Diplomarbeit

Spinal and corticospinal excitability before and after passive stretch of human plantar flexor muscles

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Graz, am 23. Juni 2018 *Daniela Kemper eh*

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List of abbreviations

ANOVA	Analysis of Variance
ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
CML	corticomotor latency
EMG	Electromyography
FWF	Fond zur Förderung der wissenschaftlichen Forschung
GABA	Gamma-Aminobutyric acid
H-Reflex	Hofman-Reflex
MEP	motor evoked potential
min	minutes
rMT	resting motor threshold
SENIAM	Surface ElectroMyoGraphy for the Non-Invasive Assessment of Muscles
SOL	soleus muscle
T-reflex	Stretch reflex (T from tendon)
T-tubules	transverse tubules
TES	transcranial electrical stimulation
TMS	transcranial magnetic stimulation

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Zusammenfassung

Hintergrund: Um den Einfluss von Dehnen auf die kortikospinale Erregbarkeit zu erforschen, hat sich die Untersuchung der Amplitude und der Latenzzeit der motorisch evozierten Potentiale (MEP) etabliert. Die spinale Erregbarkeit wird durch den Vergleich der Amplituden des Achillessehnenreflexes (T-reflex) und den H/M Quotienten analysiert. In einer vorhergehenden Studie des gleichen Projektes wurde der Einfluss 1-minütigen Dehnens bereits untersucht, zeigte keinen Effekt auf das MEP, allerdings eine Zunahme der H-reflex (Hoffmann's Reflex)-Amplitude und eine Inhibierung des T-reflexes. Ziel der vorliegenden Studie ist es, den Einfluss 5-minütigen passiven statischen Dehnens des M. soleus (SOL) auf das MEP, den T-reflex und den H-reflex zu untersuchen.

Methodik: 17 gesunde Probanden kamen an zwei Testtagen (Kontroll vs. Dehn-Bedingung). Die drei Zielparameter (T-reflex, H-reflex und MEPs) wurden an folgenden 4 Zeitpunkten am rechten SOL erhoben: Vor (Pre) und 3x nach 5-minütigem (5x60 Sek.) passivem statischen Dehnen zum individuellen maximalen Bewegungsausmaß (0, 5 und 10 Minuten danach); am Tag der Kontrollbedingung ohne Dehnung, aber 5-minütiger Beinruhehaltung statt Dehnung. Die Stimulation der MEPs wurde durch einen transkraniellen Magnetstimulator durchgeführt. Die Reflexe und die MEPs wurden mit Oberflächen-Elektromyographie aufgezeichnet. Um signifikante Unterschiede zwischen den Zeitpunkten zu finden, wurde eine Friedman Varianzanalyse mit Messwiederholung durchgeführt. Als Post-hoc-Test diente der Wilcoxon Rangsummentest.

Ergebnis: Eine signifikante ($p=0.001$) Abnahme der Amplitude des T-reflexes zeigte sich direkt nach und 5 Minuten nach der Dehn-Intervention. Sie kehrte nach 10 Minuten zum Ausgangs-Level zurück. Die Friedman ANOVA für den H/M Quotienten, die MEP Amplituden, den MEP/M_{max} Quotient und die MEP Latenzzeit ergab keine signifikanten Unterschiede ($p>0.050$) vor und nach Dehnung.

Conclusio: Es wurde gezeigt, dass eine präsynaptische Inhibierung des T-reflexes mindestens 5 Minuten andauerte, aber kein Effekt auf den H-reflex stattfand. Der unveränderte kortikale Messparameter (MEP) lässt vermuten, dass der Einfluss auf das α-Motorneuron eher unwahrscheinlich ist.

Abstract

Background: The established method to investigate influences of stretching on corticospinal excitability is the assessment of amplitudes of motor evoked potentials (MEP). The spinal excitability is investigated by comparison of amplitudes of stretch-reflex (T-reflex) and by the analysis of the H/M ratio. In a previous study of the same project, the influence of one minute stretching was already investigated and showed no effect on MEP, but inhibition of T-reflex and facilitation of H-reflex (Hoffmann's reflex). Aim of this study was to investigate whether a longer duration of 5 minutes static stretching of soleus muscle (SOL) affects MEP, T-reflex and H-reflex.

Methods: 17 healthy volunteers came on two testing days (control vs. stretching condition). The three assessments (T-reflex, H-reflex and MEPs) were carried out on right SOL at 4 time points: Before (Pre), and 3 times after 5 min (5x60s) passive static stretching at individual maximal ankle dorsiflexion (0, 5 and 10 minutes after). On the day of control condition without stretching, but 5 min rest instead. MEPs were obtained using transcranial magnet stimulation. Reflexes and MEPs were recorded by surface electromyography. In order to find significant differences between the time points, a Friedman repeated measures analysis of Variance and a Wilcoxon signed rank test were performed.

Results: A significant ($p=0.001$) decrease in the amplitude of T-reflex was found immediately after and 5 min after stretching intervention and returned to baseline values after 10 min. Friedman ANOVA for H/M ratio, MEP amplitudes, MEP/M_{max} ratio and MEP latency showed no significant differences ($p>0.050$) before and after stretch.

Conclusion: It has been shown that there is a presynaptic inhibition of T-reflex lasting at least 5 min, but no effect on H-reflex. The unchanged cortical parameter (MEP) indicates that the influence on the α-motor neuron is rather unlikely.

1 Introduction

Stretching is commonly known to improve range of movement, decrease muscle soreness and feels relaxing. This were typical answers of 331 athletes in the US to the question of effectiveness of stretching as a recovery strategy, which was also rated as the most effective recovery strategy (Crowther et al., 2017). In addition, preactivity stretching can reduce injury risk and affect the physical performance (Behm et al., 2016, McHugh and Cosgrave, 2010). Those mentioned benefits on the physical comfort are rather subjective and long lasting.

On the other hand, many studies examined the effects of stretching on the properties of contractile elements of muscle and tendon characteristics by concentrating on the morphological and mechanical changes following muscle stretch. They found modifications of the aponeurosis-tendon system (Avela et al., 2004), an increase in the maximal dorsiflexion (Guissard and Duchateau, 2004, Konrad and Tilp, 2014, Blazevich et al., 2014), decreased passive muscle stiffness (Nakamura et al., 2011, Matsuo et al., 2015) and a decrease in force (Matsuo et al., 2015).

However, there are also quantifiable short-term effects on neural level (Guissard et al., 2001, Guissard and Duchateau, 2006, Gruber et al., 2009). The spinal excitability is investigated with the comparison of amplitudes of stretch-reflex (T-reflex) and Hoffmann's reflex (H-reflex) (Guissard and Duchateau, 2006). T-reflexes (Guissard and Duchateau, 2004, Weir et al., 2005) and H-reflex (Masugi et al., 2017, Avela et al., 1999) are suppressed following muscle stretch. The established method to investigate the corticospinal excitability is the assessment of the amplitudes of motor evoked potentials (MEP) and the MEP/M_{max} ratio (Groppa et al., 2012, Weavil et al., 2015). Guissard et al. (2001) showed reduced MEPs following passive muscle stretch.

In a previous study of the same FWF[1] project, to which this study belongs, the influence of one minute stretching was already investigated and showed no effect on the corticospinal level, but inhibition of T-reflex and facilitation of the H-reflex (Budini et al., 2017) on spinal level.

The aim of this study is to investigate whether 5 minutes of static stretching of the right soleus muscle (SOL) affect the corticospinal excitability by facilitated or inhibited amplitudes of MEP, H-reflex and T-reflex or rather the normalized ratios and influences on the MEP latency.

[1] FWF: german „Fond zur Förderung der wissenschaftlichen Forschung" is the Austrian Science Fund, a funding organization for basic research

2 Background

2.1 The excitable tissues – nerve and muscle

2.1.1 Histological and anatomical basics of muscle tissue

Muscle is contractile tissue, which is classified in 3 types: smooth muscle (instriated, involuntary), skeletal muscle (striated, voluntary) and cardiac muscle (striated, voluntary) (Mescher, 2016). Since in this study the skeletal muscle soleus was investigated, the following chapter is focused specifically on the skeletal muscles. The functional unit of the skeletal muscle is the muscle fiber, an elongated, highly differentiated cell (Ovalle and Nahirney, 2013). Each cell contains several peripherally located nuclei. The cell membrane is called sarcolemma and the internal cellular portion sarcoplasm (Mescher, 2016). Outside the muscle fiber membrane, the related stem cells (satellite cells) are located (Silverthorn, 2016). The whole muscle is surrounded externally by a fascia and by three layers: The outer Epimysium, consisting of collagen fibers, separates the muscle from the surrounding tissue and converges at the end of the muscle to tendons, which connects the muscle to the bones. The central perimysium divides each muscle into separate compartments, each containing a bundle of muscle fibers (=fascicles) and is pathway for blood vessels and nerves. The inner endomysium surrounds each muscle fiber (muscle cell, see Figure 1, A), ties fibers together and contains the capillaries (Ovalle and Nahirney, 2013).

A muscle fiber is highly organized in several parallel cylindric myofibrils consisting of identical repeating series of sarcomeres. In the transmission electron microscopy, those repeating units show dark bands, called A bands (A for anisotropic) and light bands, called I bands (I for isotropic) (see Figure 1, B). The I band is bisected by a dark transverse line called Z disc (Z from german “Zwischenscheibe”). The unit between two Z discs is one sarcomere, with a length of 2.5µm in relaxed condition.

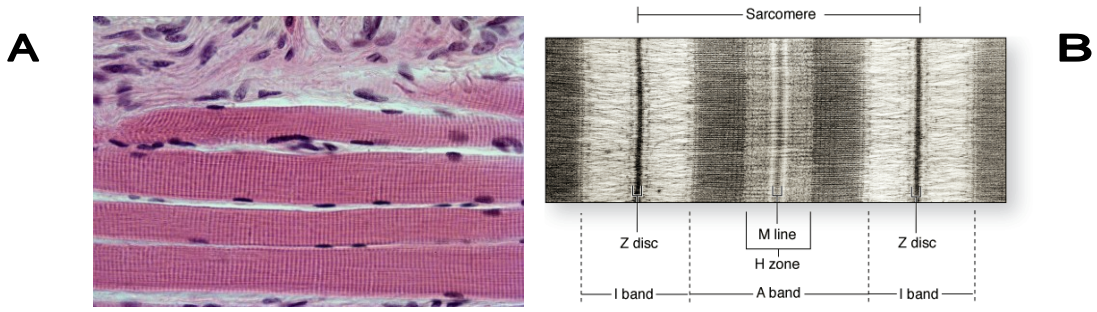


Figure 1: A) the muscle fibers appear striated by light microscopy. Responsible for the stripes are the thick and thin filaments of the sarcomeres (DVD from Hartmann et al. (2011)).
B) dark- and light-staining bands in a sarcomere seen by transmission electron microscopy (x28.000) (Mescher, 2016) © The McGraw-Hill Companies.

The myofilaments are composed of proteins. Myosin proteins form the thick, dark part of the myofilament in the center. The thin, light part of the filament at the ends of each sarcomere, are formed by the actin protein. In the middle of the A band is the H zone, the inner part of the thick filaments, which are not overlapped by the thin parts. Myosin is composed of two identical heavy chains, which are twisted constituting the tail, ending with two pairs of light chains, forming the two heads at each end of the Myosin tail (see Figure 2). Each head has two binding sides, one for adenosine triphosphate (ATP) and ATPase, one for Actin. The Actin binding site is necessary as a crossbridge between the thick and thin filaments.

Twisted G-actin and F-actin form the thin filament, containing two regulatory proteins: The Tropomyosin, wrapped between the two actin strands and the Troponin, attached to the Tropomyosin, which binds Ca^{2+}. The G-actin monomer covers a binding site for Myosin (Mescher, 2016).

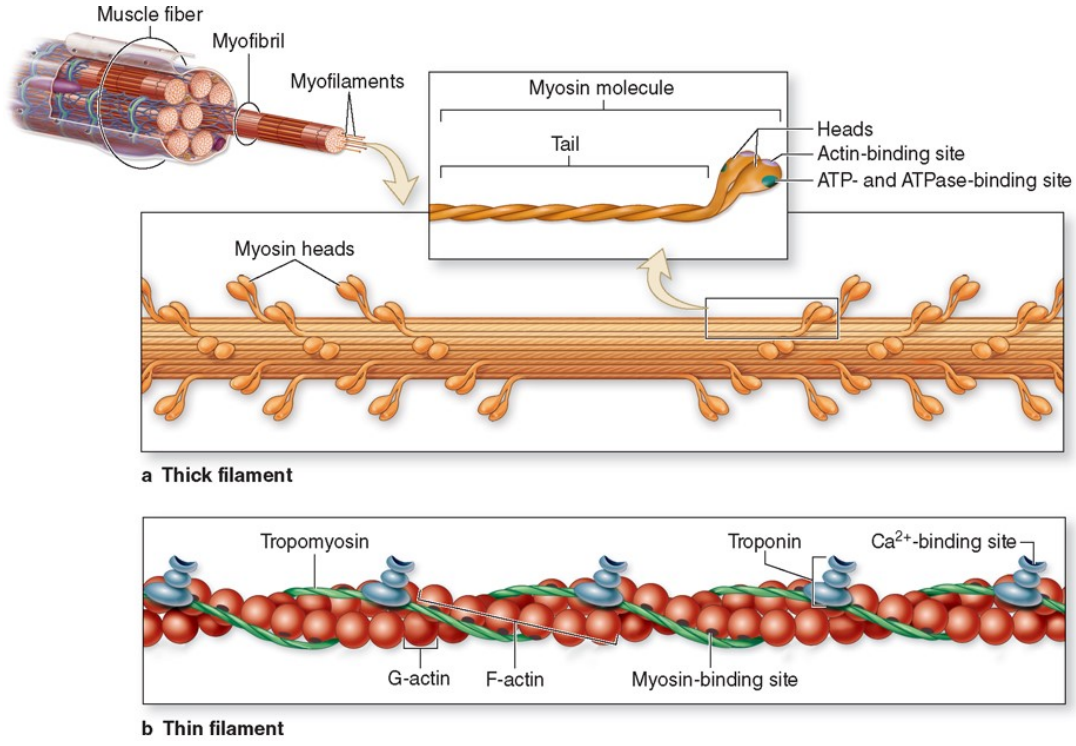


Figure 2: The thick filament (a) consisting of Myosin proteins, with two binding sides for Actin and adenosine triphosphate (ATP) or ATPase. The thin filament (b) consisting of Actin proteins, the G- and F-actin (Mescher, 2016) © The McGraw-Hill Companies.

The SOL is, together with the gastrocnemius muscle, part of the triceps surae inside the posterior compartment of the lower leg (Anderhuber et al., 2012). The SOL originates from the posterior surfaces of the fibular head, fibular collum and the tendinous arch between tibia and fibula. It inserts via the Achilles tendon together with the gastrocnemius muscle into the posterior surface of calcaneus. Together with the plantaris muscle, which runs

between the gastrocnemius and the soleus, it is part of the superficial flexor muscles. In contrast to the gastrocnemius muscle, the SOL flexes only in the upper ankle joint, whereas the gastrocnemius flexes also the knee. Both affect also the inversion of the lower ankle joint and are constantly used during walking and standing (Schünke et al., 2012, Gray, 2016). Furthermore, the SOL has an important role in the skeletal muscle pump, pumping the venous blood back to the heart (Gray, 2016). It is innervated by two branches from the the tibial nerve supplied by the first and second sacral nerve and nutritioned by the popliteal, the posterior tibial and the fibular artery (Anderhuber et al., 2012).

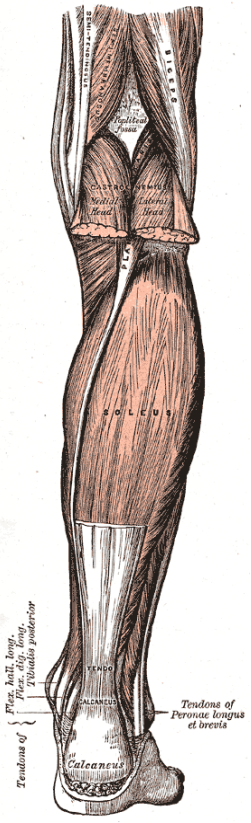


Figure 3: Soleus muscle at the back of the lower leg, superficial layer, gastrocnemius cut out (Gray, 1918).

2.1.2 Motor unit

The motor unit is the smallest functional unit consisting of one α-moto neuron and a group of muscle fibers it innervates. If the motor neuron fires, all related muscle fibers contract as one (Konrad, 2005). Depending on the fine motor action needed in a muscle, more or less muscle fibers belong to a motor neuron. E.g. extraocular muscles contain three to five muscle fibers per motor neuron, whereas in the gastrocnemius muscle, up to 2000 fibers belong to one motor neuron (Silverthorn, 2016). The strength of contraction of a muscle is dependent on the sum of the motor neurons, which come into play. This recruitment is controlled by the nervous system (Barrett et al., 2010).

2.1.3 Neurons and nervous tissue

In the present work, the spinal and corticospinal excitability is issued. The following chapter tries to illustrate the nervous tissue. The nervous tissue is composed of *nerve cells* (neurons), receiving and transmitting the incoming information, and *neuroglia* (glia cells), covering the neurons and forming the supporting frame (Hartmann et al., 2011).

Neurons exist in different types of shape, but all of them possess the same morphological characteristics. Each nerve cell, as well as the motor neuron, consists of dendrites, a cell body, an axon and terminal branches at the end of the axon, where the terminal buttons build synapses with other structures (see Figure 4). The dendrites can receive information from different sources, transfer it to the cell body, exciting the axon. The terminal branches at the end of the axon merge in synapses. Synapses are contacts between nerve cell and nerve cell or between a nerve cell and non-neuronal cell, such as muscle fibers (Pocock et al., 2017).

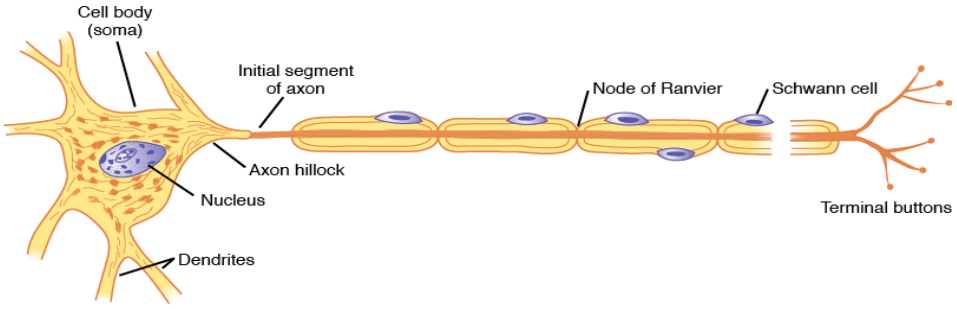


Figure 4: Motor neuron with a myelinated axon. The cell body receives information from the dendrites. The axon originates from the axon hillock and is enclosed by the Schwann cells, involved in the myelin formation. Between the Schwann cells, nodes of Ranvier are responsible for the saltatory conduction (Barrett et al., 2010) © The McGraw-Hill Companies.

There exist two types of glia cells in peripheral nervous tissue: satellite cells and Schwann cells. Satellite cells cover the surface of cell bodies and Schwann cells enclose the axons. In unmyelinated axons, several axons are embedded in the cytoplasm of one Schwann cell. In myelinated axons (see Figure 4), individual Schwann cells cover a small part of the axon, the myelin sheath. Between the individual cells of the myelin sheath occur gaps, the nodes of Ranvier. The myelin sheath plays an important role in carrying action potentials. The action potentials jump from one node to another, increasing the speed of conduction. This phenomenon is called salutatory conduction (Barrett et al., 2010). Outside of the central nerve system, the axons run in peripheral nerve trunks. Three layers of connective tissue surround each nerve. The epineurium encloses the nerve trunk. The axons run inside the epineurium in bundles called fascicles. Each fascicle, containing one or more axons, is surrounded by perineurium. Each axon inside the bundles is surrounded by endoneurium (Hartmann et al., 2011).

2.1.4 Physiology of muscle fiber contraction – from the neuromuscular junctions to the sliding filament theory

A reflex, as triggered in this study, elicits a muscle contraction. The contraction derives from the junction of a neuron and the belonging muscle fibers: the motor unit (see chapter 2.1.2). The connection, where the axon of a neuron terminates on dendrites, a cell body, an axon of another neuron or on a muscle cell, is enlarged to a terminal bouton and ends in the synapse (see Figure 5). A synaptical cleft of 20-40nm separates the surfaces of the presynaptic cell from the postsynaptic cell. Synaptic vesicles inside of the terminal bouton contain chemical transmitter. Depolarization from the axon induces the vesicles to secrete their transmitter in the synaptic cleft. If it is the synapse of a nerve cell and a muscle fiber, the transmitter is acetylcholine and the connecting part on the muscle fiber is the motor endplate. The transmitter binds to the receptors at the surface of the motor endplate (Barrett et al., 2010).

