal circuit excitability) occur before voluntary reactions, these reflex reactions may be more related to movement force rather than other factors, such as speed. On the other hand, transcranial magnetic stimulation (TMS) is a noninvasive method to investigate corticospinal excitability in human sport and training [20]. Taube et al. [18] showed that neural adaptation occurred more at the cortical level than at the spinal level through TMS-evoked motor evoked potentials (MEP) before and after balance training. Reduced MEPs and TMS-conditioned H-reflexes in the LLR but not SLR were observed during a balance perturbation task. Therefore, neural adaptation seems to occur at both spinal and supraspinal levels after balance training, depending on the timing of interest after the perturbation. Thus, responses in the SLR and LLR have been shown to differ.

Since balance control is a motor skill, balance training can also be considered motor skill learning acquisition. Rosenkranz et al. [21] showed that short-term (five sessions) training demonstrated synaptogenesis, and, thus, improved corticospinal plasticity. The activity of the primary motor cortex has been demonstrated in the early learning phase of a static balance task [22]. However, the study of Prsa et al. [23] indicated that motor skill training in a laboratory setting (e.g., moving an arm to a required target) is simpler than in 'real-life' situations, where tasks can be more complex and require conscious effort to complete. In other words, simple motor training tasks in the laboratory may be acquired quickly, even after a few trials, and in an unconscious manner. This is often referred to as 'implicit learning,' to distinguish it from 'motor skill acquisition' [24]. When using balance perturbation as a motor task, hundreds of perturbations are usually involved in one experiment, which may already induce the 'adaptation' of the central nervous system. Therefore, it is important to clarify how balance control ability improves and adapts to short-term repeated perturbation tasks.

When perturbation tasks are more challenging and unfamiliar (i.e., higher perturbation amplitude and velocity), well-coordinated and higher muscle activities are needed to maintain body balance. Subsequently, high voluntary muscle activation after LLR has been shown in our previous study [25]. However, this important time window for muscle activity has not been frequently studied previously. This voluntary muscle activity may be related to a stronger contribution of cortical drive to maintain body position/restore balance during challenging perturbation tasks [26–28]. Currently, little is known about how repeated balance training affects balance performance and voluntary activation after LLR during balance perturbation tasks, or about the contribution of the spinal and supraspinal mechanisms behind this improvement. The main purpose of this study was to investigate whether short-term motor learning leads to performance improvement during ankle transitional perturbation. A secondary purpose was to determine the neural mechanism that might modulate the improvement in balance control ability. Specifically, this study investigated (1) the effect of high-amplitude short-term balance perturbation training on balance performance, and (2) corticospinal and spinal excitability at different time points after perturbation.

2. Materials and Methods

2.1. Subjects

Fourteen subjects volunteered to participate in the study (7 males and 7 females, age: 33 ± 5 years, height: 1.71 ± 0.93 m, weight: 72.8 ± 14.2 kg, and BMI: 24.6 ± 3.5). None of the subjects had any history of neuromuscular or orthopedic diseases and all subjects were informed about the procedures and gave written informed consent. Subjects were fully introduced to the protocol and they had the opportunity to withdraw from the study at any time. An ethical statement (1267/13.00.04.00/2021) was given by the ethics board of the University and the study was performed in conformity with the declaration of Helsinki (2013).

2.2. Experimental Design

Three perturbation sessions (PS1, PS2, PS3) were conducted within 48 h intervals (see Figure 1). In PS1 and PS3, after EMG electrode setup and 5 min cycling warm-up (70 W) on a fitness ergometer (Monark, 282 E, Varberg, Sweden), 16 balance perturbations (1 set) were used to collect center-of-pressure (COP) and EMG activity data. After this, subjects were positioned in a custom-built ankle dynamometer (University of Jyväskylä, Jyväskylä, Finland) to measure the isometric maximal voluntary contraction force (MVC) of the right leg. The TMS coil was set on a hotspot and the active motor threshold (aMT) was tested when subjects sat in the ankle dynamometer. With a TMS coil attached to the head and held by the custom-built helmet [25], subjects moved carefully to the balance platform, and 10 TMS pulses were given to measure MEPs during standing rest. In the balance perturbation task with stimulation, MEPs were evoked at 40 ms and 140 ms time points after the onset of ankle movement during the balance perturbation in random order. H-reflexes were elicited at the same two time points and in random order. The stimulations were delivered during each balance perturbation (anterior and posterior directions), however, only MEPs and H-reflexes during posterior perturbations were analyzed. In PS2, 13 sets of balance perturbations (16 perturbations in each set) were given to subjects with 1–2 min rest between perturbation sets.

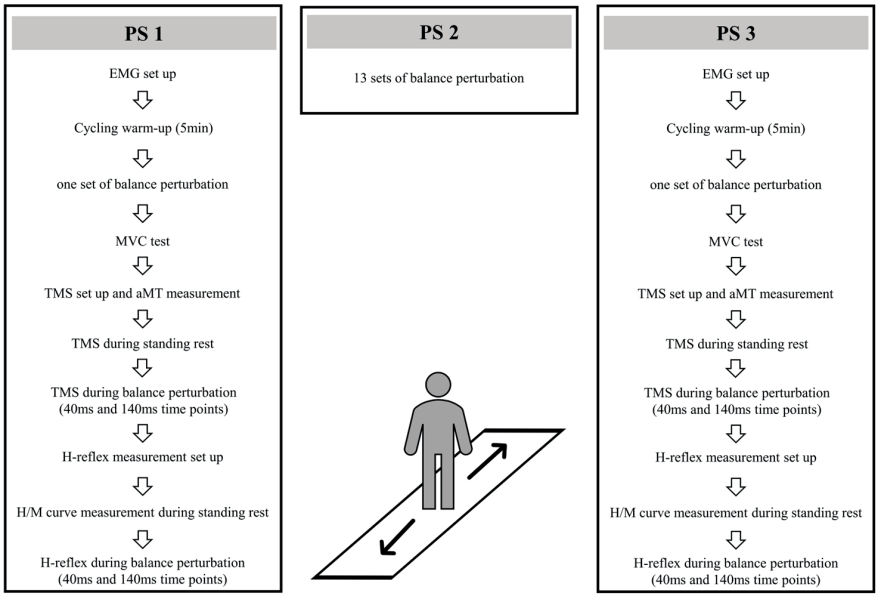

Figure 1. Experimental design in PS1, PS2, and PS3.

2.3. Isometric Maximal Voluntary Contraction

MVC was used to investigate possible muscle fatigue between sessions and to monitor muscle contraction levels during the identification of aMT (10% MVC). Subjects were positioned in a custom-built ankle dynamometer (University of Jyväskylä, Jyväskylä, Finland) to assess the MVC with the right foot on the pedal at 100° hip angle, 180° knee angle (leg fully extended) and 90° ankle angle. After the positioning procedure, the subject performed 5–7 submaximal plantarflexion trials to practice performance. MVC was performed at least three times at one-minute intervals and the highest force value was considered the MVC. If the last trial was >5% higher than the second-best, additional trials were performed until no further improvement was observed. The typical number of required maximum trials was 3–5. The force from the dynamometer pedal was measured by a strain gauge transducer sampled at 1 kHz in Spike2 (8.0) software (CED Ltd., Cambridge, UK), and the maximum MVC amplitude was analyzed.

2.4. Electromyography (EMG)

EMG was measured by bipolar electrodes (Blue Sensor, Ag/AgCl, 28 mm^2, Ambu A/S, Ballerup, Denmark) placed 2 cm below the gastrocnemius on the line of the Achilles tendon for the soleus (SOL), tibialis anterior (TA), and gastrocnemius (GM) muscles according to SENIAM guidelines [29]. In our pilot TMS study, discomfort and muscle twitch were reported by some subjects at the 140 ms (voluntary activation) time point. To reduce the potential discomfort and tension caused by high-intensity stimulation, a pseudo-monopolar setup on the SOL was used in TMS measurements, as has been used previously [30,31]. The pseudo-monopolar setup provides a better representation of the electrical characteristics of the action potentials [32], resulting in a higher MEP amplitude compared to a bipolar arrangement with the same intensity of the stimulus. In addition, according to our practical experience, the shape of the MEP is more consistent with the pseudo-monopolar setup, which is important in dynamic tasks. A disadvantage of this electrode montage is that the signal-to-noise ratio can be compromised; however, this was not a problem in the current study. One electrode was placed 2 cm below the gastrocnemius on the line of the Achilles tendon and the reference electrode was placed on the tibia at the same level. The skin was shaved, carefully abraded with sandpaper, and cleaned with alcohol. The target skin impedance was less than 5 kΩ, and if this was not the case, skin preparation was repeated. All EMG data were collected using the Neurolog EMG system (CED Ltd., Cambridge, UK), with a gain of 1000. Data were band-passed filtered (15–500 Hz) and further collected using a CED 1401 A/D-converter (CED ltd., Cambridge, UK) and Spike 2 (8.0) software (CED Ltd., Cambridge, UK) with a sampling rate of 5 kHz.

2.5. Dynamic Balance Perturbations System

Balance perturbation tasks utilized a custom-built dynamic balance device (University of Jyväskylä, Jyväskylä, Finland) modified from Piirainen et al. [33] and Hu et al. [25]. The balance perturbation system operated at 0.25 m/s, accelerating at 2.5 m/s^2 over a 0.3 m displacement. In balance perturbation tasks, 16 perturbations were performed in 1 set, with 8 anterior and 8 posterior perturbations. The order of the perturbation direction was the same in all sets. Two min rest periods were given after every perturbation set to minimize possible muscle fatigue [33]. Every perturbation was triggered when COP was below the ±5 mm level of the standing baseline for at least 1 s. This approach modified the triggering timing and ensured that the subject was always keeping the initial body position and not anticipating the upcoming perturbation. A fixation point was set on the wall 3 m from the subjects at eye level to stabilize the subjects' visual attention during measurements.

During balance perturbation tasks, COP values were collected by a force plate embedded inside the balance platform. One strain gauge sensor was located in each of the four corners of the force plate (BT4 balance platform; HUR Labs, Tampere, Finland), and data were saved and analyzed using the Coachtech-feedback system (University of Jyväskylä, Jyväskylä, Finland). COP in the anterior-posterior direction was calculated using the formula: $\mathrm{COPy} = ((\mathrm{Flf} + \mathrm{Frf}) \times 0.26 - (\mathrm{Flr} + \mathrm{Frr}) \times 0.26)/(\mathrm{Flf} + \mathrm{Frf} + \mathrm{Frr} + \mathrm{Flr})$, where F is the force value from sensors (lf = left front, rf = right front, or = right rear, lr = left rear), and 0.26 (m) is the sensor distance from the middle line.

2.6. TMS Measurement Setup

TMS was delivered using a single-pulse Magstim 200^2 stimulator with a double-cone coil (Magstim, Whitland, UK). A skin-tight (swimming) cap was placed on the head of the subject to increase friction between the coil and the scalp. The optimal TMS stimulation site for the right SOL was located on average 1 cm lateral (left) and 1 cm posterior to the cranial apex. Several stimulations were delivered to determine optimal coil placement and it was then marked by a marker pen on the cap. The aMT was the lowest stimulus intensity to elicit clear MEPs in three out of five stimulations from right ankle plantarflexion with 10% MVC [34,35]. After the confirmation of aMT, a second swimming cap with a hole in the middle of the vertex (Orca High Visibility Neoprene Swim Cap, Orca, Auckland,

New Zealand) was placed over the coil to reduce the gap and relative movement between the coil and the head. Then, a custom-made helmet (modified from an ice-hockey helmet; CCM TACK 710 JK-K, CCM Hockey, Montreal, QC, Canada) was attached to the subject's head with a chin strap. In the balance perturbation system, the TMS cable was placed on a conveyor adjacent to the safety belt conveyor on the roof and connected with the balance platform by a firm handle, which was the same as in our previous study [25]. Single-pulse TMS with a 110% intensity of aMT was delivered during standing rest and balance perturbation tasks to investigate corticospinal excitability, and 110% intensity would cause less discomfort than the higher level stimulations used in our pilot study.

During balance perturbation tasks, a constant delay (25 ms) between the platform control signal and the onset of ankle movement was reported in our previous study [25]. Therefore, 40 ms and 140 ms time points after ankle movement were defined as SLR and voluntary activation timing. MEP latency was calculated between the TMS pulse and MEP rising point during standing rest. Then, single-pulse TMS with 110% aMT was adjusted to elicit MEP arising at 40 ms and 140 ms time points.

2.7. H-Reflex Measurement Setup

For H-reflex measurements, subjects stood relaxed during the electrical stimulation setup. Electrical stimulation was administrated to the tibial nerve in the popliteal fossa. A cathode (1.5 cm × 1.5 cm) was placed over the tibial nerve, and an anode (5 cm × 8 cm) was placed above the patella. Rectangular stimulation pulses (DS7AH, Digitimer Ltd., Hertfordshire, UK) with a duration of 0.2 ms were delivered at 10 s intervals. Once the optimal site of stimulation was found, the site was marked by a marker pen, and an electrode (Blue Sensor, Ag/AgCl, 28 mm^2, Ambu A/S, Ballerup, Denmark) was placed and strapped around the subject's knee with an elastic band. An increasing intensity interval (1–5 mA) was chosen to measure the H/M recruitment curve, with at least 30 data points up to the maximal M-wave. The stimulus intensity was adjusted to 5% (±2%) of the maximum M-wave, which was used during balance perturbations to control the stimulation intensity in H-reflex measurements.

During balance perturbation tasks, the H-reflex was measured using the same protocol as in TMS trials. For the H-reflex, a successful trial from 1 perturbation set (16 perturbations) was defined as an M-wave response of 5% (±2%) of the maximal M-wave. The intensity of electrical stimulation was adjusted during perturbation trials to obtain at least five successful trials. If less than five successful H-reflex trials in a set were achieved, an extra perturbation set—four backward and four forward—was performed. For each perturbation task with stimulation, 5 successful H-reflexes were usually completed within 1 to 1.5 sets (16–24 perturbations).

2.8. Data and Statistical Analysis

COP values were analyzed in perturbation trials without stimulations. The mean standard deviation of the COP displacement curve was calculated to evaluate the general body sway (COP_SD). Peak-to-peak COP displacement (dCOP) was analyzed over a time window of 1 s before platform movement (Preparation phase; Pre), during platform movement (Active phase; Act), and 1 s from the end of platform movement (Recovery phase; Rec) (see Figure 2). The COP velocity curve was calculated by differentiating the COP curve over 20 ms windows, and then the average COP velocity of the velocity curve (vCOP) was analyzed in the same time window as dCOP (see Figure 2). Both dCOP and vCOP were normalized by the individual subject's height × weight (dCOP: mm/(m × kg); vCOP: (mm/s)/(m × kg)) according to the recommendation of Chiari et al. [36].

