FEASIBILITY OF A NOVEL MEG-COMPATIBLE PROPRIOCEPTIVE STIMULATOR OF THE KNEE JOINT

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Johdanto: Proprioseptisella afferenssilla tarkoitetaan proprioseptoreista (mm. lihasspindelit) aivoihin saapuvaa informaatiota kehon asennoista ja liikkeistä, ja se vaikuttaa merkittävästi liikkeenohjaukseen. Liikkeenohjaukseen kuuluu olennaisesti myös venytysrefleksien, kuten patellaarirefleksin toiminta. Patellaarirefleksin proprioseptisen afferenssia ei kuitenkaan ole tutkittu aivotasolla. Magnetoenkefalografialla (MEG) voidaan tutkia perifeerisen proprioseptisen stimuloinnin aikaansaamaa kortikaalista prosessointia, mutta MEG-ympäristö vaatii erityishuomioita käytettäviltä stimulointilaitteilta. Tutkimuksen tarkoituksena oli selvittää MEG:n kanssa yhteensopivan polven proprioseptisen stimulaattorin (refleksivasaran) soveltuvuutta tutkimuskäytössä sekä tuottaa alustavaa tietoa patellaarirefleksin proprioseptisen afferenssin prosessoinnista aivokuorella.

Menetelmät: Poikkileikkaustutkimukseen osallistui 15 perustervettä vapaaehtoista (28,1 ± 5,3 vuotta, 6 naista). Tutkittavien dominantin jalan (Waterloo-jalkaisuuskysely, 14 oikeajalkaista) patellajännettä stimuloitiin kahdella eri intensiteetillä, jota muutettiin refleksivasaran pudotuskorkeutta säätämällä (high- ja low-mittaus). Stimulusten kinematiikkaa tutkittiin refleksivasaraan kiinnitetyllä kiihtyvyysanturilla. Stimulaatioista seuranneita aivovasteita kuvannettiin MEG:lla. Patellaarirefleksin tuottamaa lihasaktiivisuutta rekisteröitiin pintaelektromyografialla (EMG) ja polven ojentajalihasten (m. vastus lateralis ja m. vastus medialis) reflekseistä seuranneet voimanykäykset mitattiin voima-anturilla.

Tulokset: Neljä koehenkilöä jätettiin pois lopullisista analyyseistä MEG- tai EMG-signaaleissa esiintyneiden häiriöiden vuoksi. Näin ollen 11 tutkittavalta (73 %) onnistuttiin rekisteröimään selkeät refleksi- ja aivovasteet. MEG-vasteet paikantuivat hypoteesin mukaisesti niille MEG-sensoreille, jotka sijaitsivat kontralateraalisesti somatosensorisen aivokuoren kohdalla. Stimulusten piikkimagnitudit pysyivät tasaisena läpi mittausten variaatiokertoimella tarkasteltuna ryhmätasolla (high: 11 % ja low: 10 %). Stimulusintensiteetin muutos vaikutti ainoastaan MEG-vasteiden piikkiamplitudeihin (high: 203 ± 57 fT/cm ja low: 174 ± 39 fT/cm, $p = 0,02$). MEG-vasteissa oli vähemmän ryhmätason vaihtelua (high: 28 % ja low: 22 %) verrattuna refleksivasteisiin (≥ 68 %). MEG-vasteet eivät korreloineet EMG- tai voimavasteiden kanssa ($p > 0,05$).

Johtopäätökset: Tutkimus on ensimmäinen, joka on tarkastellut patellaarirefleksin proprioseptista afferenssia aivokuorella. Polven proprioseptinen stimulaattori vaikuttaa soveltuvan tutkimuskäyttöön, sillä stimulaattori tuotti kuvannettavia lihas- ja aivotason vasteita. MEG-vasteet sisälsivät vähemmän vaihtelua kuin lihastason vasteet ja stimulusintensiteetin vaikutus havaittiin ainoastaan MEG-vasteissa. MEG vaikuttaa olevan soveltuva menetelmä venytysrefleksien proprioseptisen afferenssin tutkimiseen.

Asiasanat: magnetoenkefalografia, proprioseptiikka, sensorimotorinen, venytysrefleksi

ABSTRACT

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Introduction: Proprioceptive afference refers to the transmission of information about body's position and movements from proprioceptors to the brain. This information is crucial for sensorimotor control, with muscle stretch reflexes, such as the patellar tendon reflex, playing essential roles. Magnetoencephalography (MEG) provides a non-invasive means to examine cortical processing of the proprioceptive afference to peripheral stimuli. While evoked cortical responses have been investigated for most sensory domains, a gap exists in lower-limb proprioception research, particularly at the knee joint. In addition, the MEG environment requires special attention from the stimulation devices used, since stimulators must be designed to avoid generating interference with magnetic signals. Therefore, the objective for this study was to test the feasibility of a MEG-compatible proprioceptive stimulator (reflex hammer) of the knee joint and to provide preliminary insights into the cortical processing of proprioceptive afference of the patellar tendon reflex.

Methods: 15 healthy volunteers participated in the cross-sectional study (28.1 ± 5.3 years, 6 females). The patellar tendon of the dominant leg (Waterloo footedness questionnaire, 14 right-footed) was stimulated at two different intensities, adjusted by altering the height from which the reflex hammer was dropped (high and low measurements). The kinematics of the stimuli were examined with an accelerometer attached to the stimulator. Brain responses following the stimulations were imaged with MEG. Muscle activity resulting from the patellar tendon reflex was recorded using surface electromyography (EMG), and force responses of the knee extensor muscles (vastus lateralis and vastus medialis) were measured with a force transducer.

Results: Four participants were excluded from the final analyses due to disturbances in the MEG or EMG signals. Thus, clear reflex and brain responses were successfully recorded from 11 participants (73%). Cortical responses were localized to the MEG sensors over the somatosensory cortex, as hypothesized. The peak magnitudes of the stimuli remained consistent throughout the measurements, as assessed by the group-level coefficient of variation (high: 11% and low: 10%). When comparing the results of high and low measurements, a significant change was only observed in the amplitudes of MEG responses (high: 203 ± 57 fT/cm and low: 174 ± 39 fT/cm, $p=0.02$). There was less group-level variation in the MEG responses (high: 28% and low: 22%) compared to the reflex responses (≥68%). The MEG responses did not correlate with the EMG or force responses ($p>0.05$).

Conclusions: This study is the first to examine the proprioceptive afference of the patellar tendon reflex in the cortex. The proprioceptive stimulator appears to be feasible for research purposes, as it evoked measurable responses at both the muscle and brain levels. The MEG responses exhibited less variability than muscle-level responses, and the effect of stimulus intensity was observed only in the MEG responses. Although the study provides preliminary results on the topic, MEG appears to be a suitable method for investigating the proprioceptive afference of stretch reflexes.

Key words: magnetoencephalography, muscle stretch reflex, proprioception, somatosensory

ABBREVIATIONS

CV	coefficient of variation
EEG	electroencephalography
EMG	electromyography
EOG	electro-oculogram
HPI	head position indicator
ICA	independent components analysis
ISI	inter-stimulus interval
MEG	magnetoencephalography
SR	muscle stretch reflex
VL	vastus lateralis muscle
VM	vastus medialis muscle

CONTENTS

1 INTRODUCTION

Proprioceptive afference is a fundamental component of somatosensory function, and it refers to the transmission of information about the body's position and movements from the proprioceptors (e.g., muscle spindles) to the brain (Proske & Gandevia 2012). Proprioceptive stimulation activates the proprioceptors located in the muscles and joints, and the evoked response can be recorded using neuroimaging methods. The evoked cortical response is believed to primarily reflect the processing of proprioceptive afference. The response is visible even when cutaneous feedback is absent, suggesting that it originates from proprioceptive sources (Abbruzzese et al. 1985; Mima et al. 1996; Starr et al. 1981).

Proprioception plays a crucial role in sensorimotor control, and muscle stretch reflexes (SR), like the patellar tendon reflex, are essential components of this process. The patellar tendon reflex can be elicited by proprioceptive stimulation (e.g., percussion of the patellar tendon), triggering a rapid contraction of the quadriceps muscles. This involuntary proprioceptive response provides insight into the dynamic interplay between sensory input and motor output. Although the patellar tendon reflex was first described in the literature already over a century ago (Erb 1875; Westphal 1875), the phenomenon is not entirely clear, especially at the supraspinal level.

Despite being considered spinal, the SR should not be oversimplified, as it is more complex than initially thought (Marsden et al. 1973). Neuroimaging methods, such as magnetoencephalography (MEG), offer ways to examine central activity resulting from proprioceptive stimuli. MEG is a non-invasive brain imaging technique that records magnetic fields generated by the electrical activity of neuronal populations in the brain (Cohen 1972). While MEG is suggested to be a reliable and feasible method for investigating cortical afference for proprioception in various experimental designs (Illman et al. 2022; Mujunen et al. 2022; Piitulainen et al. 2018), the MEG environment requires special attention from the stimulation devices used since stimulators must be nonmagnetic and designed to avoid generating interference with magnetic signals.

While evoked cortical responses have been extensively investigated for most sensory domains, there is a notable gap in current somatosensory research. Neuroscientific studies on somatosensory functions have mostly focused on the upper limb, with little attention paid to

lower-limb proprioception, especially at the knee joint. Lower-limb proprioception is particularly interesting because many fundamental motor functions rely heavily on precise proprioceptive feedback from the lower limbs. Further research in this area is needed to better understand the role of lower-limb proprioception in human movement and to develop feasible and reliable methods for assessing it. Therefore, the objective of this study was to test the feasibility of a novel MEG-compatible proprioceptive stimulator of the knee joint and to provide preliminary insights into the cortical processing of proprioceptive afference of the patellar tendon reflex.

2 PROPRIOCEPTION OF THE KNEE: MUSCLE STRETCH REFLEX

Somatosensation indicates a group of sensory mechanisms that transmit signals about the body state and its physical interactions with the external environment. Somatosensation includes the senses of touch, pain, and proprioception, for instance. (Hall 2011, 571) The term 'proprioception' (from the Latin for "one's own") was first introduced by Sherrington in the early 1900s and it refers to the sense of body's position and movement (Sherrington 1907). The receptors involved in proprioception are called proprioceptors, which are types of mechanoreceptors that are found in skin, joints, tendons, and muscles. The receptors sense signals such as muscle tension and length, joint angles, and the position and movement of the trunk and limbs in space (for review, see Proske & Gandevia 2021). Without relying on visual or auditory senses, we are able to know the position and movement of our body parts due to these proprioceptive signals, which allow us to respond and react to changing circumstances. Signals from the body are essential for everyday activities, ranging from walking and reaching for objects to more complex tasks that require precise coordination. (Proske & Gandevia 2021)

Proprioception also includes spinal reflexes which are not only involuntary muscle contractions, but also serve many vital functions. Lower limb reflexes, such as patellar tendon reflex that is discussed in this work, aid in maintaining posture and balance, and have protective functions. Patellar tendon reflex is also utilized in diagnostics because it can give valuable information about the function of the human nervous system (Burke et al. 1970; Morishita et al. 2009; Zhou et al. 2023). All in all, proprioception plays a significant role in motor control and other essential functions of the body which is why proprioception has attracted interest in both clinical and research settings.

2.1 Somatosensory receptors associated with proprioception

Joint movement leads to loading and deforming of muscles, tendons, skin, and other tissues and structures associated with the joint. All these structures contain somatosensory receptors that transmit afferent information to the central nervous system when appropriate stimuli occur. Movement triggers the activation of various types of mechanoreceptors, making it complex to determine which sensors in fact influence proprioception. (Grigg 1994) Nevertheless, it appears

that many types of mechanoreceptors contribute to proprioception and thus acts as proprioceptors, although in different ways and to varying extents.

2.1.1 Muscle spindles

Of all different types of proprioceptors, muscle spindles play the most vital role in overall proprioception, and they are also the main sensors behind the SR (Proske & Gandevia 2012), as discussed in more detail in the next subchapter (2.2). Muscle spindles are fusiform encapsulated proprioceptors located in skeletal muscles which send information about the rate and velocity of length of the muscles to the central nervous system (for recent reviews, Kröger & Watkins 2021; Macefield & Knellwolf 2018). Intramuscular muscle spindles are oriented parallel to extrafusal muscle fibers, that is the 'standard' muscle fibers that constitute most of the muscle mass, are innervated by α-motoneurons and provide most of the force during contraction. Muscle spindles consist of a bundle of smaller muscle fibers called as intrafusal fibers. In contrast to extrafusal fibers, only a part of intrafusal fibers contains contractile myofilaments, and they are found predominantly in the peripheral regions of the muscle spindle. (Macefield & Knellwolf 2018)

Since much of the knowledge about mammalian muscle spindles we have today has been derived from in-depth observations made in animals (e.g., Barker 1948; Boyd 1962), these original sources are referred to in the parts where the information also applies to humans. A simplified illustration of a human muscle spindle and its innervation is shown in figure 1.

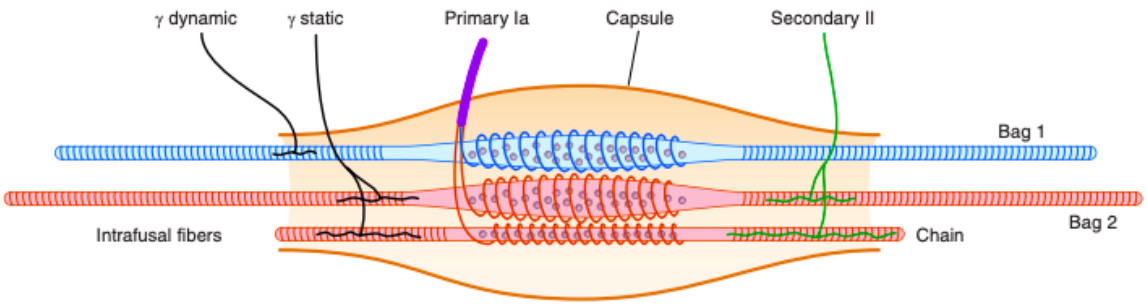

FIGURE 1. A schematic illustration of a human muscle spindle. (Adapted from Proske & Gandevia 2012).

The specialized intrafusal muscle fibers are subdivided into *nuclear bag fibers* (bag_1 and bag_2) and *nuclear chain fibers* based on their structure, function, and innervation (Boyd 1962; Cooper & Daniel 1963). Nuclear bag fibers often extend beyond the capsule of muscle spindles and attach to intramuscular connective tissue whereas nuclear chain fibers attach to the peripheral regions of the larger nuclear bag fibers (Boyd 1962). Muscle spindles are innervated by both sensory (afferent) and motor (efferent) neurons. Other types of proprioceptors have only afferent innervations, which highlights the uniqueness of muscle spindles as mechanoreceptors (Proske & Gandevia 2012).

Muscle spindles are innervated by two sensory neuron types, group Ia- and II-afferents (Barker 1948). A single group Ia-afferent rise from the primary sensory ending which makes spiral terminations around all three types of intrafusal fibers (nuclear bag_1, nuclear bag_2, nuclear chain), whereas several group II-afferents rising from secondary sensory endings terminate only nuclear bag_2 fibers and nuclear chain fibers in humans (Kennedy 1970). The primary sensory ending (Ia-afferent) terminates in the central region of a muscle spindle whereas the secondary sensory endings (II-afferents) are located next to the primary ending more towards the peripheral region, as the figure 1 illustrates. The sensory neurons arising from muscle spindles synapse also with γ-motoneurons in the spinal cord. Regarding the efferent innervation, the efferent nerves of muscle spindles consist of static (γ_s) and dynamic (γ_d) γ-motoneurons, also known as fusimotor fibers (Matthews 1962). The efferent endings terminate in the peripheral regions of muscle spindles and the synapses functions with the neurotransmitter acetylcholine (Boyd 1962). Dynamic γ-motoneurons terminate only in nuclear bag_1 fibers whereas static γ-motoneurons terminates in both nuclear bag_2 and nuclear chain fibers in humans (Kennedy 1970).

The stretch-sensing muscle spindle can be stimulated in two ways. When the whole muscle or even individual motor units are stretched, the muscle spindles stretch as well, and this excites the proprioceptors to send sensory information towards the spinal cord. The muscle spindle can also be excited without stretching of the motor units. The peripheral regions of the spindle innervated by γ-motoneurons can contract and doing so it stretches the midportion of the muscle spindle and therefore excite the receptor. (Proske & Gandevia 2012)

2.1.2 Golgi tendon organs

Even though ligaments are also deformed during joint movement, and mechanoreceptors in ligaments can respond to stretch, their potential role in SR remains controversial (Grigg 1994). Somewhat similar considerations apply to mechanoreceptors in tendons (e.g., Golgi tendon organs) even though indirect evidence suggests that they contribute to proprioception (Proske & Gandevia 2012). Golgi tendon organs are contraction-sensitive, encapsulated mechanoreceptors located in muscle-tendon junctions and innervated by Ib afferents (Hall 2011, 661).