The sarcolemma of muscle cells is a semi-permeable membrane. Ion pumps maintain an ionic equilibrium forming a resting potential of approximately -80 to -90 mV, in non-contracted condition. The inside of the cell is negatively charged (Konrad, 2005), resulting from a Na^+ surplus outside the cell and a Ka^+ surplus inside the cell. The most important ion pump here is the Na^+/Ka^+-ATPase. After the release of transmitter substances in the synapse, Na^+ flows inside the cell and results in an endplate potential. If a certain level of Na^+ influx is surpassed, the depolarization leads to an overshoot up to +30mV, the action potential (Konrad, 2005). The action potential follows the all-or-none law. The all-or-none law postulates, that if a threshold is exceeded, an action potential is produced. Any increase of the stimulus does not produce a bigger response. If the threshold is not reached, no action potential occurs (Barrett et al., 2010). It is immediately restored by a repolarization phase, dominated by Na^+ efflux. The repolarization is followed by a hyperpolarization period of the membrane (Konrad, 2005). The depolarization is transmitted to the interior of the cell by transverse-tubules (=t-tubules). T-tubules are tubular invaginations on the surface of the sarcolemma leading to a network of those narrow tubules. If a T-tubule is flanked by two terminal cisternae (sacs) of the sarcoplasmatic reticulum, they form a muscle triade. The triades play an important role in the initiation of contraction, the excitation-contraction coupling by conveying electrical impulses almost instantaneously from the sarcolemma to the cell interior. Associated with the triades is the sarcoplasmic reticulum, the storage of the intracellular Ca^{2+} (Ovalle and Nahirney, 2013). The incoming depolarization leads to a Ca^{2+} release of the sarcoplasmatic reticulum, described by the electro-mechanical coupling (Konrad, 2005).

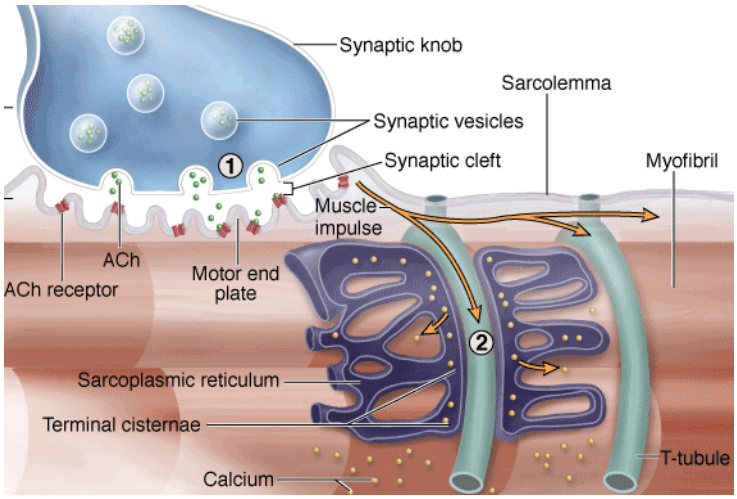


Figure 5: ① the depolarization from the nerve induces the release of acetylcholine (Ach) into the synaptic cleft, binds to the receptors in the motor end plate of the neuromuscular junction and transfers the impulse to the sarcolemma of the muscle fiber via transverse tubules (T-tubules).
② Ca^{2+} is released into the sarcoplasm (Mescher, 2016) © The McGraw-Hill Companies.

The free Ca^{2+} binds to Troponin C, uncovering the myosin-binding sites on actin, which allows the formation of cross bridges between actin and myosin. Released adenosine diphosphate (ADP) causes a conformational change of the myosin head, so the myosin head slides over the actin-filament. ATP binds to the free binding site of the myosin head, so the myosin head detaches from the actin. ATP is hydrolyzed, while inorganic phosphate is released, which again causes the attachment of the myosin head to the actin. This cycle repeats as long as Ca^{2+} and ATP stay available and causes shortening of the contractile elements of the muscle cell. The interaction from the electrical depolarization to the mechanical contraction of the filaments on molecular level is described by the excitation-contraction coupling (Barrett et al., 2010).

2.2 Descending corticospinal motor pathways

Responsible for voluntary movement control, coordination and performance is the hierarchical interaction between cerebral commands of the motor cortex, sent via the corticospinal tracts, and their implementation by the muscle system. The motor cortex is composed of the motor association cortex (=Brodmann's area 6) and the primary motor cortex (=M1 =Brodmann's area 4). The motor association cortex, including the supplementary motor cortex and the premotor cortex, is involved in the initiation of movement and projects to the primary motor cortex (Lehmann-Horn, 2007, Barrett et al., 2010). The primary motor cortex lays in the pre-central gyrus in the posterior part of the frontal lobe of the brain, rostral to the central sulcus. The different parts of the body are located in the coronal section of M1, with the head region lateral at the bottom and the

extremities medial at the top (see Figure 6). Accordingly, the body is represented minimized as a *homunculus* and in a skewed version. Areas for hands and face muscles are represented in a bigger size than e.g. the back (Lehmann-Horn, 2007).

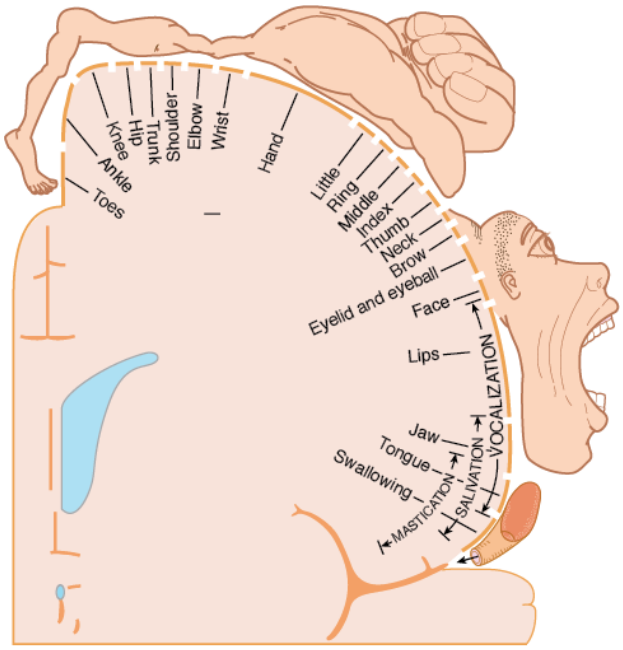


Figure 6: Motor homunculus: Different parts of the body are located in the coronal section of M1, with the head region lateral at the bottom and the extremities medial at the top. The size is proportional to the represented cortical area (Barrett et al., 2010) © The McGraw-Hill Companies (Reproduced from Penfield W, Rasmussen G: The Cerebral Cortex of Man. Macmillan, 1950).

Originating in the pre-central gyrus, the descending corticospinal motor pathways are composed of two motor neurons and optional interneurons. The upper motor neurons run ipsilateral through the internal capsule and within the midbrain, they traverse the cerebral peduncle and the pyramids, therefore the pathway is called the pyramidal tract. It synapses on the lower motor neuron in the spinal cord (Barrett et al., 2010) with the activating neurotransmitter glutamate or the inhibiting transmitter Gamma-Aminobutyric acid (=GABA) (Weber and Eisen, 2002). Depending on their location in the spinal cord, there exist lateral and ventral pyramid tracts (see Figure 7). The more lateral the axons runs, the more distal muscles are innervated, ventral located axons innervate proximal muscles. The fibers of the lateral corticospinal tract (80% of fibers) decussate in the pyramids and descend in the contralateral side of the white matter of the spinal cord, controlling the contralateral movement of the extremities by building monosynaptic connections with the spinal motor neurons (=α-motor neuron) in the anterior horn. 20% of fibers remain uncrossed and run as anterior corticospinal tract terminating on interneurons. The interneurons cross just before they leave the spinal cord and project ipsilateral or bilaterally to cervical spinal motor neurons, that control neck musculature. The interneurons synapse

with the lower motor neurons, concerned with posture, coordination or proximal muscles. Activation of the lower motor neuron triggers a contraction in the innervated muscle. Only the axon of the lower motor neuron extends outside the central nervous system, forming peripheral nerves, innervating a single motor unit (Barrett et al., 2010).

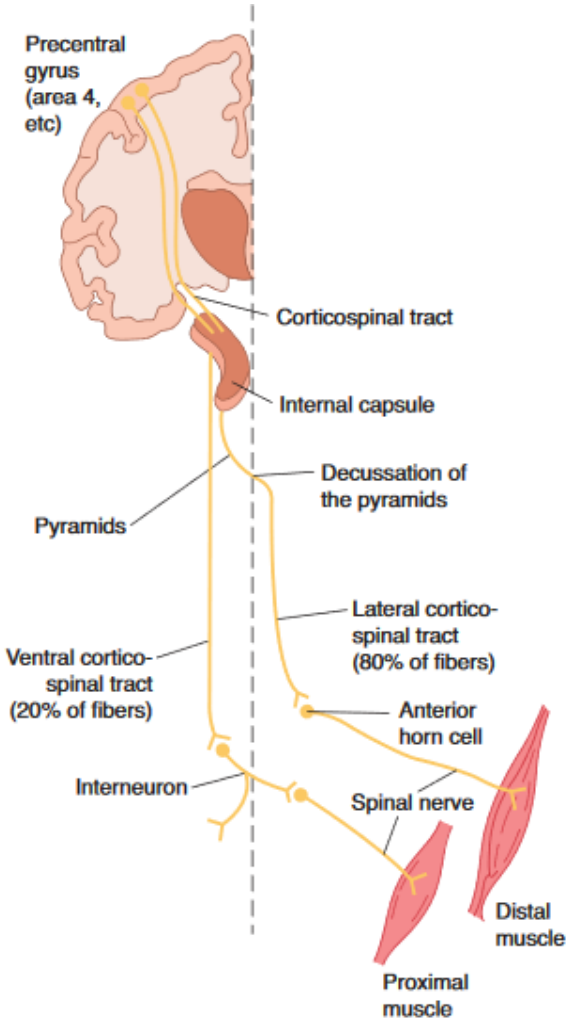


Figure 7: corticospinal tracts: The fibers of the lateral corticospinal tract (80% of fibers) decussate in the pyramids and descend in the contralateral side of the white matter of the spinal cord, building monosynaptic connections with the spinal motor neurons in the anterior horn. 20% of fibers remain uncrossed and run as anterior corticospinal tract terminating on interneurons (Barrett et al., 2010) © The McGraw-Hill Companies.

2.3 Parameters of spinal excitability

For the purpose of investigation of spinal excitability, the monosynaptic reflexes as the stretch reflex and the H-reflex are common parameters by comparing the amplitudes before and after stretch (Aagaard et al., 2002, Guissard and Duchateau, 2006). Monosynaptic reflexes are the simplest reflexes with only one synapse between the afferent and efferent neuron (Barrett et al., 2010).

2.3.1 Stretch Reflex (T-reflex)

The stretch reflex (T-reflex from T=tendon) is a monosynaptic reflex. The reflex arc runs from the muscle spindle (=affector) through the sensory neuron (Ia-afferent) to the spinal cord, where it directly synapses with the α-motoneuron (=efferent neuron). The afferent neurons reach the spinal cord via the dorsal root and exit via the ventral root (see Figure 8). Responses are excitatory postsynaptic potentials or inhibitory postsynaptic potentials at the synaptic junctions in the central nervous system, resulting in contraction of the corresponding muscle (=effector). Stimulus for the muscle spindle, as a sense organ, is stretching of the affected muscle. Many intrafusal fibers run together in a capsule, located in between the extrafusal muscle fibers. The end of an Ia-afferent neuron spirals around the non-contractile part of the muscle spindle. Each nerve fiber surrounds only one spindle, in contrast to type-II-fibers, which surround two or more spindles. The afferent nerve generates the action potential only after the all-or-none law, if a certain threshold is surpassed. The axon is myelinated, so the conduction speed is high. The transmitter is glutamate (Barrett et al., 2010, Lehmann-Horn, 2007).

But the muscle spindles have their own nerve supply for their contractile part, which is wrapped by the ends of γ-motor neurons (Barrett et al., 2010). γ-motor neurons have a smaller diameter than the α-motor neurons and they innervate several intrafusal fibers, not only one motor unit. The threshold is higher (Lehmann-Horn, 2007). Another possibility to induce the stretch reflex is the isolated contraction of the muscle spindles, following intrafusal stimulation of the γ-motor neurons. This stretches the non-contractile part of the muscle spindle and initiates the stretch reflex, as well (Barrett et al., 2010). This loop is the γ-loop, where the γ-motor neurons activate indirectly the α-motor neurons via the Ia spindle afferent activity. But under physiological conditions, the α- and γ-motor neuron are co-activated (Lehmann-Horn, 2007).

Inside the tendons in between the collagen and the connective tissue lays the golci tendon organ. The ends of the fibers are non-myelinated and lay in a capsule. Traction to the collagen stimulates the fibers. They convey to Ib-fibers, which are myelinated (Lehmann-Horn, 2007). The Ib fibers end in the spinal cord on interneurons. The golci tendon organ is responsible for the inverse stretch reflex. The more a muscle is stretched, the harder is the reflex contraction. However, when the tension reaches a very high point, the Ib fibers inhibit the α-motoneuron and this muscle relaxation as a response to strong stretch is the inverse stretch reflex. This mechanism guarantees optimal tension for the muscle (Barrett et al., 2010).

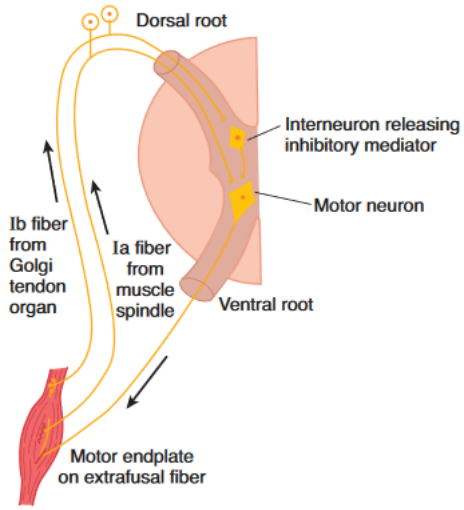


Figure 8: Stretch reflex and the inverse stretch reflex: When the muscle spindle gets stretched, e.g. by a hammer, Ia fibers, that excite the motor neuron, are activated (stretch reflex). On the other hand, stretch stimulates the Golgi tendon organ, activating Ib fibers, exciting an interneuron. With increasing stretching intensity, the Ib fibers inhibit the α-motor neuron, resulting in relaxation of the muscle (=inverse stretch reflex) (Barrett et al., 2010) © The McGraw-Hill Companies.

Inhibition of the α-motor neuron can also originate from the inhibitory effect of Renshaw-cells, spinal glycinergic inhibitory interneurons. They affect the α-motor neuron and inhibit the reflex response.

The T-reflex is facilitated by trying to pull the hands apart while the fingers are flexed into a hook-like form (Jendrassik's maneuver). This may be due to an increased excitability (reduced threshold) in consequence of a decreased inhibition (Barrett et al., 2010, Lehmann-Horn, 2007).

The ankle jerk reflex (=Achilles reflex), which is investigated in this study, is elicited by a hammer, tapping on the Achilles tendon. The associated nerve is the tibiale nerve, mediated by S1 and S2 spinal segment in the spinal cord. This triggers the triceps surae muscle to contract and results in a plantar flexion of the ankle (Weiner et al., 2010).

2.3.2 Hoffmann's reflex (H-reflex)

Measurement of the H-reflex, also a monosynaptic reflex, is used as a standardized quantitative method for the exploration of neurophysiological reflexes (Aagaard et al., 2002). It is named after the physiologist Paul Hoffmann (Lehmann-Horn, 2007). The principle consists in the electrical percutaneous stimulation of the tibial nerve in the popliteal fossa to evoke the H-reflex. The muscle response can be measured by electromyography (EMG) (Stöhr and Pfister, 2014).

The onset of a low stimulus, a short-lasting low-intensity current, activates selectively the axons of Ia afferent fibers, because of their large diameter (Aagaard et al., 2002). The way to the spinal cord, where it synapses with the efferent α-motor neuron and the α-motor

neuron travels back to the leg to contract the SOL, is long, so the response in the muscle can be recorded only with a latency of 30-40ms (see Figure 9, ② and A). The occurring wave is the H-wave. First, the amplitude of this H-wave increases with increasing stimulation intensity. The activation in this case is indirect. If the stimulation intensity gradually increases, the early direct response, with 5-10ms latency appears (see Figure 9, ① and B). This M-wave occurs in the muscle due to a direct excitation of the α-motor neuron axons under the electrode. With more increasing stimulus intensity, it comes to a collision between the direct and indirect action potentials in the α-motor neuron axon, so there is a suppression of the H wave amplitude until it completely disappears at supramaximal stimulus intensity (see Figure 9, B-D). The M-wave amplitude increases proportionally to the stimulus intensity (Lehmann-Horn, 2007, Stöhr and Pfister, 2014).

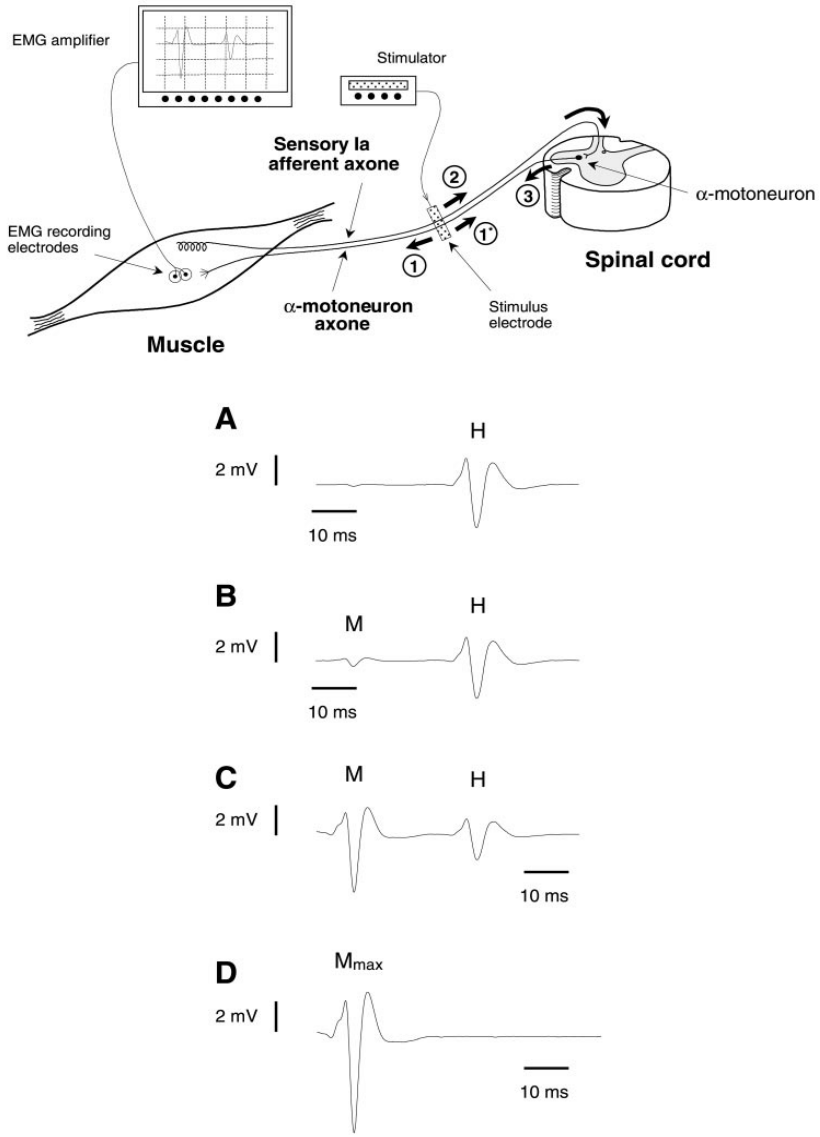


*Figure 9: lower stimuli: ②: activation of the afferent Ia fibers ③ synapses in the spinal cord to the α-motor neuron (=indirect activation → H wave (**A**)) ① Higher stimuli: The alpha motor neuron itself is elicitated (=direct activation → M wave). With increasing stimulation intensity, the direct activation (M) increases. The H wave decreases (**B**-**D**) (Aagaard et al., 2002).*

The H-reflex is not only able to assess the spinal excitability by reflecting the function of the α-motor neuron, but also by statements of neurotransmitter efficiency in Ia afferent synapses. In this way, the presynaptic inhibition can be investigated (Aagaard et al., 2002). To assess the spinal excitability, its common to calculate the H/M ratio for better interindividual comparison (Opplert et al., 2016), representing a measure for the excitability of the motor neurons (Stöhr and Pfister, 2014). Therefore the value of the amplitude of the H wave is divided through the corresponding M-wave amplitude (Opplert et al., 2016).

2.4 Transcranial magnet stimulation (TMS)

Transcranial magnet stimulation (TMS) is a noninvasive method to assess the corticospinal excitability introduced for the first time in 1985 by Barker et al. Since then, the use of this technique, especially the single pulse technique, augmented rapidly in diagnostics (Nardone et al., 2015) and treatment of various neurological and psychiatric diseases (Wagner et al., 2007). Nowadays, it is the most common method for non-invasive brain stimulation and is, beside the clinical use, a very important tool in fundamental neuroscience research (Valero-Cabre et al., 2017).

2.4.1 Device setup

TMS follows the principles of electro-magnetic induction, postulated by Faraday in 1831. Therefore, a changing magnetic field induces electrical current (Weyh and Siebner, 2007). The stimulator is composed of three main components: a capacitor, a coil and a thyristor (see Figure 10).

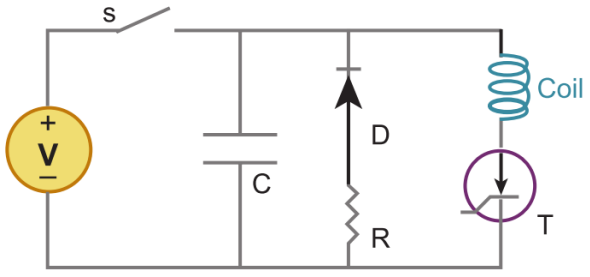


Figure 10: Simplified graphics of a single pulse, monophasic magnetic stimulator. S: Switch V: Voltage source, C: Capacitor, D: Diode, R: Resistor, T: Thyristor (Wagner et al., 2007).