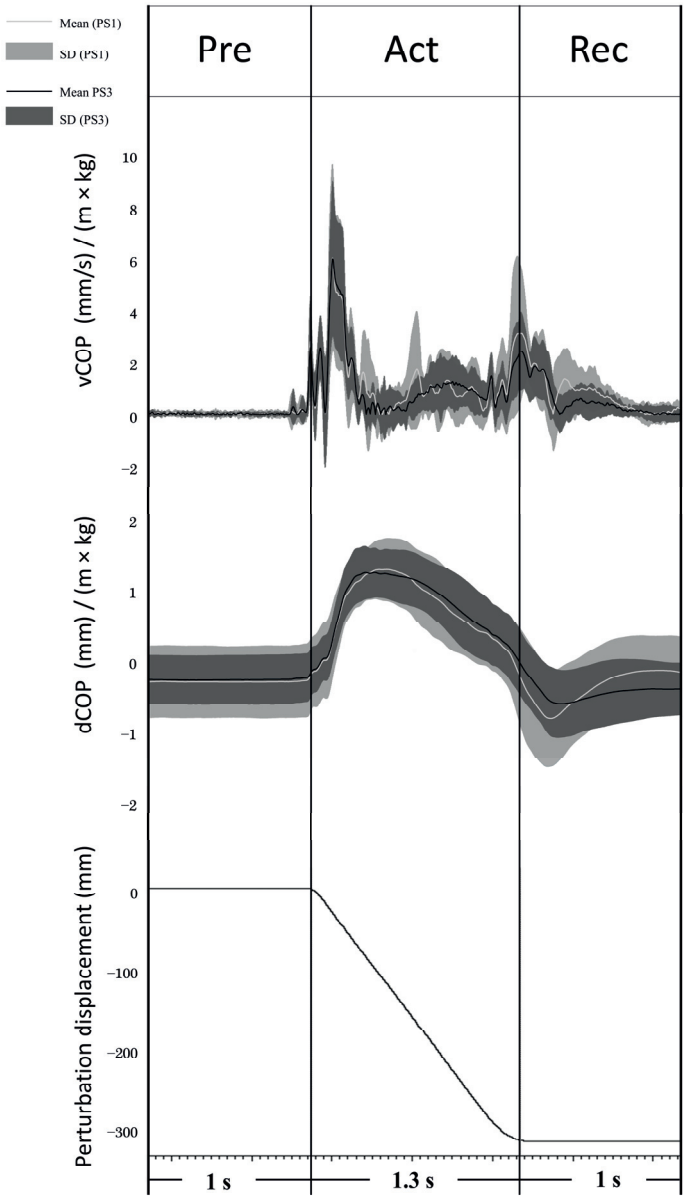

Figure 2. Average COP displacement and velocity (line) and their standard deviations (shadow) from 14 subjects in PS1 (light grey) and PS3 (black) are shown. The signal diagram (bottom part) shows the displacement of the balance perturbation (a negative value means the platform moved backward).

In the balance perturbation trials without stimulation, the average of all subjects' full wave rectified EMG data from 100 ms before the perturbation onset to 400 ms after onset was analyzed. Furthermore, EMG activity was analyzed using root mean square (RMS) over 20 ms time windows from the perturbation onset (0 ms) to 180 ms after onset.

MEPs and the H-reflex were measured during standing rest and balance perturbation. During standing rest, a clear MEP was defined to start when EMG was above the mean + 2SD level recorded 100 ms before the TMS trigger and end when below the mean-2SD level [37]. The average MEP latency and the MEP duration were analyzed during the standing rest and then used to trigger TMS during the balance perturbation. Average MEP values were determined with peak-to-peak amplitude (in mV) from 10 TMS stimulations. 4Outliers were identified from the 10 trials (±2.5 SD) and removed before analysis [38].

In balance perturbation trials, the MEP amplitude from 7–8 trials was selected when the platform moved backward and was averaged after excluding outliers (±2.5 SD), which has been shown to provide good reliability in our previous study [25]. All MEPs were normalized to the peak-to-peak value of the maximal M-wave (M_{MAX}) and presented as $\%M_{MAX}$ in the results. The H-reflex was determined as peak-to-peak amplitude and averaged from all successful trials (within 3–7% M_{MAX}) in standing rest and balance perturbation tasks. The H-reflex was also normalized to the M_{MAX}. The RMS of background EMG (BGemg) was also analyzed with a 30 ms window before TMS and H-reflex triggers and normalized to M_{MAX} (monopolar and bipolar).

In order to evaluate neural excitability changes between corticospinal and spinal levels, the MEP/H-reflex ratio (MEP/H ratio) was calculated. To explore the relationship between the changes in balance performance and corticospinal correlation from PS1 to PS3, the ΔMEP and ΔH-reflex and their correlation with Δ dCOP were analyzed by Pearson product moment correlation. Delta values were calculated (i.e., MEP, H-reflex, and dCOP, respectively) using the formula: (value (PS3)—value (PS1))/value (PS1) × 100%.

The number of participants required was based on power calculations for the expected change in mean rectified MEPs (sEMG recordings from the soleus muscle during balance perturbation). By utilizing previous data from a similar experimental setup by Hu et al. [25], we estimated that 10 subjects in each condition would provide at least 80% power (with a 95% confidence interval) to detect a 15% difference in mean rectified MEPs. This calculation assumed an SD of 10–15% between time points, with a significance level set at $p < 0.05$ (two-tailed).

Statistical analyses were conducted using JASP (Version 0.17.1). Result visualizations were performed using Prism (V9, GraphPad Software, San Diego, CA, USA). Since the original data were not normally distributed, all variables of the MEP/H ratio were processed by log transformation before statistical analyses following Nielsen's suggestion, which resulted in data being normally distributed, as assessed by Shapiro–Wilk W tests [39]. MVC, M_{MAX}, and H_{MAX}/M_{MAX} values were assessed by a paired *t*-test. Since dCOP and vCOP were analyzed by different time windows (i.e., 1 s for Pre and Rec phases but 1.3 s for the Act phase), between-session differences of dCOP, vCOP, and COP_SD were examined by paired *t*-test. To assess adaptation in corticospinal excitability during balance perturbations, MEPs, H-reflex, BGemg, and EMG activity were assessed by two-way (2 × 3) repeated-measures ANOVA with the factors SESSION (PS1 and PS3) and TIME (standing rest, 40 ms, 140 ms). When a significant F-value was observed, Mauchly's test was used to evaluate sphericity, and where the assumption was valid, F-values were reported with sphericity-assumed degrees of freedom and df error (i.e., F (sphericity-assumed df, df error)). Effect sizes for the ANOVA main effects are reported as partial eta squared (η_p^2), where 0.02, 0.13, and 0.26 are considered small, medium, and large, respectively. If significance for TIME was revealed, Bonferroni post-hoc analysis was used for pairwise comparisons between levels (i.e., standing rest, 40 ms, 140 ms). Correlations between MEP amplitude, H-reflex amplitude, and EMG activity were analyzed by Pearson product-moment correlation tests. The significance level was set at $p < 0.05$ and all results were displayed as Mean ± SD in the text and figures.

3. Results

3.1. Balance Performance during Perturbation

dCOP (mm/(m × kg)) and vCOP ((mm/s)/(m × kg)) of Pre, Act, and Rec were analyzed to explore balance performance in AP direction before, during, and after the onset of balance platform movement, respectively (see Figure 2). Both dCOP and vCOP at all phases decreased significantly from PS1 to PS3 (see Table 1). COP_SD demonstrated a significant decrease at PS3 compared to PS1 (PS1: 0.16 ± 0.05; PS3: 0.13 ± 0.06, $t_{(13)} = 2.741$, $p = 0.017$).

Table 1. dCOP (mm/(m × kg)) and vCOP ((mm/s)/(m × kg)) of Pre, Act, and Rec shown with t-value and *p*-value of the paired-t test. Hedge's g was used to interpret results with 0.2, 0.5, and 0.8, which were considered as small, medium, and large effects, respectively.

		PS1	PS3	Mean Difference	$t_{(13)}$	*p*-Value	Hedge's g
dCOP	Pre	0.09 ± 0.04	0.08 ± 0.04	0.01	2.177	0.049 *	0.217
	Act	1.14 ± 0.34	1.01 ± 0.27	0.13	2.483	0.027 *	0.395
	Rec	0.64 ± 0.21	0.44 ± 0.18	0.20	4.642	<0.001 **	0.995
vCOP	Pre	0.16 ± 0.08	0.13 ± 0.06	0.03	2.951	0.011 *	0.429
	Act	1.59 ± 0.57	1.31 ± 0.40	0.28	3.212	0.007 *	0.548
	Rec	0.97 ± 0.35	0.66 ± 0.29	0.31	5.405	<0.001 **	0.928

* ($p < 0.05$); ** ($p < 0.001$).

3.2. Corticospinal Excitability during Perturbation

There was no significant difference demonstrated in M_{MAX} (PS1: 6.79 ± 1.33 mV, PS3: 6.64 ± 1.53 mV, $t_{(13)} = 0.907$, $p = 0.381$), H_{MAX}/M_{MAX} (PS1: 48.5 ± 16.2%M_{MAX}, PS3: 47.4% ± 18.2%M_{MAX}, $t_{(13)} = 0.279$, $p = 0.785$), or MVC (PS1: 1756.9 ± 480.9 Nm, PS3: 1813.7 ± 480.9 Nm, $t_{(13)} = -2.070$, $p = 0.059$) between PS1 and PS3.

A significant main effect for time was observed for MEP amplitude (Figure 3A, $F_{(1.118, 30.897)} = 39.355$, $p < 0.001$, $\eta_p^2 = 0.602$), but there was no main effect for session ($F_{(1, 26)} = 0.817$, $p = 0.374$, $\eta_p^2 = 0.031$) or session × time interaction ($F_{(1.118, 30.897)} = 0.267$, $p = 0.650$, $\eta_p^2 = 0.010$). However, significant differences over time were observed from 40 ms to 140 ms ($p < 0.001$). In addition, MEP amplitude during standing rest was lower than 40 ms ($p = 0.012$) and 140 ms ($p < 0.001$). BGemg values demonstrated an increase at 140 ms compared to other times (Figure 3B, $p < 0.001$).

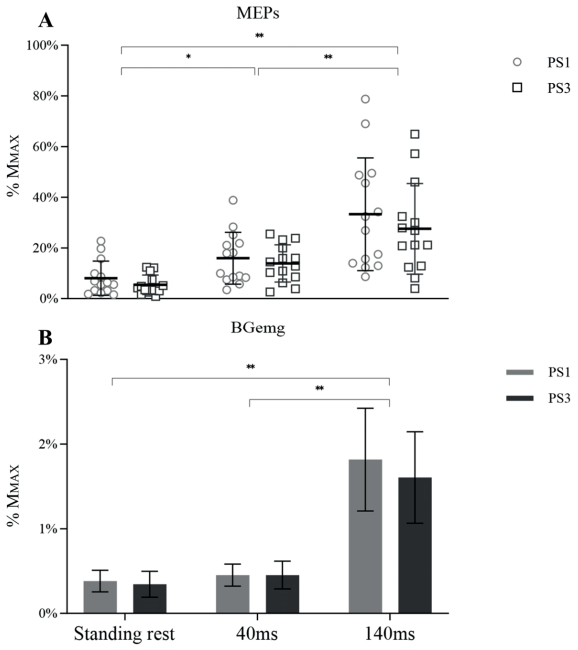

Figure 3. MEP (**A**) with BGemg (**B**) at three different times (standing rest, 40 ms, and 140 ms), in which symbols represent the MEP values of individual subjects and bar charts represent the BGemg activities from monopolar EMG setups. Significant differences between time points are marked with * ($p < 0.05$) and ** ($p < 0.001$).

Similarly, no between session difference was shown in the H-reflex ($F_{(1, 26)} = 0.048$, $p = 0.828$, $\eta_p^2 = 0.002$) or session × time interaction ($F_{(1.273, 33.099)} = 0.638$, $p = 0.466$, $\eta_p^2 = 0.024$). However, a significant main effect over time was observed (Figure 4A,

$F_{(1.273, 33.099)} = 36.269$, $p < 0.001$, $\eta_p^2 = 0.582$). Post-hoc tests showed an increased H-reflex from 40 ms to 140 ms ($p < 0.001$). Plus, the H-reflex during the standing rest was lower than 140 ms ($p < 0.001$). BGemg values demonstrated an increase at 140 ms compared with the other times (Figure 4B, $p < 0.001$).

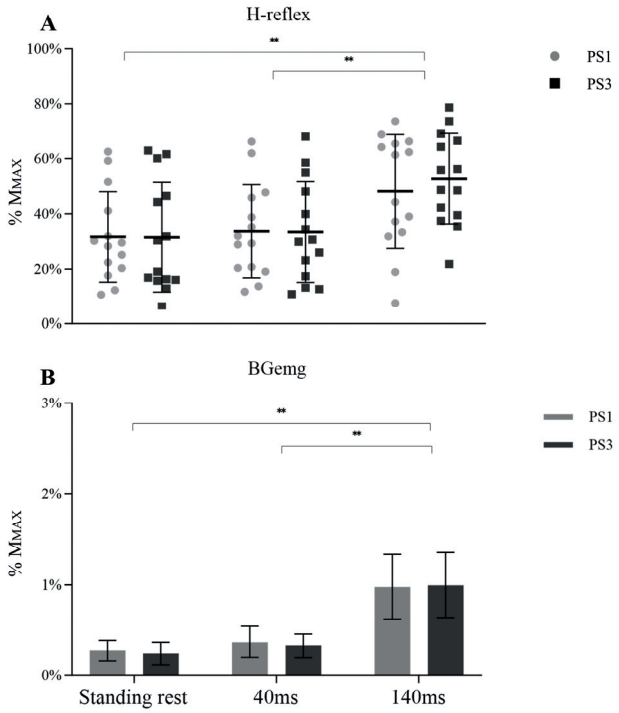

Figure 4. H-reflex (**A**) with BGemg (**B**) at three different times (standing rest, 40 ms, and 140 ms), in which symbols represent H-reflex values of individual subjects and bar charts represent the BGemg activities bipolar EMG setups. Significant differences between time are marked with "**" ($p < 0.001$).