While the receptors are sensitive to muscle contraction, stretch does not significantly activate tendon organs (Jami 1992). When it comes to stretch reflex evoked by tendon percussion, Murthy and colleagues (1978) reported that tendon taps did not significantly activate the secondary endings of the muscle spindle or the Golgi tendon organs. On the contrary, Burke and colleagues' (1983) results showed some Golgi tendon organ activity following an ankle tendon percussion. However, direct measurement of tendon organs is challenging, if not impossible, because it is difficult to apply measurable contractile forces to individual tendon organs. Additionally, selectively activating Ib-afferent fibers poses similar difficulties (Jami 1992).

2.1.3 Joint mechanoreceptors

Two types of sensory fibers arise from joint tissue: larger group II afferents (Ruffini and Paciniform endings) and smaller group III and IV afferents (Grigg 1994). The group II and IV afferents are thin, mechanically sensitive fibers, with their nerve terminals being free nerve endings (Heppelmann et al. 1990). These small afferents do not transmit information about the direction of movement but rather signal stimuli that are highly intense and potentially harmful. In fact, the III and IV afferents are primarily suggested to be pain sensors (i.e., nociceptors). (Coggeshall et al. 1983)

Ruffini end-organs are encapsulated structures within the fibrous capsule in joints (Freeman & Wyke 1967), and they are located exclusively in the posterior side of the knee joint capsule at least in cats (Grigg & Hoffman 1982). Paciniform end-organs are pressure-sensitive, thinly

encapsulated structures, and they are found around the joint capsule and periarticular connective tissue (Freeman & Wyke 1967). In animal studies, knee capsule stress is directly related to the extending the leg, and similarly, the response of the Ruffini endings is proportional to tissue stress. Therefore, the Ruffini afferents are primarily activated during extreme extensions of the knee, suggesting that they play a role in proprioception only when the joint reaches its maximum extension limit. (Grigg & Hoffman 1982) This is in line with the observations that the removal (Grigg et al. 1973) or anesthesia (Clark et al. 1979) of the joint capsule does not result in proprioceptive deficits within intermediate positions of the joint. In conclusion, it is proposed that group II afferents originating from the joint capsule contribute to proprioception only to a certain degree (Grigg 1994).

2.1.4 Cutaneous mechanoreceptors

Most of the neurophysiological evidence supporting the role of skin receptors in proprioception has come from studies involving the human hand and finger joints. The recordings done by Edin (2001) showed that the skin of the human thigh has many stretch-sensitive mechanoreceptors that could potentially pass information about knee joint positions and movements alongside with muscle spindles. Notably, aside from the hair follicle receptors, all mechanoreceptors demonstrated the capacity to convey proprioceptive information, but to different degrees (Edin 2001).

On the contrary, Clark and colleagues (1979) conducted an experiment in which they selectively blocked cutaneous afferents originating from an area surrounding the knee joint. Their findings indicated that this had minimal or negligible impact on the participants' capacity to perceive passive movements of the knee joint. The subjects' ability to accurately detect flexion, extension, and control trials remained unaffected by joint anesthesia, skin anesthesia, or a combination of both. This hypothesis is further backed up by studies by Abbruzzese et al. (1985), Mima et al. (1996), and Starr et al. (1981). The studies examined cerebral responses to proprioceptive stimuli, and the three studies shared the finding that the recorded responses persisted despite elimination of cutaneous and joint afferents due to anesthesia.

As a result, it is suggested that cutaneous (and joint afferents) play a relatively small role in proprioception. While this conclusion is reasonable, the evidence behind this topic is still

somewhat controversial, and the methods for isolating all other sensory receptors except muscle spindles is nothing but simple. Hence, the matter cannot be regarded that straightforward, but rather it seems that cutaneous afferent neurons do encode joint movements to some extent. Nevertheless, even if they do contribute to proprioception, the evidence suggests that their role is relatively minor compared to muscle spindles.

2.2 Muscle spindles and muscle stretch reflex

The SR is a fundamental function of muscle spindles, and it occurs when the length of the muscle and/or the muscle spindle increases. The stretch of the muscle also stretches the intramuscular spindles, and since they act as receptors for the rate and velocity of stretch, the sensory neurons of muscle spindles send information to the spinal cord where they synapse directly with an α-motoneuron. In turn, the α-motoneurons conduct information to the agonist and synergist muscles causing them to contract (i.e., resist the occurred stretch). In short, the SR occurs when the muscle spindle is stimulated by stretch and this induces the related muscle to contract. (Pierrot-Deseilligny & Burke 2012)

The SR can be divided into two components: 1) dynamic, short-latency and 2) static, long-latency component (Liddell & Sherrington 1924). A dynamic component of SR is caused by a rapid, sudden, and intense stimulus (stretch) of the spindles that can be demonstrated with tendon percussion. This sends strong signals to the spinal cord via primary (Ia) sensory ending and therefore a quick and profound effect, a tendon jerk, is evoked. (Hall 2011, 659) Short-latency reflexes are primarily involved in maintaining posture, preventing muscle damage, and providing rapid, automatic responses to sudden changes in muscle length. Instead, the static component occurs when the muscle is stretched slowly and corresponding weaker signals to spinal cord is sent via both sensory endings (Ia and II), and this component of the SR is quite constant. That is, muscle spindles continuously emit sensory impulses under normal conditions. (Hall 2011, 659) The background discharge depends on the muscle in question itself, but also on the length of that muscle, with stretching increasing their rate and shortening decreasing it (i.e., below normal impulse rates) (Proske & Gandevia 2012). This causes a degree of contraction of muscles, commonly known as the muscle tone. In this manner, muscle spindles smooth the muscle contractions and prevent jerkiness of movements and thus are fundamental for movement control (Hall 2011, 659).

These two components often occur simultaneously but in different time-windows, which is why the components are also referred as short latency and long latency reflexes. The dynamic component ceases within milliseconds following the stimulus, whereas the static component persist for prolonged duration (Pierrot-Deseilligny & Burke 2012). In other words, once the length of the spindle stops increasing, the impulse discharge reverts to the level of the constant static response that is still present in the signal. However, both components of the SR contribute significantly to muscle control.

The SR can be evoked mechanically by tendon percussion or by passive movement of the limb. In addition, they can also be measured using electrical stimulation to evoke H-reflexes. Traditionally it is suggested that the H-reflex would be the electrically evoked counterpart of a tendon jerk, and that these would be monosynaptic spinal reflexes. The primary distinction between the tendon jerk and H-reflex lies in the suggestion that the H-reflex bypasses the mechanisms of the receptor, relying on a group Ia volley initiated through direct electrical stimulation of the motor nerve. (Pierrot-Deseilligny & Burke 2012) Comparisons between SRs triggered by mechanical and electrical means have been conducted to examine receptor sensitivity, which in turn indicates the level of fusimotor activity (Paillard 1959; Iles 1977). Nevertheless, the pathway of the monosynaptic reflex is more complex than it might initially appear, and it is also suggested that the SR cannot be purely considered monosynaptic (Burke et al., 1983). Also, Burke and colleagues (1983) reported that the afferent volleys of mechanically and electrically evoked reflexes exhibit numerous differences, making it likely inappropriate to directly compare the H-reflex and tendon reflex as indicators of fusimotor activity.

The fusimotor system, involving afferent feedback from muscles and efferent gamma activation of muscle spindles, plays a vital part in movement. Afferent feedback is essential for maintaining steady alpha motoneuron discharges, whereas gamma drive regulates muscle spindle sensitivity. Together afferent feedback and the gamma drive cooperate to effectively manage complex motor tasks. Notably, α- and γ-motoneurons are simultaneously activated by efferent commands from the brain. Alpha-gamma coactivation is a coordinated activation of both alpha and gamma motoneurons that control extrafusal and intrafusal muscle fibers, respectively. This coordinated activation maintains the sensitivity of muscle spindles during muscle contraction, ensuring that the proprioceptive feedback remains accurate. (Pierrot-Deseilligny & Burke 2012) Activation of only α-motoneurons would decrease the activity of

Ia-afferents, while γ-activation would do the opposite. The increase of Ia-activity by γ-motoneurons occurs because the activation of a dynamic γ-motoneuron stimulates nuclear bag_1 fibers it innervates, causing the peripheral regions of the spindle to contract, and as a result, the midportion of the muscle spindle stretches, which excites the receptor and enhances the activity of the Ia-afferent (Pierrot-Deseilligny & Burke 2012).

The size of the SR is influenced by various internal mechanisms that act on the afferent and efferent volleys, and multiple factors can modify the reflex size. For example, alterations in motoneuron pool excitability, excitability of Ia-afferents, and presynaptic inhibition of Ia-terminals affect the reflex amplitude. Additionally, if large excitatory signals are emitted from the central nervous system, SR can be emphasized, and vice versa. Furthermore, the excitatory synapse between a sensory neuron and a γ-motoneuron in the spinal cord increases the sensitivity of the muscle spindle, subsequently enhancing the dynamic stretch reflex. (Pierrot-Deseilligny & Burke 2012) Hence, the SR cannot be purely regarded as a representation of the α-motoneuron excitability or another single component but is influenced by many other afferent and efferent inputs as well.

2.3 Patellar tendon reflex

The patellar tendon reflex was first described in the literature 1875 by both Wilhelm Heinrich Erb and Carl Friedrich Otto Westphal. The patellar tendon reflex is elicited by percussion of the patellar tendon with a reflex hammer which makes the quadriceps muscles to contract rapidly and thus induce a visible tendon jerk (Erb 1875; Westphal 1875). This kind of knee jerk is an example of a mechanically evoked SR. In the literature, SRs are often referred as deep tendon reflexes even though the phenomenon have little to do with tendons. That is, the proprioceptors behind the stretch reflex are the intramuscular muscle spindles as discussed in the previous subchapter. For clarity and consistency, here only the term ‘SR’ is used to describe the general phenomenon itself, and the term ‘patellar tendon reflex’ is used to describe the dynamic muscle stretch reflex that occurs in quadriceps muscles when the patellar tendon is percussed with a reflex hammer.

The patellar tendon reflex is an example of a dynamic component of a SR. Percussion of the patellar tendon stretches the quadriceps muscles and the muscle spindles lying parallel to the

extrafusal fibers. The primary ending in the intrafusal fibers is stimulated by the sudden and rapid change in stretch which leads the sensory neuron (Ia) to send action potentials to the spinal cord. The sensory neuron synapse directly with an α-motoneuron which in turn conduct action potentials to the quadriceps muscles causing it to contract. The patellar tendon reflex has a monosynaptic reflex arc which means that one sensory neuron synapses with one motor neuron in the spinal cord. (Hall 2011, 660)

2.3.1 Assessment of patellar tendon reflex

Assessment of the patellar tendon reflex has clinical value, and the phenomenon has been investigated in clinical conditions such as spasticity (Burke et al. 1970), stroke (Zhou et al. 2023), and paralysis (Morishita et al. 2009). Still, there is a lack of highly reliable gold standard method for assessing tendon reflexes. In clinical settings, the patellar tendon reflex is commonly tested by percussion of the patellar tendon with a manual reflex hammer. The visible response is evaluated using the qualitative NINDS myotatic reflex scale, which ranges from 0 to 4 (0 = reflex absent, 4 = reflex more than normal) (Hallett 1993). However, this method relies heavily on a physician's subjective judgement, and there is a controversial view on the reliability of NINDS (Litvan et al. 1996; Manschot et al. 1998). The reliability has been tested to vary from 'not acceptable' (Manschot et al. 1998) to 'moderate' (Litvan et al. 1996). Nevertheless, many of the qualitative methods employed particularly in research are found to be excessively complex and time-consuming for clinical applications.

To address these limitations, several quantitative methods for assessing patellar tendon reflex have been developed. The methodologies differ across studies regarding both the reflex stimulator and the assessment of the reflex responses. Both manually operated (e.g., Stam & van Leeuwen 1984; Toft et al. 1991) and automatic (e.g., Toft et al. 1991) reflex hammers have been used to elicit the patellar tendon reflex. Regarding the evaluation of reflex responses, the most common quantitative methods include surface electromyography (EMG) measurements to assess the two major parameters of a myotatic reflex: amplitude and latency. The latency of patellar tendon reflex is the time from the reflex hammer stimulus to the action potential recorded with EMG from quadriceps muscles. Reflex amplitudes exhibit significant intra-individual and inter-individual variability (Frijns et al. 1997; Stam & Tan 1987; Stam & van

Crevel 1989), while latency as a reflex response parameter appears to be more reliable with excellent intra-individual reliability (Frijns et al. 1997).

EMG is often combined with force measurements in reflex studies. Clarke (1965) studied the relationship between EMG and force of the isometric patellar tendon reflex response measured at the ankle, and a strong positive correlation between the EMG signal and reflex-produced isometric force was found. Neurophysiological EMG assessment has also been integrated with kinesiologic evaluation, where goniometer sensors have been used to measure kinesiological parameters, such as angular velocity, of the patellar tendon reflex (Uslu et al. 2022). Additionally, quantitative evaluation has been undertaken without neurophysiological measurements by utilizing motion analysis to assess the patellar tendon reflexes (Tham et al. 2013).

2.3.2 External factors affecting reflex responses

The monosynaptic reflex arc makes the patellar tendon reflex extremely fast. Still, there is several factors affecting the reflex responses, which must be considered when comparing the results from different reflex studies. Some of these factors are due to the subject characteristics, such as age and height. In healthy young adults, the average latency of the patellar tendon reflex has been documented to be 17–20 milliseconds (Knowlton & Britt 1963). The patellar tendon reflex latencies are affected by age (Knowlton & Britt 1963; Burke et al. 1996; Kamen & Koceja 1989) and that the latencies are positively related with height (Knowlton & Britt 1963; Stam & van Crevel 1989). Other important factor that should be considered when comparing results is EMG electrode placement.

Remote muscle contraction has been shown to enhance reflexes, and this technique (Jendrassik maneuver) has been utilized for longer than a century (Jendrassik 1883). The technique requires the subject to clasp their hands together and exert maximum force while maintaining normal breathing during the reflex stimulus. The Jendrassik maneuver increases the amplitudes and decreases the latencies in patellar tendon reflexes (Clarke 1967; Burke et al. 1996). Not only remote muscle contractions affect the reflexes but also the voluntary contraction of the target muscle (e.g., quadriceps muscle in patellar tendon reflex). However, this effect is somewhat controversial. The amplitude of the reflex has been shown to decrease in the pre-activated

muscle with the contraction level (Toft et al. 1991). Other results have suggested no difference in reflex amplitude (Pope & DeFreitas 2015; Uysal et al. 1999), but a minor decrease in latency with voluntary contraction (Uysal et al. 1999). What is more, the variability of the patellar tendon reflex amplitudes seems to decrease with pre-activation of the quadriceps muscle, even though the variation was still high in both conditions (Toft et al. 1991; Uysal et al. 1999).

Differences in reflex hammer stimulation, such as stimulus frequency and stimulus intensity, also affects the reflex responses. Inter-stimulus interval (ISI) had an impact to habituation of reflex responses in quadriceps muscles in a case study by Hollis (1971). Repeated stimuli resulted in a progressive decrement of response amplitude for both EMG and force measurements, and this habituation occurred more prominently and rapidly if the ISI was lower (Hollis 1971). It seems that the stimulus frequency should not be higher than approximately 12 hammer percussions per minute (ISI=5 seconds) to minimize habituation and the decrease in response amplitudes.

Stimulus intensity, exemplified by factors such as percussion angle (Tham et al. 2013) or deceleration during the tendon percussion (Stam & van Crevel 1989), exerts a significant impact on the evoked responses. Tham and colleagues (2013) observed a linear relationship between stimulus intensity and reflex amplitude. Furthermore, they noted that reflex latencies decreased as the stimulus strength increased. (Tham et al. 2013). Stam and van Crevel (1989) found that the relation of stimulus intensity and reflex response could be represented by a sigmoid curve: as stimulus intensity increased, the responses exhibited a rapid and extensive rise, but this rise reached a plateau after a certain level of stimulus intensity.

In summary, reflex responses are influenced by both internal and external factors. Internally, the fusimotor system and coordinated alpha-gamma coactivation play crucial roles, and various mechanisms contribute to reflex size. Externally, age, height, voluntary contractions, and reflex hammer stimulation parameters impact reflex parameters. The high variability in reflex responses poses challenges to the sensitivity of methods aiming to investigate SRs, and understanding the dynamics of the reflexes requires navigating the complex interaction of internal and external factors.

3 CENTRAL PATHWAYS AND PROCESSING OF PROPRIOCEPTION

The previous chapter discussed the peripheral dimension of proprioception, explaining the different types of receptors associated with proprioception and how they respond to different stimuli. Additionally, the chapter covered parts of the spinal level of proprioception, specifically discussing the monosynaptic reflex arc related to the patellar tendon reflex. However, the story of proprioceptive afference doesn't end at the spinal level. The different types of proprioceptors are innervated by afferent nerve fibers that convey signals to the central nervous system. This rich afferent input from various receptors is gathered in the brain, eventually forming a comprehensive neural image of the positions of our body parts, and the surrounding environment and forces acting upon it – that is, the sense of proprioception.