When the switch closes the circuit, first, the high-voltage source charges the capacitor up to 5000V. Immediately after, the capacitor discharges and activates the coil with a loop of conducting wire by the quickly changing high intensity electrical current (until about 10.000A) generating a strong, transient magnetic field of 1-3 Tesla. There are mono- and

biphasic stimulation devices available. In biphasic devices, no diode or resistor absorb the current, so the capacitor recharges in reversed polarity. Like in a resonant circuit, the capacitor discharges again, generating a magnetic field. The electrical current (Figure 11, A) shows a damped sinusoidal oscillation (Kammer and Thielscher, 2003, Wagner et al., 2007). In monophasic devices, as used in this study (see Figure 10), the diode and the resistor dissipate the current, which decreases exponentially.

From outside of the head, the brief, rapidly changing magnetic field passes skin and scull (see Figure 11, B), representing little or very little impedance, and induces an electric field and, more importantly, electric currents (see Figure 11, C) in the excitable tissues of the brain (Weber and Eisen, 2002). The more parallel the electric field is to the neurons, the more effective is the induced current. The intensity of these currents in the tissue depends on the distance to the coil (Wagner et al., 2007, Weyh and Siebner, 2007, Hallett, 2007).

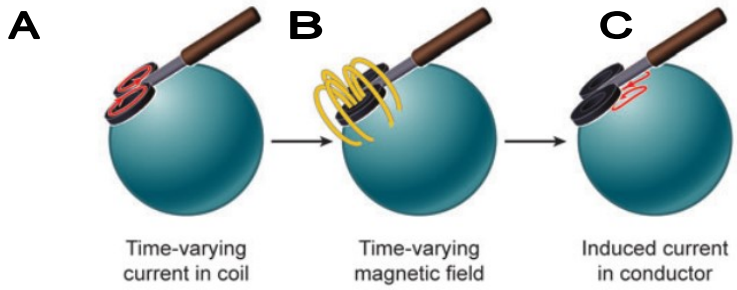


Figure 11: Current in coil induces a magnetic field, which in turn induces current in the tissue (Wagner et al., 2007)

Single-pulse devices, as we used in this study, allow a stimulus every few seconds in contrast to repetitive stimulators, producing pulse rates of many times per second. Whereas in Single-pulse devices, the thyristor eliminates reverse charging, repetitive stimulators, in contrast, allow pulse frequencies up to 100 Hz by reverse charging (Kammer and Thielscher, 2003, Wagner et al., 2007).

Most commonly used coils are single circular type coils or smaller coils, the butterfly or rather figure-eight shaped coils (see Figure 12, A and B) (Groppa et al., 2012). The circular type coils have their maximum magnetic field spread around the center of the coil and the maximum of the electrical field ring-shaped around the center of the coil. Therefore, there is no stimulation of tissue exactly under the center of the coil (Weyh and Siebner, 2007). They produce stronger fields and need more energy than the figure-eight shaped coils (Deng et al., 2014). Figure-eight/butterfly coils consist of two connected coils with the magnitude to evoke more focal stimulation, but weaker and less penetrating (Kammer and Thielscher, 2003, Weber and Eisen, 2002). The maximum of the electrical field is just under the center of the 'eight' (Weyh and Siebner, 2007).

Figure 12: different types of coils. (A) 90mm single coil (B) 70mm figure-eight-shape coil (C) 110mm double-cone coil (Magstim, 2018).

In this study, a 110mm double cone coil with two large cups with a flat central section and angled coils is used, fitting the heads shape (see Figure 12, C). This geometry allows more powerful stimulation in deeper regions of the brain, economizing energy by reducing the stimulus intensity (Magstim, 2018).

2.4.2 Limitations and side effects

Diverse side and sensory effects of TMS are described in literature. The magnetic forces deform the coil, producing a click sound as the typical artifact. There are even some dangers in relation to the use of TMS. Electrical dangers occur only in case of damaged device. The magnetic field can switch off electrical implants of the patient, as e.g. cardiac peacemakers or cochlear implants, so it must not be used in those patients. There is also the low risk of epileptic attack, but until now, it is only described for repetititve TMS (Weyh and Siebner, 2007). Epileptic seizures were reported in patients with focal epilepsy (Classen et al., 1995). But the risk for seizures induced by single-pulse TMS is very low. The risk is less than 5% only at persons with known brain pathologies (Groppa et al., 2012). For safety reasons, epilepsy, cochlear implants and pacemakers were an exclusion criterion in the present study.

Frequently, subjects report a ‘blink reflex’. It originates from direct stimulation of facial nerves and muscles by the TMS-induced electrical field (Rossini et al., 2015). Other sensorial side effects are the ‘phosphenes’, light flashes, which can occur by stimulation to much occipital, activating the visual cortex (Valero-Cabre et al., 2017, Elkin-Frankston et al., 2011).

Limitations of TMS device should be considered. A critical point is the limited focality especially in circular coils. Increase of focality is possible by the use of appropriate shapes of coils, like e.g. butterfly coils (Wagner et al., 2007). A further difficulty is the distance. The M1 area for the lower extremity is deeper in the brain than for the upper extremity (see Figure 13). The missing depth can lead to difficulties in stimulating the muscles of the

lower limb, because the magnetic field is reduced with augmenting distance to the coil (Nardone et al., 2015, Groppa et al., 2012, Deng et al., 2014). Higher intensities (Groppa et al., 2012) or adjustment of the coil can diminish this problem. In this study, the coil adjustment was chosen. Therefore, the double-cone coils have the ideal shape (Magstim, 2018).

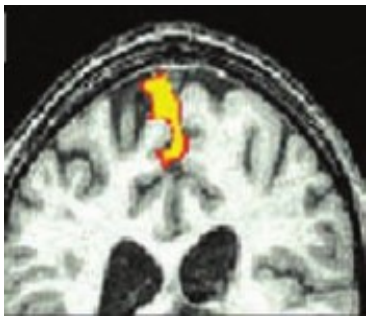


Figure 13: MR image (coronal plane) of the activated region in the precentral gyrus, induced by active movements in the ankle (dorsiflexion), showing the deep localization of the leg-representation inside the motor homunculus (Kocak et al., 2009).

2.5 Parameters of corticospinal excitability

Many authors assess the corticospinal excitability through the size of amplitudes of MEPs induced by TMS (Groppa et al., 2012, Miyata and Usuda, 2015, Budini et al., 2017) and the calculated MEP/M_{max} ratio (Weavil et al., 2015, Pearcey et al., 2014). Furthermore, the MEP latency can be taken in account, in order to examine the conduction time of the neuron (Siebner and Ziemann, 2007, Groppa et al., 2012).

2.5.1 Motor evoked potentials: Amplitudes

MEPs are evoked by stimulation of the motor cortex by transcranial electrical stimulation (TES) or TMS. TES mainly activates the proximal axons directly, which leads to D-waves, whereas TMS, which is used in this study, tends to transsynaptic excitation, generating I-waves (Groppa et al., 2012, Rossini et al., 2015).

At low-intensity stimuli of TMS, the descending motor neurons of the M1 regions are excited directly causing an early direct wave (= D wave), recordable in the peripheral muscle by EMG (Hallett, 2007, Di Lazzaro and Ziemann, 2013). At higher stimulus intensity, the transsynaptic activation initiates glutamate release in cortico-motoneural synapses, which depolarizes the postsynaptic cell membrane. If the sum of the activated volleys reach the threshold, the motor neuron is activated (Groppa et al., 2012), changeovers in the anterior horn of the spine and transfers action potentials to the target muscle (see Figure 14) inducing series of later indirect waves (= I waves) following the initial D wave (Barrett et al., 2010, Hallett, 2007). The indirect waves (see Figure 14, A)

occur at preferential time intervals (Groppa et al., 2012). These excitatory waves sum up until the threshold is reached, leading to a depolarization of the motor neuron (see Figure 14, B). The sum of all peripheral motor action potentials can be recorded in the target muscle as a MEP (see Figure 14, C) (Groppa et al., 2012, Siebner and Ziemann, 2007).

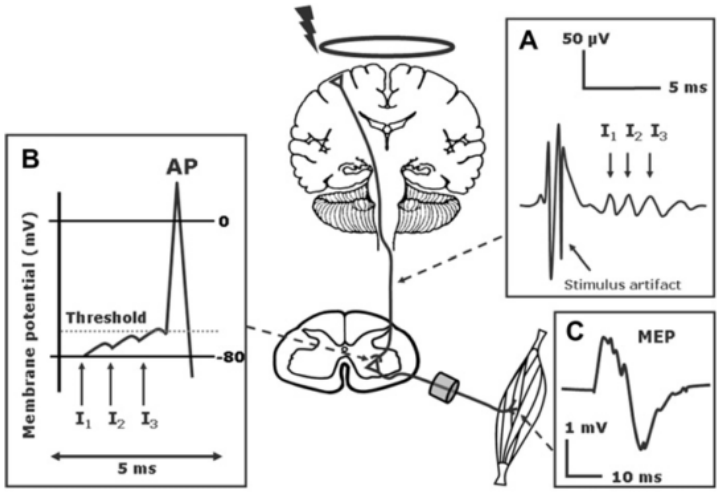


Figure 14: Formation process of the motor evoked potential (MEP). (A) TMS-induced activation of the corticospinal neurons by Indirect waves (I waves). (B) Summation at the cortico-motoneuronal synapses, leading to an action potentiak (C) resulting MEP in the target muscle recorded by sEMG (modified from Siebner and Ziemann (2007): Das TMS-Buch, Heidelberg: Springer Medizin Verlag).

The intensity of the TMS input and the excitability of spinal motor neurons determine the strength of the resulting MEP. The excitability threshold can be decreased by e.g. performing a voluntary contraction in the target muscle (Groppa et al., 2012). In this study, the participants were not allowed of any voluntary muscle contraction before, to avoid influenced muscle responses. All measurements were recorded from the relaxed muscle. The optimal position of the coil is above the hot spot, where is the highest density of corticospinal fibers to the target muscle (SOL) (Müller and Ziemann, 2007).

The interpretation of MEPs is based on the peak-to-peak amplitude from the maximal positive to the maximal negative deflection. The peak-to-peak amplitude increases with increasing stimulus intensity (Rossini et al., 2015, Groppa et al., 2012, Klampfl and Dengler, 2007). The intra-individual variability of the amplitude is very high (Wassermann, 2002). That is why always at least 12 trials are recorded and the average is calculated. To reduce interindividual variability, the MEP amplitude is normalized to the M_{max} by calculating the MEP/M_{max} ratio (Weavil et al., 2015, Groppa et al., 2012). This procedure allows the interpretation of the MEP as a parameter of the corticospinal excitability (Klampfl and Dengler, 2007). Another possibility to compare the MEPs can be the calculation of the area under the curve (Hahn et al., 2012), but most authors compare it with the peak-to-peak-amplitudes, like in this study.

2.5.2 Resting motor threshold (rMT)

The stimulus intensity in application of TMS depends on the cortical motor threshold. (Groppa et al., 2012). It is defined as 'the minimal intensity of motor cortex stimulation required to elicit a reliable MEP of minimal amplitude in the target muscle' (Rossini et al., 2015, p. 1078). The threshold mirrors the excitability of individual neurons and the membrane excitability (Hallett, 2007). The cortical motor threshold refers to the resting motor threshold (rMT) (Groppa et al., 2012). In detail, it is defined as the lowest TMS intensity needed to induce a peak-to-peak amplitude of MEPs of not less than 50μV in at least 5 out of 10 consecutive trials. There are different methods available to determine the rMT, but the most common is the relative frequency method; also used in this study, where the threshold is approximated in small intensity steps (Rossini et al., 2015, Groppa et al., 2012).

2.5.3 Motor evoked potentials: Latency

Another possible parameter for the investigation of the corticospinal excitability is the corticomotor latency (CML). The CML stands for the conduction time from cortex to the muscle and is measured from the trigger (stimulation) until the initial deflection, regardless whether positive or negative direction (Groppa et al., 2012, Nielsen, 1996). The CML is dependent of the length of the nerve fibers. The time for the muscle response in arm muscles is much shorter than for the leg muscles (Müller and Ziemann, 2007). An increased CML represents a decreased conduction velocity along the corticospinal tract (Opie et al., 2018).

2.6 Electromyography (EMG)

The common method to record MEP, H-reflex and T-reflex in the target muscle is EMG (Hahn et al., 2012, Valero-Cabre et al., 2017). EMG is useful in medical research, rehabilitation, ergonomics and sports science (Konrad, 2005). Surface EMG (surface electrodes) or intramuscular EMG (needle/fine wire electrodes) can detect electric potentials caused by activated muscle cells (Stöhr and Pfister, 2014). In this study, only non-invasive surface electrodes came to use.

2.6.1 Signal origin and detection

The EMG signal is an interference signal, superimposed of all motor unit action potentials, which are detected by the electrodes. The signal of one muscle fiber results from a depolarization-repolarization cycle forming a depolarization wave (electrical dipol), which

travels along the muscle fiber with a velocity of 2-6 m/s. The signal of one motor unit is superposed of all action potentials of the belonging fibers (see Figure 15). The final EMG signal is an interference pattern, a superposed signal of all motor units, which are beyond the electrodes, resulting in a triphasic motor unit action potential. The distribution of positive and negative amplitudes is symmetrical (Konrad, 2005).

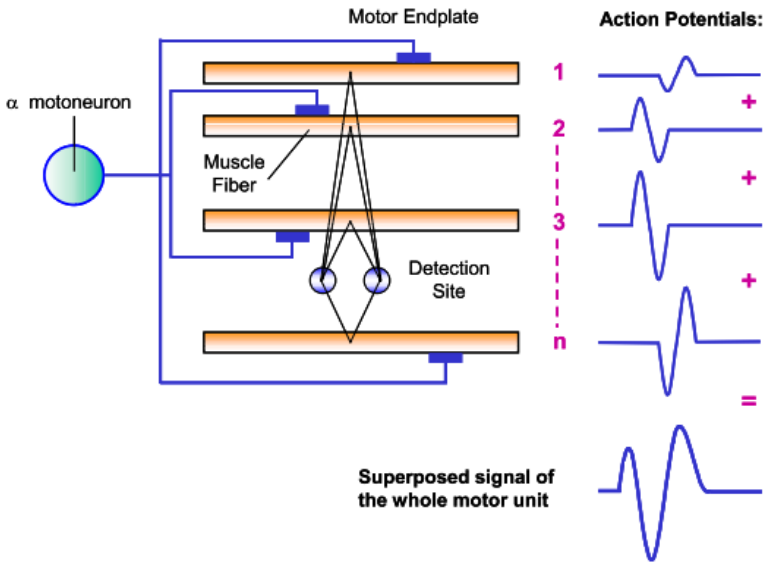


Figure 15: Superposed interference signal of all action potentials, a triphasic potential is generated (Konrad, 2005) modified from Basmajian, J., De Luca, C.: Muscles alive; Williams Wilkins, Baltimore, 1985.

Amplitude and density of the EMG signal are influenced by recruitment and firing frequency of the muscle fibers. The recruitment describes the number of activated muscle fibers. The firing frequency describes the number of action potentials per muscle fiber.

The raw EMG is unfiltered and unprocessed. The baseline of a relaxed muscle should be noise-free, dependent on the EMG amplifier. A noise-free baseline is a quality sign which ensures correct recordings of signals. The amplifiers are necessary to protect the signal from artifacts and to increase the low amplitude of the unfiltered signal. The unfiltered EMG signal is a *raw EMG*. By an analog/digital converter (A/D converter), it is transmitted by a digital signal to the computer. The sampling frequency should be at least twice as high as the maximum expected analyzed physiological frequency (Konrad, 2005).

2.6.2 Signal processing

The raw EMG gives general information about the *on-off* and the *more-less* of the contraction state of the muscle, but it is not specific enough for quantitative EMG analysis. For that purpose, the signal is processed by smoothing algorithms and filtered by low- or high-pass filter to exclude high and low frequencies. In this study, an Infinite Impulse Response Filter (iir) came to use, for an optimal high pass filtering (Konrad, 2005).

2.6.3 Factors influencing the EMG signal

There are certain external factors, which influence the EMG signal. For example, the tissue type and the tissue thickness can lead to a high interindividual difference. In addition, muscles close to the fibers of interest can interfere with the EMG signal. This phenomenon is the *physiological crosstalk*. External noise from electrical devices require the application of reference electrodes on electrically unaffected areas, such as joints or bony areas (Konrad, 2005).

2.7 Changes in spinal and corticospinal excitability following passive static muscle stretch

2.7.1 Passive static muscle stretch

"Passive stretching refers to the technique of lengthening a muscle group by slowly moving a joint to its maximal range of motion and maintaining the position for a period (usually <10s)" (Guissard and Duchateau, 2006, p. 154). On cellular level, the overlap between actin and myosin is reduced hence less cross-links between the filaments can be built (Barrett et al., 2010). The nervs lie curled in the connective tissue and are not immediately stretched, when the muscle begins to increase the length, but are maximal stretched, when it reaches the maximal range of motion. This mechanism allows the nerve fibers adapting to different joint angles (Hartmann et al., 2011). The maximal range of motion can be limited by bones, muscles (antagonist), ligaments and soft tissue. The maximal dorsiflexion in the ankle, which is measured in the present work, is mostly limited by the triceps surae muscle. Standard values for the maximal dorsiflexion in the ankle are between 20° and 30° in typical anatomy glossars (Schünke et al., 2012, Anderhuber et al., 2012). Passive muscle stretch is relevant in sports as a recovery strategy (Crowther et al., 2017), to prevent injuries (Behm et al., 2016, McHugh and Cosgrave, 2010) and to improve the flexibility (Guissard and Duchateau, 2004, Blazevich et al., 2014, Konrad and Tilp, 2014). But passive muscle stretch is also used in therapy against e.g. spasticity after strokes (Bakheit et al., 2005) or after spinal cord injuries (Nakazawa et al., 2006). In the physiotherapy in the hospital, stretching finds application, as well (Vujnovich and Dawson, 1994).

2.7.2 Spinal excitability changes following muscle stretch

It is commonly known, that passive static stretching inhibits the T-reflex. Budini et al. (2017) proved the inhibition for several minutes after stretching intervention ended. Weir et al. (2005) detected a decreased T-reflex after 5x2 minutes of stretching at 10°

dorsiflexion. Guissard and Duchateau (2004) found reduced T-reflex amplitudes of -14% in the plantar flexor muscles. Avela et al. (1999) established a decrease in the T-reflex amplitude of even 84%.

In literature, the effects *during* stretching on the H-reflex are well investigated. Many authors found a suppression of H-reflex during muscle lengthening, which restores as soon as application of stretching ended in most of the studies (Guissard and Duchateau, 2006, Guissard et al., 1988, Opplert et al., 2016, Vujnovich and Dawson, 1994, Robertson et al., 2012, Robinson et al., 1982). E.g. Guissard et al. (2001) described a reduced H-reflex amplitude during stretching of 10° and 20°. In contrast to that, Grospretre et al. (2014) found an increased H-reflex amplitude during stretching.

Investigating the influences *after* stretching is more complicated due to the quick reversibility of neuronal effects. Many authors observe an inhibition of spinal reflexes following passive static muscle stretch, as during stretching. Avela et al. (1999) reported of reduced spinal reflexes after 1 h repeated stretching of the SOL. H- and T-reflex amplitudes were as well suppressed after stretch training of the SOL in a study of Guissard and Duchateau (2004). Masugi et al. (2017) found an inhibition of the T- and H-reflex in the stretched calf muscles and even inhibitory effects on the non-stretched contralateral muscles until 5 min after stretching. Some other authors could not proof a reduced H-reflex immediately after stretching, after the joint came back into neutral position (Weir et al., 2005). Controversially, Budini et al. (2017) found a facilitation of H-Reflex immediately after stretching, lasting few seconds, corresponding to the facilitation of H-reflex observed by Grospretre et al. (2014) during stretching.

If authors found an alteration (facilitation or inhibition of the reflexes), the duration of the lasting effect remains uninvestigated in studies where the effect lasted longer than the end of the stretching application. The duration of the post effects was still significant at least 30s after stretching (Budini et al., 2017, Avela et al., 1999), but it remains unclear, how long the effect consists. In order to analyze the recovery trend, we performed post stretch measurements not only immediately after, but also at 5 and 10 minutes after stretching.

Two main variables influence the effect of stretching: The *intensity* and the *duration* of stretching. The influence of stretching duration on the H-reflex was investigated by Opplert et al. (2016), but they could not find any differences. They stretched to the maximal range of motion in the SOL, for 1x, 2x, 3x, 4x and 10x 30seconds.

The intensity, as declared in common anatomy glossars varies between 20° and 30° possible maximal dorsiflexion in the upper ankle. In a study of Masugi et al. (2017), the

amount of inhibition of H-reflex followed the intensity of dorsiflexion torque. In most studies, the investigators stretched until the point of maximal dorsiflexion (Opplert et al., 2016, Budini et al., 2017). The maximal dorsiflexion can be defined as the stretching until the point of pain or until the point of discomfort. As passive stretching to the point of pain presented no better acute advantages compared to stretching to the point of discomfort (Muanjai et al., 2017), participants in this study only had to stretch until their point of discomfort. Guissard et al. (2001) found a significant reduction in H/M ratio at 10° and 20°.