A significant main effect for time was observed in the MEP/H ratio (Figure 5, $F_{(1.592, 41.397)} = 37.174$, $p < 0.001$, $\eta_p^2 = 0.588$), but there was no main effect for session ($F_{(1, 26)} = 0.541$, $p = 0.469$, $\eta_p^2 = 0.020$) or session × time interaction ($F_{(1.592, 41.397)} = 0.704$, $p = 0.469$, $\eta_p^2 = 0.026$). Post-hoc tests demonstrated an enhanced MEP/H ratio from standing rest to 40 ms ($p < 0.001$) and standing rest to 140 ms ($p < 0.001$).

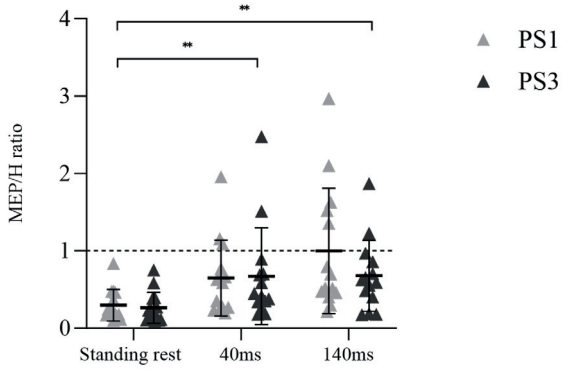

Figure 5. The ratio of MEP and H-reflex at three different times (standing rest, 40 ms, and 140 ms), in which symbols represent the values of individual subjects, while the mean values with standard deviation bars are also depicted. Significant differences between times are marked with "**" ($p < 0.001$).

3.3. Soleus Muscle Activity during Perturbation

Figure 6A shows soleus muscle activity during balance perturbation. Specifically, the RMS of EMG did not demonstrate a significant between-session difference in perturbation trials without stimulation. EMG activity was lower at the 40–60 ms window (PS1: 0.53 ± 0.20%M_{MAX}, PS3: 0.47 ± 0.21%M_{MAX}) when compared to the 140–160 ms window (PS1: 1.32 ± 0.64%M_{MAX}, PS3: 1.27 ± 0.52%M_{MAX}) ($p < 0.001$) (Figure 6B), where these windows match the timings of the stimulations.

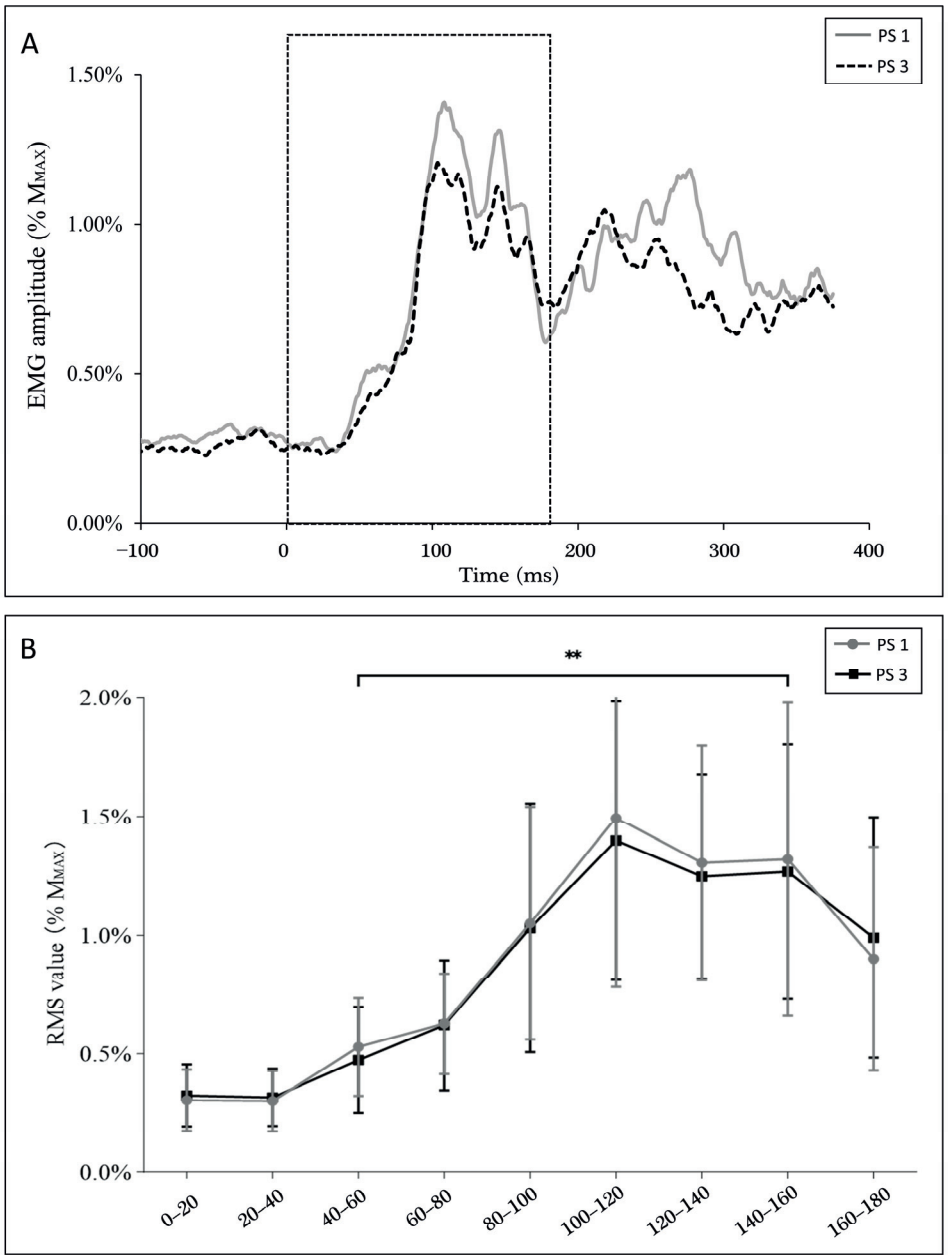

Figure 6. Average EMG activity data from all subjects in perturbation trials without stimulation (**A**). Data are normalized to M_{MAX}. RMS of EMG activity (**B**) in every 20 ms window from ankle movement (0 ms) to 180 ms, as zoomed in from the dashed square of A. Statistical significance is denoted by "**" ($p < 0.001$). There are other significant differences between the time windows, which are not marked in the figure since this study focused on 40–60 ms and 140–60 ms windows.

Additionally, a positive correlation was observed between EMG activity (40 ms–60 ms) and H-reflex (r = 0.500, p = 0.007), but not MEP (r = 0.161, p = 0.412) at 40 ms. On the contrary, EMG activity showed a positive correlation with MEP (r = 0.501, p = 0.007), but not the H-reflex (r = 0.241, p = 0.218) at 140 ms.

3.4. Correlations between Corticospinal/Spinal Excitability and Balance Performance

Figure 7 shows the correlations between changes in the displacement of COP and MEP and the H-reflex at the 40 ms time point. Δ MEP at 40 ms demonstrated a significant and positive correlation with Δ dCOP in Rec (Figure 7C). However, Δ H-reflex at 40 ms demonstrated a significant and negative correlation with Δ dCOP during the Pre (Figure 7D).

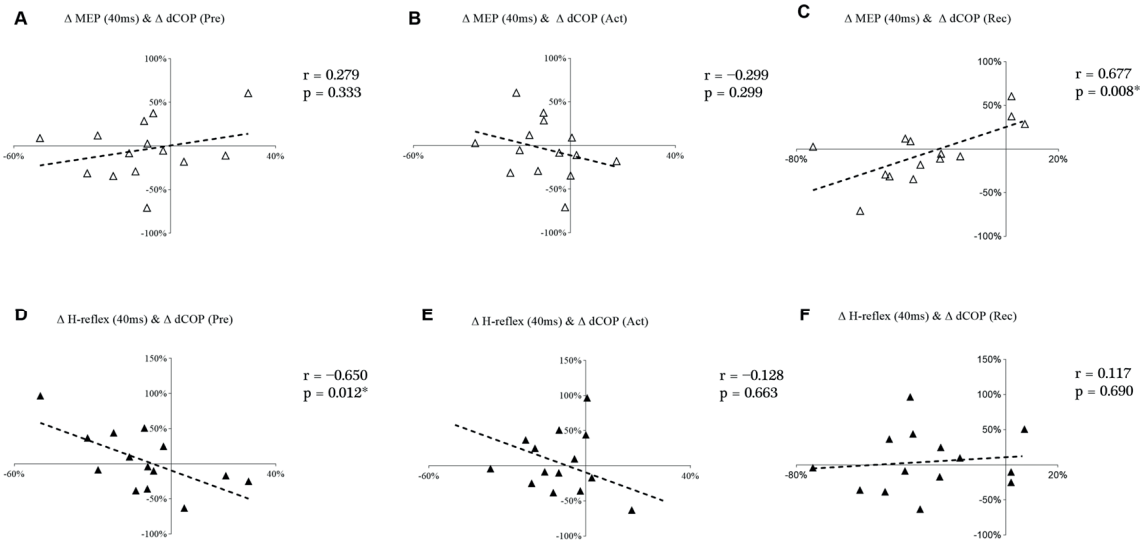

Figure 7. Scatter plots of Δ MEP with Δ dCOP at Pre (**A**), Act (**B**), and Rec (**C**); Scatter plots of Δ H-reflex with Δ dCOP at Pre (**D**), Act (**E**), and Rec (**F**). "*" is marked when $p < 0.05$.

4. Discussion

Our findings demonstrate the decreased displacement and velocity of COP in PS3, which indicates that balance performance improved during the third perturbation session. In addition, the observed positive correlation between Δ MEP (40 ms) and Δ dCOP (Rec) along with the negative correlation between Δ H-reflex (40 ms) and Δ dCOP (Pre) suggests that the decrease in COP displacement may be related to decreased corticospinal excitability and increased spinal excitability. Individual differences were shown in MEPs and H-reflexes from PS1 to PS3, but the neural adaptation for those with improved (eight subjects) balance ability was more likely to transfer from the cortical level toward the spinal level.

The balance performance adaptive process was observed as a sway reduction in both COP displacement and velocity during balance perturbation. In addition, the variability of COP decreased, and COP was close to the standing rest baseline, as shown by the reduction in COP_SD in PS3, indicating body sway. Therefore, balance perturbation training with repeated high amplitude and speed enhanced balance control performance during the third session. This was also in line with the study of Bakker et al. [15], who found that even one session of task-related balance training (but not seated cycling or rest) improved balance performance in specific balance tasks. Several studies have indicated that different types of training improve balance control ability, including sensorimotor training [12], strength training [13], and cognitive dual-task training [14]. Studies also showed that specific balance training improves postural control but not muscle strength in both young and older adults [40–42]. The results of our study demonstrated an improvement in balance

ability without an increase in muscle strength (i.e., no changes in MVC or EMG activity during the perturbation task were observed), which indicates that the balance task-related training was efficient for improving balance performance in perturbation tasks, at least for young adults (i.e., 28 years old to 42 years old in this study).

During balance perturbation tasks, both feedback control, which occurs in response to sensory feedback, and feedforward control, which refers to the anticipation of a voluntary movement, are involved in postural control [43]. In our previous study [25], balance performance may have been partly learned already following the first testing session, in which dCOP decreased from the Pre to the Rec phase after one perturbation session. It is well accepted that feedforward control is an internal model for accuracy, and does not require feedback loops (e.g., somatosensory feedback), and thus it is more related to anticipation [44]. Therefore, feedforward control might have already improved following the first perturbation session. However, a recent study with a similar setup did not show enhanced balance performance after the first perturbation session [45]. Researchers suggested that balance control ability may not be entirely acquired within just one perturbation session. On the other hand, feedback control relies on information from sensory sources. Since SLR and LLR occur during the Act phase, this study revealed significant balance performance changes in the Act phase during the third session. This suggests that feedback control may play a stronger role in balance control following perturbation training.

The increased MEP amplitude at 40 ms found in our study may be related to the anticipation of the balance system movement, even when masking strategies were in place to reduce the influence of anticipation. To avoid preparatory body position, body swaying was monitored by the perturbation system, and if COP shifted over 5 mm of baseline, perturbation would not be triggered. In the study of Mierau et al. [46], a positive potential (P1) was recorded by electroencephalography (EEG) over the centro-parietal cortical area in the early phase of a balance perturbation, which was suggested to be related to afferent feedback by the perturbation [46]. Therefore, there would not be enough time for the cortical drive to participate in the early perturbation phase. Thus, increasing MEP at 40 ms in our study may be related to feedforward control. In our data, at the late phase of the balance perturbation, an EMG peak after LLR (see Figure 6A) was observed, which can be considered a voluntary muscle activity in order to maintain body position. In the study of Nevanperä et al. [45], a significantly increased V-wave was observed after 70 ms, which indicates that supraspinal drive may be involved in the late phase of the balance perturbation. In the study of Mierau et al. [46], addressing cortical processing of sensory feedback, a negative potential (N1) was recorded with EEG during a 100–200 ms time window after the perturbation onset, which was suggested to be related to the perturbation amplitude and postural threat. The N1 amplitude demonstrated a positive correlation with the EMG activity of the gastrocnemius muscle after 100 ms. A similar positive correlation was observed in our study between EMG activity and MEP in the later phase of the balance perturbation (140 ms). Taken together with the results of Mierau et al. [46], it seems that neural activity at the cortical level is strongly related to muscle activity when trying to maintain balance in the later phases of balance perturbation.

Even though neither MEPs nor H-reflexes demonstrated changes between sessions, the decreased displacement of COP was related to a higher H-reflex but a lower MEP at the 40 ms time onset after the intervention. It seems that neural adaptation to balance perturbation may take place at both the supraspinal and spinal levels. However, the current null findings for changes in MEPs from PS1 to PS3 may also imply that the amount of training was not enough to induce any neural excitability changes cortically and, thus, the movement pattern was not likely fully learned in some subjects (e.g., 3 out of 14 subjects demonstrated increased COP). In a study by Mouthon and Taube [47], increased short-interval intracortical inhibition (SICI) was reported following two weeks (six training sessions) of balance training, showing enhanced intracortical inhibition. In addition, Lauber et al. [48] demonstrated that more cortical inhibition occurred in the initial stages of balance training but then decreased back to baseline as training progressed. Collectively,

these findings indicate that there may be a high level cortical drive at the beginning of balance training, which then shifts to a subcortical level once balance control has been acquired [49].