What about the proprioceptive afference at the higher and central level, per se? Even though the tendon reflexes are spinal, the SR overall should not be oversimplified, since at least in humans, it is not as simple as initially thought (Marsden et al. 1973). The spinal level of SR is often emphasized but the sensory receptors also inform higher parts of the nervous system of the changes in muscles (Hall 2011, 657). This matter is far from clear, but luckily neuroimaging offers ways to examine the central activity resulting from proprioceptive stimuli. Therefore, this chapter aims to clarify the central aspect of proprioception and the pathways of proprioceptive afference from the receptors to the cortex.

3.1 Afferent tracts and cortical representations for proprioception

Proprioceptive afference is transferred to the cortex through a complex somatosensory pathway that involves multiple steps and different parts of the nervous system (for reviews, see Delhaye et al. 2018 and Tuthill & Azim 2018). Afferent fibers at the periphery integrate into bundles that eventually form nerves together with efferent fibers. As the nerve approaches the spine, it divides into dorsal and ventral roots, which carry sensory and motor fibers, respectively. Afferent nerve cell bodies bundle in the dorsal root forming the dorsal root ganglia which are located along the spinal cord. Afferent axons from the dorsal root ganglia can synapse in either the same level of the spinal cord or ascend trough the dorsal column and terminate in the dorsal column nuclei of the brainstem. The dorsal column nuclei, in turn, sends signals contralaterally through the medial lemniscus to reach the thalamus. Finally, the thalamus projects this

information to the cortex. (Delhaye et al. 2018; Tuthill & Azim 2018) The conveyance of proprioceptive signals from the sensory receptors to the cortex is illustrated in figure 2.

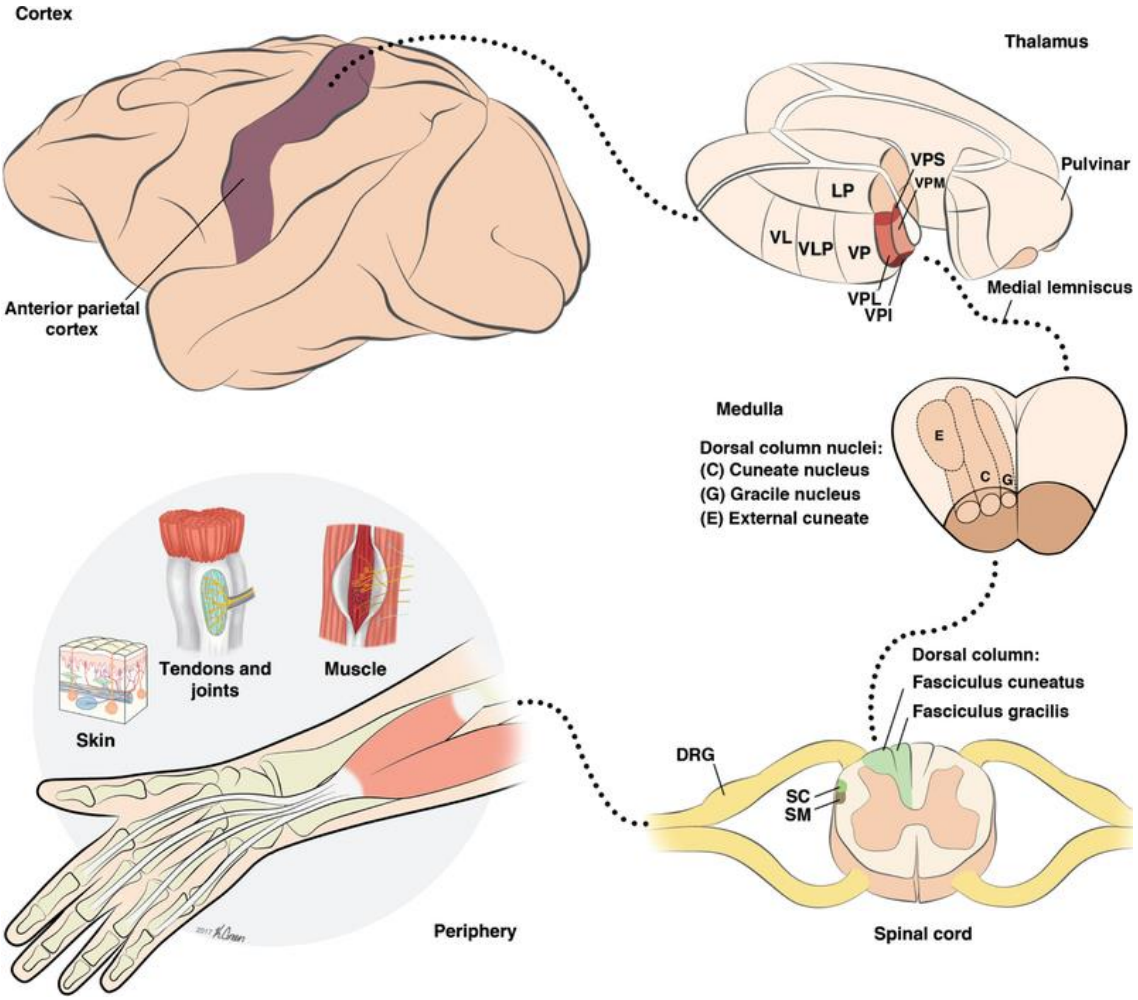

FIGURE 2. The transmission of proprioceptive information to the cortex. (Adapted from Delhaye et al. 2018).

The afferent neurons form the dorsal column tract through which the proprioceptive information is eventually conveyed to the primary somatosensory cortex (S1) (Makous et al. 1996). The signals arising from the lower body are transferred to the gracile nucleus of the dorsal column nuclei in the medulla (Delhaye et al. 2018), from where the signals are sent contralaterally to the ventroposterior complex of the thalamus, forming the medial lemniscal pathway (Rasmussen & Peyton 1948). Different parts of the ventroposterior complex of the thalamus receive different somatosensory signals, and inputs from the thalamus to the cortex can be both very divergent and convergent (Padberg et al. 2009), and the thalamic neurons project to several

areas of the S1 (Delhaye et al. 2018). In this manner, thalamus collects and processes information and projects it further either to a specific area or various areas on the cortex depending on the received input. Consequently, this afferent pathway from the periphery to the S1 via thalamus is also referred as dorsal column-medial lemniscus pathway (Tuthill & Azim 2018).

The S1, located in the anterior part of the parietal lobe, is responsible for integrating, processing, and interpreting proprioceptive information (Tuthill & Azim 2018). The S1 consist of four areas: Brodmann's areas 3a, 3b, 1, and 2. The areas lie parallel to each other along the central sulcus which extends horizontally across the brain (Kaas et al. 1979). All four areas have quite identical somatotopic representation of the contralateral body parts. That is, foot representations are near the longitudinal fissure which extends longitudinally across the brain dividing the brain into the two hemispheres, and face representations at the lateral end (Penfield & Rasmussen 1950). The somatotopic order in the S1 and the four Brodmann's areas are shown in figure 3.

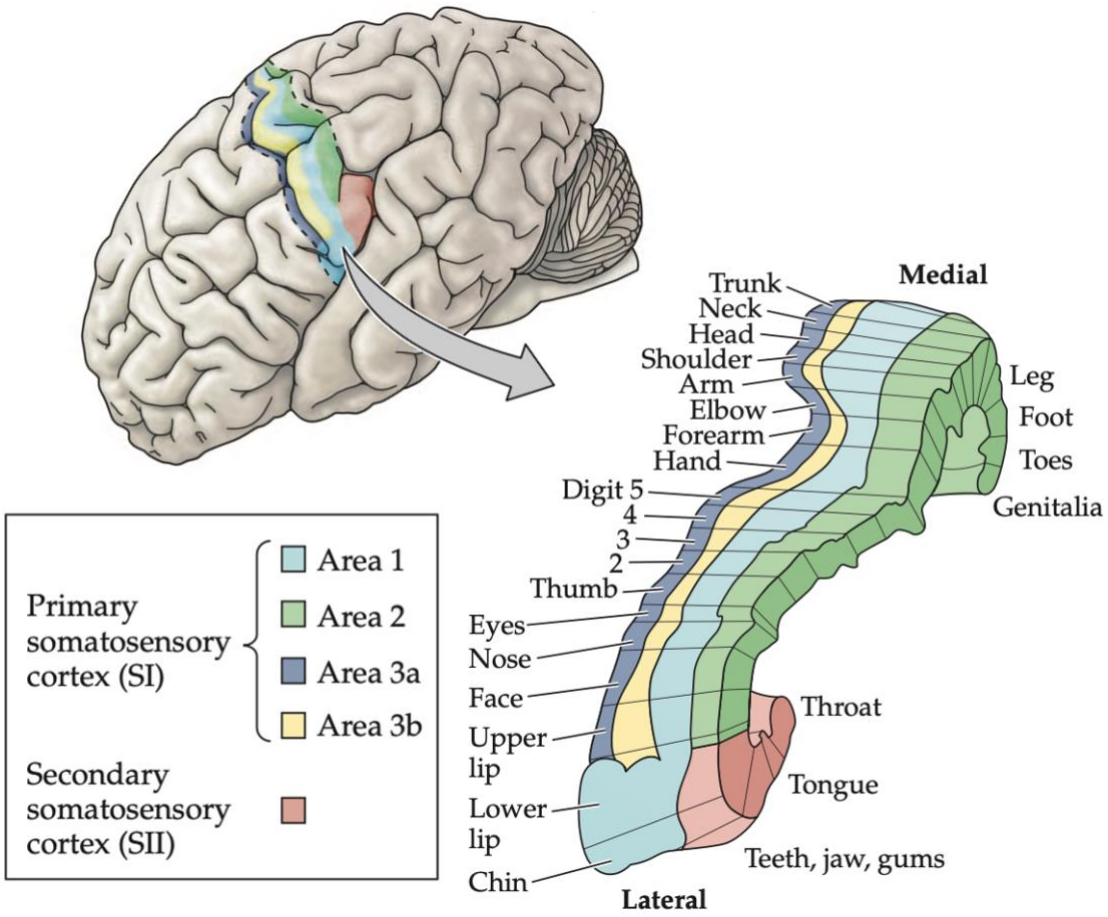

FIGURE 3. Somatotopic order in the S1. (Modified from Purves et al. 2018).

Most of the neurons in Brodmann's areas 3b and 1 exhibit cutaneous responses, whereas area 2 exhibit both cutaneous and proprioceptive responses. Area 2 has connections with areas 3a and 3b, area 1, and with primary motor cortex (M1). Area 3a responds primarily to proprioceptive stimulation and has connections with areas 1 and 2, M1, and the supplementary motor area. (Kaas 1983) Indeed, somatosensory and motor areas in the brain are functionally associated and they are both critical in executing motor tasks (Arce-McShane et al. 2016). In addition, they are located side by side in the cortex and have corresponding somatotopic arrangements. The M1 is found in the dorsal part of the frontal lobe, on the opposite side of the central sulcus from S1 (Penfield & Boldrey, 1937). Accordingly, the two regions are often examined together and referred to as the primary sensorimotor cortex (SM1) (Zhao et al., 2019), as is the case in the present study.

Proprioceptive information is further analyzed and combined with other sensory and motor information in various regions of the cortex. The S1 projects to other cortical areas, most importantly to the lateral parietal cortex and to the posterior parietal cortex. This so-called ventral pathway in the lateral parietal cortex comprises the secondary somatosensory cortex (S2) and the parietal ventral area. (Delhaye et al. 2018) The route is associated with still higher cognitive functions such as attention (Mima et al. 1998) and decision-making (Romo et al. 2002). Instead, the dorsal stream in the posterior parietal cortex includes areas 5 and 7, and this pathway is more specifically linked high-level cognitive motor plans (Andersen & Buneo 2002; Fogassi & Luppino 2005).

To summarize, proprioceptive information is transferred to the cortex through a series of neural pathways that involve sensory receptors, afferent neurons, spinal cord processing, thalamic relay, and specialized cortical regions. In addition, there are multiple complex connections between the different main somatosensory areas in the brain. This process allows us to be aware of our body's position and movement and to coordinate our movements accordingly.

3.2 Cortical responses elicited by proprioceptive stimulation

Proprioceptive stimulation activates proprioceptors located in muscles and joints, and the evoked responses can be recorded using neuroimaging methods. In the research field of cortical processing of proprioceptive signals, evoked responses refer to averaged cortical activity time-

locked with movements or other proprioceptive stimuli (Alary et al. 2002; Druschky et al. 2003). The evoked cortical responses primarily reflect the processing of proprioceptive afference and remain visible even when cutaneous feedback is absent, suggesting their origin to be from proprioceptive sources (Abbruzzese et al. 1985; Mima et al. 1996; Starr et al. 1981).

In addition to evoked responses, cortical processing of proprioception can also be examined through induced responses and corticokinematic coherence (CKC). Induced responses refer to changes in cortical activity at specific frequency bands due to movements or proprioceptive stimulation (Illman et al. 2020; Walker et al. 2020), whereas CKC refers to the coupling between cortical activity and different peripheral measures (Piitulainen et al. 2013b), mainly reflecting movement-related somatosensory proprioceptive afferent input to the SM1 (Bourguignon et al. 2015). CKC can be studied with both MEG (Bourguignon et al. 2012) and electroencephalography (EEG) (Piitulainen et al. 2020), and it has been shown to be evident in passive and active movements with insignificant effect of cutaneous input (Piitulainen et al. 2013a).

As discussed in the previous subchapter, proprioception at the central level involves several brain regions working together. Especially the 3a and 2 areas of the S1 cortex integrate and process proprioceptive information (Kaas 1983), but the M1 (Goldring & Ratcheson 1972), and the S2, supplementary motor area and PPC (Mima et al. 1996) also receive proprioceptive inputs from the periphery. That is, afferent proprioceptive feedback from the proprioceptors is processed in various brain areas but peak responses elicited by proprioceptive stimulation are predominantly located on the contralateral SM1 cortex (Alary et al. 2002; Bourguignon et al. 2012; Druschky et al. 2003; Mima et al. 1996). Evoked and induced responses for proprioceptive stimulation of the ankle joint have reported to peak in MEG sensor pairs above the contralateral foot region of the SM1 cortex (Mujunen et al. 2022). Similarly, peak induced responses (Giangrande et al. 2024b) and CKC (Giangrande et al. 2024a) for cortical processing of ankle joint proprioception have been recorded at the EEG electrode site corresponding to the foot area of the SM1. Comparable results have been reported in studies involving finger and toe movements, with CKC (Piitulainen et al. 2015) and evoked responses, induced responses, and CKC (Nurmi et al. 2023) peaking at the contralateral SM1 cortex.

Higher stimulus intensities and/or volitional muscle activation are presumed to activate more proprioceptors, thereby amplifying cortical proprioceptive processing. More comprehensive

proprioceptive stimulation has been shown to amplify cortical processing using both MEG (Hakonen et al., 2022) and fMRI (Nurmi et al., 2018). Contradictory results have also been reported, since in Nurmi et al. (2023), MEG responses remained consistent even though the movement range increased. Additionally, cortical processing of proprioceptive afference as a measure of CKC using EEG was found to be stronger with voluntary muscle activation compared to passive conditions (Giangrande et al. 2024a). Similar results, showing that the active condition produces stronger cortical processing than passive conditions, have also been reported elsewhere (Reddy et al. 2001).

3.3 Proprioceptive stimulators in neuroscientific studies

Cortical responses for proprioception can be elicited by different kinds of stimulators. Proprioceptive stimulation in neuroscientific studies typically involves either active or passive movements generated by a stimulator device. No neuroimaging studies utilizing tendon percussion as proprioceptive stimulation have been published to date. In previous MEG and fMRI studies, passive movements have been elicited either manually (i.e., by a researcher moving the participant's limb) (Druschky et al. 2003; Piitulainen et al. 2013a) or by pneumatic (Alary et al. 2002) or hydraulic devices (Yu et al. 2001). Stimulators generating active movements have also been employed (Piitulainen et al. 2013a; Reddy et al. 2001).

While most stimulators have been designed to elicit movements of the fingers (Alary et al. 2002; Druschky et al. 2003; Piitulainen et al. 2013a), studies have also investigated movements of the toes (Piitulainen et al. 2015), the elbow (Weiller et al. 1996; Yu et al. 2011), and the ankle (Dobkin et al. 2004; Francis et al. 2009; Mujunen et al. 2022). Stimulators may produce auditory noise, which can be masked by music (Alary et al. 2002) or Brownian noise (Mujunen et al. 2022), for example. In EEG studies, movement actuators have been controlled by electrical motors (e.g., Mima et al. 1996), but these devices are not MEG- or fMRI-compatible. When designing stimulators, it is important to consider the neuroimaging method used. In MEG and fMRI studies, stimulators must be nonmagnetic and should not produce mechanical or electrical artifacts in magnetic signals.