2.7.3 Corticospinal excitability changes following muscle stretch

The effect *during* stretching on the MEP was more commonly investigated. Guissard et al. (2001) described a decreased MEP amplitude due to reduced excitability of both, the cortical neurons and motor neurons, but the suppression ended as soon as stretching ended (Guissard et al., 2001). Gruber et al. (2009) discovered a decreased MEP size in biceps brachii and brachioradialis during maximal and submaximal lengthening. Since most of the study data refer to measurements of arm muscles (biceps, brachioradioalis…), the investigation of the leg muscles is necessary. The effect after passive static stretching on the corticospinal tract is still unexplored. This study wants to shed light in this chapter.

As an increased CML can detect a decrease in conduction velocity along the corticospinal tract (Opie et al., 2018), the latency is, as well, a suitable parameter to investigate the corticospinal excitability.

2.7.4 Factors influencing the spinal and corticospinal excitability negatively

There are also other influences on the excitability. Walton et al. (2003) discovered an increased H/M ratio 1 hour after caffeine capsule administration (6mg). In addition on corticospinal level, Specterman et al. (2005) observed facilitations of MEPs in the thenar muscles after intake of 46mg caffeine by an energy drink. Caffeine blocks the adenosine receptors (Barrett et al., 2010), so the increased excitatory postsynaptic neurotransmitters low the threshold for neuronal activation and enhance the spontaneous firing rate of cerebral cortical neurons (Phillis et al., 1979). Since the caffeine content of common beverages alters between 55-65mg (tea, instant coffee, coke) and 107-151mg (brewed coffee) (Bunker and McWilliams, 1979), no caffeine containing substances were allowed several hours before the measurements in the present work.

The influences of nicotine on the corticospinal excitability are not as clear as for the caffeine. Grundey et al. (2013) observed enhanced MEPs in chronic smokers and the

opposite effect for non-smokers after nicotine exposure. A cortical excitability reduction was found by Lang et al. (2008) in chronic smokers compared to non-smokers. Alkondon et al. (2000) proved GABAergic activity after stimulation of the nicotinic receptors. Depending on where exactly the interneuron synapses with the pyramidal neuron, it results in inhibition (soma or proximal axon) or disinhibition (dendrites) of the pyramidal neuron. In order to avoid influences on the neurons by nicotine, our participants were not allowed to smoke before the experiments.

Casey et al. (2014) found an influence of the sex hormone estradiol during the menstrual cycle in women. The T-reflex amplitude of the quadriceps became smaller around the time of ovulation, where the highest levels of estradiol are verified. In this study, we tried to evaluate the first day of the last menstruation of female participants, to determine their actual time point during the menstrual cycle.

In this study, 20 minutes break between the different measurements (T-reflex, H-reflex and MEP) was kept strictly in order to avoid false-positive interactions and influences between the reflexes.

As preactivation of other muscles (e.g. Jendrassik maneuver (Lehmann-Horn, 2007) or of the same muscle (Groppa et al., 2012, Hahn et al., 2012) facilitates the reflex responses by lowering the threshold, no chewing or any other movement of the body or of the soleus was allowed during recordings, to guarantee that the relaxed muscle was measured.

3 Aim of this study

The aim of this study is to investigate whether 5 minutes (5x 60seconds) static stretching of the right SOL affects the spinal and corticospinal excitability. Parameters to investigate the spinal excitability were the T-reflex and the H/M ratio. T-reflex was induced by percussion of a hammer onto the Achilles tendon and H-reflex by a probe (electrical stimulation) in the popliteal fossa. The corticospinal excitability was assessed by examining the MEP amplitudes and MEP latency. MEPs were obtained by TMS. Reflexes and MEPs were recorded by EMG. The test time points were before (Pre), immediately after (Post0) and, to investigate the recovery, 5 (Post5) and 10 minutes (Post10) after the stretching intervention or, in case of control condition, after a control rest. Expected effects are inhibition of T- and H-reflex (Budini et al., 2017, Masugi et al., 2017). During stretching, MEP sizes tend to decline (Gruber et al., 2009, Guissard et al., 2001). But the effects on MEP after stretch and the duration of potential effects after stretching remain still unclear, because of the long duration of the stretching intervention (5x 60seconds).

4 Methods

4.1 Study population

A collective of 17 healthy volunteers (9 female, 8 male; 22.8 ± 2.3 years; 68.7 ± 15.3 kg) with no history of neurological or psychological diseases (in particular epilepsy), no metal implants in their head (except dental prosthesis) and no electronical implants (cardiac peacemaker, neurostimulator or cochlear implant) were recruited. Additional exclusion criteria were pregnancy and acute injuries of the joints or the muscle tendon apparatus. Volunteers were instructed about the study protocol, the planned measurements and gave their informed consent prior to participation (Appendix 7.3 and 7.2). They were recruited with aid of recruitment leaflets in the medical university and the University of Sports Sciences (Appendix 7.1), so the participants consisted of sports and medical students. They were asked to avoid intense physical activity on the testing day and to refrain from intake of any caffeine or nicotine containing substances 2h before the procedures (Walton et al., 2003, Phillis et al., 1979).

Table 1 shows the mean values (MV), standard deviation (SD), and minimum and maximum of the physical characteristics of the participants. For more detailed information see Table 4 (Appendix 7.4).

Table 1: Physical characteristics of the participants (MV: Mean value, SD: standard deviation, Min: Minimum, Max: Maximum).

	Age	Weight (kg)	Height (m)
MV	22.8	69	1.74
SD	2.3	15	0.12
Min	20	46	1.59
Max	28	95	1.95

The present study adheres to the principles of the Declaration of Helsinki by the World Medical Association. The Ethics Committee of the university of Graz approved the project (Number: GZ. 39/77/63 ex 2013/14). It is the last part of a 3-year-founded FWF project.

4.2 Procedures

The measurements took place from January the 30 to March the 24 in the laboratory of the Sports Institute of the Karl-Franzens-University in Humboldtstr. 46, 8010 Graz. In a crossover design, the procedure was split into two randomized sessions lasting ca. 3 hours, with the stretching condition on the one day and the control condition on another day in a

random order, with a minimum of 24h between the two appointments. During control condition they acted as their own control.

4.3 Preparation

The preparation started with the determination of maximal dorsiflexion (at the stretching session only) followed by EMG skin preparation and electrode placement. After that continued the determination of rMT and the assessment of H- and M-Max by a ramp protocol (Figure 16).

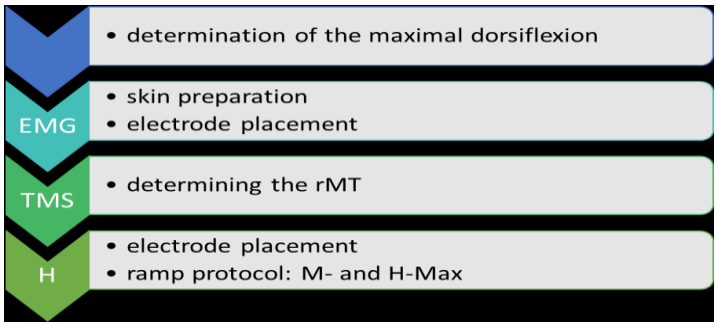


Figure 16: Preparation summary: maximal dorsiflexion, EMG, TMS, M- and H-Max (EMG: electromyography, TMS: transcranial magnet stimulation, rMT: resting motor threshold, H: Hofman reflex).

4.3.1 Maximal dorsiflexion

At the beginning of the stretching session only, the maximal dorsiflexion was quantified. For this purpose, the volunteers sat on the dynamometer chair (Con-Trex Multijoint, CMV AG, Dübendorf, Switzerland) in a semi-recumbent position (trunk at 70°) with the right leg extended at the knee, the foot laying on a foot-plate, the ankle fixed at 100° and the ankle angel set at 10° plantar flexion deviating from a neutral position at 90°. The participants were asked to operate the footplate rotation by the push of a button until reaching a position, at which the soleus was uncomfortably but not painful stretched. After that, mechanical stops were set at the defined position of maximal dorsiflexion for safety reasons. This passive maximal dorsiflexion was recorded.

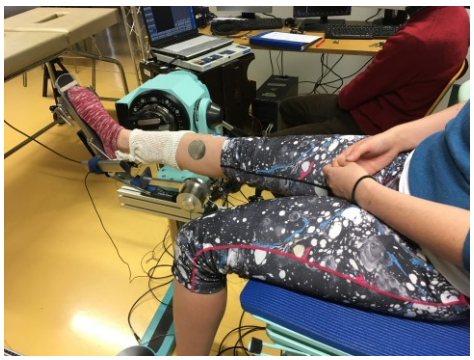


Figure 17: Participant sitting in the dynamometer chair, while determining the maximal dorsiflexion.

4.3.2 Electromyography

EMG was recorded from right SOL and TA as a reference muscle. The skin preparation followed the common guidelines from SENIAM (Surface ElectroMyoGraphy for the Non-Invasive Assessment of Muscles*)* (Merletti et al.): Therefore, after shaving the skin of the muscle belly at the place where the electrodes should be positioned, the skin was roughed by a pumice stone to remove dead skin and subsequently wiped with alcohol, to guarantee a stable skin contact and low impedance. EMG (custom made) from SOL was recorded in monopolar configuration with the first electrode (Blue Sensor^{N}; Ambu® Ballerup, Denmark) positioned right under the lateral portion of the gastrocnemius and the second electrode positioned on the Achilles tendon (see Figure 18 left). For the TA, two electrodes were positioned parallel to muscle fibers direction with an inter-electrode distance of approximately 2 cm on the muscle belly (Konrad, 2005). The ground electrode (STIMEX adhesive gel electrode) was placed on the front side of the tibial bone (see Figure 18 right) Skin preparation for the ground electrode correspond to the skin preparation described above for the EMG.

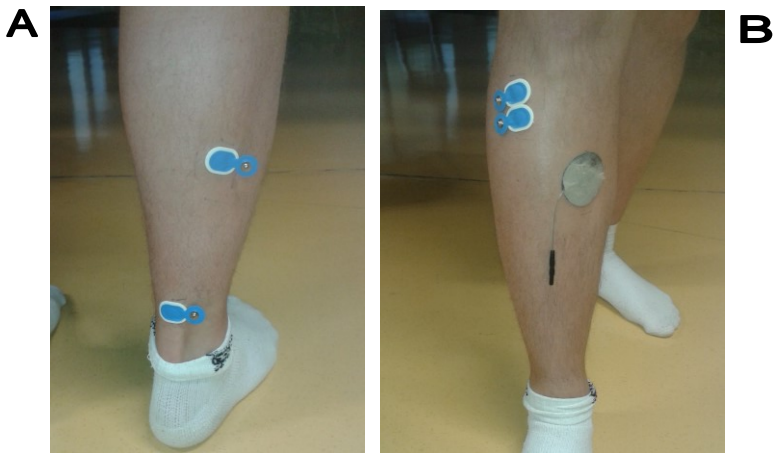


Figure 18: Soleus (A) and tibialis anterior (B): Placement of the self-adhesive surface electrodes. Ground electrode position is shown on the right photo (B).

4.3.3 Transcranial magnetic stimulation

With the TMS (Magstim 200², The Magstim Company ltd, Whitland, Wales, UK), the magnetic field with monophasic current pulses from anterior to posterior was externally applied to the cortex by a 110mm double-cone coil (Figure 19, right), which was positioned 1-2 cm left of the longitudinal fissure on the M1 area of the left hemisphere, where the plantar flexor muscles are represented. In order to find a correct stimulation intensity for each volunteer, in a first step, the individual rMT was determined. The rMT was defined as the lowest TMS intensity needed to induce a peak-to-peak amplitude of MEPs in the soleus of not less than 50µV in at least 5 out of 10 consecutive trials (Rossini

et al., 1994). The starting intensity amounted 30%, a subthreshold intensity. In this manner, the subjects got used to the feeling of the TMS. The coil position varied slightly to approximate the optimal hot spot on the M1 area. When no response was found, the intensity was raised in 5% steps until a motor response (amplitude >50µV) could be observed. This intensity was additionaly adjusted by decreasing the stimulation intensity in 1% steps. The position of this hot spot was marked on a cap, which the volunteers were wearing during the whole experiment, to guarantee, that the position of the coil rested constant. The following MEP amplitudes were recorded at 120% of the individual rMT. A volunteer sitting in the dynamometer chair during TMS measurement is shown in Figure 19 (left).

Figure 19: left: Volunteer sitting in the dynamometer chair with the foot on the footplate during TMS measurement.
right: coil.

4.3.4 Peripheral nerve stimulation: H-reflex and M-wave: recruitment ramp

The optimal position for the stimulation electrode was tested with the subjects laying prone on a medical examination table. The anode (5x9cm, STIMEX adhesive gel electrode) was positioned on the patellar tendon. The tibial nerve was probed in the popliteal fossa in order to find the lowest possible stimulation intensity where an H-reflex could be observed in the soleus. At this position, the cathode connected to a constant current stimulator (KeyPoint® 2-channel, Alpine Biomed ApS, Skovlunde, Denmark) was placed. After that, two full H-M stimulation ramps with rectangular pulses of 1.0 ms duration were performed with the subjects sitting in the dynamometer chair. The stimulation frequency was set at a random interval between 7 and 9 seconds. Stimulation intensity appropriate to produce a visible M-wave and an H-wave in the ascending part of the recruitment curve was determined in order to be used for the following part of the experiment. The volunteers

were asked to report any sensation of leg formication in which case the experiment continued only after the participants felt recovered again.

4.3.5 T-reflex

A reflex hammer driven by a motor (Type GDRX 075, Magnet-Schultz, Memmingen, Germany) was fixed at the dynamometer chair under the ankle more specifically the Achilles tendon about 3-4 cm above its insertion on the calcaneus to trigger the T-reflex (see Figure 20). The motor delivered an electrical output with the information about rotation enabling observation and recording of hammer movement and acceleration.

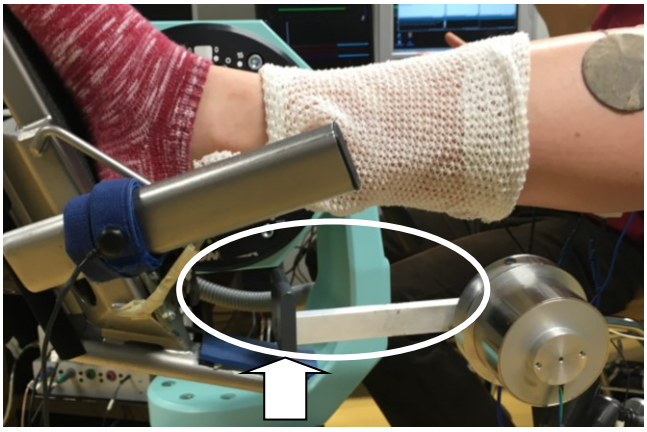


Figure 20: reflex hammer (inside the circle) positioned under the Achilles tendon. Arrow turns into the direction, in which the reflex hammer blows.

4.4 Assessment procedures

The experiment started with the baseline measurement consisting in 12 stimulations of the H-Reflex, T-Reflex and the TMS (order randomized) controlled by a computer system. The interval between the 12 stimulations was also random between 7 and 9 seconds as for the ramp protocol. Following baseline measurement, the volunteer rested for 20 minutes, standing for part of this time, then sitting. The standing time was calculated as the difference from 20 minutes minus the H-M ramps time, in this way, the sitting time was equivalent to the time during which the volunteer sat down before starting the recordings. During the sitting time, the participants had their leg in the elongated position as during the preparation time.

Each session (H-Reflex, T-Reflex, TMS, order randomized) started with a Pre-measurement (Pre) with 12 trials at each condition. After that, the intervention was carried out for 5 minutes. In case of control condition, the participants sat relaxed in the dynamometer chair, knees extended and foot laying on the footplate. In case of stretching

condition, the soleus was stretched 5 times for 1 minute by footplate rotation until the point of maximal dorsiflexion, as determined before (see 4.3.1).

Immediately after the foot plate returned back into position, a post measurement (Post0) followed, then after 5 min rest (Post5) and after further 5 min rest (Post10). During the 5 min rest in between the post measurements, the participants sat relaxed with their leg bend to not less then 90°. The experimental procedure is summarized in

Figure 21.

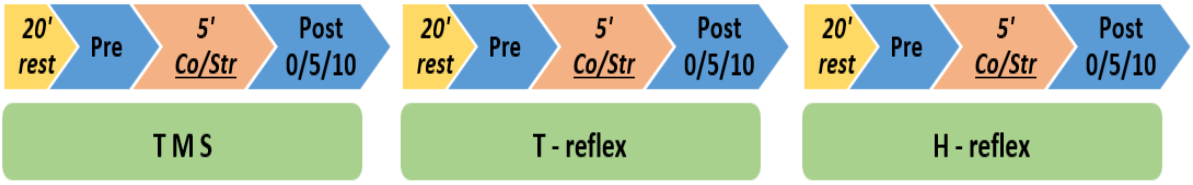


Figure 21: Experimental procedure of control rest (Co) or stretching (Str) condition: 20 minutes rest consists of 10 minutes standing, than 10 minutes sitting position in the dynamometer.
blue arrows: time points of measurements (at least 12 trials each) Pre: before, Post0: immediately after, Post5: 5 minutes after, Post10: 10 minutes after.
During rest in between the post measurements: sitting position in the dynamometer, leg relaxed, bend to not less then 90°. Order of transcranial magnetic stimulation (TMS), Stretch-reflex (T-reflex) and Hofmanns-reflex (H-reflex) randomized.

During measurements, the participants were not allowed to do any other muscle activity as e.g. chewing or arm and leg movements and were asked to keep their eyes closed or fix a spot in front of them. This was necessary to avoid enhanced reflex responses caused by the preactivation of the same or other muscles (see Jendrassik's maneuver (Barrett et al., 2010, Lehmann-Horn, 2007)).

4.5 Data recording und processing

The signals (EMG, torque, displacement, trigger and motor output were recorded by the software DEWESoft 7.1.1 (DEWESoft d.o.o, Trbovlje, Slovenia) at a sampling frequency of 10kHz, amplified (x180), band-pass filtered (5–1000 Hz), transmitted by an A/D Converter (16bit) and high-pass filtered by an iir Bessel filter. The data were pre-processed in MatLab (7.8.90.347 R2009a, MATLAB®, The MathWorks, Inc, Natick, USA) by determining the Min and Max of the M and H-wave, MEP and T-Reflex. In this way, the peak-to-peak amplitude in all measurements (M-wave, H-wave, T-Reflex, MEP) could be determined. An example is demonstrated in Figure 22. To reduce inter-subject variability, this amplitudes were normalized to their corresponding M max (Hahn et al., 2012) and the MEP/M_{max} ratio was calculated (Groppa et al., 2012) as well as the H/M ratio (Opplert et al., 2016) in Microsoft Excel® 2013 (Microsoft Corporation, Redmond, USA). To

determine the M_{max}, the ramps were analyzed and the 3-5 highest measured values, followed by no higher value were chosen to calculate the MV. H-waves were checked for consistency and those related to an M wave showing peak-to-peak amplitude exceeding the target stimulation intensity by ±2 standard deviations were discarded. Because of this criterion 1 subject had to be excluded.

The delta variation for the H/M ratio and the T-reflex was determined. For this purpose, the Post measurements were made in relation with their Pre measurements and calculated in percent for further investigation.

In addition, the latency (CML) was assessed in MatLab for the TMS measures. Therefore, the latency was defined as the first deflection from baseline EMG after stimulation. The deflection could be negative or positive (see Figure 22, A and B). The mean value of the stimulations per time point was calculated in Microsoft Excel®.

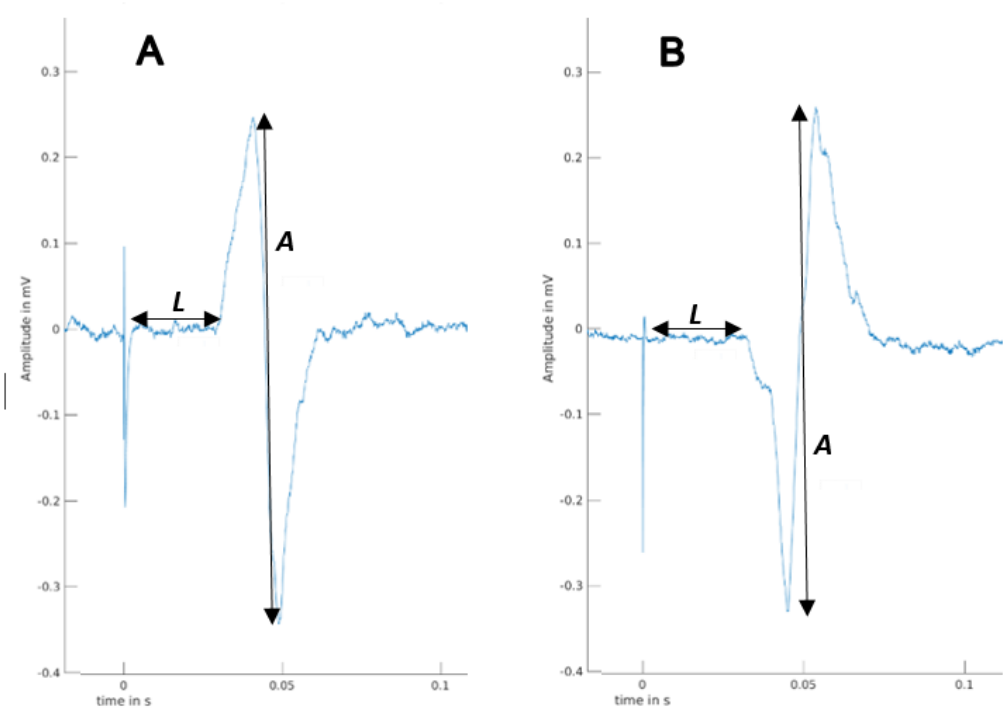


Figure 22: Determination of latency (L) and amplitudes (A) of MEPs of two different participants: A: MEP deflection direction is first positive; B: MEP deflection direction is first negative.