In the current study, only single-pulse TMS at one stimulation intensity was used. Therefore, future studies should consider using paired-pulse TMS to determine intracortical inhibition and facilitation at different delays during balance perturbation tasks. On the other hand, it should also be noted that the adaptation of automaticity-related neural excitability may occur at the spinal level according to modulation of H-reflex to the demands of the task [50]. Previous studies have shown decreasing soleus H-reflex amplitude when human body balance is challenged (i.e., from lying to standing [51], or from standing to walking [52]). In our study, reduced COP indicated that balance control ability was improved after balance perturbation training. In addition, the MEP/H ratio in the majority of subjects (12 out of 14) was lower in PS3 when compared to PS1 (see Figure 5), suggesting that maintaining balance was not as challenging as before. Finally, a study by Bakker et al. [15] found that 30 min balance training improved balance performance, but no neural adaptations such as altered MEP amplitude or increased SICI, as hypothesized, were observed. Researchers speculated that other brain areas, such as the cerebellum, may underlie balance performance improvement [15]. Considering the lack of MEP or H-reflex changes in our study from PS1 to PS3, and the relationship of Δ MEP and Δ H-reflex with Δ dCOP at the 40 ms time point, these findings may imply that subcortical circuit activity could be a crucial factor in balance control, contributing to the enhancement of balance performance in PS3.

Some limitations should be mentioned regarding this study. Large variabilities were observed in both MEP and H-reflex amplitudes when responding to balance perturbation tasks. This variability may have led to the lack of significant differences between sessions. Therefore, more subjects in a future study would be ideal. Despite utilizing a random perturbation order and COP monitor to reduce anticipation and body sway before perturbation onset, it was very difficult to rule out all anticipation influences. Due to a similar order and time interval between the perturbations of each set, it is still possible that subjects could have learned some pattern, which might be one of the reasons for the increasing MEP amplitude at the 40 ms time point.

5. Conclusions

Improved balance control, shown by decreased COP displacement and velocity, was observed in session three. However, no significant group-level neural adaptation was shown at the supraspinal or spinal level. A correlation between ΔCOP and the ΔMEP/ΔH-reflex may imply potential corticospinal excitability changes in parallel with balance performance improvement on an individual level. Based on our findings, it appears that only a few sessions are required to demonstrate motor learning and/or neural adaptation. Although neither the precise driver nor mechanism of adaptation could be identified in the present study, our evidence suggests that repeated short-term balance training led to modifications from cortical to more subcortical functioning.

Author Contributions: N.H., J.A. and J.M.P. designed the experiment. N.H. performed the data analysis; S.W. performed the statistical method. N.H. and J.A. wrote the initial draft. J.M.P., S.W. and D.J.K. commented on and modified the manuscript. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: This study was approved by the ethical statement (1267/13.00.04.00/2021) given by the ethics board of the University and the study was performed in conformity with the declaration of Helsinki (2013).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: All data that support the findings of this study are available upon request according to the GDPR principles. Please contact the corresponding author: Nijia Hu (email: nijia.n.hu@jyu.fi).

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Conflicts of Interest: The authors declare no conflict of interest.

References

1. Pollock, A.S.; Durward, B.R.; Rowe, P.J.; Paul, J.P. What Is Balance? *Clin. Rehabil.* **2000**, *14*, 402–406. [CrossRef] [PubMed]
2. Horak, F.B.; Shupert, C.L.; Dietz, V.; Horstmann, G. Vestibular and Somatosensory Contributions to Responses to Head and Body Displacements in Stance. *Exp. Brain Res.* **1994**, *100*, 93–106. [CrossRef] [PubMed]
3. Jacobs, J.V.; Horak, F.B. Cortical Control of Postural Responses. *J. Neural Transm. Vienna* **2007**, *114*, 1339–1348. [CrossRef] [PubMed]
4. Ivanenko, Y.; Gurfinkel, V.S. Human Postural Control. *Front. Neurosci.* **2018**, *12*, 171. [CrossRef]
5. Rasman, B.G.; Forbes, P.A.; Tisserand, R.; Blouin, J.-S. Sensorimotor Manipulations of the Balance Control Loop–Beyond Imposed External Perturbations. *Front. Neurol.* **2018**, *9*, 899. [CrossRef]
6. Petersen, N.; Christensen, L.O.D.; Morita, H.; Sinkjær, T.; Nielsen, J. Evidence That a Transcortical Pathway Contributes to Stretch Reflexes in the Tibialis Anterior Muscle in Man. *J. Physiol.* **1998**, *512*, 267–276. [CrossRef]
7. Taube, W.; Schubert, M.; Gruber, M.; Beck, S.; Faist, M.; Gollhofer, A. Direct Corticospinal Pathways Contribute to Neuromuscular Control of Perturbed Stance. *J. Appl. Physiol.* **2006**, *101*, 420–429. [CrossRef]
8. Fitzpatrick, R.C.; Taylor, J.L.; McCloskey, D.I. Ankle Stiffness of Standing Humans in Response to Imperceptible Perturbation: Reflex and Task-Dependent Components. *J. Physiol.* **1992**, *454*, 533–547. [CrossRef]
9. Schieppati, M.; Nardone, A.; Siliotto, R.; Grasso, M. Early and Late Stretch Responses of Human Foot Muscles Induced by Perturbation of Stance. *Exp. Brain Res.* **1995**, *105*, 411–422. [CrossRef]
10. Schieppati, M.; Nardone, A. Medium-Latency Stretch Reflexes of Foot and Leg Muscles Analysed by Cooling the Lower Limb in Standing Humans. *J. Physiol.* **1997**, *503*, 691–698. [CrossRef]
11. Kurtzer, I.L. Long-Latency Reflexes Account for Limb Biomechanics through Several Supraspinal Pathways. *Front. Integr. Neurosci.* **2015**, *8*, 99. [CrossRef]
12. Taube, W.; Gruber, M.; Gollhofer, A. Spinal and Supraspinal Adaptations Associated with Balance Training and Their Functional Relevance. *Acta Physiol.* **2008**, *193*, 101–116. [CrossRef] [PubMed]
13. Piirainen, J.M.; Cronin, N.J.; Avela, J.; Linnamo, V. Effects of Plyometric and Pneumatic Explosive Strength Training on Neuromuscular Function and Dynamic Balance Control in 60–70 year Old Males. *J. Electromyogr. Kinesiol.* **2014**, *24*, 246–252. [CrossRef] [PubMed]
14. Li, K.Z.; Roudaia, E.; Lussier, M.; Bherer, L.; Leroux, A.; McKinley, P.A. Benefits of Cognitive Dual-Task Training on Balance Performance in Healthy Older Adults. *J. Gerontol. Ser. Biomed. Sci. Med. Sci.* **2010**, *65*, 1344–1352. [CrossRef] [PubMed]
15. Bakker, L.B.; Nandi, T.; Lamoth, C.J.; Hortobágyi, T. Task Specificity and Neural Adaptations after Balance Learning in Young Adults. *Hum. Mov. Sci.* **2021**, *78*, 102833. [CrossRef] [PubMed]
16. Gruber, M.; Taube, W.; Gollhofer, A.; Beck, S.; Amtage, F.; Schubert, M. Training-Specific Adaptations of H- and Stretch Reflexes in Human Soleus Muscle. *J. Mot. Behav.* **2007**, *39*, 68–78. [CrossRef]
17. Schubert, M.; Beck, S.; Taube, W.; Amtage, F.; Faist, M.; Gruber, M. Balance Training and Ballistic Strength Training Are Associated with Task-Specific Corticospinal Adaptations. *Eur. J. Neurosci.* **2008**, *27*, 2007–2018. [CrossRef]
18. Taube, W.; Gruber, M.; Beck, S.; Faist, M.; Gollhofer, A.; Schubert, M. Cortical and Spinal Adaptations Induced by Balance Training: Correlation between Stance Stability and Corticospinal Activation. *Acta Physiol.* **2007**, *189*, 347–358. [CrossRef]
19. Correia, J.P.; Vaz, J.R.; Domingos, C.; Freitas, S.R. From Thinking Fast to Moving Fast: Motor Control of Fast Limb Movements in Healthy Individuals. *Rev. Neurosci.* **2022**, *33*, 919–950. [CrossRef]
20. Moscatelli, F.; Messina, A.; Valenzano, A.; Monda, V.; Salerno, M.; Sessa, F.; La Torre, E.; Tafuri, D.; Scarinci, A.; Perrella, M.; et al. Transcranial Magnetic Stimulation as a Tool to Investigate Motor Cortex Excitability in Sport. *Brain Sci.* **2021**, *11*, 432. [CrossRef]
21. Rosenkranz, K.; Kacar, A.; Rothwell, J. Differential Modulation of Motor Cortical Plasticity and Excitability in Early and Late Phases of Human Motor Learning. *J. Neurosci.* **2007**, *27*, 12058–12066. [CrossRef] [PubMed]
22. Egger, S.; Wälchli, M.; Rüeger, E.; Taube, W. Short-Term Balance Consolidation Relies on the Primary Motor Cortex: A RTMS Study. *Sci. Rep.* **2023**, *13*, 5169. [CrossRef]
23. Prsa, M.; Thier, P. The Role of the Cerebellum in Saccadic Adaptation as a Window into Neural Mechanisms of Motor Learning. *Eur. J. Neurosci.* **2011**, *33*, 2114–2128. [CrossRef] [PubMed]
24. Krakauer, J.; Hadjiosif, A.; Xu, J.; Wong, A.; Haith, A. Motor Learning. In *Comprehensive Physiology*; John Wiley & Sons: Hoboken, NJ, USA, 2019; Volume 9, pp. 613–663. [CrossRef]

25. Hu, N.; Avela, J.; Kidgell, D.J.; Nevanperä, S.; Walker, S.; Piirainen, J.M. Reliability of Transcranial Magnetic Stimulation and H-Reflex Measurement during Balance Perturbation Tasks. *Front. Physiol.* **2022**, *13*, 957650. [CrossRef] [PubMed]
26. Freyler, K.; Gollhofer, A.; Colin, R.; Brüderlin, U.; Ritzmann, R. Reactive Balance Control in Response to Perturbation in Unilateral Stance: Interaction Effects of Direction, Displacement and Velocity on Compensatory Neuromuscular and Kinematic Responses. *PLoS ONE* **2015**, *10*, e0144529. [CrossRef]
27. Pruszynski, J.A.; Kurtzer, I.; Scott, S.H. The Long-Latency Reflex Is Composed of at Least Two Functionally Independent Processes. *J. Neurophysiol.* **2011**, *106*, 449–459. [CrossRef]
28. Wälchli, M.; Tokuno, C.D.; Ruffieux, J.; Keller, M.; Taube, W. Preparatory Cortical and Spinal Settings to Counteract Anticipated and Non-Anticipated Perturbations. *Neuroscience* **2017**, *365*, 12–22. [CrossRef]
29. Hermens, H.; Freriks, B.; Merletti, R.; Hägg, G.; Stegeman, D.F.; Blok, J.H.; Hägg, G.; Blok, W.J. *SENIAM 8: European Recommendations for Surface Electromyography*; Roessingh Research and Development: Enschede, The Netherlands, 1999.
30. Blazevich, A.J.; Kay, A.D.; Waugh, C.; Fath, F.; Miller, S.; Cannavan, D. Plantarflexor Stretch Training Increases Reciprocal Inhibition Measured during Voluntary Dorsiflexion. *J. Neurophysiol.* **2012**, *107*, 250–256. [CrossRef]
31. Kirk, B.J.C.; Trajano, G.S.; Pulverenti, T.S.; Rowe, G.; Blazevich, A.J. Neuromuscular Factors Contributing to Reductions in Muscle Force After Repeated, High-Intensity Muscular Efforts. *Front. Physiol.* **2019**, *10*, 783. [CrossRef]
32. Rodriguez-Falces, J.; Place, N. End-of-Fiber Signals Strongly Influence the First and Second Phases of the M Wave in the Vastus Lateralis: Implications for the Study of Muscle Excitability. *Front. Physiol.* **2018**, *9*, 162. [CrossRef]
33. Piirainen, J.M.; Linnamo, V.; Cronin, N.J.; Avela, J. Age-Related Neuromuscular Function and Dynamic Balance Control During Slow and Fast Balance Perturbations. *J. Neurophysiol.* **2013**, *110*, 2557–2562. [CrossRef] [PubMed]
34. Sen, C.B.A.; Fassett, H.J.; El-Sayes, J.; Turco, C.V.; Hameer, M.M.; Nelson, A.J. Active and Resting Motor Threshold Are Efficiently Obtained with Adaptive Threshold Hunting. *PLoS ONE* **2017**, *12*, e0186007. [CrossRef]
35. Groppa, S.; Oliviero, A.; Eisen, A.; Quartarone, A.; Cohen, L.G.; Mall, V.; Kaelin-Lang, A.; Mima, T.; Rossi, S.; Thickbroom, G.W.; et al. A Practical Guide to Diagnostic Transcranial Magnetic Stimulation: Report of an IFCN Committee. *Clin. Neurophysiol.* **2012**, *123*, 858–882. [CrossRef]
36. Chiari, L.; Rocchi, L.; Cappello, A. Stabilometric Parameters Are Affected by Anthropometry and Foot Placement. *Clin. Biomech.* **2002**, *17*, 666–677. [CrossRef]
37. Hirano, M.; Kubota, S.; Koizume, Y.; Tanaka, S.; Funase, K. Different Effects of Implicit and Explicit Motor Sequence Learning on Latency of Motor Evoked Potential Evoked by Transcranial Magnetic Stimulation on the Primary Motor Cortex. *Front. Hum. Neurosci.* **2016**, *10*, 671. [CrossRef] [PubMed]
38. Avenanti, A.; Paluello, I.M.; Bufalari, I.; Aglioti, S.M. Stimulus-Driven Modulation of Motor-Evoked Potentials during Observation of Others' Pain. *NeuroImage* **2006**, *32*, 316–324. [CrossRef]
39. Nielsen, J.F. Logarithmic Distribution of Amplitudes of Compound Muscle Action Potentials Evoked by Transcranial Magnetic Stimulation. *J. Clin. Neurophysiol.* **1996**, *13*, 423–434. [CrossRef] [PubMed]
40. Buchner, D.M.; Cress, M.E.; De Lateur, B.J.; Esselman, P.C.; Margherita, A.J.; Price, R.; Wagner, E.H. The Effect of Strength and Endurance Training on Gait, Balance, Fall Risk, and Health Services Use in Community-Living Older Adults. *J. Gerontol. A Biol. Sci. Med. Sci.* **1997**, *52*, M218–M224. [CrossRef]
41. Howe, T.E.; Rochester, L.; Neil, F.; Skelton, D.A.; Ballinger, C. Exercise for Improving Balance in Older People. *Cochrane Database Syst. Rev.* **2011**, *11*. [CrossRef]
42. Kurz, I.; Gimmon, Y.; Shapiro, A.; Debi, R.; Snir, Y.; Melzer, I. Unexpected Perturbations Training Improves Balance Control and Voluntary Stepping Times in Older Adults—A Double Blind Randomized Control Trial. *BMC Geriatr.* **2016**, *16*, 58. [CrossRef]
43. Dietz, V.; Trippel, M.; Ibrahim, I.K.; Berger, W. Human Stance on a Sinusoidally Translating Platform: Balance Control by Feedforward and Feedback Mechanisms. *Exp. Brain Res.* **1993**, *93*, 352–362. [CrossRef]
44. Kawato, M. Internal Models for Motor Control and Trajectory Planning. *Curr. Opin. Neurobiol.* **1999**, *9*, 718–727. [CrossRef] [PubMed]
45. Nevanperä, S.; Hu, N.; Walker, S.; Avela, J.; Piirainen, J.M. Modulation of H-Reflex and V-Wave Responses during Dynamic Balance Perturbations. *Exp. Brain Res.* **2023**, *241*, 1599–1610. [CrossRef] [PubMed]
46. Mierau, A.; Hülsdünker, T.; Strüder, H.K. Changes in Cortical Activity Associated with Adaptive Behavior during Repeated Balance Perturbation of Unpredictable Timing. *Front. Behav. Neurosci.* **2015**, *9*, 272. [CrossRef]
47. Mouthon, A.; Taube, W. Intracortical Inhibition Increases during Postural Task Execution in Response to Balance Training. *Neuroscience* **2019**, *401*, 35–42. [CrossRef] [PubMed]
48. Lauber, B.; Gollhofer, A.; Taube, W. What to Train First: Balance or Explosive Strength? Impact on Performance and Intracortical Inhibition. *Scand. J. Med. Sci. Sports* **2021**, *31*, 1301–1312. [CrossRef]
49. Logan, G.D. On the Use of a Concurrent Memory Load to Measure Attention and Automaticity. *J. Exp. Psychol. Hum. Percept. Perform.* **1979**, *5*, 189–207. [CrossRef]
50. Adkins, D.L.; Boychuk, J.; Remple, M.S.; Kleim, J.A. Motor Training Induces Experience-Specific Patterns of Plasticity across Motor Cortex and Spinal Cord. *J. Appl. Physiol.* **2006**, *101*, 1776–1782. [CrossRef]
51. Mynark, R.G.; Koceja, D.M. Down Training of the Elderly Soleus H Reflex with the Use of a Spinally Induced Balance Perturbation. *J. Appl. Physiol.* **2002**, *93*, 127. [CrossRef]