4 MAGNETOENCEPHALOGRAPHY (MEG)

MEG is a safe and non-invasive brain imaging technique that gives a possibility to study brain activity by recording the magnetic fields generated by the electrical activity of neuronal populations in the brain (Cohen 1972). The first time MEG was used for studying human brain was in 1968 by researcher David Cohen from the Massachusetts Institute of Technology. For comparison, first article presenting results of EEG, which is used for many of the same purposes as MEG, was published 40 years before the Cohen's study (Berger 1929). The technology behind MEG has improved substantially over time, and there are two major turning points in this development. The first was the invention of ultrasensitive sensors (SQUID-sensors) by Silver and Zimmerman in 1965, which were successfully utilized in human studies a few years later (Cohen 1972). The second drastic improvement was the invention of the whole-scalp system by Ahonen and colleagues in 1993, which made possible to cover the measurements of the whole brain.

The key strengths of MEG include excellent temporal resolution of under one millisecond. Magnetic fields are not distorted by the anatomical structures of the skull and scalp, which means that magnetic permittivity is the same between different tissues (Baillet 2017). In comparison, EEG signals are profoundly affected by the difference in electrical conductivity between tissues like scalp, skull, and cerebrospinal fluid (Lopes da Silva 2013). Therefore, the spatial resolution of MEG is fairly more precise compared EEG, which is cheaper and thus more commonly used method within neuroscience.

Other advantages of MEG in contrast to EEG include faster and easier subject preparation. Technique of EEG requires good contact with electrodes and scalp and that is why some kind of medium substance such as gel is needed to put into every electrode, and researchers must manually verify that the impedance of all the EEG electrodes are low enough. (Gross 2019). Nevertheless, MEG and EEG should not be accounted as competing methods, but rather think they are complementary to each other. The methods could and should be used together whenever it is possible and when it adds extra value to the research (Hari et al. 2018). However, it should not be forgotten that EEG and MEG measures different physical quantities, microvolts and femtoteslas, respectively, and that the direct comparison between these could be more complicated than initially thought (Baillet 2017).

Although MEG has its own limitations, the method is useful both in clinical settings and in research. It is possible to identify the neural sources of evoked potentials and spontaneous activity, such as brain rhythms. (Cohen 1972). The method allows us to study neurophysiology and gain better knowledge how human brain functions. There are several high-quality review articles regarding MEG that are cited in this work, and which can be recommended for further reading (e.g., Baillet 2017; Gross 2019; Gross et al. 2013; Hari et al. 2018; Lopes da Silva 2013).

4.1 Neurophysiology behind MEG signal

MEG as a neuroimaging methos is based on the fundamental physical principle that electrical currents create magnetic fields *(Maxwell's equations)*, and thus the magnetic fields recorded by MEG directly reflect the underlying neuronal currents. During neural activity, the movement of ions creates electrical currents, which MEG can detect and localize by measuring the resulting magnetic fields. (Gross 2019) It is estimated that 10.000–50.000 neurons are required to produce signals that can be detected with MEG (Baillet 2017). The amplitude of MEG signal depends not only on the number of activated neurons, but also on temporal synchrony and spatial alignment (Gross 2019). MEG signals lie on the frequency band of 0.5–1000 Hz, with the range of 1–80 Hz being most typical (Baillet 2017).

Figure 4 illustrates the connection between neural electrical currents and magnetic induction, as well as the neural sources of MEG signal. Magnetic induction is perpendicular to the electrical currents in the neurons and as mentioned, this induction can be recorded outside the scalp. There are two main sources of the recorded magnetic fields: the postsynaptic potentials and action potentials. The sum of postsynaptic potentials is thought to be the main generator of MEG signals. Furthermore, MEG is most sensitive to detect activity of the cortex rather than deeper structures of the brain. (Baillet 2017) Cortical neuron types include pyramidal cells, which tend to be perpendicular to the cortical surface (Hämäläinen et al. 1993). This means that also the electrical currents flow perpendicular to cortical surface.

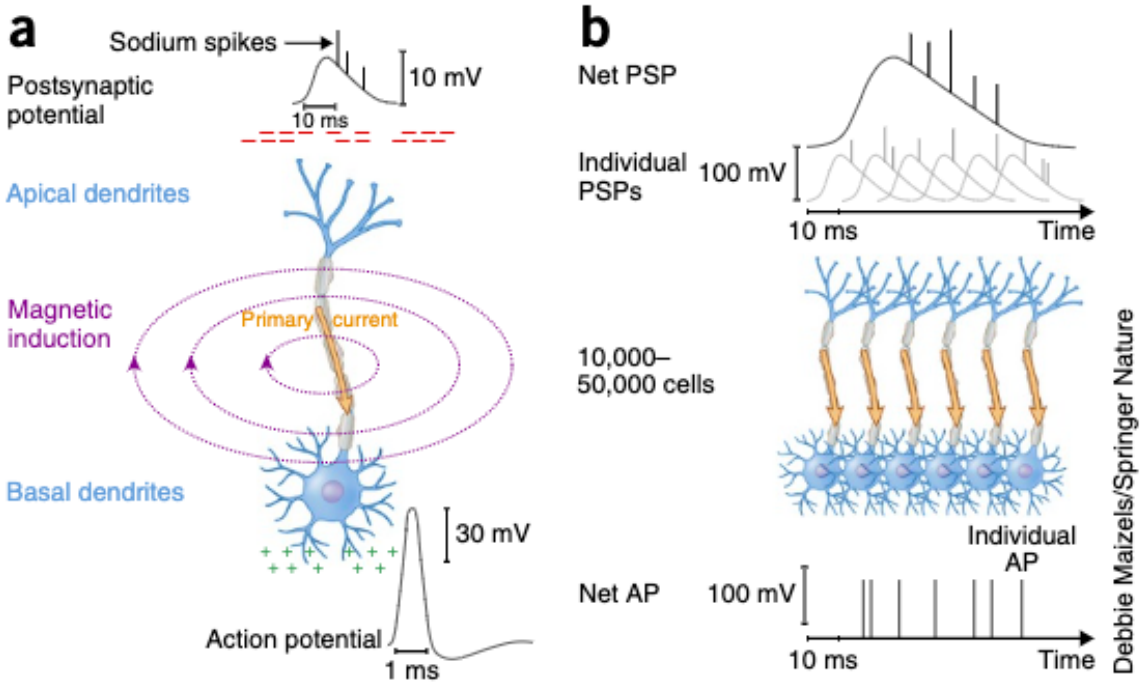

FIGURE 4. Neural sources of MEG signals. (a) Electrical current and its connection to magnetic induction: Magnetic induction is perpendicular to the primary current in the neurons. Both postsynaptic potentials (PSP) and action potentials (AP) have effects on MEG signals. (b) Even though individual APs are stronger than individual PSPs, the net effect of PSPs are the main source of MEG signals. (Adapted from Baillet 2017).

4.2 Instrumentation, data collection, and data analysis

Magnetic fields produced by brain are very weak compared to external magnetic fields, such as the steady magnetic field of the earth. To detect and record these weaker magnetic signals special instrumentation is needed. An example of MEG instrumentation and hardware is shown in figure 5. The state-of-the-art instrumentation includes approximately 300 extremely sensitive SQUID-sensors coupled with pick-up coils that are used for recording magnetic fields produced by brain. MEG hardware must be kept in a magnetically and electrically shielded room to minimize contamination from other magnetic fields. (Gross 2019) There are also other ways to reduce the nuisance such as the noise from elevators and car traffic. For example, magnetometers and gradiometers are used to emphasize signals coming from the brain with respect to environmental noise (Baillet 2017).

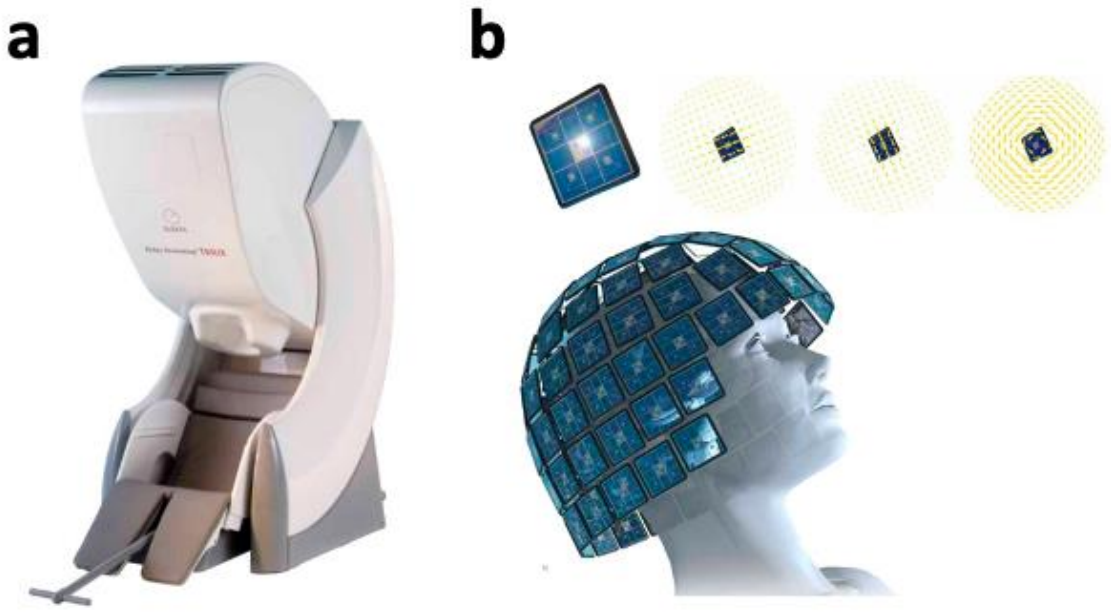

FIGURE 5. MEG instrumentation: Elekta Neuromag® TRIUX™. (a) The dewar, where the SQUID sensors are positioned in a helmet-shaped cryogenic and thermally insulated container. (b) Each sensor element includes three independent sensors with different sensitivity patterns, and one magnetometer and two gradiometers, which improve the signal quality. (Modified from Medical Expo Catalogs – Elekta Neuromag® TRIUX™, 17.4.2023).

The sensors of MEG do not need to be attached to the scalp because magnetic induction travels through the air. Instead, the sensors are at least two centimeters from the scalp because they need to be thermally insulated (Baillet 2017). In these whole-head systems, sensors are positioned in a helmet-shaped cryogenic and thermally insulated container called a dewar. The dewar contains 70–100 liters of liquid helium which temperature is kept in -269°C. In this almost absolute zero temperature, some materials lose all their resistance and thus become superconducting. This superconducting temperature minimizes thermal noise and reduces signal loss and therefore enhances data quality. (Baillet 2017; Gross 2019)

Sample sizes in MEG studies are often rather small, on average around twenty (Gross 2019). Proper data collection is a vital part of MEG studies because it greatly affects data quality. Gross et al. (2013) have gathered good practices for conducting and reporting MEG research. Before entering the shielded room, the subject needs to remove all wearable metallic objects, such as jewelry. Also, researchers need to discuss the procedures and safety issues with the subject. It is quite usual to combine MEG recordings with acquisition of other types of signals,

e.g., ECG (electro-cardiogram) and EOG (electro-oculogram). Simultaneous collection of these types of signals are recommended because eye movements and blinks as well as the magnetic field of the heart generates artifacts in the MEG data. (Gross et al. 2013)

Before the measurement itself, "empty room" recordings without the subject (only the instrumentation inside the shielded room) are recommended to help to identify the noise level of the environment. The subject's head should be centered inside the helmet as close to the surface of the walls as possible, and subject must be instructed to keeping still and avoiding excessive eye movements and blinks during the measurement. When the subject is completely prepared and instrumentation is ready for the measurements, short (approximately 2 minutes) "resting state" recordings are recommended to gain baseline data before the start of the actual experiment or paradigm. (Gross et al. 2013)

Regarding the data acquisition, use of sufficient sampling frequency and online data monitoring are important. Sampling frequency should be at least two times the highest frequency of interest according to Nyquist frequency (Hari et al. 2018) and often 3–4 times higher frequency is a valid option (Gross et al. 2013). Online monitoring is essential since the data quality can be assessed in real-time and artifacts and their sources can be identified (Gross et al. 2013). It is easier to try to prevent artifacts before and during measurements than fixing them afterwards during data analysis (Hari et al. 2018).

The analysis of MEG signals is rather time-consuming. Data analysis begins with preprocessing, which purpose is to handle the artifacts and get the raw data to the point it can be further analyzed. First, visual examination is done to assess data quality, and the possible bad channels are removed (Hari et al. 2018). Independent components analysis (ICA) is a widely used computational method used especially in preprocessing due to its ability to identify artifacts (Lopes da Silva 2013). Furthermore, proper filtering needs to be selected, because filters can remove or attenuate signals in certain frequency bands (Hari et al. 2018). Notch filter with the frequency of the power line (50 Hz in Finland) are often recommended. Analysis after preprocessing depends heavily on the research design and the research questions and there are numerous different MEG analyses. Some of the most used analyses are covered in the Gross et al. (2013) article, and the reader are recommended to explore the paper for further information.

4.3 Validity and reliability of MEG

MEG exhibits reduced sensitivity to neural currents oriented radially (i.e., those aligned from brain's center to the surface). Instead, the tangential currents produce a perpendicular magnetic induction that reach pick-up coils outside the scalp. (Baillet 2017) This phenomenon is illustrated in figure 6 in a simplified manner. MEG's sensitivity to tangential current flows leads to the suggestion that it mainly measures the activity from the cortical fissures. Fortunately, all primary sensory regions are located within the fissures of the cortex (Penfield & Rasmussen 1950). However, this may not be the whole truth, and there is some evidence that radial currents are not as silent in MEG as initially thought (Hillebrand & Barnes 2002). Still, this limitation of MEG's sensitivity, or insensitivity, is important to acknowledge.

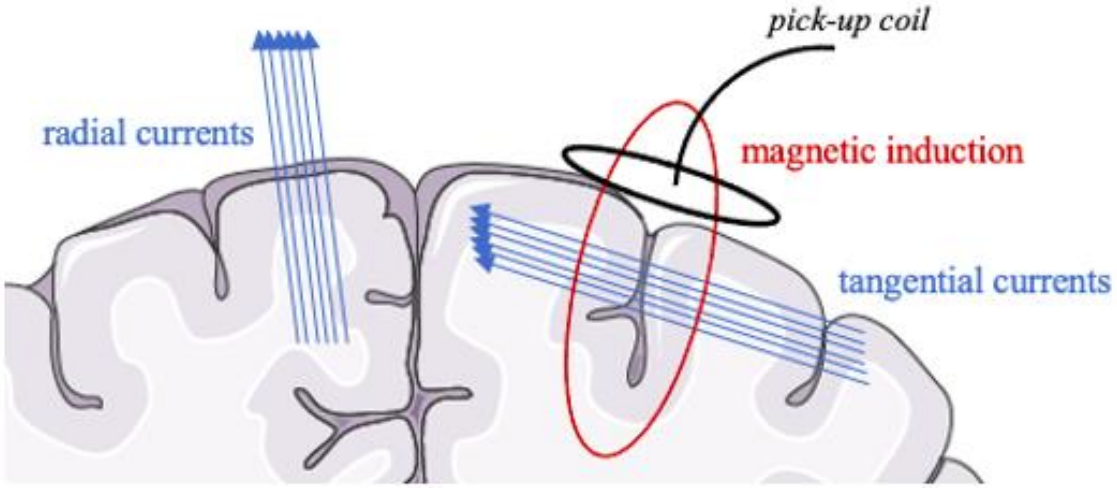

FIGURE 6. MEG is less sensitive to radial neural currents since only the tangential neural currents produce a magnetic field outside the scalp.