4.6 Statistics

The statistical analysis was performed using the automated statistical software tool SPSS for Windows Software (IBM® SPSS® Statistics, Version 23, IBM Corporation, Armonk, USA). The p-value was set at 0.05. Because of the small sample, all data were tested for normality by Shapiro-Wilk test. The difference between the rMT at the two different days (conditions) and the M_{max} at the two different days (conditions) were determined by a t-

test, the confidency interval was 95%. The differences between the time points and conditions were determined using a Friedman repeated measures ANOVA because of a failed normal distribution. A significant ANOVA result requires a subsequently passed post-hoc-test. As post-hoc test served the Wilcoxon signed rank test (non normal distribution) to compare individual pairs and required a Bonferroni-Holm adjustement to avoid a multiple comparisons problem (Bühner and Ziegler, 2009).

As “the logarithmic transformation of amplitude data provides a better approximation to a normal distribution” (Nielsen, 1996, p. 430), the amplitude data of MEPs were transformed by taking their natural logarithms.

5 Results

A group of 17 volunteers was tested on two different days: On one day, the stretching condition (5x60s passive static stretching at individual maximal ankle dorsiflexion) and on the other day the control condition without any stretching. The three assessments (T-reflex, H-reflex, TMS) were carried out at 4 time points: Before (Pre), and 3 times after the condition (Post0, Post5, Post10). MEPs were obtained using TMS. The T-reflex was induced by a hammer and the H-reflex by a stimulation electrode. Reflexes and MEPs were recorded by surface EMG on the right SOL and TA.

5.1 Maximal dorsiflexion

The maximal dorsiflexion in the right ankle of the subjects ranged between 18.6° and 39.7° (MV±SD: 29.87±6.14°). Men had in average 28.55 ± 6.76° and women 31.93 ± 5.97° maximal dorsiflexion. For detailed values of the individual participants, see Table 4 in the appendix.

5.2 T-reflex

Three subjects were rejected from analysis because of missing T-reflex responses and one subject because of outlier values.

5.2.1 T-reflex amplitudes

The T-reflex amplitude of Pre measurement within the stretching condition amounted 4.75±4.69mV. Immediately after stretching (Post0), it decreased to 1.48±1.28mV and increased after 5 min (Post5) to 4.36±4.52mV and after 10 min (Post10), it was 4.32±4.69mV. The T-reflex amplitude of Pre measurement within the control condition was 4.59±5.03mV. Immediately after the control rest (Post0), it decreased to 3.78±4.71mV, increased after 5 min (Post5) to 4.12±4.97mV and again after 10 min (Post10) to 4.32±4.26mV (see Figure 23).

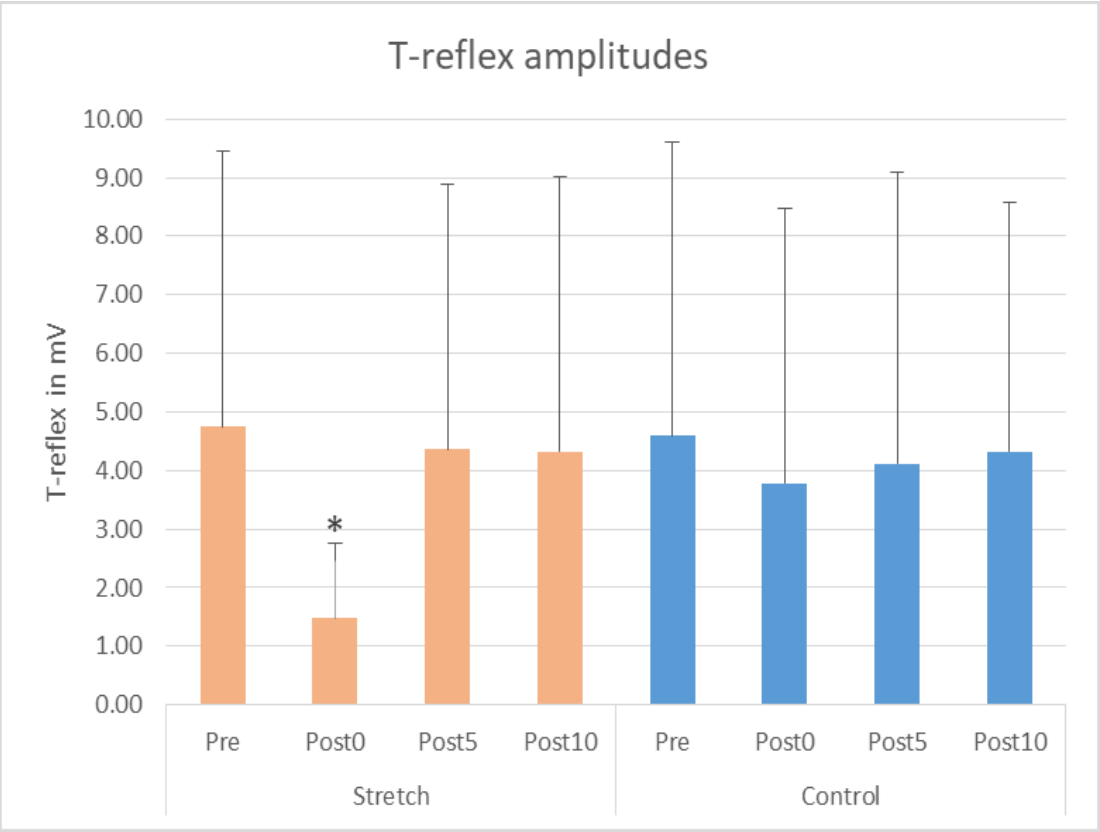


Figure 23: Comparison of the T-reflex amplitudes between the time points (Pre, Post0, Post5, Post10) and the two conditions stretch and control (MV+SD).
** Post0 of the stretching condition was significant to Pre, Post5 and Post10 of the stretching condition, furthermore significant to the Post0 measurement of the control condition.*

Data of the amplitudes at the different time points were not normally distributed (Shapiro-Wilk: $p<0.05$), hence the non parametric Friedman repeated measures ANOVA was performed. The Friedman test was significant ($\chi^2=23.85$, $p=0.001$) between the two conditions and the eight time points (see Table 7 and Table 8 in the appendix). To find significant differences, a Wilcoxon test with a Bonferroni Holm correction was executed. No differences could be found between the control measurement and their belonging time points during stretching condition, except Post0 at stretching condition to Post0 at control condition ($p=0.028$; $Z=-2.197$). But a significant difference (*) could be found between Pre-Post0 ($p=0.002$; $Z=-3.059$) and Pre-Post5 ($p=0.012$; $Z=-2.510$) of the stretching condition. After 10 Minutes (Post10), the amplitude returned to baseline measurement (Pre) ($P=0.480$; $Z=-0.706$). There was also a significant result between Post0 to Post 5 ($p=0.004$; $Z=-2.903$) and Post0 to Post10 ($p=0.002$; $Z=-3.059$) (see Table 9 in the appendix).

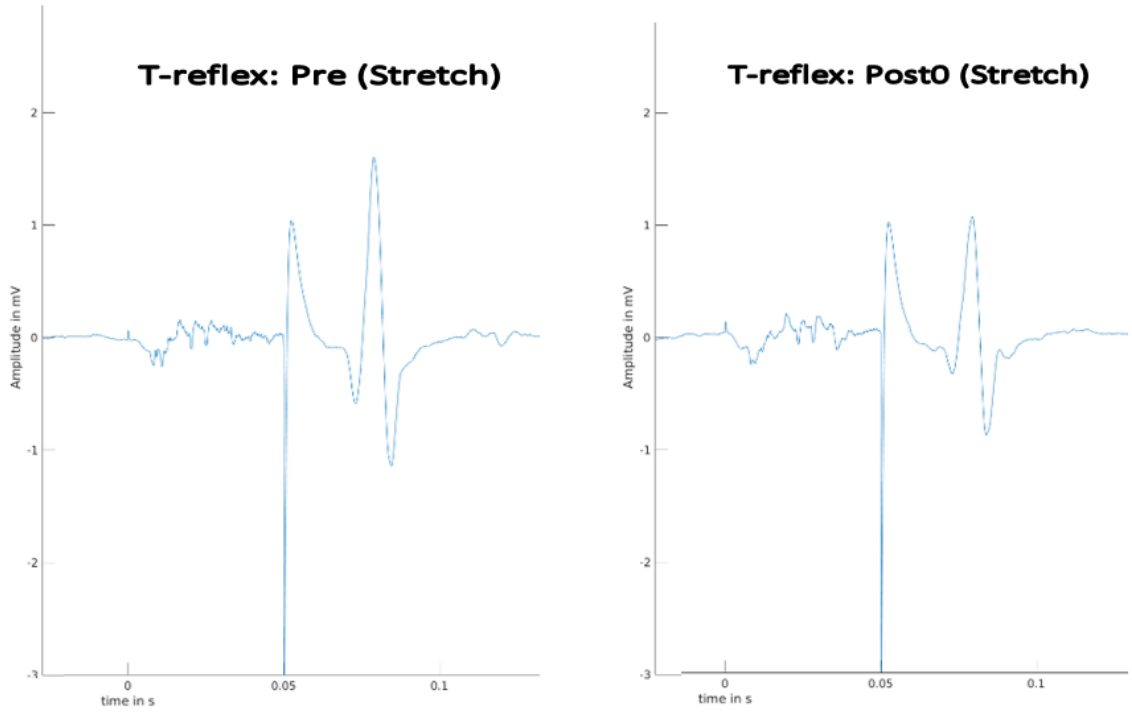


Figure 24: Raw data of one participant. The inhibition of the T-reflex is apparent in the decreased amplitude after stretch.

5.2.2 T-reflex delta variation

The Post measurements (Post0, Post5, Post10) of control and stretching condition were made in relation with their Pre measurement and were calculated in percent of the Pre measurement for further investigation:

$$\text{Delta variation} = \frac{Post0/5/10 - Pre}{Pre}$$

After stretching, the amplitude declined to -57.61±32.15% (Post0) of the Pre measurement. After 5 minutes (Post5), the decrease amounted only -16.17±19.88% of the Pre measurement. 10 minutes after (Post10), the amplitude was at baseline level with -11.25±28.53% of Pre measurement. The values at control condition did not differ very much. Immediately after control rest, the amplitude decreased -4.43±44.46%, 5 minutes after 3.69±63.65% and 10 minutes after 0.43±62.76% (see Figure 25).

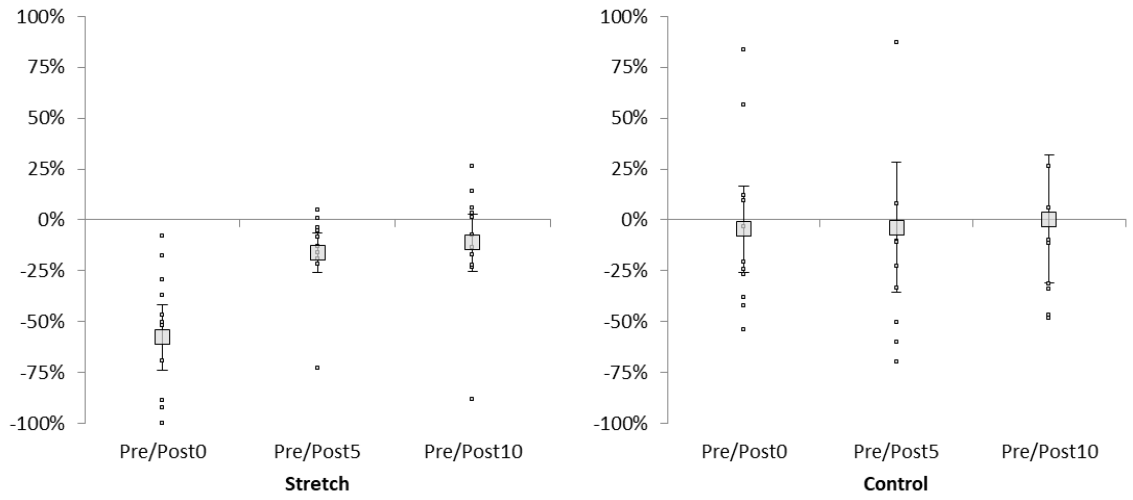


Figure 25: Delta variation Pre-Post stretching and control for each time point (Post0, Post5, Post10) after intervention for individual subjects (small markers) and the group MV ± SD (large markers).

The Shapiro-Wilk showed no normal distribution ($p<0.05$). The Friedman ANOVA showed a significant difference between the time points and the two conditions ($\chi^2=19.159$; $p=0.002$) (see Table 10 and Table 11 in the appendix). As post-hoc test served the Wilcoxon test with the Bonferroni Holm correction. A significant difference could be found immediately after stretching (Post0) to immediately after control rest ($p=0.005$; $Z= -2.803$), immediately after to 5 minutes after ($p=0.004$; $Z=-2.903$) and to 10 minutes after ($p=0.002$; $Z=3.059$) (see Table 12 in the appendix).

5.3 H-reflex

One participant has been excluded because of outlier values. Participants tolerated the stimulations well without reporting any side effects.

5.3.1 H/M ratio

The H-reflex amplitude was normalized to the corresponding M (H/M ratio). Within stretching condition, the ratio increased from 24.81±25.06 (Pre) to 26.61±24.45 (Post0) and decreased after 5 min to 24.02±21.90 (Post5) and finally to 22.80±23.18 (Post10). Within control condition, the H/M ratio stayed at the same level from Pre measurement (22.49±23.85) to Post0 (22.78±25.29), Post5 (22.92±24.95) and Post10 (22.05±23.16) (see Figure 26).

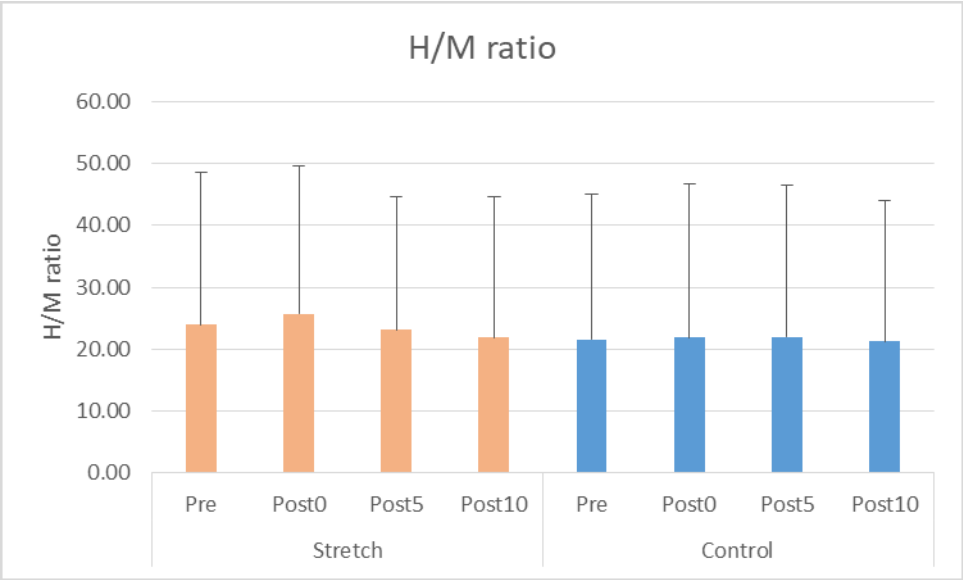


Figure 26: Comparison of the H/M ratio between the timepoints (Pre, Post0, Post5, Post10) and the two conditions stretch and control (MV+SD).

The Shapiro-Wilk showed no normal distribution ($p<0.05$), so the Friedman test was performed ($\chi^2=7.792$; $p=0.351$), showing no significances between the time points (see Table 13 and Table 14 in the appendix).

5.3.2 Delta variation H/M ratio (%)

For the further analysis, the Post measurement (Post0) of control and stretching condition were made in relation with their Pre measurement and were calculated in percent. The delta variation immediately after stretching amounted 15.55±22.04%, 5 minutes after (Post5) 3.48±44.14% and 10 minutes after (Post10) -0.94±32.39%. At control condition, the delta variation was 0.93±10.51% immediately after control rest (Post0), 6.17±30.81% 5 minutes after (Post5) and 3.07±21.82% 10 minutes after (Post10) (see Figure 27).

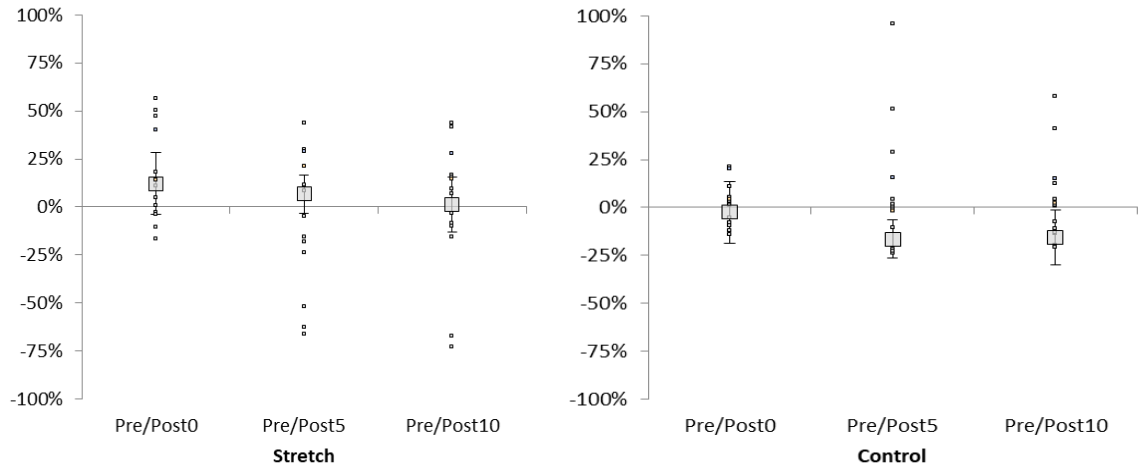


Figure 27: Delta variation Pre-Post stretching and control for each time point (Post0, Post5, Post10) after intervention for individual subjects (small markers) and the group MV ± SD (large markers).

To compare the stretching and control condition and the different time points, a Shapiro-Wilk test was performed first, to determine a normal distribution (no normal distribution; $p<0.05$). The Friedman ANOVA showed a not- significant result ($\chi^2=6.786$; $p=0.237$) (see Table 15 and Table 16 in the appendix).

5.4 Corticospinal excitability

The corticospinal excitability was determined by changes in the MEP peak-to-peak amplitudes, changes in MEP/M_{max} ratio and the latency of the MEP signal. All participants tolerated the TMS stimulations well without reporting any side effects. One participant had to be excluded because of missing valid M_{max} values.

5.4.1 Resting motor threshold (rMT)

The rMT on the day of stretching condition was 40.00±6.94% of stimulator output and on the day of control condition, it amounted 39.76±6.78% of stimulator output. The Shapiro-Wilk test showed a normal distribution ($p>0.05$), which allows a t-test. The t-test showed no difference between the rMTs on the two different conditions ($t=0.365$; $p=0.720$). More detailed values are presented in Table 5, Table 17 and Table 18 in the appendix.

5.4.2 M_{max}

The M_{max} measured before the stretching condition was 27.21±5.65mV and measured before the control condition 26.35±5.29mV. The two conditions were normal distributed ($p>0.05$), so a t- test was executed and showed no difference between the M_{max} between the two testing days and conditions ($t=-0.840$; $p=0.414$). For detailed values see Table 6, Table 19 and Table 20 in the appendix.

5.4.3 Data MEP amplitudes and normalized to M_{max}

Table 2: Data of MEP amplitudes and normalized to M_{max} (MV ± SD) (MV: Mean value; SD: standard deviation; MEP: Motor evoked potential).

		MEP peak-to peak amplitude (MV±SD)	MEP/M_{max} ratio (MV±SD)
Stretch	Pre	0.53 ± 0.27	0.020 ± 0.011
	Post0	0.40 ± 0.16	0.015 ± 0.007
	Post5	0.49 ± 0.24	0.018 ± 0.009
	Post10	0.53 ± 0.23	0.020 ± 0.010
Control	Pre	0.42 ± 0.18	0.016 ± 0.009
	Post0	0.38 ± 0.13	0.015 ± 0.007
	Post5	0.47 ± 0.19	0.018 ± 0.008
	Post10	0.47 ± 0.23	0.019 ± 0.010

5.4.4 MEP amplitudes

Figure 28 shows the mean values of the different time points investigated. The MEP amplitude of Pre measurement within the stretching condition amounted 0.53±0.27mV. Immediately after stretching (Post0), it decreased to 0.40±0.16mV and increased after 5 min (Post5) to 0.49±0.24mV and after 10 min (Post10) again to 0.53±0.23mV. The MEP amplitude of Pre measurement within the control condition was 0.42±0.18mV. Immediately after the control rest (Post0), it decreased to 0.38±0.13mV and increased after 5 min (Post5) to 0.47±0.19mV and was after 10 min (Post10) to 0.47±0.23mV.