52. Capaday, C.; Stein, R.B. Amplitude Modulation of the Soleus H-Reflex in the Human during Walking and Standing. *J. Neurosci.* **1986**, *6*, 1308–1313. [CrossRef]

III

MODULATIONS OF CORTICOSPINAL EXCITABILITY FOLLOWING RAPID ANKLE DORSIFLEXION IN SKILL- AND ENDURANCE-TRAINED ATHLETES

by

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ORIGINAL ARTICLE

Modulations of corticospinal excitability following rapid ankle dorsiflexion in skill- and endurance-trained athletes

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Abstract

Purpose Long-term sports training, such as skill and endurance training, leads to specific neuroplasticity. However, it remains unclear if muscle stretch-induced proprioceptive feedback influences corticospinal facilitation/inhibition differently between skill- and endurance-trained athletes. This study investigated modulation of corticospinal excitability following rapid ankle dorsiflexion between well-trained skill and endurance athletes.

Methods Ten skill- and ten endurance-trained athletes participated in the study. Corticospinal excitability was tested by single- and paired-pulse transcranial magnetic stimulations (TMS) at three different latencies following passive rapid ankle dorsiflexion. Motor evoked potential (MEP), short-latency intracortical inhibition (SICI), intracortical facilitation (ICF), and long-latency intracortical inhibition (LICI) were recorded by surface electromyography from the soleus muscle.

Results Compared to immediately before ankle dorsiflexion (Onset), TMS induced significantly greater MEPs during the supraspinal reaction period (~ 120 ms after short-latency reflex, SLR) in the skill group only (from 1.7 ± 1.0 to 2.7 ± 1.8%M-max, $P = 0.005$) despite both conditions being passive. ICF was significantly greater over all latencies in skill than endurance athletes ($F_{(3, 45)} = 4.64$, $P = 0.007$), although no between-group differences for stimulations at specific latencies (e.g., at SLR) were observed.

Conclusion The skill group showed higher corticospinal excitability during the supraspinal reaction phase, which may indicate a "priming" of corticospinal excitability following rapid ankle dorsiflexion for a supraspinal reaction post-stretch, which appears absent in endurance-trained athletes.

Keywords Physical exercise · Training adaptation · Stretch reflex · Transcranial magnetic stimulation · Corticospinal excitability

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Abbreviations

ANOVA	Analysis of variance
EMG	Electromyography
ICF	Intracortical facilitation
LICI	Long-latency intracortical inhibition
LLR	Long-latency reflex
LTP	Long-term potentiation
MEP	Motor evoked potential
MLR	Medium-latency reflex
M-max	Maximum compound action potential
PAS	Paired associative stimulation
rMT	Resting motor threshold
SICI	Short-latency intracortical inhibition
SLR	Short-latency stretch reflex
SOL	Soleus muscle
TA	Tibialis anterior muscle
TMS	Transcranial magnetic stimulation

Introduction

Corticospinal plasticity, the ability of the brain to modify neuronal connections, is essential for learning, motor control, improved memory, and recovery from brain injury (Kantak et al. 2012). It can be modified by conditions such as visual, auditory, and proprioception information through adapting the neural connections (Pascual-Leone et al. 2005). Motor training is a process of acquiring information from external sources and accomplishing movement, which is intrinsically associated with neuroplasticity. Long-term sports training improves corticospinal plasticity for motor learning (Hötting and Röder 2013; Singh et al. 2016). Meanwhile, different categories of training such as endurance training and skill training seem to modify the neural system differently (Schlaffke et al. 2014).

Endurance training aims to increase the capacity of continuous motor output by repeating the same movement sequence and, therefore, increasing the efficiency of the movement (Barnes and Kilding 2015). Endurance training increases cognition and neuroplasticity in several brain regions such as the cerebellum, hippocampus, and cerebral cortex via different mechanisms of global affection, such as altered blood volume in the brain and lactate induces elevation of neural growth factors and, but does not alter specific motor map organization or synapse number (synaptogenesis), which is produced by motor learning (Thomas et al. 2012; Taubert et al. 2015). At the spinal level, the excitability of the motor neurons is known to adapt after long-term endurance training (Koceja et al. 2004). Following rapid toe movement, well-trained swimmers demonstrated higher spinal excitability than non-trained individuals (Ogawa et al. 2009), and similar results were later presented in endurance runners (Ogawa et al. 2012). In general, long-term endurance training results in enhanced spinal excitability and provides increased blood flow, and oxygen delivery through angiogenesis, to brain regions, but appears not to participate directly in the modulation of synaptic number or topology (Churchill et al. 2002; Taubert et al. 2015; Chen et al. 2019).

On the other hand, skill training is defined as the acquisition and subsequent refinement of novel movement sequences (Adkins et al. 2006). According to neuroimaging studies, when learning a new specific exercise (e.g., dancing, gymnastics) that triggers motor skill learning processes, greater neural networks are activated within the brain area of the focused task compared with simple movements (e.g., grasping and moving small objects) (Papale and Hooks 2018; Ungerleider et al. 2002). A transcranial magnetic stimulation (TMS)-based experiment showed that skill-trained athletes (dancing, gymnastics, and figure skating) have higher capacity for corticospinal plasticity of the test-relevant muscle (soleus) compared to endurance-trained athletes (cross-country skiing, orienteering), (Kumpulainen et al. 2015). During a single motor skill learning session, there is an increase in corticospinal excitability in the area controlling the corresponding limb (Suzuki et al. 2012), which may be related to decreasing cortical inhibitory neurotransmission (Kolasinski et al. 2019). From previous studies, both single session of skill training seems to modify cortical behavior, and long-term skill training has been shown to result in changes of corticospinal excitability and different cortical responsiveness versus endurance training (Suzuki et al. 2012; Perez et al. 2004).

One method to investigate the effects of interventions on corticospinal plasticity is the stretch reflex test, where neural responses are recorded by surface electromyography (EMG) (Hagbarth 1967). Such a method can be combined with stimulation methods, such as TMS, to determine the contribution of different parts of the neural system to the reflex response (Budini et al. 2017). This stretch reflex contraction occurs naturally in locomotion (i.e., stretch-shortening cycle), but the exposure to and fatigue induced by these actions during training (Avela and Komi 1998) could be hypothesized to be different between skill- and endurance athletes leading to differential corticospinal responses. For a healthy human, an imposed dorsiflexion of the ankle joint leads to a series of clear responses in the EMG of the stretched muscles. The main response, with an onset latency at 40−50 ms, is called the short-latency stretch reflex (SLR) and is mediated by a monosynaptic reflex loop (Fellows et al. 1993; Lee and Tatton 1975). The classic view is that SLR is "purely" under spinal control, whereas only the long-latency reflex (LLR ~ 90 ms) (Dietz et al. 1984) can be influenced by cortex behavior (Petersen et al. 1998) based on latencies likely for a transcortical loop (Evarts 1973). This view is supported by TMS-evoked MEP responses not affecting SLR or medium-latency reflex (MLR), but being facilitated at LLR_2 (after ~ 120 ms) (Taube et al. 2008). Based on the movements performed during training, e.g., endurance athletes performing repetitive stretch–shortening cycles while skill athletes perform regularly changing movement patterns, it is reasonable to assume that skill- and endurance athletes differ in their corticospinal control of movement and that this may become apparent in different phases following muscle stretch.

The aim of the current study was to explore the contribution of/and the underlying corticospinal mechanisms mediating motoneuronal responses to stretch reflex of skill- and endurance-trained athletes by recording MEPs in the soleus muscle. Both skill and endurance training are known to lead to neuronal adaptation and mechanisms of neuronal modulation in short-term sports training has been explored

(Kolasinski et al. 2019), but there are contentions about how the different types of long-term training affects neuroplasticity. Whether muscle stretch influences corticospinal facilitation/inhibition differently in endurance- and skill-trained athletes and, thus the mechanism(s) behind natural movement remain unknown. It was hypothesized that endurance-trained athletes would show more prominent modulation at SLR, while skill-trained athletes would show higher modulation after SLR (SLR + 120 ms).

Methods

Participants and ethical approval

Ten endurance-trained athletes: seven males and three females (mean ± standard deviation: 25 ± 3 years, 70 ± 9 kg, 176 ± 8 cm) and ten skill-trained athletes: 1 male and 9 females (22 ± 3 years, 67 ± 8 kg, 165 ± 8 cm) volunteered to participate in this study. There is evidence showing that no difference exists in resting MEP between males and females (Pitcher et al. 2003). Thus, the different contribution of genders between the groups should not bias the results. Training background information was collected by a questionnaire. The endurance group had trained endurance sports on average 12 ± 3 years for 11 ± 3 h per week. Three participants practiced cross-country skiing, two long-distance running, three triathlon, and two swimming. The skill group had trained skill sports on average 13 ± 3 years for 9 ± 1 h per week. Four participants practiced aerobic gymnastics, three esthetic group gymnastics, two martial arts, and one dancing. None of the participants had any history of neuromuscular or orthopedic diseases and all participants were informed about the procedures and gave written informed consent. The study was approved by the ethics board of the university and the study was performed in conformity with the Declaration of Helsinki. Participants were asked not to train 12 h before measurements and not have any caffeine on the measurement day to avoid interference with the TMS protocol (Turco et al. 2020).

Experimental design

There were two test sessions in this study, a single-pulse session and a paired-pulse session in that order. Before the first testing session, subjects were familiarized with both TMS and the ankle perturbations. On each test occasion, participants were positioned on a custom-built ankle dynamometer (University of Jyvaskylä, Finland) with the hip at 120° and the right knee in a fully extended position of 180°. The right foot ankle was set at 90° and rested on a pedal of the dynamometer. A seat belt restricted movement of the upper body and straps secured the right thigh and foot. Hands were resting and held together during the measurement. After the positioning procedure, the maximum compound action potential (M-max) of the resting soleus muscle was measured first. The participant contracted the ankle submaximally several times for warmup and then performed three maximal isometric plantarflexion actions with 2 min rest between trials. The highest force value from the three trials was considered as the maximal voluntary contraction (MVC). Resting stretch reflex of the soleus muscle (10 trials) and TMS (10 trials) was performed separately to calculate the latency of SLR and the latency of MEP before the experiment trials. This allowed precise arrival of the MEP to the soleus muscle coinciding with the desired stretch reflex latencies for each individual participant. During separate test sessions, MEPs of soleus muscle were elicited in four conditions: at the beginning of the pedal movement (Onset), at SLR (SLR), 120 ms after SLR in a passive condition (p120), and 120 ms after SLR while plantar flexing the ankle to 25% of MVC (a120). All single-pulse trials were performed during one test session and then following 5 days, all paired-pulse trials were performed in a second testing session. One endurance subject completed the paired-pulse but not the single-pulse testing session, meaning $n = 10$ for paired-pulse but $n = 9$ for single-pulse data in the endurance group.