MEG is also less sensitive to detect deep and mid brain activity since the signal-to-noise ratio is lower when the source is deep in the brain (Hillebrand & Barnes 2002). However, models to record deep brain activity has been introduced (Attal et al. 2007), and there is also some experimental evidence that deep and mid brain activity could be detected with optimized paradigms and signal extraction techniques (e.g., Roux et al. 2013; Cornwell et al. 2008). Unlike MEG, EEG is rather sensitive to both tangential and radial components (Lopes da Silva 2013). This can be accounted as advantage or disadvantage depending on the neural source in interest. MEG signals depend heavily on the positioning of the pick-up coils relative to neural sources. The pick-up coils' placement varies between different manufacturers' instrumentation and are

also relative to the head position within the MEG helmet. That is why monitoring of head position and movements during measurements is important. This is achieved by online video monitoring so that the researchers are able to see and register the movement of the subject. In addition, MEG systems have software to measure head position in real-time in the helmet, and offline software solutions are also available. (Baillet 2017)

The validity of MEG, that is if the method measures what it should measure, has been mostly estimated by evaluating sensitivity. Also, often MEG studies rely on theoretical and physical models and the validity is then estimated by researchers themselves (i.e., face validity). Validity can also be quantitatively assessed in study designs where MEG is compared with EEG. Sensitivity to cortical and subcortical sources of MEG and EEG was recently investigated by Piastra et al. (2020). The results showed that MEG is more sensitive than EEG to the majority of cortical sources and that MEG could also detect tangential sources from subcortical brain areas. EEG was more sensitive to radial and deep sources, as expected. The research group concluded that EEG and MEG are in fact complimentary methods because their different sensitivities for different sources and should be used together if possible. Furthermore, if both methods cannot be used simultaneously, researchers and clinicians should decide the best method precisely for their experiment or diagnostics. (Piastra et al. 2020)

It is noteworthy that the assessment of reliability must be done separately for different phenomena and study designs in interest. It is not possible to test the overall reliability of MEG, but rather investigate if MEG is reliable method for specific study designs. Reliability of MEG has been tested in several experimental designs, and it is shown to be a highly reproducible method for investigating different brain functions. Even though MEG responses typically exhibit large inter-individual variation, within-individual variation is showed to be low in movement-related oscillations (Espenhahn et al. 2017), resting-state oscillations (Martín-Buro et al. 2016), corticokinematic coherence (Piitulainen et al. 2018), somatosensory responses of upper limb (McCusker et al. 2021), and proprioceptive responses of the ankle joint (Mujunen et al. 2022) and of the hand (Illman et al. 2022). Since, MEG is suggested to be a capable tool for tracking individual brain activity of different domains over longer periods of time in longitudinal studies. In the field of research where the interest usually lies in averages, paired samples analyses are suggested to be used to overcome the large inter-individual variability (Gross et al 2013).

5 PURPOSE OF THE STUDY AND RESEARCH QUESTIONS

The purpose of the present study was to test the feasibility of a MEG-compatible proprioceptive stimulator of the knee joint and to provide preliminary insights into the cortical processing of proprioceptive afference of the patellar tendon reflex. The primary focus of this study was 1) to evaluate the feasibility of the stimulator, including its ability to produce quantifiable cortical responses. This evaluation also encompassed investigating the stability of the stimuli produced by the device, as well as exploring within-subject and between-subject variability of the evoked responses. The secondary focus was 2) to investigate whether stimulus intensity influenced the evoked responses, and 3) to examine the associations between evoked cortical and reflex responses. Consequently, three research questions and hypotheses were formulated:

Research question 1. Is the proprioceptive stimulator feasible for producing quantifiable cortical responses?
Hypothesis: The stimulator is expected to elicit stable stimuli and produce quantifiable MEG responses. Within-subject variability of evoked responses is anticipated to be lower than between-subject variability.
Rationale: Previous studies have shown different stimulators' capability to produce quantifiable MEG responses, and peak responses elicited by proprioceptive stimulation are located on the contralateral SM1 cortex (Mujunen et al. 2022; Nurmi et al. 2023; Piitulainen et al. 2015). Reflex amplitudes exhibit significant within-subject and between-subject variability (Frijns et al. 1997; Stam & Tan 1987; Stam & van Crevel 1989; Toft et al. 1991; Uysal et al. 1999), while latency as a reflex response parameter appears to be more reliable (Frijns et al. 1997).

Research question 2. Does stimulus intensity affect cortical and reflex responses?
Hypothesis: Increased stimulus intensity is expected to strengthen all evoked responses (MEG, EMG, and force responses).
Rationale: Higher stimulus intensities are presumed to activate more proprioceptors, thereby amplifying evoked responses. Regarding cortical responses, more comprehensive proprioceptive stimulation has been shown to amplify cortical processing using both MEG (Hakonen et al. 2022) and fMRI (Nurmi et al. 2018), but contradictory results have also been reported (Nurmi et al. 2023). Regarding reflex responses, prior studies have shown that higher stimulus intensity leads to larger reflex responses (Stam & van Crevel 1989; Tham et al. 2013), with Tham et al. (2013) noting a linear relationship between stimulus intensity and reflex

amplitude, and Stam and van Crevel (1989) observing a plateau in reflex responses beyond a certain level of stimulus intensity.

Research question 3. Are cortical responses associated with reflex responses?
Hypothesis: It is hypothesized that there is a positive association between MEG responses and EMG and force responses.
Rationale: Proprioceptive stimulation activates proprioceptors located in muscles and joints (Abbruzzese et al. 1985; Mima et al. 1996; Starr et al. 1981), and the evoked responses can be recorded using MEG (e.g., Alary et al. 2002; Bourguignon et al. 2012; Druschky et al. 2003). Proprioceptive signals travels both to the spinal cord for reflexive responses and to the cortex for higher-level processing (Tuthill & Azim 2018). Given that both MEG and reflex responses are to some extent influenced by proprioceptive stimulation, it's plausible that they exhibit a positive association.

6 MATERIALS AND METHODS

6.1 Subjects

Fifteen healthy volunteers participated in the study (14 right-foot dominant, 28.1 ± 5.3 years, 6 females) that underwent from November 2023 to February 2024. Participants were recruited through various channels, including web posts and face-to-face communication. A recruitment advertisement for the study was shared with potential participants, outlining that volunteers were sought for research purposes and providing a brief overview of the study. To meet inclusion criteria, participants were required to have a normal walking ability and to fall within the age range of 18 to 50 years. Exclusion criteria comprised individuals with recent knee injuries or pain, musculoskeletal/neural diseases, and those using medications affecting the central nervous system. Additionally, participants were required to be free from metal objects in the head area and internal medical devices to prevent interference with MEG signal quality.

The informed consent process emphasized that participation was voluntary, ensuring that individuals were aware of their right to withdraw from the study at any point without consequences. The lack of direct personal benefit and absence of compensation for participation were also made clear. Before participation, all participants were informed about the potential risks and the possible discomfort linked with the measurements. All subjects gave their written informed consent to participate in the study. The procedures of the study were approved by the University of Jyväskylä ethics committee and were carried out according to the declaration of Helsinki.

6.2 Study design and experimental protocol

The present study consisted of one research session, which was held in the Centre for Interdisciplinary Brain Research, University of Jyväskylä. The research session included filling out the Waterloo footedness questionnaire (appendix 1) and one MEG session (figure 7). The MEG session consisted of two resting measurements (first eyes open, second eyes closed), and two subsequent measurements where the knee joint was stimulated with two different intensities. The result of the footedness questionnaire determined the side of stimulation.

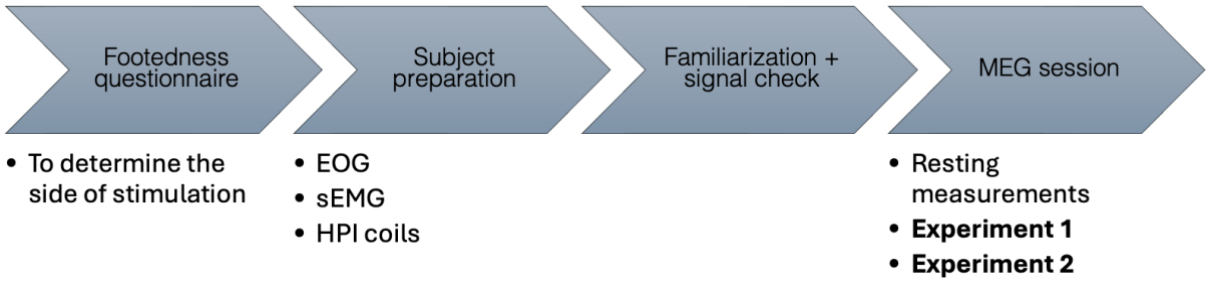

FIGURE 7. Overview of the research session.

Before the MEG session, EOG and EMG electrodes, five head position indicator (HPI) coils, as well as one ground electrode were taped on the skin of the subject. The skin was rubbed with an alcohol pad before attaching the EOG electrodes. The patellar tendon reflex was initially tested using a hand-held reflex hammer and a reference point was marked on top of the patellar tendon to assist setting the MEG-compatible reflex hammer at the correct position before the measurements. The stimulation reference point and EMG placement on top of vastus lateralis and vastus medialis muscles are shown in figure 8.

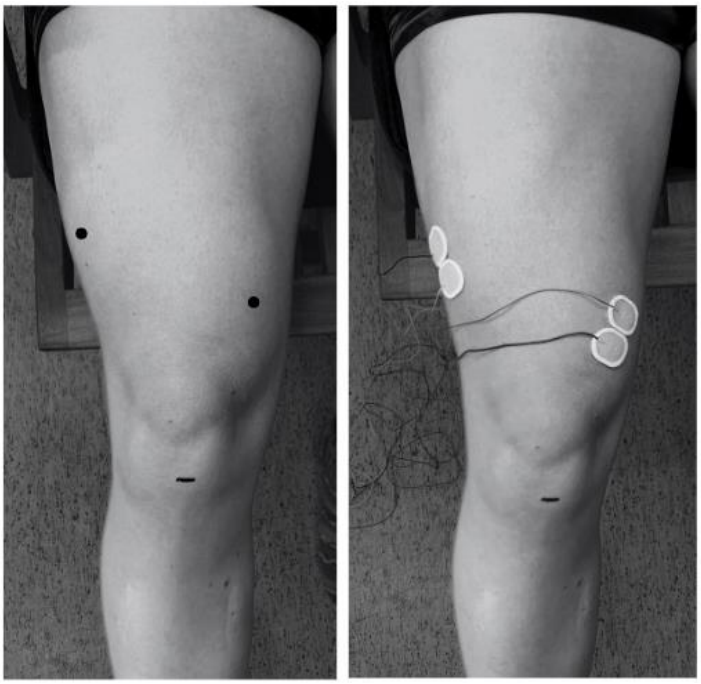

FIGURE 8. Placement of the EMG electrodes on top of the vastus lateralis and vastus medialis muscles, and the reference point of the patellar tendon stimulation location.

EMG electrodes were attached after the body hair from small parts of the other thigh was removed with a razor, the dead skin cells were removed with a piece of sandpaper, and the skin was sanitized with an alcohol pad. HPI coils were taped on the forehead of the subject below

the hairline and behind the ears. Thereafter, the head-coordinate system was digitized using a digitizer (Isotrak, Polhemus, Colchester, VT, USA). During the digitization, the researcher gently rested the tip of the digitization pen on the anatomical landmarks (nasion and pre-auricular points) and the HPI coils and pressed the button on the pen. Then, the head shape was digitized by gently stroking the point of the pen over several spots of the head while pressing the button of the pen.

MEG measurements were conducted in a magnetically shielded room (Magnetical Shielding Cabin, VACOSHIELD, Vacuumschmelze GmbH & Co. KG, Hanau, Germany), equipped with video and intercom systems that allowed the researchers to see, hear, and communicate with the subjects. The subject sat comfortably in a chair connected to the MEG device with their head in a helmet-like MEG dewar. The participant's arms were positioned on top of a pillow placed on their lap. The participant's other leg was attached to the chair with a Velcro belt so that the position of the leg remained the same throughout the measurement. Visual distractions were eliminated by using a sheet of paper so that the subjects could not see the reflex hammer, the researcher operating it, or their lower limbs. The set-up is illustrated in figure 9.

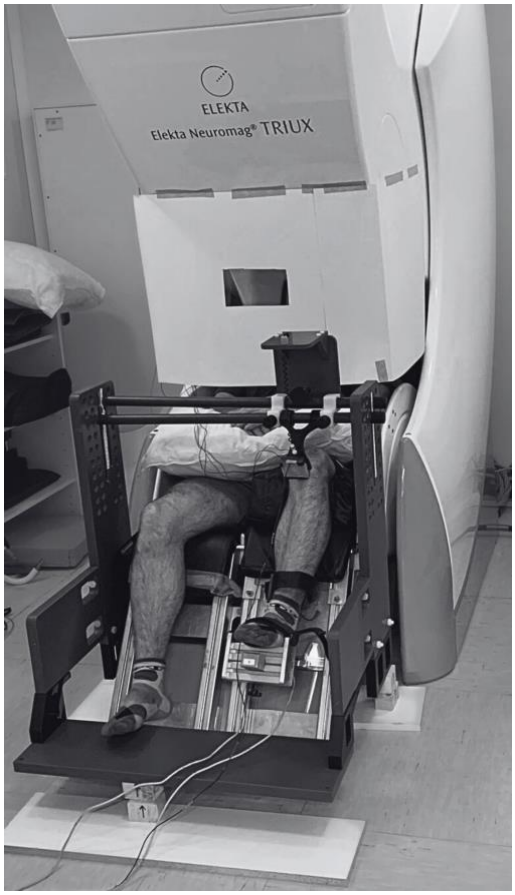

FIGURE 9. The experimental setup of the measurements.

Since head movements tend to occur due to reflex responses, pieces of foam rubber were placed between the subject's forehead and the outer surface of the dewar and secured with a net cap. This helped to keep the head still during the stimulations. During the MEG measurements, participants were instructed to focus their vision on a marker positioned in front of them on the wall, and to keep their head still and avoid extensive eye blinks during the measurements to minimize signal disturbance.

The MEG session included two measurements with proprioceptive stimulations of the knee joint with a manually guided MEG-compatible reflex hammer. One researcher was inside the shielded room with the participants during the proprioceptive stimulations, guiding the stimulation device. The stimuli were controlled using Presentation software version 21.1 (Neurobehavioral Systems Inc., Albany, CA, USA) to deliver visual signals (a light at the end of an optical fiber) to the MEG room allowing the hammer operator to know when to drop the hammer. To block the minor auditory disturbances stemming from the reflex hammer, subjects were instructed to wear earplugs. Any residual noise was further suppressed by Brownian noise played through flat panel speakers. The subjects confirmed their inability to hear any external noise.

6.3 MEG-compatible knee joint stimulator

A non-magnetic proprioceptive stimulator (reflex hammer) of the knee was designed and developed in the faculty of Sport and Health Sciences in the University of Jyväskylä by researchers and a specialized laboratory engineer. The hammer was operated manually by a researcher. The hammer was dropped through an arc using gravity to stimulate the patellar tendon. After the hammer had percussed the patellar tendon once, the researcher captured the hammer and moved it to its original upright position. The hammer could be moved in horizontal and vertical plane to find the right placement for the stimulations. With some subjects, the margin of adjustment in the vertical plane were not enough. In these cases, extra blocks were placed underneath the device, as seen in the previous figure. The custom-made stimulator is shown in figure 10.

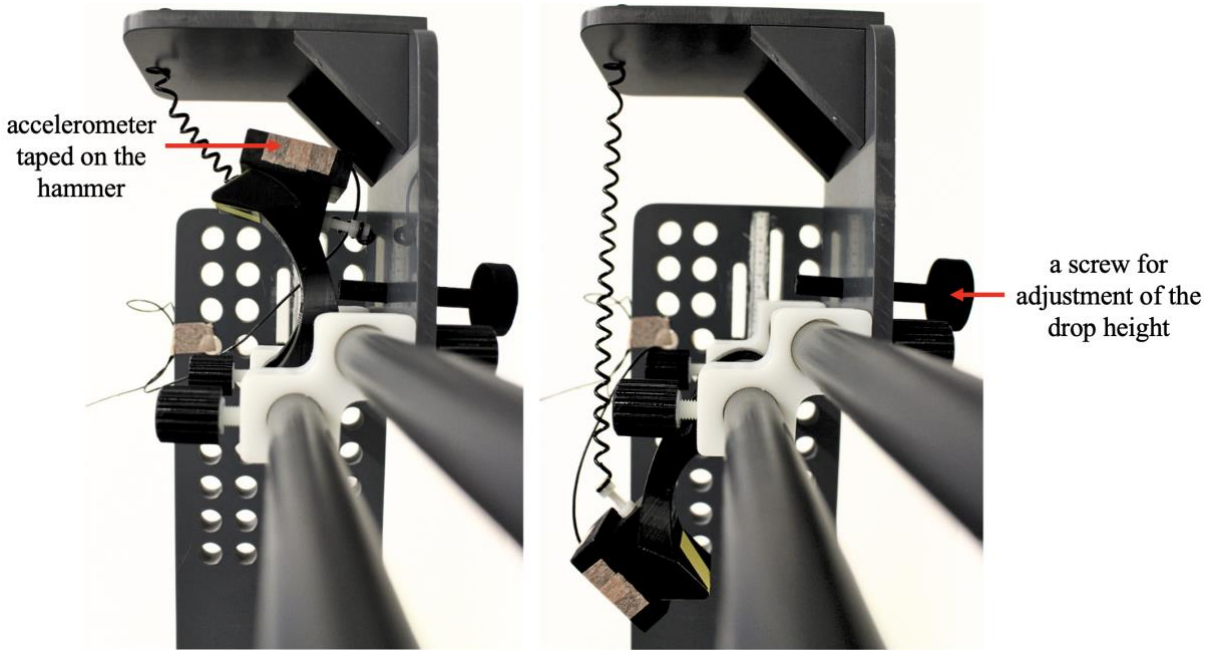

FIGURE 10. Manual MEG-compatible knee joint stimulator.