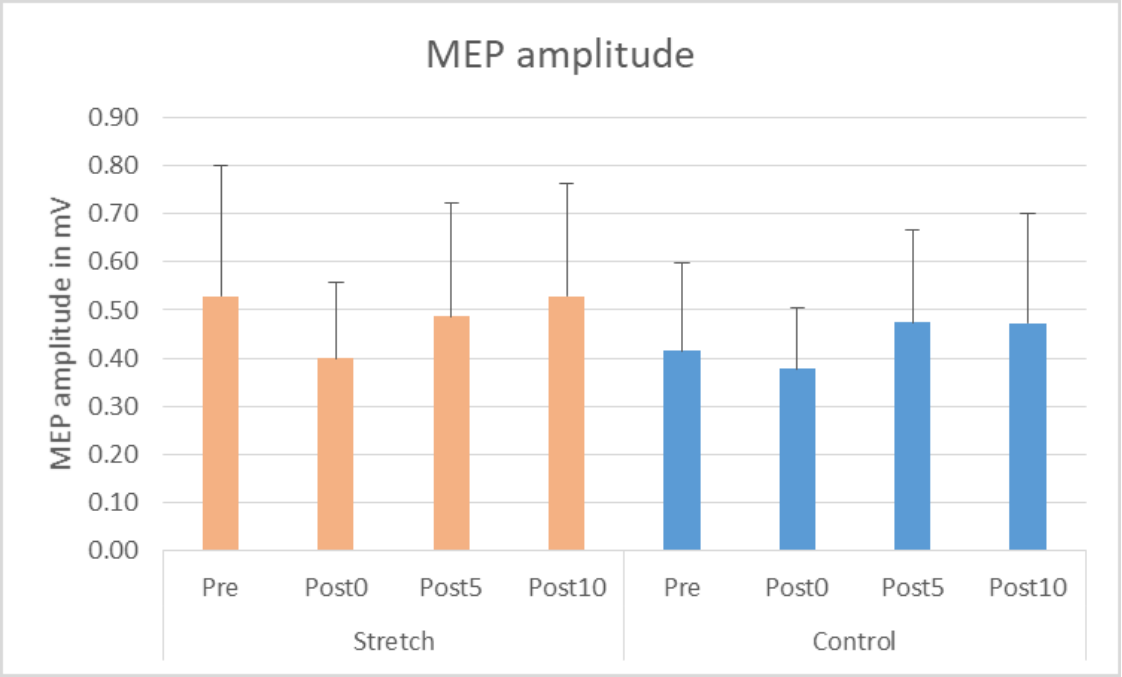


Figure 28: Comparison of the MEP amplitudes between the timepoints (Pre, Post0, Post5, Post10) and the two conditions stretch and control (MV + SD).

Data of the amplitudes at the different time points were not normally distributed (Shapiro-Wilk: $p<0.05$), hence the non parametric Friedman repeated measures ANOVA was performed. The Friedman test showed no significant differences ($\chi^2=5.833$, $p=0.559$) between the two conditions and the 8 time points (see Table 21 and Table 22 in the appendix).

Figure 29 showes a high alteration of the amplitudes, indicating a high interindividual variability. For that reason, the MEP amplitudes were normalized to the M_{max}.

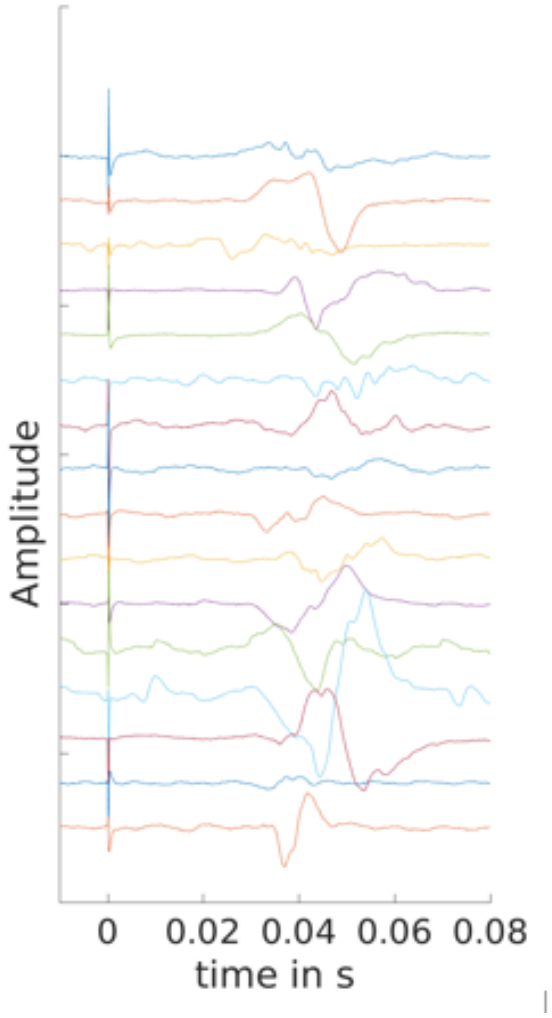


Figure 29: motor evoked potentials of 16 different participants at the same time point (Pre-Control) with high alteration in peak-to-peak amplitudes apparent.

5.4.5 Normalized to M_{max} (MEP/M_{max} ratio)

After normalization of the MEP to the M_{max}, the Pre measurement at the day of stretching condition was 0.020±0.011. Immediately after stretching (Post0), the amplitude decreased to 0.015±0.007 and increased after 5 minutes (Post5) to 0.018±0.009 and after 10 minutes (Post10) again to 0.020±0.010. The Pre measurement within the control condition was 0.016±0.009. Immediately after the control rest (Post0), it decreased to 0.015±0.007 and increased after 5 minutes (Post5) to 0.019±0.008 and decreased again after 10 minutes (Post10) to 0.019±0.010 (see Figure 30).

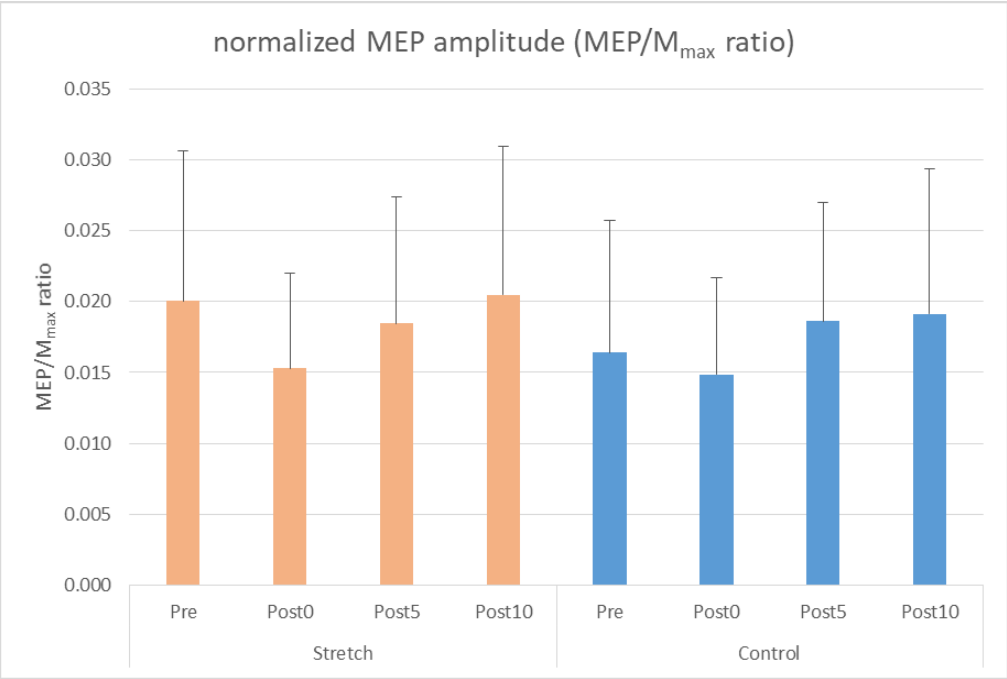


Figure 30: Comparison of MEP/M_{max} ratio between the timepoints (Pre, Post0, Post5, Post10) and the two conditions stretch and control (MV + SD).

The Shapiro-Wilk showed again a not normal distribution ($p<0.05$). A non parametric Friedman repeated measures ANOVA was not significant ($\chi^2=5.444$, $p=0.606$) between the 8 time points and the 2 conditions (see Table 23 and Table 24 in the appendix).

5.4.6 MEP latency

The time latency during stretching condition varied from Pre to Post0 from 31.81±3.39ms to 32.83±3.42ms. After 5 minutes (Post5), the latency lasted 31.42±3.68ms. 10 minutes after stretching (Post10), the MEP began 32.09±3.72ms after stimulation. On the day of control condition, the latency augmented from 31.59±3.94ms before (Pre) to 32.29±4.06ms immediately after stretching (Post0). 5 minutes after stretching (Post5), the latency lasted 32.04±3.83ms and 32.00±3.91 after 10 minutes (Post 10) (see Figure 31).

Table 3: Data of MEP latency (MEP: motor evoked potential.

		MEP Latency in ms (MV±SD)
Stretch	Pre	31.81 ± 3.39
	Post0	32.83 ± 3.42
	Post5	31.42 ± 3.68
	Post10	32.09 ± 3.72
Control	Pre	31.59 ± 3.94
	Post0	32.29 ± 4.06
	Post5	32.04 ± 3.83
	Post10	32.00 ± 3.91

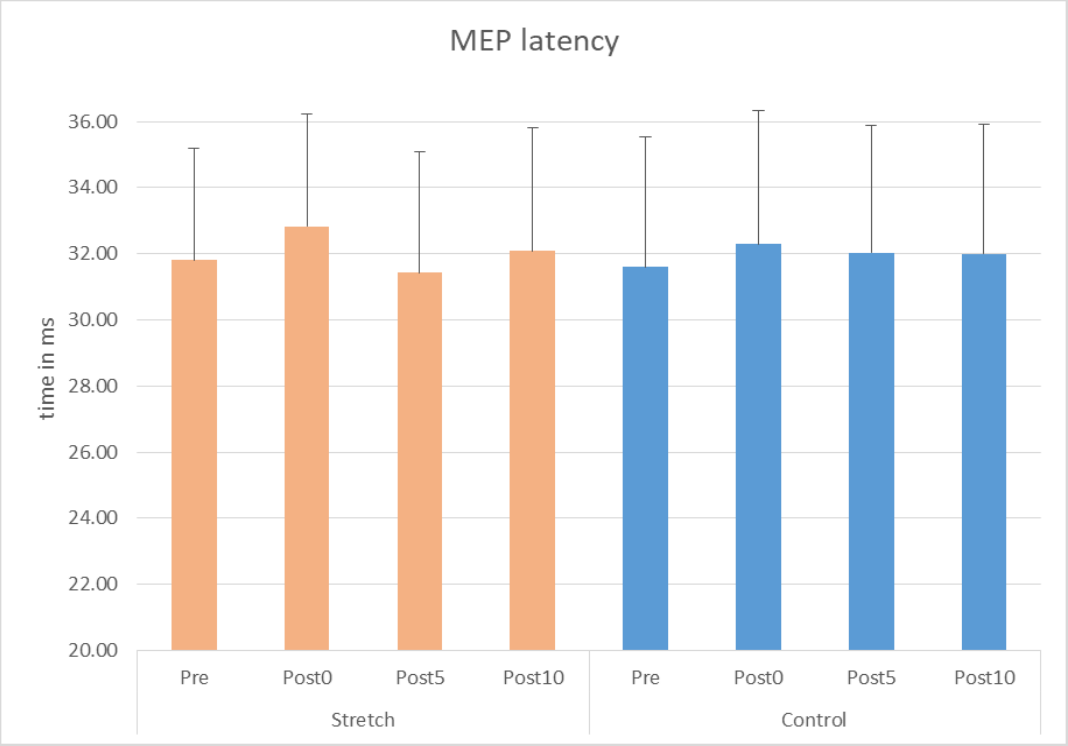


Figure 31: Comparison of the MEP latency between the timepoints (Pre, Post0, Post5, Post10) and the two conditions stretch and control (MV+SD).

The Shapiro-Wilk showed no normal distribution ($p<0.05$), so the non parametric Friedman repeated measures ANOVA was executed and showed no significances ($\chi2=10.76$, $p=0.149$) between the 8 time points and the 2 conditions (see Table 25 and Table 26 in the appendix).

6 Discussion

T-reflex, H-reflex and MEPs on right SOL of 17 healthy volunteers were tested immediately after, 5 and 10 min after 5 min (5x60s) static stretching at individual maximal ankle dorsiflexion. On a second day, the control testing without stretching was carried out. MEPs were obtained using TMS. Reflexes and MEPs were recorded by surface EMG. The aim was to investigate if 5 minutes static stretching of SOL affects the corticospinal excitability by a facilitated or inhibited amplitude and latency of MEPs, and if it influences the amplitudes of T-reflex and H-reflex. The results on spinal level showed a presynaptic inhibition of T-reflex lasting at least 5 min, but no effect on H-reflex following 5 minutes passive static muscle stretch. The results of MEP amplitudes and MEP latency showed that corticospinal excitability remains unchanged.

6.1 T-reflex

The assessment of T-reflex amplitudes and delta variations of T-reflex (relation to Pre-measurement) showed a strongly inhibited T-reflex in the right SOL following 5 min passive static stretching to the point of individual maximal dorsiflexion. The inhibiting effect lasted up to 5 minutes and returned to baseline values 10 minutes after stretching.

An inhibition of T-reflex amplitude was already found in literature (Guissard and Duchateau, 2004, Masugi et al., 2017, Avela et al., 1999, Weir et al., 2005, Budini et al., 2017). Budini et al. (2017) attributed the inhibited T-reflex after 1 minute of stretching to a decrease in muscle spindle sensitivity on presynaptic level with a recovery trend in between 150-300s. In the present work of 5 minutes stretching, the T-reflex shows the recovery trend only between 5 and 10 minutes, indicating, that a longer applied stretching duration results in a longer lasting inhibition of the T-reflex.

Rossi-Durand (2002) suggested that spindle stretch-sensitivity and resting discharge contribute to changes in the T-reflex.

Responsible for an inhibition of the T-reflex is the decrease in muscle spindle sensitivity (Ia fibers elicited) (Budini et al., 2017, Rossi-Durand, 2002) or another mechanism based on the golci tendon organ. The Ib fibers, elicited by the golci tendon organ, inhibit the α-motor neuron via interneurons in the spinal cord (Barrett et al. 2010) (see Figure 8).

6.2 H-reflex

In the present study, no significant effects after passive static stretching on the H-reflex were found. Interpretation and statistical analysis were difficult concerning a high interindividual variability, especially due to a high SD. Furthermore, it has to be considered, that increasing stretching angles effect a linear reduction of H/M ratio, which was assessed by Guissard et al. (1988). In this study, the range of motion differed between -18.6°, the smallest, to -39.7°, the highest, assuming to influence the results. But the Pre-Post matches, made in this study should minimize this influence.

The findings in this study, that stretching did not provoke any significant changes in the H/M ratio disagreed with the results of many previous studies, which found an inhibited H-reflex (Guissard and Duchateau, 2006, Robertson et al., 2012, Robinson et al., 1982, Masugi et al., 2017, Avela et al., 1999) as already described in chapter 2.3.2. A reason can be the longer duration of stretch in our study and the smaller number of participants.

However, in this study, the H-reflex immediately after stretching showed slight facilitation, which resembles to the findings of Budini et al. (2017) with the distinction that in the present study, the facilitation was not significant. In this way, it more corresponds to the results of Opplert et al. (2016), who did not found any changes in the H/M ratio up to 30s stretching duration.

The anatomical structures, which can be causal for a change in the H-reflex, are presynaptic (muscle spindles, Golci organs and Ia afferent fibers) or postsynaptic (α-motor neuron) (Guissard et al., 2001) (see chapter 2.3.2):

Presynaptic explanation for the mostly depressed H-reflex is the presynaptic inhibition of the Ia afferent fibers (Guissard and Duchateau, 2006, Robinson et al., 1982). This inhibition leads to a decrease of the excitatory drive from the Ia afferent fibers onto the α-motoneurons (Avela et al., 1999) originating from intramuscular receptors as the Golci tendon organ or the muscle spindles (Robinson et al., 1982). For Kohn et al. (1997), the most probable explanation for the H-reflex depression consists in the presynaptic inhibition. Budini et al. (2017) explained their facilitation of the H-reflex by the decrease in muscle sensitivity.

While the T-reflex, as a mechanically stimulated reflex was observed to be inhibited after stretching, the H-reflex, as an electrically stimulated reflex showed no significant effect. Such a divergent result was already described by Rossi-Durand (2002), who found a facilitated T-reflex and a facilitated or depressed H reflex after mental effort. It allows the assumption that the H-reflex and the T-reflex are influenced by different physiological

mechanisms, which influences the amplitude. Rossi-Durand (2002) attributes the changes in the T-reflex to the muscle spindle sensitivity and the changes in the H-reflex to post-activation depression.

A postsynaptic inhibition is caused by an activation of spinal cord interneurons (Renshaw, Ib…) (Kohn et al., 1997). Guissard et al. (2001) assume after their study with H-reflex and MEP measurements at different angles during stretching, that presynaptic mechanisms are primarily involved in weak stretching amplitudes, whereas postsynaptic mechanisms are more important in stronger stretching amplitudes. Vujnovich and Dawson (1994) determined a reduced H-reflex amplitude during stretching. They explained it with the inhibitory effect on the α-motor neuron during stretching. To examine this theory, in this study the effects on the MEPs were also considered, which showed no significant inhibition, making an influence on the level of the α-motor neuron rather unlikely.

6.3 MEP

The examination of MEP amplitude and MEP/M_{max} ratio showed no significant changes in the MEP peak-to-peak amplitudes in the right SOL following 5 min passive static stretching to the point of individual maximal dorsiflexion. There was a slight depression of the amplitudes immediately after stretching for the peak-to-peak amplitudes and for the MEP/M_{max} ratio, as well, but not significant. A depression of MEP amplitudes was already found *during* stretching (Guissard et al., 2001, Gruber et al., 2009). However, the investigation of the MEP amplitudes and MEP latency *after* passive static stretching is not well investigated in literature.

After logarithmising the MEP data (Nielsen, 1996), the repeated measures ANOVA showed no significant differences between the time points ($p>0.050$), so this analysis was not further pursued.

One reason for the non-significant result of the MEP amplitudes in this study is the high interindividual variability. We found a high variation of amplitudes between the participants. Standard deviation reached maximal values of 56% of the mean (see Figure 29). Kiers et al. (1993) assume that the variability in MEP size originates in ‘constant, rapid, spontaneous fluctuations in corticospinal and segmental motoneuron excitability levels’ (p. 415). Beside this spontaneous random variability (Kiers et al., 1993), other influences on the amplitude were already reported in the literature. Pitcher et al. (2003) found a decrease in amplitude related to age and a higher amplitude variability in women, but this sex related differences had a low effect and were not as strong as the age influence.

Ellaway et al. (1998) attributed the wide range of variablity in the thenar and wrist extensor muscles more to the cortical, than to the spinal level.

The decrease in MEP size after stretch, found in other studies, refers to postsynaptic inhibitory mechanisms (Guissard and Duchateau, 2006) or spinal glycinergic inhibitory interneurons, the Renshaw-cells (Barrett, 2010). The fact that the MEPs were uneffected by stretching in this work showed that an influence on the cortical neuron or the α-motor neuron was rather unlikely.

In measurement of conduction time (latency), no significant effect after stretching was found. We did not expect changes in the latency, because changes in the latency indicate pathologies in the spinal tract (Barrett et al., 2010).

6.4 Evaluation of the methods

The test set up cannot consider all possibilities at the same time. Unknown factors occurred during or after the examination. Since differences in the stretch response during the menstrual cycle were already found in women by Casey et al. (2014), the data during the menstrual cycle of the female subjects were collected in this study. But two of the nine women did not know the first day of their last menstruation, and two had oligomenorrhea or rather amenorrhea, in this way, only four women would have been available for analysis. This is the reason why the hormone cycles could not be considered.

Some further limitations in the present work should be taken into account. Unexpected problems appeared after analyzing the data. In particular the T-reflex could not always be triggered by the hammer, reducing the number of analyzed participants.

Participants had a wide range of possible range of motion from -18.6°, the smallest, to -39.7°, the highest. But in literature no differences between more-flexible and less-flexible volunteers could be found (Blazevich et al., 2012).

No side effects were reported, the participants tolerated the procedures very well. Except for some T-reflex measurements, the reflex responses were triggered in almost 100% of the trials. It was possible to present the effects on spinal and corticospinal level with non-invasive electrophysiological methods.

6.5 Summary and outlook

In conclusion, the results on spinal level show a significant long lasting inhibition of T-reflex, but no effect on the H-reflex following 5 minutes passive static muscle stretch of the plantar flexor muscles. The results of MEP amplitudes and MEP latency showed that

corticospinal excitability remains unchanged. This indicates that the influence on the cortical neuron or the α-motor neuron is rather unlikely, but the effect on the muscle spindles on presynaptical level induces an inhibited T-reflex, but no effect on the H-reflex. Further investigations are needed to distinguish between the different mechanisms of the presynaptical changes (T-reflex changed, H-reflex not changed), especially immediately after the stretching condition.

Spasticity in patients with neurological disorders after stroke (Bakheit et al., 2005) or after injuries of the spinal cord (Nakazawa et al., 2006) derives from hyperexcitability of the corticospinal tract (Masugi et al., 2017). The clinical benefit of stretching of the affected muscles (Vujnovich and Dawson, 1994) occurred via inhibition of the presynaptic reflexes, which represent a decrease of the spinal excitability. Further research is needed to confirm the results on corticospinal level because in this study, no effect after stretching could be proved, but several studies already investigated a decrease in the MEP amplitudes during stretching in the upper and lower extremity (Guissard et al., 2001, Gruber et al., 2009). Moreover, the effects in aged patients, suffering from e.g. stroke should be investigated.