Recordings

EMG measurements were performed by bipolar electrodes (Blue Sensor N, Ag/AgCl, 28 mm^2, Ambu A/S, Ballerup, Denmark) placed 2 cm below the gastrocnemius on the line of the Achilles tendon for soleus muscle (SOL) and over the belly for tibialis anterior muscle (TA) at 1/3 of the distance between the fibula and medial malleolus. A reference electrode was placed on the ipsilateral medial malleolus. Before electrode placement, skin under the electrodes was shaved, abraded with sandpaper, and cleaned with alcohol to reduce the resistance below 5 kΩ. EMG signals were amplified (1000×) by a preamplifier (NL824; Digitimer, Welwyn Garden City, UK), and then band-pass filtered (10−1000 Hz) by another preamplifier (NL900D/NL820A; Digitimer Ltd., UK). Reaction forces from the dynamometer pedal were measured by a piezoelectric crystal transducer (Kistler Holding, Winterthur, Switzerland). EMG was sampled at 5 kHz and reaction forces were sampled at 1 kHz via a 16-bit AD converter (CED power 1401, Cambridge Electronics Design Limited, UK). Spike2 software (CED, Cambridge, UK) was used for all online data collection and offline analyses.

M-max was measured for MEP normalization purposes. M-wave was elicited with an electrical stimulator (DS7AH, Digitimer Ltd., Hertfordshire, UK) in the right soleus muscle by stimulating the posterior tibial nerve. The stimulus was a square-wave pulse of 1 ms duration. The anode electrode was placed above the patella. The cathode was placed in the

popliteal fossa and moved until the best position for eliciting the M-wave with participants in standing position was found. It was then fixed to that position throughout the experiment. The M-max was tested in the experimental position and a further 20% of current was used once a plateau in response was observed (120% M-max stimulation intensity).

TMS stimulations

TMS was delivered using a paired-pulse Magstim 200^2 stimulator with a double cone coil (Magstim, Whitland, UK). To investigate corticospinal excitability, single-pulse TMS with 120, 140, and 150% intensity of resting motor threshold (rMT) were delivered during the four conditions. To investigate intracortical facilitation/inhibition, short-interval intracortical inhibition (SICI), intracortical facilitation (ICF), and long-interval intracortical inhibition (LICI) were measured during the four conditions. SICI was elicited by paired-pulse TMS stimulation with a suprathreshold TMS pulse (120% intensity of rMT) after a subthreshold TMS pulse (80% intensity of rMT) at 3 ms inter-stimulus interval. Similarly, ICF (15 ms inter-stimulus interval) and LICI (50 ms inter-stimulus interval) were produced using the same sub- to suprathreshold intensities (Kujirai et al. 1993; Ziemann et al. 1996; Wassermann et al. 1996).

The optimal TMS stimulus site for the right soleus muscle was located on average 1 cm lateral (left) and 1 cm posterior to the cranial apex. Several stimulations were delivered to determine optimal coil placement and it was then marked by a marker pen on the scalp of the participant. rMT was defined as the lowest stimulus intensity to elicit clear MEPs in three out of five trials. Ten TMS stimulations with 120% of rMT intensity were delivered to calculate the latency of MEP. In the single-pulse session, ten TMS stimulations were given with different intensities (120, 140 and 150% of rMT) randomly for the four conditions. There were 5–8 s intervals between each TMS stimulation in each trial and 2 min rest between conditions. In the paired-pulse session, each condition included ten TMS stimulations with 120% rMT single-pulse as the test MEP, and different paired-pulse paradigms (SICI, ICF and LICI). In passive trials, participants were asked to perform an attention task, which consisted of counting down from 200 silently. In active trials, participants were asked to focus on a line marking 25% MVC on a screen in front of them and perform plantar flexion to follow the force line throughout the trial.

Stretch reflex induced by rapid ankle dorsiflexion

The stretch reflex of the right soleus muscle was elicited by a motor-driven ankle dynamometer (Faculty of Sport and Health Sciences, University of Jyväskylä, Finland) with dorsiflexion (rotational magnitude: 4°, speed: 3.5°rad/s). Stretch reflexes were measured while participants sat relaxed in the dynamometer chair. When ten stretch reflexes were measured, the latency of SLR was calculated in Spike2 software using the average of the waveforms. The latency of SLR was defined as the time between the onset of a digital trigger of pedal movement and the start of the ascending EMG signal.

Data and statistical analyses

In the single-pulse session, the peak-to-peak amplitude of the soleus MEPs and stretch reflex were determined, averaged over the ten trials, and normalized to M-max. MEP amplitude from stimulations with 120, 140 and 150% of rMT, respectively, did not differ between groups. Consequently, during off-line analyses, the data from all stimulus intensities were averaged and defined as 'MEP_{AVG}', thereby increasing the number of trials per condition to 30. MEP_{AVG} at SLR condition was compared with stretch reflex values without stimulation to demonstrate the SR/ MEP_{AVG} ratio. In the paired-pulse session, the peak-to-peak amplitude of conditioned MEP was compared to the test MEP. SICI, ICF, and LICI were expressed as a percentage of the test MEP with the following formula: (conditioned MEP/ test MEP) × 100. A higher ICF percentage represents more facilitation, while higher SICI and LICI percentage values represent less intracortical inhibition when comparing conditions.

Statistical analyses were conducted using IBM SPSS 20.0 (SPSS, Chicago, USA). All variables were processed by log transformation prior to statistical analyses, which resulted in the data being normally distributed as assessed by Shapiro–Wilk's *W* tests. Baseline differences between the groups for training years, MVC, M-max, stretch reflex, and rMT were tested by independent sample *t* tests. TMS-induced responses were assessed by a two-way repeated measures ANOVA with within-subject factor of four levels (Onset, SLR, p120, and a120) and between-subject factor groups of two levels (endurance and skill). Mauchly's test was used to evaluate sphericity, and where the assumption was valid *F* values were reported with sphericity-assumed degrees of freedom and df error (i.e., $F_{(\text{sphericity-assumed df, df error})}$). MEP_{AVG} and LICI violated the assumption of sphericity and so *F* values were reported along with their Greenhouse–Geisser adjustments (i.e., $F_{(\text{Greenhouse–Geisser adjusted df, df error})}$). When a significant *F* value for Condition was observed, Bonferroni post hoc tests were run for the four conditions (Onset, SLR, p120, and a120). Effect sizes for the ANOVA main effects are reported as partial eta squared (η_p^2), where 0.02, 0.13, 0.26 are considered small, medium and large, respectively. Correlations between MEP_{AVG} and stretch reflex, MVC and MEP_{AVG} were analyzed for non-log transformed MEP values and stretch reflex values using the Spearman's rank correlation test. The

significance level was set at $P = 0.05$ and all results were displayed as mean ± SD.

Results

There were no differences between groups in training years (endurance group: 11 ± 3 years; skill group: 13 ± 3 years, $P = 0.330$), rMT (endurance group: 54 ± 7% stimulator output; skill group: 47 ± 8% stimulator output, $P = 0.055$) or MVC (endurance group: 297 ± 67 Nm; skill group: 227 ± 86 Nm, $P = 0.140$).

Single-pulse MEPs

A significant main effect for condition was observed (Fig. 2, $F_{(1.971, 33.51)} = 83.908$, $P < 0.001$, $\eta_p^2 = 0.832$), but there was no main effect for group (Fig. 1C $F_{(1, 17)} = 0.532$, $P = 0.476$, $\eta_p^2 = 0.030$) or group × condition interaction ($F_{(1.971, 33.51)} = 1.88$, $P = 0.169$, $\eta_p^2 = 0.100$). MEP_{AVG} in the endurance group was 1.6 ± 0.8%M-max (Onset), 10.7 ± 9.4%M-max (SLR), 2.1 ± 1.3%M-max (p120), and 10.0 ± 5.0%M-max (a120). MEP_{AVG} in the skill group was 1.7 ± 1.0%M-max (Onset), 14.4 ± 6.4%M-max (SLR), 2.7 ± 1.8%M-max (p120), and 8.9 ± 5.9%M-max (a120).

Significant differences over time (i.e., between conditions) were observed from Onset to SLR, SLR to p120, p120 to a120 and Onset to a120 for both groups (Fig. 2, $P < 0.01$). In addition, there was a significant difference between Onset and p120 ($P = 0.005$), and SLR and a120 ($P = 0.024$) in the skill group only (Fig. 2).

There was a strong correlation between MVC and Onset MEP_{AVG} in the skill group ($r = 0.790$, $P = 0.007$, $N = 10$, Fig. 3), but no relationship was observed for the endurance group ($r = -0.417$, $P = 0.265$, $N = 9$).

SR/ MEP_{AVG} ratio revealed that the increase in MEP_{AVG} from Onset to SLR was partly affected by the presence of stretch reflex, and there were no differences between two groups (endurance = 1.8 ± 0.8; skill = 1.3 ± 1.0). However, the correlation of MEP_{AVG} and stretch reflex showed a strong relationship in the endurance group (Fig. 4, $r = 0.733$, $P = 0.025$, $N = 9$), but not in the skill group (Fig. 4, $r = 0.212$, $P = 0.556$, $N = 10$).

Paired-pulse MEPs

SICI showed a significant main effect for condition ($F_{(3, 42)} = 5.154$, $P = 0.004$, $\eta_p^2 = 0.269$), but not between groups ($F_{(1, 14)} = 0.409$, $P = 0.533$, $\eta_p^2 = 0.028$) or group × condition interaction ($F_{(3, 42)} = 1.074$, $P = 0.370$, $\eta_p^2 = 0.071$). Post hoc (Bonferroni) tests for SICI did not reveal significant differences between conditions for each group separately (endurance group: Onset vs. SLR $P = 1.000$; skill group: e.g., Onset vs. SLR $P = 0.081$).

ICF showed a significant main effect for condition ($F_{(3, 45)} = 4.64$, $P = 0.007$, $\eta_p^2 = 0.236$) and for group ($F_{(1, 15)} = 6.163$, $P = 0.025$, $\eta_p^2 = 0.291$). There was no group × condition interaction ($F_{(3, 45)} = 0.455$, $P = 0.715$, $\eta_p^2 = 0.029$). However, post hoc tests for ICF did not show significant differences between conditions in either group.

There were no main effects observed for LICI (condition: $F_{(1.892, 28.386)} = 2.186$, $P = 0.133$, $\eta_p^2 = 0.127$; group: $F_{(1, 15)} = 3.925$, $P = 0.066$, $\eta_p^2 = 0.207$) (Table 1).

Discussion

This study investigated changes in corticospinal excitability at different latencies relative to rapid dorsiflexion between skill- and endurance-athlete groups. As planned, the passive ankle dorsiflexion led to a stretch reflex in the soleus muscle, which is an important part of proprioceptive processing and results in afferent feedback to both spinal and supraspinal centers. It was hypothesized that the endurance-trained athletes would show more prominent corticospinal modulations at SLR, while skill-trained athletes would show higher modulation during the period where a supraspinal reaction to the movement is prominent (SLR + 120 ms). In line with the hypothesis, the present study showed higher MEPs at p120 in the skill group. However, in opposition to the hypothesis, the endurance group did not demonstrate more prominent corticospinal modulation at SLR. Finally, MVC was strongly correlated with resting MEPs in the skill group, which was not the case in the endurance group. On the other hand, a strong correlation between stretch reflex and MEPs was observed at SLR in the endurance group but not in the skill group.

In the present study, MEPs at p120 were higher than at Onset only in the skill group. p120 took place 120 ms after SLR, which was approximately at the latency of the second long-latency reflex (LLR_2) reported by Taube et al. (2008). The increased MEPs at LLR_2 indicated modulation of corticospinal excitability, while reduced H-reflex at the same time point suggested that this modulation was cortical in nature (Taube et al. 2008). At this phase, there is sufficient time to allow different pathways, including cortical and spinal, to contribute to the recorded MEPs' facilitation and inhibition. Greater MEPs in the skill group 120 ms after SLR suggests that they have a greater or more long-lasting facilitation of corticospinal excitability than endurance-trained athletes after rapid ankle dorsiflexion, even in a passive condition. One important suggestion on the mechanisms of motor learning-induced cortical plasticity is that synaptic connections at the cortical level are modified through LTP (Friedman and Donoghue 2000).

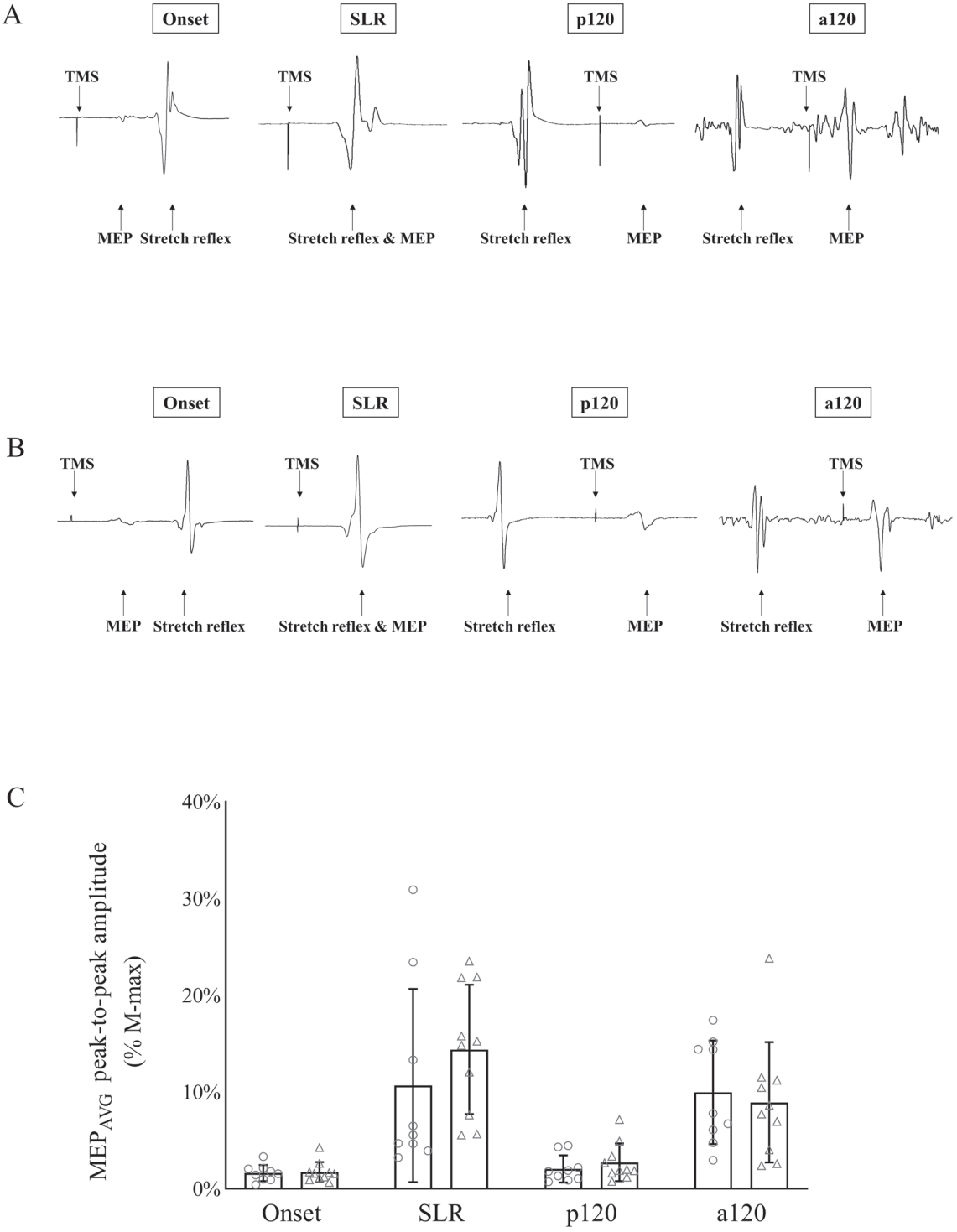

Fig. 1 Raw EMG signals showing stretch reflex responses and MEP induced during the four conditions (Onset, SLR, p120, a120) from single TMS trials in one endurance-trained subject (**A**) and skill-trained subject (**B**). Group-level MEP_{AVG} responses during the four conditions (**C**). There was no difference shown in MEP_{AVG} between groups. Individual values are shown by symbols (open circle = endurance group, open triangle = skill group)