6.4 Data collection

MEG. MEG signals were acquired using a 306-channel whole-scalp neuromagnetometer (Elekta Neuromag TRIUXTM, Elekta Oy, Helsinki, Finland). Eye blinks were captured with EOG, and a ground electrode was attached to the side of the neck. All signals were sampled at 1000 Hz with a passband of 0.1–330 Hz. Continuous head position identification was conducted with the five HPI coils during the recordings to monitor the subject's head position relative to the MEG sensors.

Peripheral signals. Peripheral signals (EMG, force, and acceleration) were low-pass filtered at 330 Hz and sampled at 1 kHz, and time-locked to MEG signals. Acceleration of the reflex hammer was recorded with a 3-axis accelerometer (ADXL335 iMEMS Accelerometer, Analog Devies Inc. Norwood, MA, USA) attached on the top of the hammer.

6.5 Data analysis

Preprocessing of MEG signals. Preprocessing was done similarly to Mujunen et al. (2022), where the raw data of MEG signals were initially examined to identify noisy MEG sensors.

After the removal of noisy sensors, temporally extended signal-space separation algorithm (tSSS, MaxFilter 3.0 software, Elekta Neuromag Oy, Helsinki, Finland) was employed. This approach included compensating for head movement to diminish external disturbances. MEG signals were filtered between 1 and 40 Hz using a zero-phase finite impulse response filter (firwin in SciPy; Hamming window) and decomposed into 30 components using fast ICA algorithm using MNE Python software. The noise components associated with cardiac activity and eye blinks were discerned by analyzing the time-series data and topographical maps of the independent components and were removed from the final data, with typically 2–3 such components per participant.

MEG, EMG, force and kinematic analysis. The high and low experimental conditions were analyzed in an identical manner. Acceleration signals were filtered with 1 Hz high-pass filter, after which the peak magnitude of acceleration was calculated. This peak magnitude was used to determine the timing of stimulation (hammer percussion). After determining the timing of the stimulation, both MEG and EMG signals were divided into two-second epochs, one second before stimulation and one second after stimulation. Before EMG signal analysis, the signals were filtered with 1 Hz high-pass filter, after which the peak-to-peak amplitude of the SR was calculated for each epoch of both EMG signals, along with the standard deviation and coefficient of variation of these amplitudes. A similar analysis method was used for force signals, which were filtered into a 1–80 Hz band. The final selected amplitudes of the SR and force impulse for further analysis were the averages of 100 stimulation trials.

MEG signals were filtered into a 1–95 Hz band, after which epochs (n=100) were averaged relative to the stimulus onset (hammer percussion). After averaging, gradiometer pairs were combined into a vector sum, and then the peak gradiometer pair was selected from the sensors in the parietal area. The peak gradiometer pair was the pair of sensors with the highest evoked response amplitude in the parietal area. The same gradiometer pair was used for both high and low measurements per subject in the analyses. The latency of the evoked response is the time from the stimulus onset to the peak value of the response.

Statistical analysis. Data were analyzed using IBM SPSS 28.0 Statistic software (IBM, United States) and Microsoft Office Excel 365 (Microsoft Corporation, United States). Normality of data were checked using Shapiro-Wilk test ($n<50$). Since all the parameters were not normally distributed, and the sample size was relatively small, non-parametric statistical tests were used

(i.e., Wilcoxon, Spearman correlation). To estimate the stability of the measures, coefficient of variation (CV) for acceleration and evoked responses was calculated to describe between-subject and within-subject variability within session. To examine the associations between the measures, correlations (Spearman) were calculated. To investigate if the stimulus intensity affected the cortical MEG responses, force responses, and/or EMG responses, Wilcoxon signed-rank test for paired samples was used.

7 RESULTS

Figure 11 illustrates the data of a representative subject, presenting the characteristics of the recorded responses in the present study. The data included stimulus kinematics, muscle stretch reflexes represented by both EMG activity and force responses, and the MEG responses.

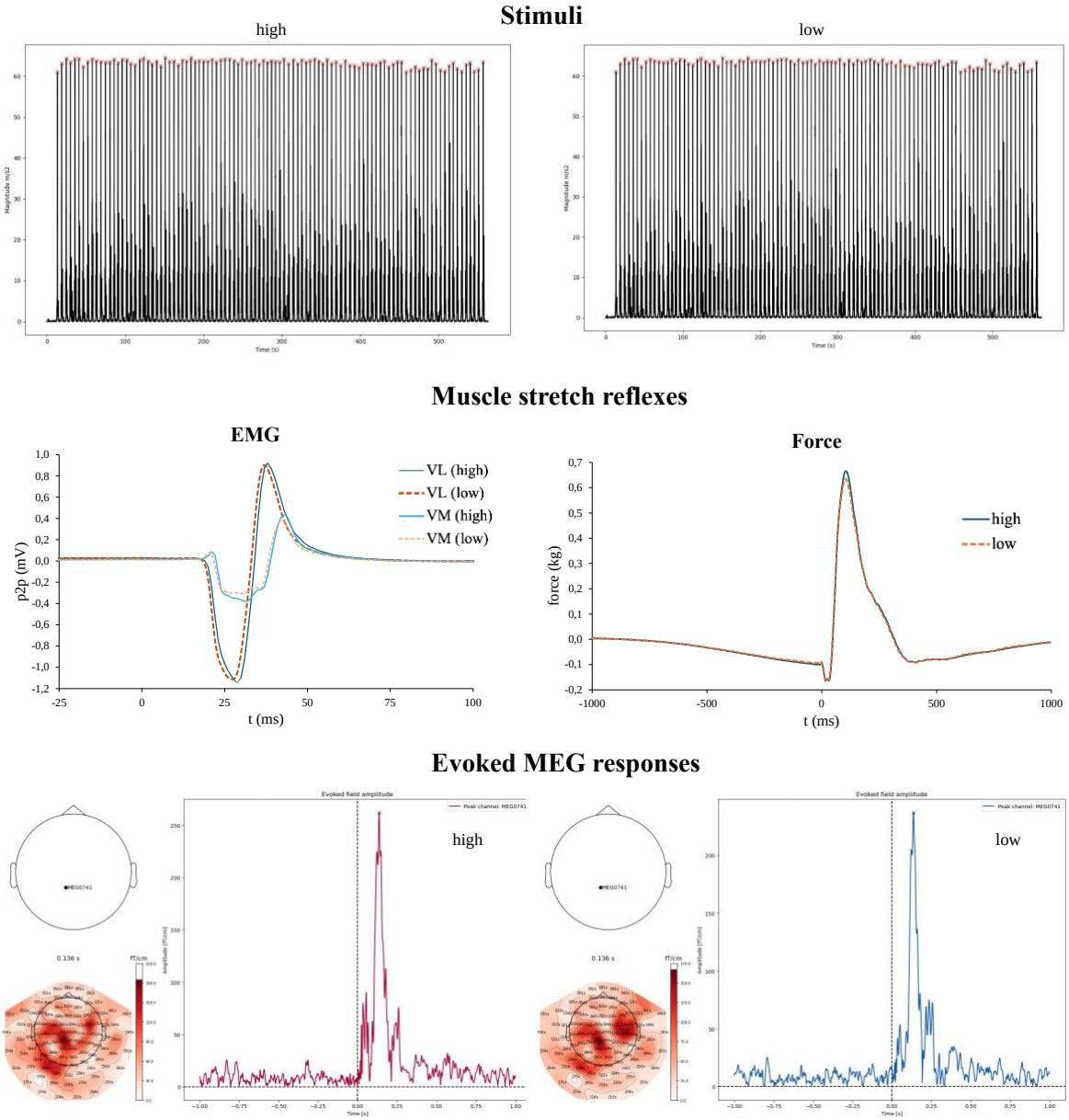

FIGURE 11. Stimuli kinematics and evoked responses in a representative subject. In all subfigures, magnitudes are plotted on the y-axis, while time is shown on the x-axis. The topmost subfigure displays the peak magnitudes of acceleration of the stimuli (depicted by red crosses) throughout the measurements. Evoked EMG and force responses are shown in the middle of the figure, while the evoked MEG responses are displayed at the bottom.

The representative subject in the figure 11 exhibited physiological responses both in the periphery (EMG and force responses) and at the central level (MEG responses). To be included in the final analyses, subjects' recorded responses needed to demonstrate clear physiological characteristics while including no major artifacts that would affect the physiological responses. Consequently, four participants were excluded from the statistical analyses due to disturbances in either MEG signals and/or EMG stretch reflex responses. As a result, clear muscle stretch reflexes and evoked MEG responses produced by proprioceptive stimulation could be recorded from 11 participants, accounting for 73% of the initial sample of 15. Therefore, all the upcoming results presented here have a sample size of 11. Despite the exclusion of these four subjects, the sample characteristics remained relatively consistent regarding age and gender distributions. All subjects in the final sample were right-foot dominant, with an average age of 28.3 ± 6.1 years, including 5 females.

7.1 Stimuli kinematics

Both measurements (high and low) consisted of 100 stimuli each. The ISI was 5.5 ± 0.03 seconds for the high measurement and 5.5 ± 0.02 seconds for the low measurement. There was no significant difference in ISIs between the two measurements (p=0.18). Intra-session variability was low, observed both within-subject and between-subject levels. Within-subject variability is described in table 1. The CV was 1.9 ± 1.7% in the high measurement and 1.9 ± 1.2% in the low measurement. Between-subject variability was 10.8% and 9.9% in the high and low measurements, respectively.

TABLE 1. Within-subject variability of the stimuli[a].

	High	**Low**
mean (min–max)	58 (45.6–64.0) m/s^2	55 (47.0–61.9) m/s^2
SD (min–max)	1 (0.4–3.3) m/s^2	1 (0.3–2.6) m/s^2
CV (mean ± SD)	1.9 ± 1.7%	1.9 ± 1.2%

[a] Peak magnitude of acceleration of the stimuli.

The peak magnitudes of the stimuli in the two measurements did not differ significantly from each other (high: 58 ± 6 m/s^2 and low: 55 ± 5 m/s^2, p=0.13). The acceleration signal of the

reflex hammer contained one peak and is illustrated in figure 12. The figure displays the group-level grand average and standard deviations of the time-series of the stimuli.

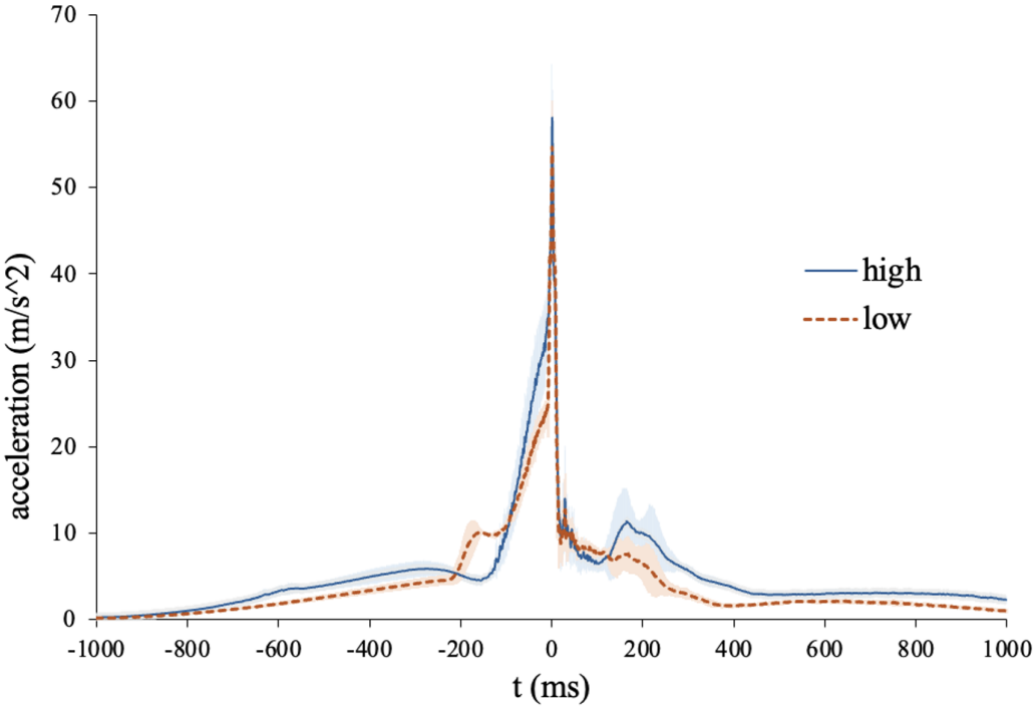

FIGURE 12. Group-level grand averages (lines) and standard deviations (shades) of the stimuli in the high and low measurements.

7.2 Evoked responses

To start with the MEG responses, figure 13 shows the group-level topographies of the evoked responses, peaking at the sensors on top of the somatosensory cortex at 67 ± 36 milliseconds and 81 ± 48 milliseconds after stimulation onset in the high and low measurements, respectively. Additionally, figure 13 illustrates the grand average time-series of the evoked fields. The MEG data quality was generally very good in the final sample. Among the 11 subjects, peak responses could be observed in four different but adjacent gradiometer pairs in the parietal area. Consequently, the peak gradiometer pair represented the pair of MEG sensors with the highest evoked response amplitude in this region. Despite the occurrence of artifacts and/or multiple peaks in MEG data within some subjects, robust responses were still identifiable on physiologically relevant MEG channels in the parietal area. See appendix 2 for more information about selecting peak gradiometer pairs from the parietal area.

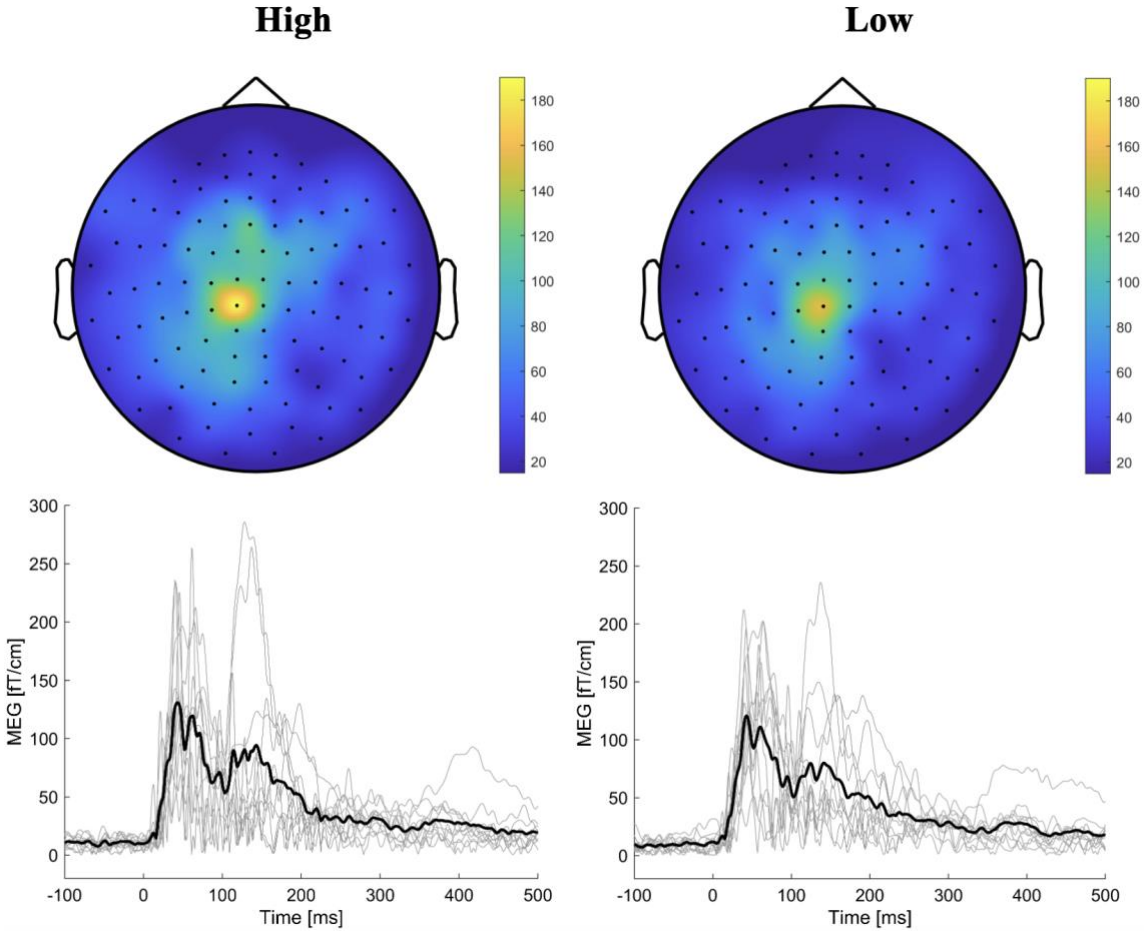

FIGURE 13. Group-level topographies and grand average time-series of evoked fields in high and low measurements.