Finally, stretching stays the most effective recovery strategy in sports due to the improvement of range of motion, flexibility, the decrease in muscle soreness (Guissard and Duchateau, 2004, Crowther et al., 2017, Blazevich et al., 2014, Konrad and Tilp, 2014) and to prevent injuries (Behm et al., 2016, McHugh and Cosgrave, 2010). Therefore more studies are indicated, to compare the effects after stretching in sportsmen to non-sportsmen and to reduce the stretching duration again, to investigate in a more practical orientation.

References

AAGAARD, P., SIMONSEN, E. B., ANDERSEN, J. L., MAGNUSSON, P. & DYHRE-POULSEN, P. 2002. Neural adaptation to resistance training: changes in evoked V-wave and H-reflex responses. *J Appl Physiol (1985),* 92**,** 2309-18.

ALKONDON, M., PEREIRA, E. F., EISENBERG, H. M. & ALBUQUERQUE, E. X. 2000. Nicotinic receptor activation in human cerebral cortical interneurons: a mechanism for inhibition and disinhibition of neuronal networks. *J Neurosci,* 20**,** 66-75.

ANDERHUBER, F., STREICHER, J. & PERA, F. 2012. *Waldeyer Anatomie des Menschen,* Graz, De Gruyter Studium.

AVELA, J., FINNI, T., LIIKAVAINIO, T., NIEMELA, E. & KOMI, P. V. 2004. Neural and mechanical responses of the triceps surae muscle group after 1 h of repeated fast passive stretches. *J Appl Physiol (1985),* 96**,** 2325-32.

AVELA, J., KYROLAINEN, H. & KOMI, P. V. 1999. Altered reflex sensitivity after repeated and prolonged passive muscle stretching. *J Appl Physiol (1985),* 86**,** 1283-91.

BAKHEIT, A. M., MAYNARD, V. & SHAW, S. 2005. The effects of isotonic and isokinetic muscle stretch on the excitability of the spinal alpha motor neurones in patients with muscle spasticity. *Eur J Neurol,* 12**,** 719-24.

BARKER, A. T., JALINOUS, R. & FREESTON, I. L. 1985. Non-invasive magnetic stimulation of human motor cortex. *Lancet,* 1**,** 1106-7.

BARRETT, K., BROOKS, H., BOITANO, S. & BARMAN, S. 2010. *Ganong's review of medical physiology,* United States, McGraw-Hill Companies.

BEHM, D. G., BLAZEVICH, A. J., KAY, A. D. & MCHUGH, M. 2016. Acute effects of muscle stretching on physical performance, range of motion, and injury incidence in healthy active individuals: a systematic review. *Appl Physiol Nutr Metab,* 41**,** 1-11.

BLAZEVICH, A. J., CANNAVAN, D., WAUGH, C. M., FATH, F., MILLER, S. C. & KAY, A. D. 2012. Neuromuscular factors influencing the maximum stretch limit of the human plantar flexors. *J Appl Physiol (1985),* 113**,** 1446-55.

BLAZEVICH, A. J., CANNAVAN, D., WAUGH, C. M., MILLER, S. C., THORLUND, J. B., AAGAARD, P. & KAY, A. D. 2014. Range of motion, neuromechanical, and architectural adaptations to plantar flexor stretch training in humans. *J Appl Physiol (1985),* 117**,** 452-62.

BUDINI, F., GALLASCH, E., CHRISTOVA, M., RAFOLT, D., RAUSCHER, A. B. & TILP, M. 2017. One minute static stretch of plantar flexors transiently increases H reflex excitability and exerts no effect on corticospinal pathways. *Exp Physiol,* 102**,** 901-910.

BÜHNER, M. & ZIEGLER, M. 2009. *Statistik für Psychologen und Sozialwissenschaftler*, Pearson Studium.

BUNKER, M. L. & MCWILLIAMS, M. 1979. Caffeine content of common beverages. *J Am Diet Assoc,* 74**,** 28-32.

CASEY, E., HAMEED, F. & DHAHER, Y. Y. 2014. The muscle stretch reflex throughout the menstrual cycle. *Med Sci Sports Exerc,* 46**,** 600-9.

CLASSEN, J., WITTE, O. W., SCHLAUG, G., SEITZ, R. J., HOLTHAUSEN, H. & BENECKE, R. 1995. Epileptic seizures triggered directly by focal transcranial magnetic stimulation. *Electroencephalogr Clin Neurophysiol,* 94**,** 19-25.

CROWTHER, F., SEALEY, R., CROWE, M., EDWARDS, A. & HALSON, S. 2017. Team sport athletes' perceptions and use of recovery strategies: a mixed-methods survey study. *BMC Sports Sci Med Rehabil,* 9**,** 6.

DENG, Z. D., LISANBY, S. H. & PETERCHEV, A. V. 2014. Coil design considerations for deep transcranial magnetic stimulation. *Clin Neurophysiol,* 125**,** 1202-12.

DI LAZZARO, V. & ZIEMANN, U. 2013. The contribution of transcranial magnetic stimulation in the functional evaluation of microcircuits in human motor cortex. *Front Neural Circuits,* 7**,** 18.

ELKIN-FRANKSTON, S., FRIED, P., RUSHMORE, R. J. & VALERO-CABRE, A. 2011. From qualia to quantia: a system to document and quantify phosphene percepts elicited by non-invasive neurostimulation of the human occipital cortex. *J Neurosci Methods,* 198**,** 149-57.

ELLAWAY, P. H., DAVEY, N. J., MASKILL, D. W., RAWLINSON, S. R., LEWIS, H. S. & ANISSIMOVA, N. P. 1998. Variability in the amplitude of skeletal muscle responses to magnetic stimulation of the motor cortex in man. *Electroencephalogr Clin Neurophysiol,* 109**,** 104-13.

GRAY, H. 1918. Anatomy of the human body, by Henry Gray. 20th ed., thoroughly rev. and re-edited by Warren H. Lewis. Philadelphia: Lea & Febiger.

GRAY, H. 2016. *In:* STANDRING, S. (ed.) *Gray's Anatomy - The anatomical basis of clinical Practice.* 41 ed. London: Elsevier Limited.

GROPPA, S., OLIVIERO, A., EISEN, A., QUARTARONE, A., COHEN, L. G., MALL, V., KAELIN-LANG, A., MIMA, T., ROSSI, S., THICKBROOM, G. W., ROSSINI, P. M., ZIEMANN, U., VALLS-SOLE, J. & SIEBNER, H. R. 2012. A practical guide to diagnostic transcranial magnetic stimulation: report of an IFCN committee. *Clin Neurophysiol,* 123**,** 858-82.

GROSPRETRE, S., PAPAXANTHIS, C. & MARTIN, A. 2014. Modulation of spinal excitability by a sub-threshold stimulation of M1 area during muscle lengthening. *Neuroscience,* 263**,** 60-71.

GRUBER, M., LINNAMO, V., STROJNIK, V., RANTALAINEN, T. & AVELA, J. 2009. Excitability at the motoneuron pool and motor cortex is specifically modulated in lengthening compared to isometric contractions. *J Neurophysiol,* 101**,** 2030-40.

GRUNDEY, J., FREZNOSA, S., KLINKER, F., LANG, N., PAULUS, W. & NITSCHE, M. A. 2013. Cortical excitability in smoking and not smoking individuals with and without nicotine. *Psychopharmacology (Berl),* 229**,** 653-64.

GUISSARD, N. & DUCHATEAU, J. 2004. Effect of static stretch training on neural and mechanical properties of the human plantar-flexor muscles. *Muscle Nerve,* 29**,** 248-55.

GUISSARD, N. & DUCHATEAU, J. 2006. Neural aspects of muscle stretching. *Exerc Sport Sci Rev,* 34**,** 154-8.

GUISSARD, N., DUCHATEAU, J. & HAINAUT, K. 1988. Muscle stretching and motoneuron excitability. *Eur J Appl Physiol Occup Physiol,* 58**,** 47-52.

GUISSARD, N., DUCHATEAU, J. & HAINAUT, K. 2001. Mechanisms of decreased motoneurone excitation during passive muscle stretching. *Exp Brain Res,* 137**,** 163-9.

HAHN, D., HOFFMAN, B. W., CARROLL, T. J. & CRESSWELL, A. G. 2012. Cortical and spinal excitability during and after lengthening contractions of the human plantar flexor muscles performed with maximal voluntary effort. *PLoS One,* 7**,** e49907.

HALLETT, M. 2007. Transcranial magnetic stimulation: a primer. *Neuron,* 55**,** 187-99.

HARTMANN, M., PABST, M.-A. & DOHR, G. 2011. *Zytologie, Histologie und mikroskopische Anatomie - Licht- und elektronenmikroskopischer Bildatlas,* Wien, Facultas Verlags- und Buchhandels AG.

KAMMER, T. & THIELSCHER, A. 2003. Physikalische und physiologische Grundlagen der transkraniellen Magnetstimulation. *Nervenheilkunde,* 22**,** 168-76.

KIERS, L., CROS, D., CHIAPPA, K. H. & FANG, J. 1993. Variability of motor potentials evoked by transcranial magnetic stimulation. *Electroencephalogr Clin Neurophysiol,* 89**,** 415-23.

KLAMPFL, K. & DENGLER, R. 2007. Motoneuronerkrankungen. *Das TMS-Buch - Handbuch der transkranielle Magnetstimulation.* Heidelberg: Springer Medizin Verlag: H.R. Siebner

U. Ziemann.

KOCAK, M., ULMER, J. L., SAHIN UGUREL, M., GAGGL, W. & PROST, R. W. 2009. Motor homunculus: passive mapping in healthy volunteers by using functional MR imaging--initial results. *Radiology,* 251**,** 485-92.

KOHN, A. F., FLOETER, M. K. & HALLETT, M. 1997. Presynaptic inhibition compared with homosynaptic depression as an explanation for soleus H-reflex depression in humans. *Exp Brain Res,* 116**,** 375-80.

KONRAD, A. & TILP, M. 2014. Effects of ballistic stretching training on the properties of human muscle and tendon structures. *J Appl Physiol (1985),* 117**,** 29-35.

KONRAD, P. 2005. The ABC of EMG - A Practical Introduction to Kinesiological Electromyography.

LANG, N., HASAN, A., SUESKE, E., PAULUS, W. & NITSCHE, M. A. 2008. Cortical hypoexcitability in chronic smokers? A transcranial magnetic stimulation study. *Neuropsychopharmacology,* 33**,** 2517-23.

LEHMANN-HORN, F. 2007. Motorische Systeme. *In:* SCHMIDT, R. & LANG, F. (eds.) *Physiologie des Menschen.* 30 ed. Heidelberg: Springer Medizin Verlag.

MAGSTIM. 2018. *Description 110mm Double Cone Coil* [Online]. Available: https://www.magstim.com/product/16/110mm-double-cone-coil [Accessed 20th jan 2018].

MASUGI, Y., OBATA, H., INOUE, D., KAWASHIMA, N. & NAKAZAWA, K. 2017. Neural effects of muscle stretching on the spinal reflexes in multiple lower-limb muscles. *PLoS One,* 12**,** e0180275.

MATSUO, S., SUZUKI, S., IWATA, M., HATANO, G. & NOSAKA, K. 2015. Changes in force and stiffness after static stretching of eccentrically-damaged hamstrings. *Eur J Appl Physiol,* 115**,** 981-91.

MCHUGH, M. P. & COSGRAVE, C. H. 2010. To stretch or not to stretch: the role of stretching in injury prevention and performance. *Scand J Med Sci Sports,* 20**,** 169-81.

MERLETTI, R., RAU, G., DISSELHORST-KLUG, C., STEGEMANN, D.-F. & HÄGG, G. M. *The SENIAM project (Surface ElectroMyoGraphy for the Non-Invasive Assessment of Muscles* [Online]. Available: http://www.seniam.org/ [Accessed 26.11.2017 2017].

MESCHER, A. L. 2016. *Junqueira's Basic Histology - Text and Atlas,* U.S., McGraw Hill Education.

MIYATA, K. & USUDA, S. 2015. Changes in corticospinal excitability with short-duration high-frequency electrical muscle stimulation: a transcranial magnetic stimulation study. *J Phys Ther Sci,* 27**,** 2117-20.

MUANJAI, P., JONES, D. A., MICKEVICIUS, M., SATKUNSKIENE, D., SNIECKUS, A., SKURVYDAS, A. & KAMANDULIS, S. 2017. The acute benefits and risks of passive stretching to the point of pain. *Eur J Appl Physiol*.

MÜLLER, F. & ZIEMANN, U. 2007. Bestimmung der zentralmotorischen Leitungszeit. *In:* SIEBNER, H. & ZIEMANN, U. (eds.) *Das TMS-Buch - Handbuch der transkranielle Magnetstimulation.* Heidelberg: Springer Medizin Verlag.

NAKAMURA, M., IKEZOE, T., TAKENO, Y. & ICHIHASHI, N. 2011. Acute and prolonged effect of static stretching on the passive stiffness of the human gastrocnemius muscle tendon unit in vivo. *J Orthop Res,* 29**,** 1759-63.

NAKAZAWA, K., KAWASHIMA, N. & AKAI, M. 2006. Enhanced stretch reflex excitability of the soleus muscle in persons with incomplete rather than complete chronic spinal cord injury. *Arch Phys Med Rehabil,* 87**,** 71-5.

NARDONE, R., HOLLER, Y., BRIGO, F., ORIOLI, A., TEZZON, F., SCHWENKER, K., CHRISTOVA, M., GOLASZEWSKI, S. & TRINKA, E. 2015. Descending motor pathways and cortical physiology after spinal cord injury assessed by transcranial magnetic stimulation: a systematic review. *Brain Res,* 1619**,** 139-54.

NIELSEN, J. F. 1996. Logarithmic distribution of amplitudes of compound muscle action potentials evoked by transcranial magnetic stimulation. *J Clin Neurophysiol,* 13**,** 423-34.

OPIE, G. M., CIRILLO, J. & SEMMLER, J. G. 2018. Age-related changes in late I-waves influence motor cortex plasticity induction in older adults. *J Physiol*.

OPPLERT, J., GENTY, J. B. & BABAULT, N. 2016. Do Stretch Durations Affect Muscle Mechanical and Neurophysiological Properties? *Int J Sports Med,* 37**,** 673-9.

OVALLE, W. K. & NAHIRNEY, P. C. 2013. *Netter's essential histology,* Philadelphia, Elsevier Saunders.

PEARCEY, G. E., POWER, K. E. & BUTTON, D. C. 2014. Differences in supraspinal and spinal excitability during various force outputs of the biceps brachii in chronic- and non-resistance trained individuals. *PLoS One,* 9**,** e98468.

PHILLIS, J. W., EDSTROM, J. P., KOSTOPOULOS, G. K. & KIRKPATRICK, J. R. 1979. Effects of adenosine and adenine nucleotides on synaptic transmission in the cerebral cortex. *Can J Physiol Pharmacol,* 57**,** 1289-312.

PITCHER, J. B., OGSTON, K. M. & MILES, T. S. 2003. Age and sex differences in human motor cortex input-output characteristics. *J Physiol,* 546**,** 605-13.

POCOCK, G., RICHARDS, C. & RICHARDS, D. 2017. *Human Physiology,* New York, Oxford University Press.

ROBERTSON, C. T., KITANO, K., KOCEJA, D. M. & RILEY, Z. A. 2012. Temporal depression of the soleus H-reflex during passive stretch. *Exp Brain Res,* 219**,** 217-25.

ROBINSON, K. L., MCCOMAS, A. J. & BELANGER, A. Y. 1982. Control of soleus motoneuron excitability during muscle stretch in man. *J Neurol Neurosurg Psychiatry,* 45**,** 699-704.

ROSSI-DURAND, C. 2002. The influence of increased muscle spindle sensitivity on Achilles tendon jerk and H-reflex in relaxed human subjects. *Somatosens Mot Res,* 19**,** 286-95.

ROSSINI, P. M., BARKER, A. T., BERARDELLI, A., CARAMIA, M. D., CARUSO, G., CRACCO, R. Q., DIMITRIJEVIC, M. R., HALLETT, M., KATAYAMA, Y., LUCKING, C. H. & ET AL. 1994. Non-invasive electrical and magnetic stimulation of the brain, spinal cord and roots: basic principles and procedures for routine

clinical application. Report of an IFCN committee. *Electroencephalogr Clin Neurophysiol,* 91**,** 79-92.

ROSSINI, P. M., BURKE, D., CHEN, R., COHEN, L. G., DASKALAKIS, Z., DI IORIO, R., DI LAZZARO, V., FERRERI, F., FITZGERALD, P. B., GEORGE, M. S., HALLETT, M., LEFAUCHEUR, J. P., LANGGUTH, B., MATSUMOTO, H., MINIUSSI, C., NITSCHE, M. A., PASCUAL-LEONE, A., PAULUS, W., ROSSI, S., ROTHWELL, J. C., SIEBNER, H. R., UGAWA, Y., WALSH, V. & ZIEMANN, U. 2015. Non-invasive electrical and magnetic stimulation of the brain, spinal cord, roots and peripheral nerves: Basic principles and procedures for routine clinical and research application. An updated report from an I.F.C.N. Committee. *Clin Neurophysiol,* 126**,** 1071-107.

SCHÜNKE, M., SCHULTE, E., SCHUMACHER, U., VOLL, M. & WESKER, K. 2012. *Prometheus Lernatlas der Anatomie - Allgemeine Anatomie und Bewegungssystem*.

SIEBNER, H. R. & ZIEMANN, U. 2007. Hirnstimulation - Physiologische Grundlagen. *In:* SIEBNER, H. R. & ZIEMANN, U. (eds.) *Das TMS-Buch - Handbuch der transkranielle Magnetstimulation.* Heidelberg: Springer Medizin Verlag.

SILVERTHORN, D. U. 2016. Muscles. *In:* CHURCHMAN, K. (ed.) *Human physiology - an integrated approach.* 7 ed. Harlow: Pearson Education Limited.

SPECTERMAN, M., BHUIYA, A., KUPPUSWAMY, A., STRUTTON, P. H., CATLEY, M. & DAVEY, N. J. 2005. The effect of an energy drink containing glucose and caffeine on human corticospinal excitability. *Physiol Behav,* 83**,** 723-8.

STÖHR, M. & PFISTER, R. 2014. *Klinische Elektromyographie und Neurographie - Lehrbuch und Atlas,* Stuttgart, W. Kohlhammer GmbH.

VALERO-CABRE, A., AMENGUAL, J. L., STENGEL, C., PASCUAL-LEONE, A. & COUBARD, O. A. 2017. Transcranial magnetic stimulation in basic and clinical neuroscience: A comprehensive review of fundamental principles and novel insights. *Neurosci Biobehav Rev,* 83**,** 381-404.

VUJNOVICH, A. L. & DAWSON, N. J. 1994. The effect of therapeutic muscle stretch on neural processing. *J Orthop Sports Phys Ther,* 20**,** 145-53.

WAGNER, T., VALERO-CABRE, A. & PASCUAL-LEONE, A. 2007. Noninvasive human brain stimulation. *Annu Rev Biomed Eng,* 9**,** 527-65.

WALTON, C., KALMAR, J. & CAFARELLI, E. 2003. Caffeine increases spinal excitability in humans. *Muscle Nerve,* 28**,** 359-64.

WASSERMANN, E. M. 2002. Variation in the response to transcranial magnetic brain stimulation in the general population. *Clin Neurophysiol,* 113**,** 1165-71.

WEAVIL, J. C., SIDHU, S. K., MANGUM, T. S., RICHARDSON, R. S. & AMANN, M. 2015. Intensity-dependent alterations in the excitability of cortical and spinal projections to the knee extensors during isometric and locomotor exercise. *Am J Physiol Regul Integr Comp Physiol,* 308**,** R998-1007.

WEBER, M. & EISEN, A. A. 2002. Magnetic stimulation of the central and peripheral nervous systems. *Muscle Nerve,* 25**,** 160-75.

WEINER, W. J., GOETZ, C. G. & SHIN, R. K. 2010. *Neurology for the Non-neurologist*, Lippincott Williams & Wilkins.

WEIR, D. E., TINGLEY, J. & ELDER, G. C. 2005. Acute passive stretching alters the mechanical properties of human plantar flexors and the optimal angle for maximal voluntary contraction. *Eur J Appl Physiol,* 93**,** 614-23.

WEYH, T. & SIEBNER, H. R. 2007. Hirnstimulation - technische Grundlagen. *In:* SIEBNER, H. R. & ZIEMANN, U. (eds.) *Das TMS-Buch - Handbuch der transkranielle Magnetstimulation.* Heidelberg: Springer Medizin Verlag Heidelberg.

7 Appendix

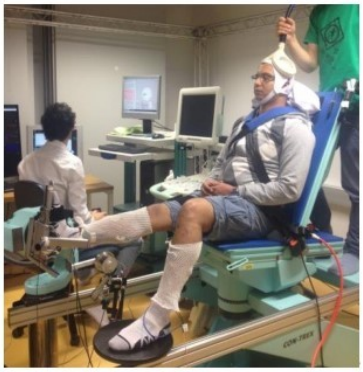
7.1 Recruitment leaflet

ProbandInnen für Stretching-Studie gesucht (Aufwandsentschädigung € 30)!

Ziel des Projekts ist es, die kurzfristigen Effekte der statischen Dehnmethode auf neuromuskuläre Parameter (Muskelaktivität) zu untersuchen.