LTP is a prerequisite for synaptogenesis and, thus, skill training has, indeed, been shown to lead to synaptogenesis of the motor cortex (Adkins et al. 2006). PAS, which is an artificial intervention pairing electrical stimulation of somatosensory nerves and TMS of the corresponding area of the motor cortex, can produce LTP-like plasticity in the

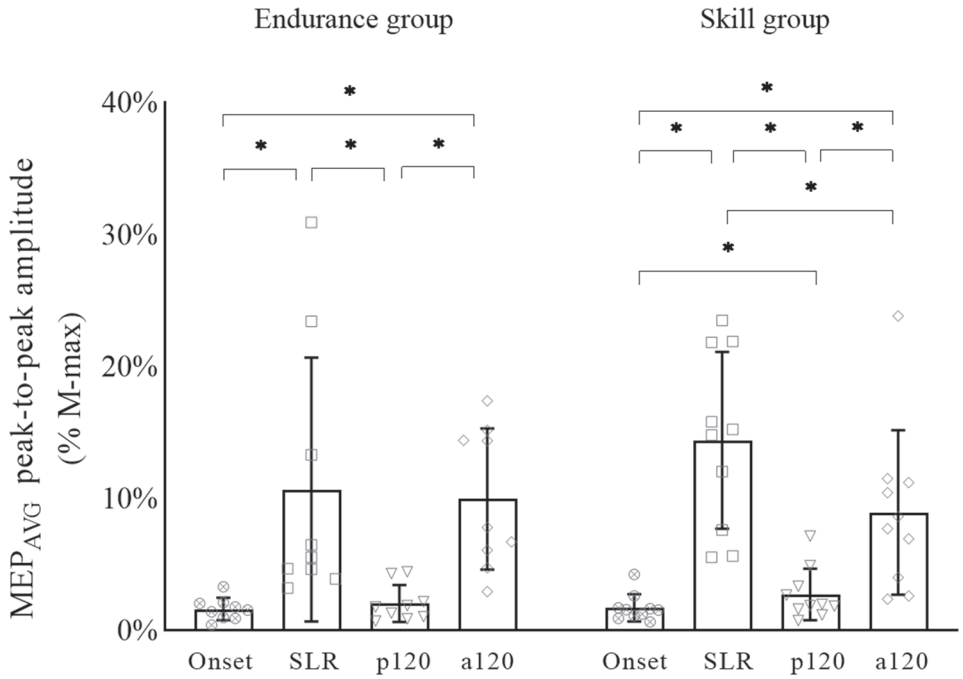

Fig. 2 Group-level MEP_{AVG} responses during the four conditions and within-subject statistical comparisons. In the skill group, there were differences between each condition. There was no difference shown in the endurance group between Onset and p120 or SLR and a120 conditions. Values of all participants are shown by symbols for each condition ('' = Onset, open square = SLR, open inverted triangle = p120, open diamond = a120). asterisk = significant difference ($P < 0.05$) between conditions

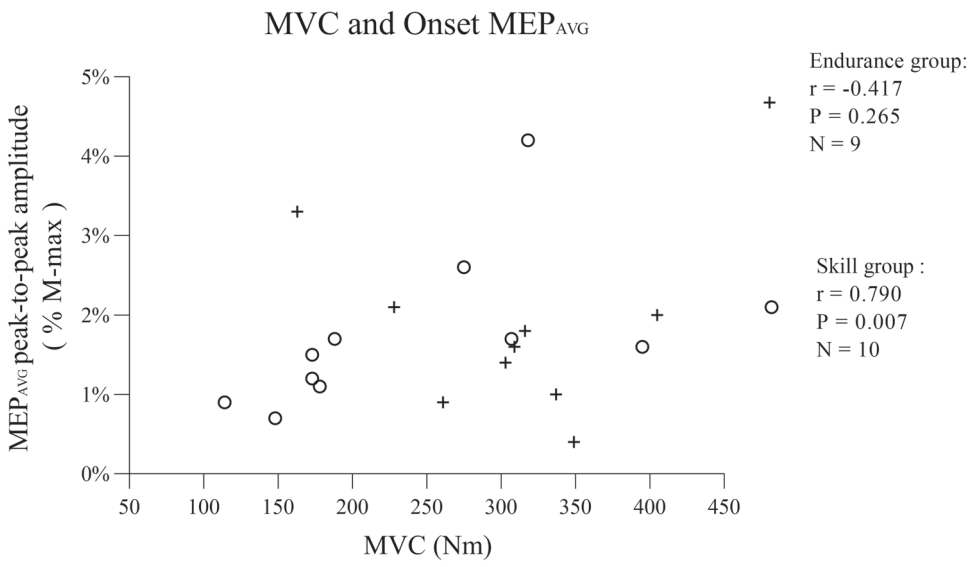

Fig. 3 Scatter plot of MVC (Nm) and Onset MEP_{AVG} (%M-max) in two groups (endurance group = '+', skill group = 'O'). Data from the skill group ($N = 10$) showed a positive correlation ($P = 0.007$). Data from the endurance group ($N = 9$) did not reach statistical significance ($P = 0.265$)

synapse. The amount of PAS-induced LTP-like plasticity increase depends on the number of active synapses. Therefore, a PAS intervention has been used as a measure of corticospinal plasticity (Lazzaro et al. 2009). In a study by Kumpulainen et al. (2015), PAS induced increased MEP in skill athletes, but not in endurance athletes or untrained adults, revealing higher corticospinal plasticity and greater synaptogenesis at the cortical level of skill-trained athletes. Previous findings suggest that skill training results in increasing adaptability of corticospinal plasticity and, thus, skill-trained athletes may preferentially rely more on cortical sources for voluntary movement as was the case in the present study at p120 and a120 condition.

It is important to note that there was no evidence of voluntary muscular activity prior to the stretch or after the stretch reflex response had abated in the passive trials (i.e., the muscle was silent). We speculate that this was a 'priming' mechanism in the skill group to modulate top-down responses by motor programs stored in the central nervous system after the rapid perturbation (Pierrot-Deseilligny & Burke, 2005). Possible explanations are as follows: first, for skill-trained athletes, there are more voluntary movement changes in training and competition, which need to be controlled by the motor cortex, cerebella, or somatosensory association cortex (Kurtzer 2015; Suminski et al. 2007). Second, central control has been exposited in the processing

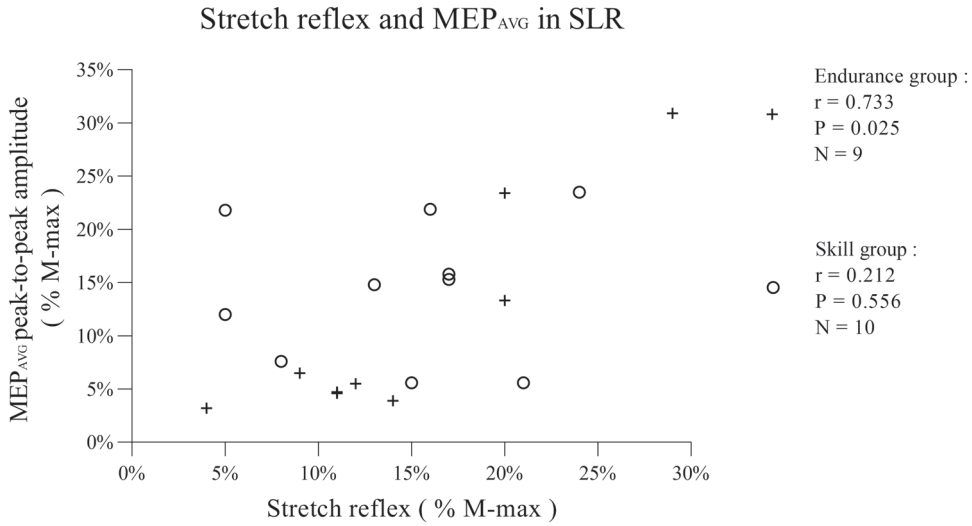

Fig. 4 Scatter plot of stretch reflex (%M-max) and MEP_{AVG} (%M-max) in two groups (Endurance group = '+', Skill group = 'O'). There was a significant positive correlation observed in the endurance group ($P = 0.025$), but not in the skill group ($P = 0.556$)

Table 1 SICI, ICF and LICI at different conditions as a percentage of the test MEP (mean ± SD)

	Paired pulse	Onset (%)	SLR (%)	p120 (%)	a120 (%)
Endurance group	SICI	50.6 ± 21.0	67.3 ± 23.0	69.4 ± 32.3	94.2 ± 35.7
	ICF	166.2 ± 90.2	97.9 ± 32.3	149.6 ± 33.8	115.2 ± 34.9
	LICI	114.3 ± 32.8	106.9 ± 29.8	141.8 ± 70.9	104.0 ± 28.2
Skill group	SICI	46.4 ± 27.9	114.8 ± 39.7	69.2 ± 37.3	86.6 ± 26.9
	ICF	185.0 ± 51.3	139.5 ± 56.4	157.7 ± 42.9	130.4 ± 35.6
	LICI	130.4 ± 45.8	128.1 ± 23.6	146.7 ± 44.8	108.9 ± 43.9

of, e.g., expected postural response (Horak et al. 1989). Thus, following the perturbation, the skill athletes may have been 'primed' for a voluntary response after the rapid ankle movement. In support of this contention, a strong positive correlation between MVC and resting MEPs was observed in the skill group only. MVC force is dependent on recruitment of motor units and the force-producing capacity of muscle fibers. A higher MEP value is related to higher excitability of motor cortical output cells and motor neurons during voluntary contraction (Taylor et al. 2002). Therefore, for skill-trained athletes, corticospinal excitability plays an important part in voluntary movement and is possibly observed in our enhanced p120 MEP_{AVG} during the phase where supraspinal reaction would be possible as a cortical adaptation to a top-down strategy in response to rapid ankle dorsiflexion.

Weaker, but supporting, evidence for greater reliance on cortical involvement in skill athletes was found in ICF. During all conditions, average ICF values were higher in the skill than endurance group and a significant main effect for the group was observed, indicating that ICF was higher. Nevertheless, there were no (pairwise) statistical between-group differences at any condition, which dilutes confidence in making such inferences. The cortical mechanisms of ICF are not fully clear. Increased ICF is known to be strongly influenced by decreased GABAergic inhibition or a separate increase in glutamatergic facilitation (McGinley et al. 2010; Ziemann 2003). Since the two receptors of GABAergic inhibition, $GABA_A$ and $GABA_B$, influence SICI and LICI, respectively (McDonnell et al. 2006; Kujirai et al. 1993), and that no differences in SICI or LICI were observed, it may be that potentially higher ICF in skill athletes was due to glutamatergic facilitation. While this is speculative, glutamatergic facilitation is one of the important molecular mechanisms for LTP, and as such, a greater ICF would support Kumpulainen et al. (2015) findings that skill athletes have higher corticospinal plasticity than endurance athletes.

Even though LICI showed facilitation (i.e. > 100% of test MEP) in the present study, this has been previously shown to occur when 50 ms inter-stimulus interval is employed in the assessment of LICI (Valls-Solé et al. 1992; Di Lazzaro et al. 2002). This presumably occurs because of increased post-synaptic excitability elicited by the conditioning stimulus or stimulus-induced activity in subcortical regions (Bolden et al. 2017; Valls-Solé et al. 1992; Di Lazzaro et al. 2002). However, the present study is not able to determine the precise mechanisms underpinning this finding.

At SLR, SR/ MEP_{AVGs} ratio was used to normalize MEPs with stretch reflex responses to reveal whether TMS has an additive effect on EMG amplitude, which was expected to demonstrate a between-group difference in the present

study. However, although the ratio in each group (endurance group = 1.8, skill group: = 1.3) was raised (i.e., > 1), no significant between-group differences were observed. In a previous study between endurance athletes and a non-trained group, endurance runners demonstrated higher monosynaptic reflex excitability by enhanced stretch reflex response, which highlights enhanced modulation of spinal excitability after long-term endurance training (Ogawa et al. 2012). In the present study, there were also no between-group differences observed for SICI or LICI at SLR. These findings imply that, in the non-motor control task (i.e., resting muscle), corticospinal modulation did not affect SLR differently between training groups.

On the other hand, SLR MEP_{AVG} showed a strong correlation with stretch reflex in the endurance group only. The monosynaptic spinal loop likely contributes more to corticospinal excitability than the supraspinal loop in the fast response phase after rapid ankle dorsiflexion. Possible reasons for the relationship in endurance athletes are as follows: training that includes repetitive stretch–shortening cycle actions was the predominant training form of eight out of this study's ten endurance athletes (cross-country skiing, long-distance running, and triathlon). It is widely known that training-induced muscle and spinal motor neuron adaptation occurs from such a stimulus (Churchill et al. 2002; Taubert et al. 2015; Avela and Komi 1998). In rapid ankle movement, i.e., stance and swing phase in running, fast spinal loop modulation helps to keep balance during body oscillations since modulations via cortical processes would be too slow (Tahayori and Koceja 2012). In agreement with this hypothesis, a study performed using the H-reflex method revealed that after a long period of typical endurance training (well-trained swimmers in the study), athletes demonstrated greater spinal excitability (i.e., increased H-reflex) than non-trained individuals (Ogawa et al. 2009). However, the present study was unable to determine whether spinal excitability is indeed higher in endurance training athletes compared with skill training athletes through direct comparisons. There were some individuals in the endurance group seemingly specifically adapted for high excitability post-stretch from our results, and perhaps the lack of between-group differences may have been due to high within-group variance and low sample size.