The descriptive statistics of the MEG, force, and EMG responses are presented in table 2, which also includes the comparison of the means between high and low measurements. In addition, figure 14 illustrates the comparison of the evoked responses in high and low measurements using box plots. A statistically significant difference between stimulus intensities was found only in amplitudes of MEG responses ($p=0.02$), whereas no such differences in amplitudes were observed in force ($p=0.11$) or EMG responses (VL: $p=0.48$ and VM: $p=0.86$). Peak-to-peak EMG amplitudes were greater in VL compared to VM at both intensities. Additionally, the latencies did not significantly differ from each other between stimulus intensities at either the brain level ($p=0.46$) or the muscle level (VL: $p=0.25$ and VM: $p=0.16$).

TABLE 2. Descriptive statistics of the evoked responses and comparison of the means between high and low measurements.

	High		Low		
	mean ± SD	min–max	mean ± SD	min–max	p-value[a]
MEG					
amplitude (fT/cm)	203 ± 57	120–283	174 ± 39	101–237	0.02*
latency (ms)	67 ± 36	30–140	81 ± 48	40–160	0.46
Force					
force (kg)	0.9 ± 0.6	0.2–2.1	0.8 ± 0.5	0.1–1.8	0.11
EMG (VL)					
amplitude (mV)	0.86 ± 1.00	0.08–2.96	0.75 ± 0.73	0.06–2.00	0.48
latency (ms)	27 ± 5	18–35	26 ± 3	22–30	0.25
EMG (VM)					
amplitude (mV)	0.46 ± 0.38	0.06–1.29	0.46 ± 0.41	0.04–1.45	0.86
latency (ms)	28 ± 5	21–39	27 ± 4	19–33	0.16

[a] Wilcoxon signed-rank test for paired samples for comparison of high and low measurements.

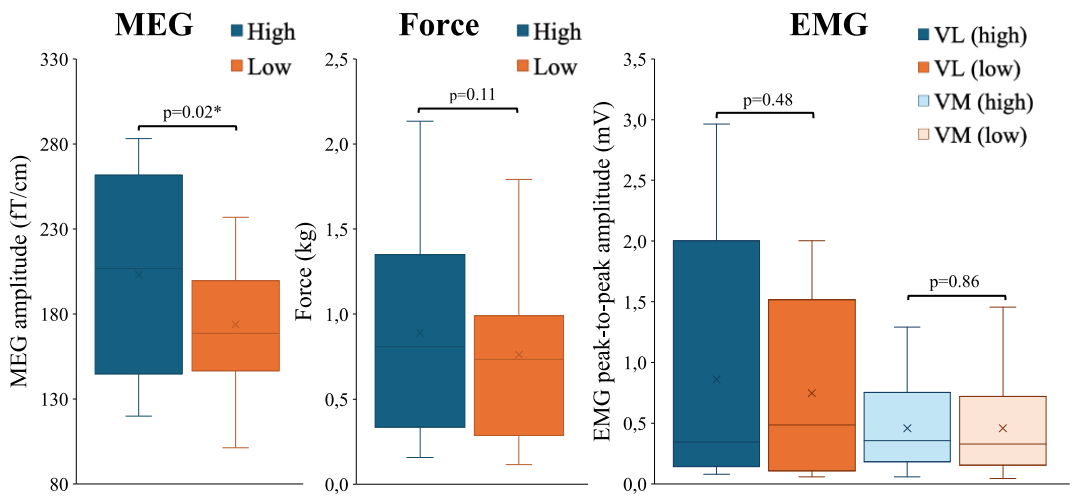

FIGURE 14. Comparison of evoked responses between high and low measurements. *=statistical significance at $p<0.05$.

The CV for evoked responses was calculated to estimate between-subject and/or within-subject variability, thereby assessing the stability of the parameters within sessions. The group-level CV for peak MEG amplitude was 22% in the low measurement and 28% in the high

measurement. Group-level CVs for EMG amplitudes and force responses were notably higher with a minimum of 68%, indicating substantial variability. Between-subject CVs for EMG latencies in the high measurement were 17% and 19% for VL and VM, respectively. In the low measurement, the corresponding values were 11% and 16%. Within-subject variability for force and EMG responses is presented in table 3. Within-subject variability was generally excellent for EMG latencies, while EMG amplitudes exhibited considerable variability even within subjects. Force responses were mostly at an acceptable level of variability.

TABLE 3. Within-subject variability of force and peak-to-peak EMG amplitudes.

	High (CV, %)					**Low** (CV, %)				
subject	F	VL amp	VM amp	VL lat	VM lat	F	VL amp	VM amp	VL lat	VM lat
1	14	29	31	13	14	61	30	31	4	20
2	31	62	58	7	12	30	64	57	5	10
3	16	50	35	24	13	20	69	37	29	15
4	21	41	52	33	17	35	61	50	19	6
5	15	33	24	2	6	24	42	31	3	3
6	15	22	33	10	3	15	29	30	6	4
7	12	20	19	6	2	14	36	24	8	2
8	18	22	32	2	2	19	25	36	2	2
9	21	25	57	4	3	20	25	46	3	2
10	14	46	44	12	8	18	56	74	19	11
11	26	62	84	9	7	12	60	84	7	9
mean±SD	18±6	38±16	43±19	11±9	8±5	24±14	45±17	45±19	9±9	8±6

CV=(SD/x̄)*100, <10% excellent, 10–20% good, 20–30% fair, > 30% poor
amp=amplitude, lat=latency, F=force, VL=m. vastus lateralis, VM=m. vastus medialis

Table 4 presents correlations (Spearman correlation coefficient, r_s) between the parameters in both high and low measurements. Acceleration parameters did not show significant correlations with any of the other parameters. Peak MEG amplitudes in high and low measurements exhibited a positive correlation with each other (r_s=0.70, $p<0.05$). The EMG amplitude parameters showed positive correlations across the two different muscles. Furthermore, most EMG amplitude parameters were positively associated with force responses.

TABLE 4. Correlations between parameters in high and low measurements, including proprioceptive stimuli, peak MEG amplitudes, peak EMG amplitudes, and force responses.

	high ACC	high MEG	high VL	high VM	high F	low ACC	low MEG	low VL	low VM	low F
high ACC	1									
high MEG	0.57	1								
high VL	0.04	0.33	1							
high VM	0.17	0.06	0.76**	1						
high F	-0.04	0.15	0.77**	0.63*	1					
low ACC	0.28	0.28	0.25	0.03	0.35	1				
low MEG	0.31	0.70*	0.02	-0.19	-0.38	0.12	1			
low VL	0.07	0.38	0.96***	0.83**	0.79**	0.26	0.00	1		
low VM	0.13	0.19	0.70*	0.87***	0.55	-0.20	-0.11	0.77**	1	
low F	0.04	0.09	0.72*	0.84**	0.89***	0.22	-0.38	0.82**	0.69*	1

[a] Correlations were estimated with Spearman's rank correlation coefficient (2-tailed).
[b] *<0.05, **<0.01 ***<0.001.
[c] ACC=acceleration peaks of the stimuli, MEG=peak MEG amplitude, F=force, VL=peak-to-peak EMG amplitude from m. vastus lateralis, VM=peak-to-peak EMG amplitude from m. vastus medialis.

In figure 15, the topmost scatter plots illustrate correlations between EMG amplitudes (VL and VM muscles) and force responses in both high and low measurements. The strongest correlations were observed between VL and force in both measurements (high: r_s=0.82, $p<0.01$ and low: r_s=0.77, $p<0.01$). The correlations between VM and force were 0.69 and 0.63 in low and high measurements, respectively, significant at $p<0.05$. The lower scatterplot demonstrates a positive correlation between the peak MEG amplitude in high measurement and the peak MEG amplitude in low measurement (r_s=0.70, $p<0.05$).

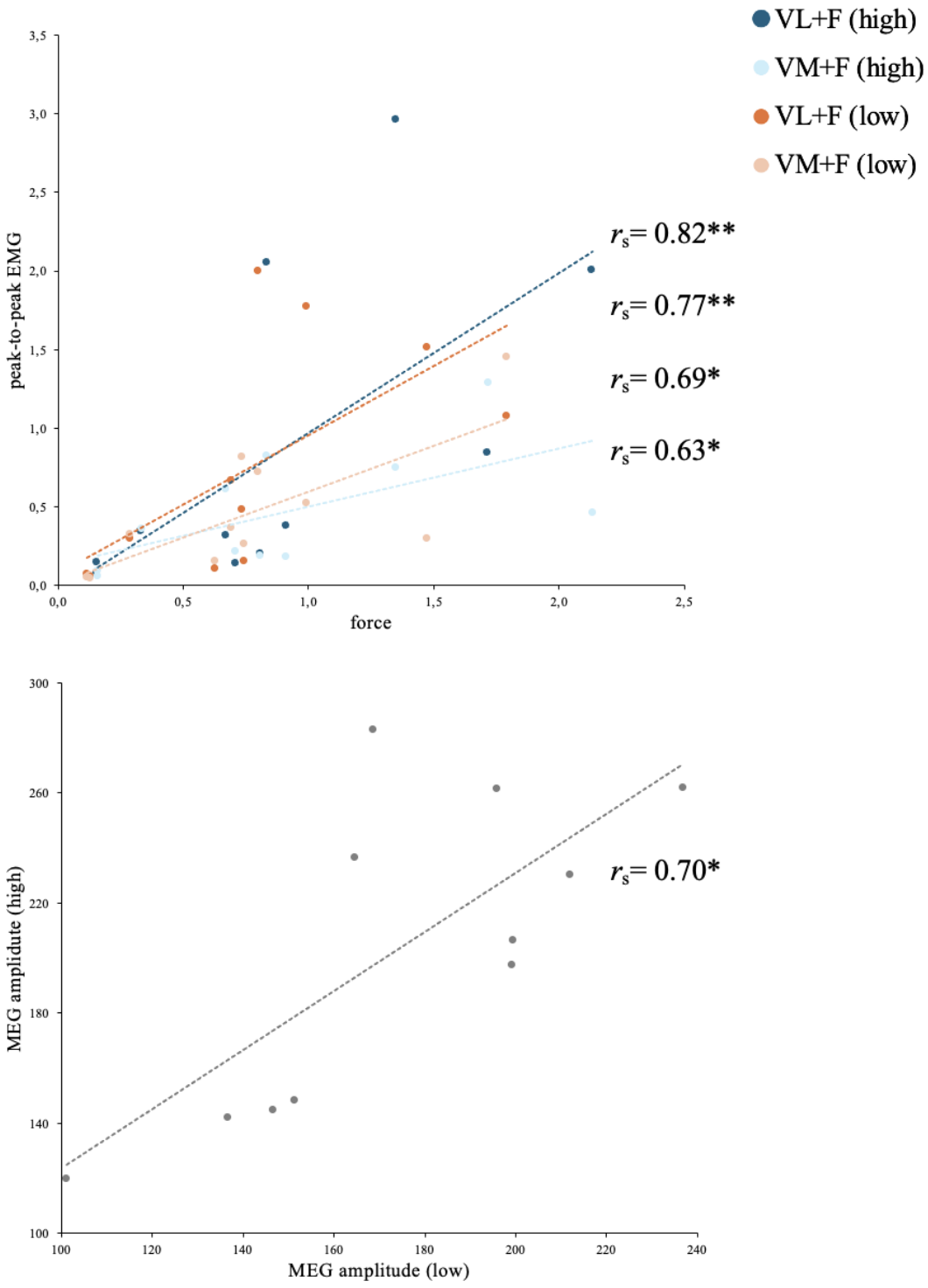

FIGURE 15. Scatter plots illustrating correlations between peak-to-peak EMG amplitudes and force responses (top) and the positive correlation between peak MEG amplitudes in high and low measurements (down).

8 DISCUSSION

The objective of this study was to assess the feasibility of a novel MEG-compatible proprioceptive stimulator of the knee joint and to provide preliminary insights into the cortical processing of proprioceptive afference of the patellar tendon reflex. This study was the first to investigate the processing of proprioceptive afference of the patellar tendon reflex in the cerebral cortex.

The main findings of this study demonstrated that the MEG-compatible proprioceptive stimulator produced stable stimuli within sessions, and it was possible to elicit measurable physiological responses both at muscle and brain levels. Consequently, the novel proprioceptive stimulator seems to be feasible for research purposes. In addition, there was less inter-subject variability in the cortical responses compared the peripheral responses, and MEG was more sensitive in detecting changes in stimulus intensity compared to EMG and force transducer. Thus, MEG appears to be a suitable method for investigating the processing of proprioceptive afference of the patellar tendon reflex.

8.1 Feasibility

The primary focus of this study was to evaluate the feasibility of the stimulator, including its ability to produce quantifiable cortical and peripheral responses, and the stability of the stimuli it generates. The variability of the other measures (i.e., cortical, muscle activity, and force measures) was also investigated. It was hypothesized that the stimulator would elicit stable stimuli and produce corresponding quantifiable MEG, EMG, and force responses, with within-subject variability of evoked responses expected to be lower than between-subject variability.

To be included in final analyses, subjects' recorded responses at both the peripheral and cortical level were required to demonstrate clear physiological characteristics without major artifacts that could affect the physiological responses. Consequently, four participants were excluded from the analyses due to disturbances observed in either MEG signals or EMG stretch reflex responses. As a result, clear muscle stretch reflexes and corresponding evoked MEG responses elicited by proprioceptive stimulation could be recorded from 11 participants, accounting for 73% of the initial sample of 15 subjects.

The exclusion of a few subjects from the final analyses was due to disturbances observed in either MEG or EMG signals, but these disturbances were not likely attributed to the stimulator itself, as the stimuli kinematics did not differ from those of included subjects. In addition, most of the subjects in the final sample exhibited excellent signal quality, with no electric or magnetic artifacts arising from the stimulation device or the external environment. Although some subjects exhibited stimulus-locked artifacts in the MEG signals, these artifacts could be distinguished from the physiological responses. Overall, the custom-made proprioceptive stimulator successfully elicited quantifiable cortical MEG responses and peripheral EMG and force responses for majority of subjects, and thus the methods employed in this study appear to be feasible.

8.1.1 Stimulator and stimuli kinematics

Before thoroughly discussing the evoked responses, it is relevant to first address the stimulator and the stimuli it produced. The custom-made stimulator was manually operated by a researcher, with the hammer dropped through an arc using gravity to stimulate the patellar tendon. After the hammer struck the tendon once, the researcher captured it and returned the hammer to its original upright position. The manual guidance of the device was reported to be rather simple and feasible. To block the minor auditory noise stemming from the reflex hammer, subjects were instructed to wear earplugs. Any residual noise was further suppressed by Brownian noise played through flat panel speakers. The subjects confirmed their inability to hear any external noise, and no auditory-evoked fields elicited by sound was noticed in the MEG results.

Each measurement included 100 stimuli and lasted approximately nine minutes. Given the variable nature of the evoked responses (reflex responses: e.g., Stam & van Crevel 1989 and MEG responses: e.g., Mujunen et al. 2022) this substantial number of stimuli was necessary. In a prior study, repeated tendon percussions have been reported to result in a progressive decrement of response amplitudes for both EMG and force responses, and this habituation seems to occur more prominently if the ISI is lower than five seconds (Hollis 1971). Although this finding stems from a case study, the results of the present study support this, as an ISI of 5.5 seconds was sufficient for the responses not to significantly diminish during the 100 stimulations, indicating no significant habituation. Additionally, the ISI in the present study

included slight intentional jitter, potentially contributing to the prevention of habituation. In conclusion, the number of stimuli and ISI utilized were sufficient to generate clear responses for further analysis.

Each stimulation produced one peak in the acceleration signal, reflecting the percussion of the hammer on the patellar tendon. The acceleration peak magnitudes were well reproducible within session both within and between individuals. Despite slightly lower within-subject variability, all CV values were at a very good level. Thus, the stimuli generated by the device were stable and reproducible within the sessions.

While stimuli were stable, there is room for further improvements in the stimulator device. Although the development of the stimulator was not the objective of this study and, for that reason, the development process is not described here, the device could be enhanced. Despite the hammer being movable in both horizontal and vertical planes to allow for optimal placement for stimulation, there were instances where the margin of adjustment in the vertical plane was insufficient. Hence, the stimulator should be redesigned to allow for a wider range of vertical movement. In addition, in some measurement sessions, the hammer did not move as smoothly as usual. This issue occurred particularly in the initial measurements (i.e., the first subjects' measurements), during higher stimulus intensity stimulations. This likely contributed to the observation that in three individual cases, the acceleration peaks in high measurement were not greater compared to the low measurement. Despite precise piloting of the experimental setup, the operator may have become more familiar with the device over time. Thus, additional practice for the operator and structural refinements to the hammer component of the device could further improve the stimulation.