Diese vom österreichischen Forschungsfonds (FWF) geförderte Studie bietet einen Einblick in modernste wissenschaftliche Messverfahren, die Muskelphysiologie und das Bewegungswissenschaftliche Labor am Institut. Die Aufwandsentschädigung für Ihre Zeit beträgt € 30.

Alle Messverfahren (Elektromyogramm, Transkranielle Magnetstimulation, Elektrostimulation, Dehnreflex) sind nicht-invasiv und gehören zu den Standardverfahren in der Bewegungswissenschaft bzw. Neurophysiologie (siehe Bild). Manche Messungen könnten zu Beginn als unangenehm empfunden werden, sind aber völlig ungefährlich.



Die Messungen werden an 2 Messtagen durchgeführt, einmal mit und einmal ohne Stretching.
Die Eingewöhnungstestung dauert pro Messtag jeweils ca. 20-30 Minuten. Für die Messung der neuromuskulären Parameter werden jeweils ca. 2,5 Stunden benötigt. Die zwei Messungen sind so geplant, dass alle Messungen innerhalb einer Woche abgeschlossen sind.

Bei Interesse bzw. weiteren Fragen bitte ich Sie, eine der untenstehenden Personen zu kontaktieren:

Daniela Kemper
Email: daniela.kemper@stud.medunigraz.at

Dr. Francesco Budini (English only)
Tel: +43-316-380 2333
Email: francesco.budini@uni-graz.at

7.2 TMS questionnaire

TMS-Abklärung

Nur von VL auszufüllen!

Kennzahl: ..... Datum: ..... VL: .....

Bitte ausfüllen!

Name in Druckbuchstaben .....

Geburtsdatum: ..... männlich ☐ weiblich ☐

Ich hatte:

Schädelverletzung	ja ☐	nein ☐
Gehirnerschütterung	ja ☐	nein ☐
Augenverletzung an der Metallsplitter beteiligt waren	ja ☐	nein ☐
Sonstige Verletzung an der Metallsplitter beteiligt waren	ja ☐	nein ☐
Rückenmark Operation	ja ☐	nein ☐
Synkope (kurzzeitige Ohnmacht)	ja ☐	nein ☐

Ich habe:

Epilepsieerkrankung	ja ☐	nein ☐
Epilepsieerkrankung in der Familie	ja ☐	nein ☐
Aneurysma Clip	ja ☐	nein ☐
Herzschrittmacher	ja ☐	nein ☐
Neurostimulator	ja ☐	nein ☐
Cochlear Implantat	ja ☐	nein ☐
Metallische Implantate im Körper	ja ☐	nein ☐
Hörgerät	ja ☐	nein ☐
Zahnspange, Retainer	ja ☐	nein ☐
Tätowierung, Permanentes Make-up	ja ☐	nein ☐
Metallische Platte, Nägel etc. im Körper	ja ☐	nein ☐

Ich bin:

schwanger	ja ☐	nein ☐

Sonstiges: Medikamente: ja ☐..... nein ☐

Ich habe bereits an einer: TMS Studie teilgenommen	ja ☐	nein ☐
MRI Studie teilgenommen	ja ☐	nein ☐

7.3 Informed consent

Einverständniserklärung

Studie: Effekte statischen Dehnens auf die neuromuskulären Eigenschaften des Muskel-Sehnen-Apparates

Testleiter: Univ. Prof. Mag. Dr. Markus Tilp

Diese Einverständniserklärung soll Ihnen einen Einblick in diese Studie geben und Sie darüber aufklären, was Sie erwarten wird. Wenn Sie Fragen haben oder weiteres über diese Studie erfahren möchten, können Sie sich gerne an das Testpersonal wenden. Bitte nehmen Sie sich ausreichend Zeit, um diesen Text genau durchzulesen.

Ziel der Studie:
Das Ziel der Studie besteht darin, die kurzfristigen Effekte statischen Dehnens zu überprüft. Im speziellen werden Veränderungen der mechanisch-morphologischen Eigenschaften des Muskel-Sehnen-Apparates und neuromuskuläre Parameter der Wadenmuskulatur untersucht.

Ablauf der Studie:
Für die Messungen werden Sie gebeten, sich auf ein Dynamometer zu setzen. Ihren rechten Fuß legen Sie in der Fußhalterung des Gerätes ab. Auf Ihrem rechten Unterschenkel werden Elektromyografie(EMG)-elektroden zur Überwachung der muskulären Aktivität angebracht.
Über ihrem Kopf wird eine Spule zur transkraniellen Magnetstimulation (TMS) platziert, über die die Wadenmuskulatur innerviert wird. Über die EMG-Elektroden wird die Stimulationsantwort des Muskels gemessen, um die zentralnervösen Eigenschaften zu bestimmen. Im Anschluss wird der Wadenmuskel direkt am Nerv elektrisch stimuliert, um über die verschiedene Parameter (M-wave, H-reflex und F-wave) Informationen über die Eigenschaften des peripheren Nervensystems zu erlangen. Ein Reflexhammer wird verwendet, um Reflexmessungen durchzuführen. Die 3 Versuchsanordnungen (TMS, H-Reflex und Hammerreflex) werden in 3 Blöcken hintereinander durchgeführt mit einer Kontrollmessung davor und jeweils 20 Minuten Pause dazwischen. Dadurch ergibt sich eine Versuchsdauer von ca. 2 Stunden und 30 Minuten. Innerhalb der Blöcke findet eine fünfminütige Dehnintervention statt. Diese wird passiv durch das Dynamometer durchgeführt. Nach der Dehnintervention werden die Messungen 3x wiederholt: direkt danach, 5 und 10 Minuten danach. An einem weiteren Tag wird das gesamte Experiment wiederholt, allerdings findet keine Dehnintervention statt, sondern 5 Minuten Pause als Kontrolle. Der Gesamtaufwand für Sie beträgt somit ca. 5 Stunden verteilt auf 2 Tage.
Nach Ablauf aller Messungen erhalten Sie am 2. Testtag 30 Euro Entschädigung für den Zeitaufwand.

Unannehmlichkeiten und Risiken:
Während der Messungen sind keine Unannehmlichkeiten oder Risiken zu erwarten. Unter Umständen kann es zu leichten Ermüdungserscheinungen des Testbeines kommen. Selten wird die Untersuchung als unangenehm empfunden. Sollten Sie sich

während der Messung unsicher fühlen, bitten wir Sie, dies sofort dem Testpersonal mitzuteilen. Bitte bleiben Sie nach der Untersuchung noch eine halbe Stunde in der Nähe des Labors, um sich von der Testung vollständig zu erholen.
In dieser Studie kommt die transkranielle Magnetstimulation (TMS) zur Anwendung. Durch Applikation einzelner Stromimpulse über dem motorischen Hirnareal kommt es dabei zu kurzzeitigen lokalen Nervenerregungen, die mit messbaren Muskel-antworten assoziiert sind. Die TMS ist eine nicht-invasive Untersuchungstechnik die als sicher und nebenwirkungsfrei gilt. Bis auf eventuelle leichte, vorübergehende Kopfschmerzen, sind für die Teilnehmer dabei aber keine gesundheitlichen Risiken, Beschwerden und Begleiterscheinungen zu erwarten. Aus Sicherheitsgründen gelten dabei folgende Ausschlusskriterien:

- neurologische und psychische/psychiatrische Erkrankungen (insb. Epilepsie)
- Metallimplantate im Kopf (außer Zahnersatz)
- elektronische Implantate (Herzschrittmacher, Kochlearimplantat)
- Schwangerschaft

Datenschutz:
Ihre persönlichen Testergebnisse obliegen strenger Geheimhaltung und werden nur Ihnen und den bearbeitenden Wissenschaftlern bereitgestellt. Bei einer Veröffentlichung der Studie kann es durch ein spezielles Kodierungsverfahren zu keiner Identifizierung von einzelnen Probanden kommen.

Freiwilligkeit:
Alle Testteilnehmer nehmen an der Studie freiwillig teil und haben das Recht jederzeit die Teilnahme ohne Begründung zu widerrufen.

Ihre Unterschrift bestätigt, dass Sie die Inhalte der Einverständniserklärung verstanden haben.

Sollte es zu weiteren Fragen, Anregungen oder Beschwerden kommen wenden Sie sich bitte an den Testleiter Univ.Prof. Mag. Dr. Markus Tilp +43 316/ 380 8332 oder markus.tilp@uni-graz.at

Falls Sie Fragen über Ihre Rechte als möglicher Testteilnehmer dieser Studie haben, wenden Sie sich bitte an die Ethikkommission der Universität Graz +43 316/380 oder ethikkommission@uni-graz.at

Unterschrift Proband	Datum
Unterschrift Testleiter	Datum
Unterschrift Zeuge	Datum

7.4 Detailed participant information

Table 4: detailed participant information (No: Number, g: gender, MD: maximal dorsiflexion, m: male, f: female, MV: Mean value, SD: standard deviation, Min: Minimum, Max: Maximum)

No	g	age	MD	weight (kg)	height (m)
1	m	23	-25.2	83	1.83
2	m	22	-21.8	64	1.83
3	m	22	-37.9	83	1.82
4	m	23	-18.6	77	1.81
5	m	20	-27.7	82	1.9
6	m	28	-29.4	94	1.83
7	m	27	-36.9	95	1.95
8	m	21	-30.9	80	1.86
9	f	22	-22.9	54	1.67
10	f	22	-32.5	60	1.6
11	f	25	-23.4	60	1.59
12	f	22	-31.7	46	1.7
13	f	20	-32.1	60	1.67
14	f	20	-37.9	56	1.65
15	f	23	-30.9	58	1.6
16	f	25	-39.7	66	1.62
17	f	22	-28.3	50	1.65
MV		22.8	-29.9	68.7	1.74
SD		2.3	6.1	15.3	0.12
Min		20	-39.7	46	1.59
Max		28	-18.6	95	1.95

7.5 rMT of the individual participants

Table 5: individual values of the participants on the day of stretching and control condition in % of the stimulator output (MV: Mean value; SD: standard deviation; Min: Minimum; Max: Maximum)

No	Stretch	Control
1	42	39
2	38	41
3	48	46
4	29	27
5	40	42
6	30	32
7	32	37
8	31	31
9	41	40
10	45	45
11	45	39
12	40	40
13	54	57
14	36	36
15	42	40
16	39	38
17	48	46
MV	40.00	39.76
SD	6.94	6.78
Min	29	27
Max	54	57

7.6 M_{max} detailed values

Table 6: individual values of the participants on the day of stretching and control condition in mV (MV: Mean value; SD: standard deviation)

No	Stretch	Control
1	28.31	27.49
2	26.94	32.53
3	17.54	19.42
4	31.69	28.45
5	19.93	5.35
6	35.13	23.28
7	28.62	29.61
8	27.07	31.02
9	21.80	21.10
10	23.89	22.61
11	22.79	22.64
12	36.17	34.11
13	19.34	17.42
14	30.42	24.34
15	34.40	35.51
16	21.80	24.79
17	29.43	27.22
MV	26.78	25.11
SD	5.74	7.23
Min	17.54	5.35
Max	36.17	35.51

7.7 SPSS Outputs

7.7.1 T-reflex amplitudes

Table 7: SPSS output: Shapiro-Wilk test of T-reflex amplitudes on the two testing conditions (S: Strech, C: Control) and 4 time points (Pre, Post0, Post5, Post10)

Tests auf Normalverteilung

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistik	df	Signifikanz	Statistik	df	Signifikanz
Pre_C	,326	9	,007	,746	9	,005
Post_00_C	,318	9	,009	,728	9	,003
Post_05_C	,345	9	,003	,770	9	,009
Post_10_C	,330	9	,005	,790	9	,016
Pre_S	,289	9	,030	,865	9	,110
Pre_00_S	,215	9	,200*	,942	9	,605
Pre_05_S	,272	9	,054	,906	9	,288
Pre_10_S	,250	9	,109	,877	9	,147

*. Dies ist eine untere Grenze der echten Signifikanz.

a. Signifikanzkorrektur nach Lilliefors

Table 8: SPSS output: non-parametric Friedman ANOVA

Statistik für Test[a]

N	9
Chi-Quadrat	23,852
df	7
Asymptotische Signifikanz	,001

a. Friedman-Test

Table 9: SPSS output: Wilcoxon test pair wise without the Bonferroni Holm correction

Statistik für Test[a]

	Pre_S - Pre_C	Post_00_S - Post_00_C	Post_05_S - Post_05_C	Post_10_S - Post_10_C	Post_00_ S - Pre_S	Post_05_ S - Pre_S	Post_10_ S - Pre_S	Post_05_S - Post_00_S	Post_10_S - Post_00_S
Z	-,357[b]	-2,197[c]	-,533[b]	-1,988[b]	-3,059[c]	-2,510[c]	-,706[c]	-2,903[b]	-3,059[b]
Asymptotische Signifikanz (2-seitig)	,721	,028	,594	,047	,002	,012	,480	,004	,002

a. Wilcoxon-Test

b. Basiert auf negativen Rängen.

c. Basiert auf positiven Rängen.

7.7.2 T-reflex delta variation

Table 10: SPSS output: Shapiro-Wilk test of T-reflex delta variations on the two testing conditions (S: Strech, C: Control)

Tests auf Normalverteilung

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistik	df	Signifikanz	Statistik	df	Signifikanz
Pre_Post00_C	,194	9	,200*	,897	9	,236
Pre_Post05_C	,256	9	,092	,851	9	,076
Pre_Post10_C	,244	9	,131	,745	9	,005
Pre_Post00_S	,170	9	,200*	,949	9	,674
Pre_Post05_S	,316	9	,010	,718	9	,002
Pre_Post10_S	,252	9	,104	,832	9	,047

*. Dies ist eine untere Grenze der echten Signifikanz.

a. Signifikanzkorrektur nach Lilliefors

Table 11: SPSS output: non-parametric Friedman ANOVA of T-reflex delta variations

Statistik für Test[a]

N	9
Chi-Quadrat	19,159
df	5
Asymptotische Signifikanz	,002

a. Friedman-Test

Table 12: SPSS output: Wilcoxon test pair wise without the Bonferroni Holm correction

Statistik für Test[a]

	Pre_Post00_S - Pre_Post00_C	Pre_Post05_S - Pre_Post05_C	Pre_Post10_S - Pre_Post10_C	Pre_Post05_S - Pre_Post00_S	Pre_Post10_S - Pre_Post00_S	Pre_Post10_S - Pre_Post05_S
Z	-2,803[b]	-,059[b]	-,059[b]	-2,903[c]	-3,059[c]	-,706[c]
Asymptotische Signifikanz (2-seitig)	,005	,953	,953	,004	,002	,480

a. Wilcoxon-Test

b. Basiert auf positiven Rängen.

c. Basiert auf negativen Rängen.

7.7.3 H/M ratio

Table 13: SPSS output: Shapiro-Wilk test of the different time points of H/M_{max} ratio

Tests auf Normalverteilung

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistik	df	Signifikanz	Statistik	df	Signifikanz
Pre_C	,342	16	,000	,702	16	,000
Post_00_C	,327	16	,000	,700	16	,000
Post_05_C	,284	16	,001	,719	16	,000
Post_10_C	,277	16	,002	,725	16	,000
Pre_S	,258	16	,005	,730	16	,000
Post_00_S	,273	16	,002	,743	16	,001
Post_05_S	,208	16	,063	,836	16	,009
Post_10_S	,270	16	,003	,731	16	,000

a. Signifikanzkorrektur nach Lilliefors

Table 14: SPSS output: non-parametric Friedman ANOVA

Statistik für Test[a]

N	16
Chi-Quadrat	7,792
df	7
Asymptotische Signifikanz	,351

a. Friedman-Test

7.7.4 H/M ratio delta variation

Table 15: SPSS output: Shapiro-Wilk test of H/M ratio delta variations on the two testing conditions (S: Strech, C: Control)

Tests auf Normalverteilung

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistik	df	Signifikanz	Statistik	df	Signifikanz
Pre_Post00_C	,144	16	,200*	,942	16	,368
Pre_Post05_C	,274	16	,002	,796	16	,002
Pre_Post10_C	,166	16	,200*	,875	16	,033
Pre_Post00_S	,212	16	,053	,918	16	,159
Pre_Post05_S	,117	16	,200*	,960	16	,668
Pre_Post10_S	,197	16	,097	,897	16	,073

*. Dies ist eine untere Grenze der echten Signifikanz.

a. Signifikanzkorrektur nach Lilliefors

Table 16: SPSS output: non-parametric Friedman ANOVA

Statistik für Test[a]

N	16
Chi-Quadrat	6,786
df	5
Asymptotische Signifikanz	,237

a. Friedman-Test

7.7.5 rMT

Table 17: SPSS output: Shapiro-Wilk test of resting motor thresholds (rMT) on the two testing conditions (S: Strech, C: Control)

Tests auf Normalverteilung

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistik	df	Signifikanz	Statistik	df	Signifikanz
rMT_Stretch	,111	17	,200*	,966	17	,752
rMT_Control	,135	17	,200*	,945	17	,384

*. Dies ist eine untere Grenze der echten Signifikanz.

a. Signifikanzkorrektur nach Lilliefors

Table 18: SPSS output: t-test of the control condition (C) vs stretching condition (S): resting motor threshold (rMT)

Test bei gepaarten Stichproben

		Gepaarte Differenzen					T	df	Sig. (2-seitig)
		Mittelwert	Standardabweichung	Standardfehler des Mittelwertes	95% Konfidenzintervall der Differenz				
					Untere	Obere			
Paaren 1	rMT_Stretch - rMT_Control	,23529	2,65823	,64472	-1,13144	1,60203	,365	16	,720

7.7.6 M_{max}

Table 19: SPSS output: Shapiro-Wilk test of Control and Stretching Condition of the M_{max}

Tests auf Normalverteilung

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistik	df	Signifikanz	Statistik	df	Signifikanz
Control	,116	16	,200*	,976	16	,928
Stretch	,106	16	,200*	,967	16	,785

*. Dies ist eine untere Grenze der echten Signifikanz.

a. Signifikanzkorrektur nach Lilliefors

Table 20: SPSS output: T-test M_{max}

Test bei gepaarten Stichproben

		Gepaarte Differenzen					T	df	Sig. (2-seitig)
		Mittelwert	Standardabweichung	Standardfehler des Mittelwertes	95% Konfidenzintervall der Differenz				
					Untere	Obere			
Paaren 1	Control - Stretch	-,86250	4,10819	1,02705	-3,05160	1,32660	-,840	15	,414

7.7.7 MEP peak-to-peak amplitudes

Table 21: SPSS output: Shapiro-Wilk test of Motor evoked potential (MEP) peak-to-peak amplitudes

Tests auf Normalverteilung

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistik	df	Signifikanz	Statistik	df	Signifikanz
Pre_S	,241	12	,053	,843	12	,030
Post_00_S	,144	12	,200*	,968	12	,894
Post_05_S	,210	12	,149	,908	12	,202
Post_10_S	,124	12	,200*	,961	12	,794
Pre_C	,259	12	,026	,823	12	,017
Post_00_C	,144	12	,200*	,920	12	,289
Post_05_C	,126	12	,200*	,954	12	,702
Post_10_C	,176	12	,200*	,939	12	,482

*. Dies ist eine untere Grenze der echten Signifikanz.

a. Signifikanzkorrektur nach Lilliefors

Table 22: SPSS output: Friedman test of Motor evoked potential (MEP) peak-to-peak amplitudes

Statistik für Test[a]

N	12
Chi-Quadrat	5,833
df	7
Asymptotische Signifikanz	,559

a. Friedman-Test

7.7.8 MEPs Normalized to M_{max}

Table 23: SPSS output: Shapiro-Wilk test of normalized motor evoked potentials (MEP): MEP/M_{max} ratio

Tests auf Normalverteilung

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistik	df	Signifikanz	Statistik	df	Signifikanz
Pre_S	,240	12	,055	,855	12	,043
Post_00_S	,130	12	,200*	,956	12	,723
Post_05_S	,160	12	,200*	,921	12	,291
Post_10_S	,159	12	,200*	,916	12	,252
Pre_C	,253	12	,033	,703	12	,001
Post_00_C	,219	12	,115	,772	12	,005
Post_05_C	,195	12	,200*	,915	12	,248
Post_10_C	,211	12	,146	,904	12	,177

*. Dies ist eine untere Grenze der echten Signifikanz.

a. Signifikanzkorrektur nach Lilliefors

Table 24: SPSS output: Friedman test of normalized motor evoked potentials (MEP): MEP/M_{max} ratioMEP latency

Statistik für Test[a]

N	12
Chi-Quadrat	5,444
df	7
Asymptotische Signifikanz	,606

a. Friedman-Test

7.7.9 MEP latency

Table 25: SPSS output: Shapiro-Wilk test of data of MEP latency

Tests auf Normalverteilung

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistik	df	Signifikanz	Statistik	df	Signifikanz
Pre_S	,174	17	,183	,929	17	,209
Post_00_S	,176	17	,169	,919	17	,141
Post_05_S	,200	17	,068	,899	17	,066
Post_10_S	,181	17	,143	,919	17	,142
Pre_C	,236	17	,013	,853	17	,012
Post_00_C	,170	17	,200*	,889	17	,044
Post_05_C	,168	17	,200*	,919	17	,144
Post_10_C	,141	17	,200*	,921	17	,156

*. Dies ist eine untere Grenze der echten Signifikanz.

a. Signifikanzkorrektur nach Lilliefors

Table 26: SPSS output: Friedman test of data of MEP latency

Statistik für Test[a]

N	17
Chi-Quadrat	10,775
df	7
Asymptotische Signifikanz	,149

a. Friedman-Test