Some study limitations should be considered. Due to COVID-19 and quarantine policies of the laboratory, we were only able to recruit and complete testing for 20 participants. Therefore, this sample size may not have been sufficient to determine between-group differences considering the large variabilities of MEP amplitudes when responding to rapid ankle dorsiflexion. Although it is not possible to perform a priori sample size estimation for novel measurements, the convention within the field is that typical sample sizes per group are approximately 15 (e.g., Kumpulainen et al. 2015; Wächli et al. 2017). Therefore, only the clearest differences in corticospinal plasticity between skill- and endurance athletes may have reached the level of statistical significance in the present study. This may explain, for example, significant main effects for IC, but no significant differences when post hoc tests were performed to ascertain specific differences between conditions. It is also possible that different loading patterns may induce differences in corticospinal plasticity between sports. For example, the two swimmers included in the present study may have added variance to the results given that their sport does not include stretch–shortening cycle actions in the triceps surae muscles through repetitive ground contact as in running. We suggest that sport and training characteristics should be considered when recruiting athletes as participants if the research involves corticospinal responses during motor tasks. Finally, information regarding the exact phase of the menstrual cycle was not collected in the present study. It is currently debatable whether testing during different menstrual cycle phase would influence the data (Ansdell et al. 2019; El-Sayes et al. 2019), but it may be pertinent to consider in the future.

Conclusion

This study observed a similar pattern of corticospinal modulation, as revealed by MEP_{AVG}, in long-term trained endurance- and skill athletes during and following rapid ankle dorsiflexion. However, corticospinal excitability (MEP_{AVG}) was enhanced 120 ms after muscle stretch in skill-trained athletes, together suggesting a 'priming' of corticospinal excitability during the supraspinal reaction phase. Our skill-trained athletes demonstrated a positive relationship between MEP amplitude and MVC, supporting the view that some reliance on corticospinal excitability for voluntary action is particularly important for skill athletes.

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Authors' contributions NH, JA, JMP, and SW: contributed to the conception and design of the study. NH and JA: organized the database. NH, DJK, and SW: performed and oversaw the statistical analyses. NH and SW: drafted the original manuscript. All authors read, revised, and approved the submitted version of the manuscript.

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Availability of data and material All data and materials of this study support published claims and comply with field standards.

Code availability Not applicable.

Declarations

Conflicts of interest The authors declare no conflicts of interest.

Ethics approval This study is approved by the ethics board of the university and the study was performed in conformity with the Declaration of Helsinki.

Consent to participate All participants were informed about the procedures and gave written informed consent before the experiment.

Consent for publication All participants gave permission to publish their data to the journal.

References

Adkins DL, Boychuk J, Remple MS, Kleim JA (2006) Motor training induces experience-specific patterns of plasticity across motor cortex and spinal cord. J Appl Physiol 101(6):1776–1782. https://doi.org/10.1152/japplphysiol.00515.2006

Ansdell P, Brownstein CG, Škarabot J (2019) Menstrual cycle-associated modulations in neuromuscular function and fatigability of the knee extensors in eumenorrheic women. J Appl Physiol 126(6):1701–1712. https://doi.org/10.1152/japplphysiol.01041.2018

Avela J, Komi PV (1998) Interaction between muscle stiffness and stretch reflex sensitivity after long-term stretch-shortening cycle exercise. Muscle Nerve 21(9):1224–1227. https://doi.org/10.1002/(sici)1097-4598(199809)21:9%3c1224::aid-mus19%3e3.0.co;2-r

Barnes KR, Kilding AE (2015) Running economy: measurement, norms, and determining factors. Sports Med Open 1(1):8. https://doi.org/10.1186/s40798-015-0007-y

Bolden LB, Griffis JC, Pati S, Szaflarski JP (2017) Cortical excitability and neuropsychological functioning in healthy adults. Neuropsychologia 102:190

Budini F, Gallasch E, Christova M, Rafolt D, Rauscher AB, Tilp M (2017) One minute static stretch of plantar flexors transiently increases H reflex excitability and exerts no effect on corticospinal pathways. Exp Physiol 102(8):901–910. https://doi.org/10.1113/ep086374

Chen K, Ge R, Cheng Y, Dai Y (2019) Three-week treadmill training changes the electrophysiological properties of spinal interneurons in the mice. Exp Brain Res 237(11):2925–2938. https://doi.org/10.1007/s00221-019-05647-3

Churchill JD, Galvez R, Colcombe S, Swain RA, Kramer AF, Greenough WT (2002) Exercise, experience and the aging brain. Neurobiol Aging 23(5):941–955. https://doi.org/10.1016/s0197-4580(02)00028-3

Di Lazzaro V, Oliviero A, Mazzone P, Pilato F, Saturno E, Insola A, Visocchi M, Colosimo C, Tonali PA, Rothwell JC (2002) Direct demonstration of long latency cortico-cortical inhibition in normal subjects and in a patient with vascular parkinsonism. Clin Neurophysiol 113(11):1673–1679. https://doi.org/10.1016/s1388-2457(02)00264-x

Dietz V, Quintern J, Berger W (1984) Cerebral evoked potentials associated with the compensatory reactions following stance and gait perturbation. Neurosci Lett 50(1):181–186. https://doi.org/10.1016/0304-3940(84)90483-X

El-Sayes J, Turco CV, Skelly LE, Nicolini C, Fahnestock M, Gibala MJ, Nelson AJ (2019) The effects of biological sex and ovarian hormones on exercise-induced neuroplasticity. Neuroscience 410:29–40. https://doi.org/10.1016/j.neuroscience.2019.04.054

Evarts EV (1973) Motor cortex reflexes associated with learned movement. Science 179(4072):501–503. https://doi.org/10.1126/science.179.4072.501

Fellows SJ, Dömges F, Töpper R, Thilmann AF, Noth J (1993) Changes in the short- and long-latency stretch reflex components of the triceps surae muscle during ischaemia in man. J Physiol 472(1):737–748. https://doi.org/10.1113/jphysiol.1993.sp019970

Friedman D, Donoghue JP (2000) Learning-induced LTP in neocortex. Science 290(5491):533–536

Hagbarth KE (1967) EMG studies of stretch reflexes in man. Electroencephalogr Clin Neurophysiol: Suppl 25:74–79

Horak FB, Diener HC, Nashner LM (1989) Influence of central set on human postural responses. J Neurophysiol 62(4):841–853. https://doi.org/10.1152/jn.1989.62.4.841

Hötting K, Röder B (2013) Beneficial effects of physical exercise on neuroplasticity and cognition. Neurosci Biobehav Rev 37(9):2243–2257. https://doi.org/10.1016/j.neubiorev.2013.04.005

Kantak SS, Stinear JW, Buch ER, Cohen LG (2012) Rewiring the brain: potential role of the premotor cortex in motor control, learning, and recovery of function following brain injury. Neurorehabil Neural Repair 26(3):282–292. https://doi.org/10.1177/1545968311420845

Koceja DM, Davison E, Robertson CT (2004) Neuromuscular characteristics of endurance- and power-trained athletes. Res Q Exerc Sport 75(1):23–30. https://doi.org/10.1080/02701367.2004.10609130

Kolasinski J, Hinson EL, Divanbeighi Zand AP, Rizov A, Emir UE, Stagg CJ (2019) The dynamics of cortical GABA in human motor learning. J Physiol 597(1):271–282. https://doi.org/10.1113/JP276626

Kujirai T, Caramia MD, Rothwell JC, Day BL, Thompson PD, Ferbert A, Wroe S, Asselman P, Marsden CD (1993) Corticocortical inhibition in human motor cortex. J Physiol 471(1):501–519. https://doi.org/10.1113/jphysiol.1993.sp019912

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. Eur J Appl Physiol 115(5):1107–1115

Kurtzer IL (2015) Long-latency reflexes account for limb biomechanics through several supraspinal pathways. Front Integr Neurosci. https://doi.org/10.3389/fnint.2014.00099

Lazzaro VD, Dileone M, Pilato F, Profice P, Oliviero A, Mazzone P, Insola A, Capone F, Ranieri F, Tonali PA (2009) Associative motor cortex plasticity: direct evidence in humans. Cereb Cortex 19(10):2326–2330

Lee RG, Tatton WG (1975) Motor responses to sudden limb displacements in primates with specific CNS lesions and in human

patients with motor system disorders. Can J Neurol Sci Le J Can Des Sci Neurol 2(3):285–293. https://doi.org/10.1017/s0317167100020382

McDonnell MN, Orekhov Y, Ziemann U (2006) The role of GABA(B) receptors in intracortical inhibition in the human motor cortex. Exp Brain Res 173(1):86–93. https://doi.org/10.1007/s00221-006-0365-2

McGinley M, Hoffman RL, Russ DW, Thomas JS, Clark BC (2010) Older adults exhibit more intracortical inhibition and less intracortical facilitation than young adults. Exp Gerontol 45(9):671–678. https://doi.org/10.1016/j.exger.2010.04.005

Ogawa T, Kim GH, Sekiguchi H, Akai M, Suzuki S, Nakazawa K (2009) Enhanced stretch reflex excitability of the soleus muscle in experienced swimmers. Eur J Appl Physiol 105(2):199–205. https://doi.org/10.1007/s00421-008-0890-8

Ogawa T, Kawashima N, Suzuki S, Nakazawa K (2012) Different modulation pattern of spinal stretch reflex excitability in highly trained endurance runners. Eur. J. Appl Physiol 112(10):3641–3648. https://doi.org/10.1007/s00421-012-2351-7

Papale AE, Hooks BM (2018) Circuit changes in motor cortex during motor skill learning. Neuroscience 368:283–297. https://doi.org/10.1016/j.neuroscience.2017.09.010

Pascual-Leone A, Amedi A, Fregni F, Merabet LB (2005) The plastic human brain cortex. Annu Rev Neurosci 28:377–401. https://doi.org/10.1146/annurev.neuro.27.070203.144216

Perez MA, Lungholt BK, Nyborg K, Nielsen JB (2004) Motor skill training induces changes in the excitability of the leg cortical area in healthy humans. Exp Brain Res 159(2):197–205

Petersen N, Christensen LOD, Morita H, Sinkjær T, Nielsen J (1998) Evidence that a transcortical pathway contributes to stretch reflexes in the tibialis anterior muscle in man. J Physiol 512(1):267–276. https://doi.org/10.1111/j.1469-7793.1998.267bf.x

Pitcher JB, Ogston KM, Miles TS (2003) Age and sex differences in human motor cortex input-output characteristics. J Physiol 546(Pt 2):605–613. https://doi.org/10.1113/jphysiol.2002.029454

Schlaffke L, Lissek S, Lenz M, Brüne M, Juckel G, Hinrichs T, Platen P, Tegenthoff M, Schmidt-Wilcke T (2014) Sports and brain morphology—A voxel-based morphometry study with endurance athletes and martial artists. Neuroscience 259:35–42. https://doi.org/10.1016/j.neuroscience.2013.11.046

Singh AM, Neva JL, Staines WR (2016) Aerobic exercise enhances neural correlates of motor skill learning. Behav Brain Res 301:19–26. https://doi.org/10.1016/j.bbr.2015.12.020

Suminski AJ, Rao SM, Mosier KM, Scheidt RA (2007) Neural and electromyographic correlates of wrist posture control. J Neurophysiol 97(2):1527–1545. https://doi.org/10.1152/jn.01160.2006

Suzuki M, Kirimoto H, Onishi H, Yamada S, Tamaki H, Maruyama A, Yamamoto J-i (2012) Reciprocal changes in input–output curves of motor evoked potentials while learning motor skills. Brain Res 1473:114–123. https://doi.org/10.1016/j.brainres.2012.07.043

Tahayori B, Koceja D (2012) Activity-dependent plasticity of spinal circuits in the developing and mature spinal cord. Neural Plast 2012:964843. https://doi.org/10.1155/2012/964843

Taube W, Leukel C, Schubert M, Gruber M, Rantalainen T, Gollhofer A (2008) Differential modulation of spinal and corticospinal excitability during drop jumps. J Neurophysiol 99(3):1243–1252. https://doi.org/10.1152/jn.01118.2007

Taubert M, Villringer A, Lehmann N (2015) Endurance exercise as an "endogenous" neuro-enhancement strategy to facilitate motor learning. Front Hum Neurosci. https://doi.org/10.3389/fnhum.2015.00692

Taylor JL, Petersen NT, Butler JE, Gandevia S (2002) Corticospinal transmission after voluntary contractions. Adv Exp Med Biol 508:435–441. https://doi.org/10.1007/978-1-4615-0713-0_49

Thomas AG, Dennis A, Bandettini PA, JohansenBerg H (2012) The effects of aerobic activity on brain structure. Front Psychol 3:86

Turco CV, Arsalan SO, Nelson AJ (2020) The influence of recreational substance use in tms research. Brain Sci 10(10):751. https://doi.org/10.3390/brainsci10100751

Ungerleider LG, Doyon J, Karni A (2002) Imaging brain plasticity during motor skill learning. Neurobiol Learn Mem 78(3):553–564. https://doi.org/10.1006/nlme.2002.4091

Valls-Solé J, Pascual-Leone A, Wassermann EM, Hallett M (1992) Human motor evoked responses to paired transcranial magnetic stimuli. Electroencephalogr Clin. Neurophysiol 85(6):355–364. https://doi.org/10.1016/0168-5597(92)90048-g

Wassermann EM, Samii A, Mercuri B, Ikoma K, Oddo D, Grill SE, Hallett M (1996) Responses to paired transcranial magnetic stimuli in resting, active, and recently activated muscles. Exp Brain Res 109(1):158–163. https://doi.org/10.1007/bf00228638

Ziemann U (2003) Pharmacology of TMS. Suppl Clin Neurophysiol 56:226–231

Ziemann U, Rothwell JC, Ridding MC (1996) Interaction between intracortical inhibition and facilitation in human motor cortex. J Physiol 496(3):873–881. https://doi.org/10.1113/jphysiol.1996.sp021734