8.1.2 Evoked cortical responses

The group-level topographies of the evoked responses indicated that the cortical responses peaked at sensors over the somatosensory cortex, and the peak amplitudes were robust. These findings suggest that the proprioceptive stimulator effectively elicited cortical responses time-locked with the stimuli. Although precise source localization was not possible due to the lack of anatomical magnetic resonance images, the observed responses were located at the sensors over the SM1, which aligns with prior research (Mujunen et al. 2022; Nurmi et al. 2023;

Piitulainen et al. 2015). Notably, the location appeared slightly posterior within the parietal area, which may be attributed to the placement of foam rubbers on top of forehead that were used to stabilize subjects' heads during MEG recordings.

The MEG data quality was generally good in the final sample, and the time-series of evoked fields looked appropriate. Peak responses were observed in four different but adjacent gradiometer pairs in the parietal area. That is, the peak gradiometer pair represented the pair of MEG sensors with the highest evoked response amplitude in this region. Some participants expressed stimulus-locked artefacts in the MEG data, likely due to minor head movements despite stabilization techniques. Regardless of the presence of artifacts and non-relevant peaks in some subjects' data, robust responses were still clearly identifiable on physiologically relevant MEG channels in the parietal area. Excluded subjects included three subjects whose MEG data did not include a relevant peak at all, or it could not be separated from the disturbances.

The CVs for peak MEG amplitudes within sessions were calculated to estimate between-subject variability, thereby assessing the reliability of this parameter. The peak MEG amplitudes exhibited an acceptable amount of variability. Due to the complexity of computing further MEG analysis, within-subject variability could not be determined in the present study, and thus, these results are going to be reported elsewhere. However, previous research indicates that intra-subject variability of evoked MEG responses tends to be substantially lower than inter-subject variability (Mujunen et al. 2022). Since the between-subject CVs observed in the present study were on an acceptable level, it is presumable that also the within-subject variability was rather low.

The presence of stimulus-locked artifacts in some participants' MEG data, likely resulting from minor head movements, highlights the importance of stabilization techniques during data collection in tendon percussion studies. Despite these artifacts, the observed responses exhibited characteristic topographies and appropriate time-series profiles, indicative of physiological cortical activity related to proprioceptive afference. Consequently, the evoked MEG responses likely reflected cortical processing of proprioceptive stimuli. In summary, the proprioceptive stimulator demonstrated efficacy in eliciting quantifiable cortical MEG responses, providing valuable insights into the cortical processing of proprioceptive afference. Further studies incorporating anatomical imaging and improved stabilization techniques would

enhance the precision and accuracy of cortical localization, thereby advancing the understanding of sensorimotor integration in proprioception.

8.1.3 Evoked peripheral responses

SRs were evaluated by EMG and force responses. These peripheral measures were recorded primarily to determine if an appropriate reflex response was elicited by the stimulator, which could then be compared to the MEG responses. Three of the excluded subjects elicited remarkably small SR responses or the EMG signal included disturbances. The subjects in the final sample exhibited clear SR responses both in EMG activity and force response. EMG amplitudes were greater in the vastus lateralis compared to the vastus medialis. The EMG latencies were approximately 27 milliseconds for both muscles in the two measurements, which is a bit higher that latencies reported by both Knowlton & Britt (1963) and Frijns et al. (1997). However, there are several factors affecting the reflex responses which must be considered when comparing the results from different SR studies, such as age, height, position of the subject, and EMG electrode placement. Still, both the amplitudes and latencies of SRs were somewhat comparable to the results reported by Frijns et al. (1997).

Reflex amplitudes have been reported to exhibit significant intra-individual and inter-individual variability (Frijns et al. 1997; Stam & Tan 1987; Stam & van Crevel 1989), while latency as a reflex response parameter appears to be more reliable (Frijns et al. 1997). This was supported by the results from the present study, since latencies exhibited lower variability than amplitudes. In this study, group-level CVs for EMG and force responses were notably higher than for MEG responses. Overall, within-subject variability was generally excellent for EMG latencies and acceptable for force measures, while EMG amplitudes exhibited considerable variability even within subjects. The between-subject variability was higher in all parameters, as expected, with only latency reaching an acceptable level of variability.

In conclusion, the proprioceptive stimulator effectively elicited clear peripheral responses in most subjects. SR responses are influenced by a combination of internal and external factors, contributing to their high variability (Stam and Tan 1987). Internally, for example the fusimotor system and coordinated alpha-gamma coactivation contribute to reflex size and variability (Pierrot-Deseilligny & Burke 2012). Externally, factors such as age, height (Knowlton & Britt,

1963), muscle contraction (Toft et al. 1991; Uysal et al. 1999), and reflex hammer stimulation parameters (Tham et al. 2013) impact reflex responses. Although most peripheral measures demonstrated instability in this study, recording them was advantageous in verifying the occurrence of SRs. Without such verification, evaluating corresponding cortical measures would have been more uncertain.

8.2 Effects of stimulus intensity

The secondary focus of this study was to investigate whether stimulus intensity influenced evoked responses, including MEG, EMG, and force responses. Higher stimulus intensity was expected to strengthen MEG responses, as more comprehensive stimulation is presumed to activate more proprioceptors, thereby amplifying the processing of proprioceptive afference (Hakonen et al. 2022; Nurmi et al. 2018). Peripheral responses were anticipated to be emphasized as well, since prior studies have shown that higher stimulus intensity leads to larger reflex responses (Stam & van Crevel 1989; Tham et al. 2013).

When discussing the effects of stimulus intensity, it should be noted that although the peak magnitudes of acceleration were higher in the high measurement, the difference between the two measurements was not statistically significant. One factor that could have affected this was the functioning of the hammer at higher intensities. As discussed earlier, in some of the high measurements, the hammer did not move as smoothly as in the low-intensity measurements. To explore the effects of stimulus intensity more precisely, alterations to the stimulator should be made.

A statistically significant difference between stimulus intensities was found in the amplitudes of MEG responses, consistent with the hypothesis and findings from Hakonen et al. (2022) and Nurmi et al. (2018), despite differences in methods and experimental designs compared to the present study. A similar trend was observed in force and muscle activity measures although the changes were not statistically significant. The fact that no significant differences were observed in force or EMG responses between the two stimulus intensities was contrary to expectations and prior research (Stam & van Crevel 1989; Tham et al. 2013), The high variability in EMG amplitudes may have contributed to the lack of observed effects of stimulus intensity. However,

these findings suggest that MEG may be more robust in detecting changes in stimulus intensity compared to peripheral measures.

8.3 Associations between evoked responses

The other secondary focus of this study was to examine the associations between evoked cortical and peripheral responses. It was hypothesized that there would be a positive correlation between MEG responses and EMG and force responses, since proprioceptive stimulation activates proprioceptors located in muscles and joints (Abbruzzese et al. 1985; Mima et al. 1996; Starr et al. 1981), and the evoked responses can be recorded using MEG (e.g., Alary et al. 2002; Bourguignon et al. 2012; Druschky et al. 2003). Proprioceptive signals travel both to the spinal cord for reflexive responses and to the cortex for higher-level processing (Tuthill & Azim 2018). Given that both MEG and reflex responses are influenced by proprioceptive stimulation, it was plausible that they exhibit a positive association.

However, this hypothesis was not supported by the results, as no significant correlations were observed between MEG responses and the peripheral responses. This lack of correlation suggests that sensorimotor integration and processing of proprioceptive afference are complex processes that involves multiple neural mechanisms and is influenced by various factors. Proprioceptive information is transferred to the cortex through a series of neural pathways that involve sensory receptors, afferent neurons, spinal cord processing, thalamic relay, and specialized cortical regions (Delhaye et al. 2018). In addition, the variability particularly in the peripheral measures may have concealed some underlying correlation between cortical and peripheral responses, making it more challenging to detect significant associations. Additionally, measurement limitations, such as the inability of MEG recordings to capture and distinguish all the different currents in the bran (Baillet 2017), mean that the full complexity of sensorimotor processing cannot be assessed with MEG alone. The indirect nature of surface EMG and force measurements further contributes to the difficulty in establishing direct associations.

Correlation analyses were also done to see how the same parameters act in the two different measurements. Analyses showed that subjects who exhibited higher MEG responses in the high measurement, exhibited also higher MEG responses in the low measurement. Same occurred

with the peripheral measures, since EMG amplitudes and force responses in high and low measurements correlated significantly with each other. Furthermore, most EMG amplitude parameters were positively associated with force responses in the measurements. The strongest correlations were observed between VL and force in both measurements. This aligns with findings by Clarke (1965), who examined the relationship between EMG and force of the isometric patellar tendon reflex response measured at the ankle, and as a result, a high positive correlation between the EMG signal and reflex-produced isometric force was found.

8.4 Strengths and limitations

This study had several strengths. It introduced a novel MEG-compatible proprioceptive stimulator for assessing cortical responses to proprioceptive stimuli, addressing the need for specialized equipment for eliciting dynamic SRs in neuroscience research. The stimulator generated stable stimuli, and evoked quantifiable cortical and peripheral responses and produced no electric or magnetic artifacts. By combining MEG, EMG, and force measurements, the study provided preliminary insights into the cortical and peripheral processing of proprioceptive afference of the patellar tendon reflex. The stimulator, after some minor improvements, has future potential to be used in the research of the sensorimotor system in both healthy subjects and various clinical groups.

The present study also included limitations. Although the utilized methods were successful and feasible for most of the subjects, a small number of participants were excluded from the final analyses due to disturbances in MEG or EMG signals, potentially limiting the generalizability of the findings. The study did not incorporate anatomical magnetic resonance imaging, obstructing localization of cortical responses. Peripheral measures, particularly EMG amplitudes, exhibited substantial variability both within and between subjects, potentially confounding the interpretation of associations with cortical responses. In addition, although altering the drop height changed the kinematics of the stimuli, the change was not sufficient to achieve statistical significance. Nevertheless, both intensities were able to produce clear responses at both cortical and muscle levels. Despite these limitations, the study provides valuable insights into proprioceptive processing and highlights the potential of the MEG-compatible proprioceptive stimulator for advancing our understanding of sensorimotor integration in human proprioception.

8.5 Conclusions

The findings of this study demonstrate the feasibility of a novel MEG-compatible proprioceptive stimulator. The stimulator device produced repeatable stimuli, and it was possible to elicit measurable physiological responses both at muscle and brain levels. The observed lower variability in MEG responses compared to muscle-level responses within measurement sessions underscores the sensitivity and reliability of MEG in detecting subtle changes in proprioceptive processing. Hence, MEG appears to be a suitable method for investigating the processing of proprioceptive afference of muscle stretch reflexes.

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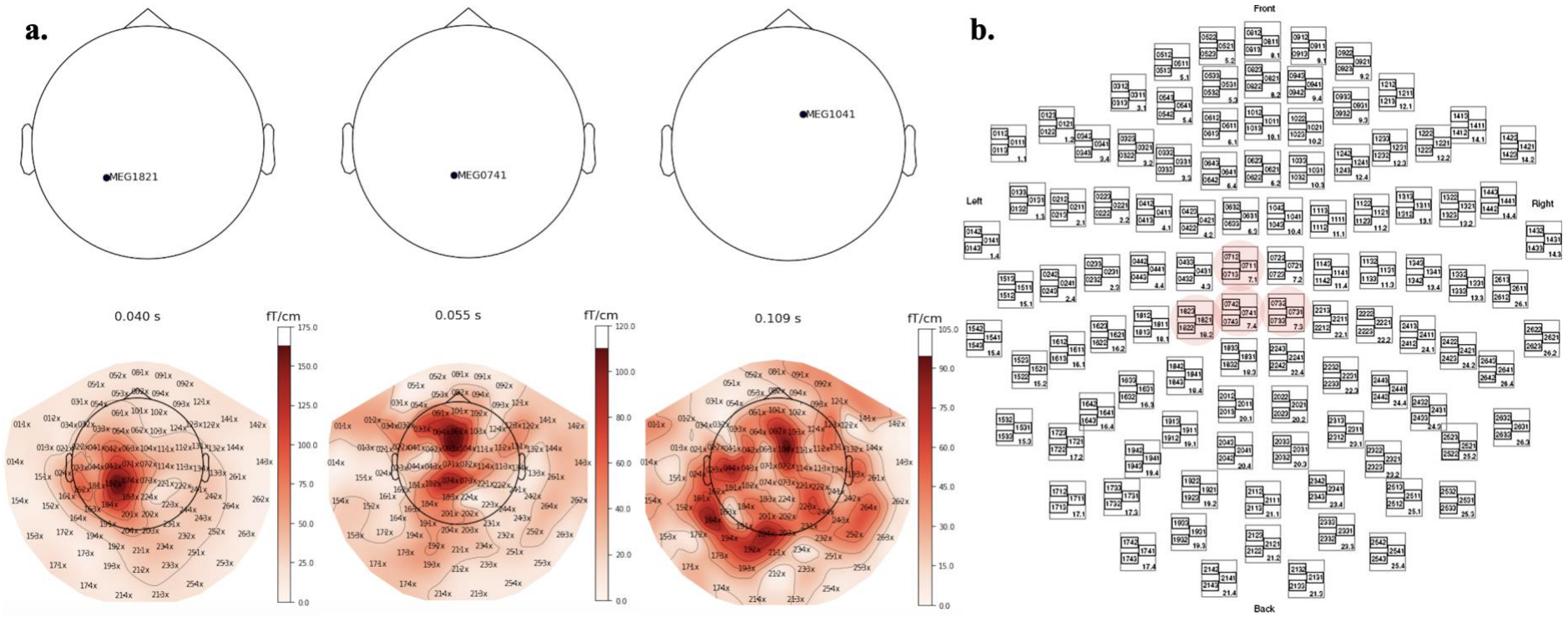
Appendix 1. Waterloo footedness questionnaire.

Vastaa alla oleviin kysymyksiin niin tarkasti kuin pystyt. Ympyröi yksi vastausvaihtoehdoista jokaisen kysymyksen kohdalla. Yritä kuvitella mielelläsi kysymyksessä esitetyn tehtävän suorittaminen, tarvittaessa voit myös kokeilla tehtävän suorittamista pantomiimina.

	Aina vasemmalla	**Yleensä vasemmalla**	**Kummallakin jalalla yhtä usein**	**Yleensä oikealla**	**Aina oikealla**
Kummalla jalalla potkaisisit paikallaan olevan pallon kohti edessäsi olevaa kohdetta?	-2	-1	0	1	2
Jos sinun pitäisi seistä yhdellä jalalla, kummalla seisoisit?	-2	-1	0	1	2
Kumpaa jalkaa käyttäisit hiekan tasoittamiseen hiekkarannalla?	-2	-1	0	1	2
Jos sinun pitäisi nousta tuolille seisomaan, kumman jalan nostaisit tuolille ensimmäisenä?	-2	-1	0	1	2
Kummalla jalalla tallaisit nopeasti liikkuvan ötökän päälle?	-2	-1	0	1	2
Jos sinun pitäisi tasapainoilla yhdellä jalalla rautatiekiskon päällä, kumpaa jalkaa käyttäisit?	-2	-1	0	1	2
Jos sinun pitäisi poimia varpaidesi avulla marmorikuula lattialta, kumpaa jalkaa käyttäisit?	-2	-1	0	1	2
Jos sinun pitäisi hyppiä yhdellä jalalla, kumman jalan valitsisit?	-2	-1	0	1	2
Kummalla jalalla auttaisit painamaan lapiota maahan?	-2	-1	0	1	2
Rennosti seisoessa ihmiset yleensä laittavat suurimman osan painosta toisen jalan päälle, jolloin toinen jalka koukistuu hieman. Kummalle jalalle asetat enemmän painoa ensin?	-2	-1	0	1	2
Oletko vaihtanut jostain syystä (esim. loukkaantuminen) valintaasi oikean ja vasemman jalan välillä yllä mainituissa tilanteissa?	EI	KYLLÄ			
Onko sinulle koskaan annettu erityistä harjoitusta tai suositusta käyttää tiettyä jalkaa yllä mainituissa tilanteissa?	EI	KYLLÄ			
Jos vastasit KYLLÄ kysymyksiin 11 tai 12, voitko antaa tarkemman selityksen:					

Appendix 2. MEG analysis: Selecting peak gradiometer pairs from the parietal area.



(a) Topographies of three individual subjects: The first subject exhibited the peak response in physiologically relevant sensors in the parietal area. The second subject's highest peak response was found outside the parietal area, but a slightly smaller peak was found in physiologically relevant sensors. This peak was manually adjusted to be included in the final analyses. The third subject was excluded from the final analyses due to the absence of peak responses in relevant sensors. (b) Among the 11 subjects comprising the final sample, peak responses were observed in four different, yet adjacent, gradiometer pairs within the parietal area, as highlighted in red.